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Habitat preference of three-spined stickleback juveniles in experimental conditions and in wild eelgrass

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Long-term population dynamics of three-spined stickleback Gasterosteus aculeatus *in the White Sea during the 20th century has patterns similar to that of eelgrass* Zostera marina. *In this study we address possible mechanisms of such association through analysis of spatial distribution of juvenile stickleback in the wild and their substrate preferences in experimental conditions. Samples from different habitats (*Z. marina, Fucus *spp.) in 13 localities of Kandalaksha Bay have shown that the juvenile sticklebacks occurred mainly in the eelgrass beds. Their density was significantly lower in fucoids. In the experiments, carried out in August 2008–2009, the juveniles were offered the following types of substrates: brown macroalgae (dense and scarce), eelgrass and control (no substrate). In the experiments sticklebacks also showed a tendency to prefer eelgrass to fucoids, even if the density of the latter was higher. This may explain their higher densities associated with eelgrass in the wild.*

Keywords: behaviour, distribution, fucoids, *Gasterosteus aculeatus*, juveniles, White Sea, *Zostera marina*

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INTRODUCTION

Peripheral populations are usually less numerous than core ones and exhibit a higher variability in population size (Vecutich & Waite, 2003). The subarctic White Sea is a distribution boundary for a number of boreal species. Three-spined stickleback *Gasterosteus aculeatus* L. is a typical boreal species (Wootton, 1984; Zyuganov, 1991) and in the White Sea they show an outstanding variation of population size on the centennial timescale. They were extremely numerous in the 1930s, then drastically declined by the 1960s, and again considerably increased in numbers since the late 1990s. Such a change of abundance coincided with climate changes, which suggests that increased temperature is an important reason for stickleback population growth in the White Sea (Lajus *et al.*, 2013a).

Climate can affect species abundance directly or indirectly. Direct impact of climate change can be shown in a decrease or increase of species when considering growth rates, reproduction success, disease resistance etc. (Portner & Farrell, 2008). The indirect effect of the climate is mediated by interactions of one species with another affected by the climate change (Hansson, 1984; Harrington *et al.*, 1999).

During the reproduction period the White Sea three-spined sticklebacks are associated with eelgrass *Zostera marina* L. for which the White Sea is the northernmost distribution limit (Short *et al.*, 2010). Eelgrass show patterns of long-term fluctuations similar to stickleback: they were abundant in the 1930s and 40s, but declined in the early 1960s, when large areas previously covered with *Z. marina* disappeared (Vekhov, 1992). Recovery of eelgrass started in 1966 (Vekhov, 1970), i.e. earlier than that of stickleback. It is suggested that climate-related decline of eelgrass was the main reason for the concurrent decline of stickleback populations (Telegin, 1999). It appears that the stickleback population interacts with eelgrass against a background of the mentioned population fluctuations of both species synchronous with climate changes, which brings a special interest to analysis of this interaction.

Eelgrasses, due to their high biomass and productivity, are known to enhance the marine environment, providing suitable habitats for many marine organisms (Hemminga & Duarte, 2000; Lee *et al.*, 2001). Fish in different stages of their life cycle use eelgrass for various purposes: as spawning grounds, temporary nurseries, feeding areas, permanent habitat or shelter from predation (Kikuchi & Peres, 1977; Jackson *et al.*, 2001; Nakaoka, 2005). Some studies show, for example, a significant role of eelgrass for several fish species such as the pipe fish *Syngnathus leptorhynchus*, the crescent gunnels *Pholis laeta*, and the shiner perch, *Cymatogaster aggregata* (Murphy *et al.*, 2000). In the White Sea we have observed more intensive stickleback spawning in the eelgrass beds than in the other habitats (Lajus *et al.*, 2013b).

At the same time, our recent study in Kandalaksha Bay of the White Sea has shown a relatively high abundance of the stickleback spawners during the breeding season not only in the eelgrass beds, but also in brown macroalgae (*Fucus* spp. and *Ascophylium nodosum*) and in small littoral pools with green algae (Lajus *et al.*, 2011, Ivanova *et al.*, 2016 in press). Since many adults are found in these habitats, we might assume that the fish may also spawn there. Larvae however are found mostly in eelgrass, as our preliminary results showed (Shatskikh *et al.*, 2010). According to the literature,

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juveniles start to organize shoals and undertake active migrations ~3 weeks after hatching (Gomeliuk, 1976). This may result in different patterns of distribution compared with adults.

The main goal of this work is to analyse the spatial distribution of the juvenile three-spined stickleback in relation to different types of aquatic vegetation in the wild, and to study their preference for different types of substrate in experimental conditions.

The following hypotheses have been tested:

1) Stickleback juveniles are associated with eelgrass beds. To support this, presence and abundance of juveniles were monitored during several field seasons in eelgrass beds and alternative habitats of the Keret' Archipelago.
2) Stickleback juveniles actively prefer eelgrass over the fucoids. To challenge this we have performed experiments with different substrates such as eelgrass and fucoids of different density. Prior to performing the experiments we addressed several methodological questions:
   (i) Does the juvenile distribution depend on position of substrates in the experimental tank?
   (ii) What time is required for stabilization of juvenile distribution?
   (iii) Does the distribution differ in the light and dark?
   (iv) Does the habitat where the fish were originally caught influence their preferences for a certain type of substrate in the experimental tank?

MATERIALS AND METHODS

Material collection and storage

Material for this study was obtained from field sampling in different habitats of Keret' Archipelago, the White Sea. Experiments were carried out at the Marine Biological Station of St. Petersburg State University (Keret' Archipelago, Kandalaksha Bay) (66°17.41′N 33°38.45′E) in August 2008–2009 (Shatskikh *et al.*, 2010).

MEASURING DENSITY OF JUVENILES IN THE WILD

Field samples were collected in August 2008–2014 every 10 days with a small beach seine (7.5 m in length, 1.5 m in height, and 120 m² catchment area) from low tide to semi-range of tide. A seine was used with a wing mesh of 5 mm, the mesh of the seine's centre was 3, and 1 mm in a purse. The catching efficiency (the ratio of fish caught related to the total number of fish in the catching area) of the gear was accepted as 0.6 (T. Ivanova, M. Ivanov, D. Lajus, unpublished data) and assumed to be equal in different habitats.

Samples were collected from 13 sites with different types of vegetation: beds of eelgrass *Z. marina* of various density; fucoids *Fucus* sp. and *A. nodosum*; and mixed eelgrass and brown algae (Figure 1 and Table 1). Maximal depth at a 30 m distance from the shoreline in sampling sites was about 2–3 m, it was somewhat deeper in sites dominated by fucoids. In all sites, sublittoral vegetation was quite abundant, although during low tide fucoids and, to a lesser extent, eelgrass, were dried. Vegetation, other fish species and three-spined stickleback adults were removed from each catch, and only juveniles of the three-spined stickleback were left for further analysis. The total volume of each sample was measured, and one or two subsamples of 0.05 l each were taken from the initial sample. In the case when the total volume of the sample was less than 0.05 l, all juveniles were taken for analysis. Density of stickleback within the 30 m-wide inshore zone was estimated in two ways:

- Individuals per square metre: $D_1 = N \times S^{-1} \times CE^{-1}$.
- Individuals per kilometre of shoreline: $D_2 = N \times L^{-1} \times CE^{-1} \times 1000$ m.

where N is the number of fish in the total sample (ind), S is catching area (most often 120 m²), L is length of the beach seine (m), CE is the catching efficiency.

The samples were kept at a temperature of −5 to −10°C up to 3 weeks before the analyses.

COLLECTING OF JUVENILES FOR THE EXPERIMENTS

Juveniles for the experiments were caught in three different habitats in the inshore zone: (i) unvegetated area and sparse growth of fucoids right near the Marine Biological Station (designated at Figure 1 with acronym 'MBS') in 2008; (ii) dense eelgrass *Z. marina* beds (site 1, Figure 1) in 2009; (iii) growth of fucoids (site 13, Figure 1) in 2009. Juveniles were kept for a week in the experimental tank or aquaria. The juvenile total length ranged from 6.0 to 22.8 mm (mean and

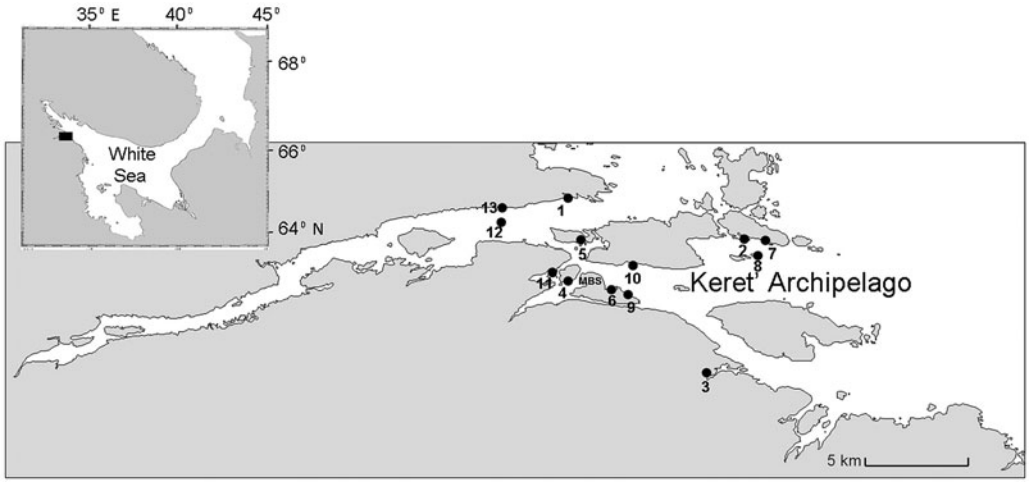

Fig. 1. Sampling area in Keret' Archipelago.

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Table 1. Characteristics of sampling sites and density of *Gasterosteus aculeatus* juveniles in Keret' Archipelago in mid-August. Dash '—' designates absence of data.

Habitat	No of site at Figure 1	Biomass of vegetation, kg m^{-2}	Density of juvenile stickleback, ind m^{-2}		
			2008	2009	2014
Eelgrass	1	>5	171.3	29.4	218.7
Eelgrass	2	3.5	15.7	37.0	156.1
Eelgrass	3	2.0	–	–	6.3
Eelgrass	4	1.0	17.2	5.7	–
Eelgrass	5	0.5	–	–	42.7
Eelgrass	6	1.0	0.3	–	101.1
Fucoids	7	–	–	0.6	–
Fucoids	8	–	0	0.75	0
Fucoids	9	–	0	–	–
Fucoids	10	–	0	1.35	0.15
Fucoids	11	–	–	–	8.1
Fucoids	12	–	–	7.0	–
Fucoids	13	–	–	9.9	0.15

standard error, 11.5 ± 0.03 mm, N = 4028) in 2008, and from 7.9 to 25 mm (15.6 ± 0.04 mm, N = 2626) in 2009.

Research facility and performing experiments

In the experiments we studied distribution of juveniles in different substrates. The study was achieved through a series of three preparatory experiments.

The following substrates were used: Z – eelgrass (*Z. marina*) with 70% ground vegetation cover, which corresponded to natural eelgrass biomass of 2100 g m^{-2}; F1 – brown algae (*F. vesiculosus*), with 70% ground vegetation cover, which corresponded to biomass of 800 g m^{-2}, this type of substrate was used due to the fact that the plant cover of fucoids and eelgrass differ in nature; to evaluate the influence on juvenile preference with bed cover regardless of the type of vegetation, a sparse cover of fucus was used, which approximately corresponded to natural eelgrass beds in terms of ground cover; F2 – brown algae (*F. vesiculosus*), with 100% ground vegetation cover which corresponded to natural *F. vesiculosus* biomass of 3700 g m^{-2}; E – empty cuvette – with no substrates, ground vegetation cover 0%.

All experiments were carried out in a fibreglass tank 450 × 70 × 50 cm^3 (Figure 2). The bottom of the tank was covered with a thin layer of sand. The tank was separated into two parts with a wooden frame with a gauze warren to avoid moving the juveniles from one half of the tank to another. This allowed us to conduct two experiments at the same time in identical conditions. The tank was positioned outdoors in the shade. Running seawater came to one part of the tank and flowed out from the other side (Figure 2). The seawater was obtained from a depth of 8 m. The water temperature and salinity were monitored on a daily basis. The temperature during experiments ranged from +11.4 to +16.0°C in 2008 and from 10.0 to 12.3°C in 2009. Experiments which were compared directly to each other were carried out at temperatures which differed by not more than 2°C. Salinity ranged from 22 to 24 ppt, and was similar to that in the sea during the summer.

GENERAL EXPERIMENTAL DESIGN

Substrates were put into 4–12 cuvettes of 27 × 35 cm. The cuvettes were set up in the tank at the gauze warren with the entrance, made of a metal frame of 27.5 × 35.5 cm with a height of 60 cm. The cuvette was set so that when the cage frame was raised above the water level, the cuvette and juveniles from adjacent area were inside the cage. At a set time, the cages were simultaneously raised. Fish were removed from the cage and placed in a bowl with sea water for further photographing. Then they were returned to the experimental tank. Fish were counted and measured using digital imaging and Image Tool software. These operations were replicated two or three times (depending on the type of the experiment). Four series of the experiments were conducted.

In the experimental tank we created a juvenile density similar to that observed in the wild. In 2008, the density of juveniles in the inshore zone in August was, on average, 1020 ind m^{-2}; in the corresponding experiment, juvenile density was 750 ind m^{-2}. In 2009, juvenile density in eelgrass beds varied from 120 to 200 ind m^{-2}; in the experiment it was set at 160 ind m^{-2}.

The mortality of juveniles during the experiment was 0.4–4.0% a day. In all series of the experiments, except for series 1, the number of juveniles was kept constant by adding an adequate number of live fish to replace the dead at the end of the day. In 2008 (series 1) juvenile mortality was, on average, 4% a day. In this experiment live fish were not added. Instead, a correction factor was used equal to the mean number of juveniles to their number at a particular

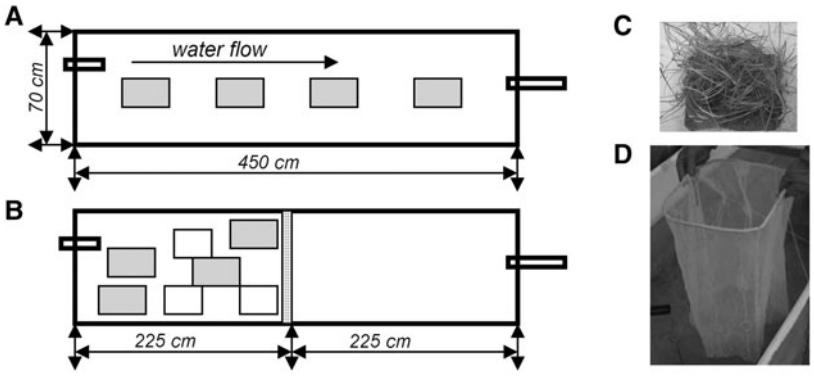

Fig. 2. Scheme of experiments: (A) setting up substrates for series 1, and (B) for series 2–4, (C) cuvette with eelgrass, (D) the lifted gauze cage, cuvette with substrate is inside.

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moment of the time. All the numbers of juveniles obtained were multiplied based on this correction factor.

Series 1. Does the juvenile distribution depend on position of substrates in the experimental tank?
This experiment tested whether the distribution of juveniles was related to the position of the substrate in the tank. In a case of absence of such association, we would need further experiments with different combinations of shelter positions. The experiments were conducted from 11–18 August 2008. Four cuvettes were placed into the experimental tank. Two of them contained planted fucoid (F2), and the other two remained empty (E). The position of the cuvettes was changed (numbers 1–4 along the direction of water flow). Each experiment was performed in triplicate. Juvenile substrate preference could depend on the position of the cuvette relative to the place of water inflow to the tank, the degree of proximity to the edges of the tank, different illumination of various parts of the tank, and other factors. The duration of the experiments was 15 h (from 21:00 to 12:00) or 7 h (from 13:00 to 20:00).

Series 2. What time is required for stabilization of juvenile distribution in the shelters?
In this experiment we determined how the distribution of juveniles changed in the tank depending on the duration of the experiment, with the aim to determine the minimal time sufficient for the experiment. A series of experiments was conducted on 11–12 August 2009 with four cuvettes with eelgrass (Z). After the cages were installed in the tank, the water in it was gently stirred to achieve a uniform spread of the fish, and left for a certain time period, after which the cages were raised. The duration of experiments were 10 min, 30 min, 1, 3, 5 and 9 h in series on 11 August and 10 min, 3 and 5 h on 12 August.

Series 3. Does the distribution differ in light and dark times of the day?
In this series we studied distribution of juveniles in the shelters depending on the time of day. Two experiments were conducted on 11–12 and 21–22 August 2009, each lasting for 24 h, terminating at 00:00, 4:00, 08:00, 12:00, 16:00 and 20:00. In August in the White Sea region darkness persists from 21:00 in the evening to 5:00 in the morning. Four cuvettes with the same substrate – eelgrass (Z) were used.

Series 4. Do juvenile stickleback actively prefer eelgrass to fucoids?
The main purpose of this series was to determine the preferable type of substrates for stickleback juveniles. Two experiments were conducted on 14–15 and 22–23 August 2009. The total exposure time in each experiment was 48 h. We tested substrate preference four times, with two experiments starting from 0.00 to 2.00, and finishing at 14.00, and two others starting from 14.00 to 16.00 and finishing at 0.00.

The average length of juveniles in the first series was 16.0 ± 0.33 and in the second series it was 16.7 ± 0.31 mm. Three types of substrates were offered: eelgrass (Z), fucus with different ground vegetation cover (F1 and F2, see above) and three empty cuvettes (control). Each half of the tank had nine cuvettes, and each of the three types of substrate was placed in two cuvettes. In addition, three empty cuvettes for reference were placed. We measured number of juveniles in two cuvettes with the same substrate. Therefore, we counted juveniles in 16 cuvettes with three types of substrate (eelgrass, dense and spare fucoids) from two habitats of origin (eelgrass and fucoids), 96 cuvettes in total.

In this series we also addressed the question of whether the habitat where the fishes were caught influences the preferences under experimental conditions. Two halves of the tank contained juveniles caught in a thicket of eelgrass or in thickets of fucoids. The goal was to study whether juveniles from different habitats have different substrate preferences.

Length measurements

Juveniles were placed into a pan and photographed with a scale-size indicator. The body length and number of juveniles were measured using the free UTHSCSA Image Tool program, available at ftp://maxrad6.uthscsa.edu. In different situations live or dead fish were used, for which the length was measured in different ways because in living fish only the total length including the tail fin (L_2, mm) could be measured, whereas in the dead fish length from the tip of the snout up to beginning of the caudal fin (L_1, mm) was measured. To intercalibrate the measurements, we performed measurements of both lengths on 65 individuals, and based on those data obtained the following empirical equation ($R^2 = 0.92$), which was used in further analyses:

$$L_1 = 0.901 \times (L_2) - 0.229.$$

Statistical analysis

All statistical testing used STATISTICA software. To compare density of juveniles in different sites during 3 years we used two-way ANOVA, using habitat type and year as factors. We analysed experimental data by two-way and three-way ANOVA with significance level 0.05. In each case the mean and standard error are provided.

RESULTS

Spatial distribution and population dynamics

Usually the hatchlings of the three-spined stickleback first appear in the second half of July, with the mass hatching taking place in early August. As shown in Table 2, the typical pattern is decrease of juvenile density during August and most of the juveniles left the coastal zone during September. So the number of juveniles in the site 1 (Figure 1) with dense eelgrass beds dropped by the end of August by 4 times in 2007, 77 times in 2008, 26 times in 2010, and 6.2 times in 2011 and 2012. However, there were days when the number of juveniles in mid-August was lower than at the end of the month, which is likely caused by different weather conditions. In fucoids, the number of juveniles during August decreased more profoundly, for instance, at station 13 the number of juveniles in 2009 dropped by 115 times, in 2010 by 8 times, and in 2014 by 2100 times.

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Table 2. Density of juveniles *Gasterosteus aculeatus* (ind m^{-2}) during August in two monitoring sites with different habitats in Keret' Archipelago.

Site	Date	2007	2008	2009	2010	2011	2012	2013	2014
Eelgrass (1)	01.08			183		15.6	46.5		134
	08.08	51	3177	302	162	4	43.5		
	16.08	45	718	30					
	24.08		171	167	79	7	28.5		218.7
	28.08	10.5	41			0.6	7.5		
Brown algae (13)	01.08								316
	08.08			69	8.6			2.5	
	16.08			9.9					
	24.08			0.6	1.1				0.15

Distribution of juveniles during August showed high spatial heterogeneity (Figure 1, Table 1). The maximum number of juveniles was found in the densest eelgrass beds (site 1); while in the more sparse beds (site 9) the number of juveniles was the lowest. In fucoids, juveniles were either entirely absent, or were very rare (densities were 0.15–9.9 ind m^{-2}). Juveniles abundance was higher in eelgrass than in brown algae every year. Two-way ANOVA, where the factors were the 'year' (2008, 2009, 2014) and 'habitat' (eelgrass, fucoids) showed that the first factor did not cause significant effect $F_{2,18} = 1.25$, $P > 0.05$ but the second factor had a significant influence $F_{1,18} = 7.0$, $P < 0.01$, indicating that juveniles prefer eelgrass.

It should be noted that visual observations of juveniles from the boat in the study area also confirmed preference of juvenile sticklebacks to eelgrass beds, even if they were very small in size.

Experimental studies

SERIES 1. TESTING THE HYPOTHESIS `THE JUVENILE DISTRIBUTION DEPENDS ON SUBSTRATES´

Analysis shows that the position of the cuvette does not play a role, as two-way ANOVA, where the factors were the 'position of the cuvette' and 'substrate type' showed that the first factor effect is not significant ($F_{3,40} = 2.1$, $P > 0.05$), and the second factor had a significant influence ($F_{1,40} = 30.1$, $P < 0.0001$), indicating that juveniles prefer fucoids over empty cells (Figure 3).

Taking into account the results of this experiment, in future, different substrates will be placed in the tank randomly, assuming that the distribution of juveniles is not associated with the position of the shelter, but depends on its type.

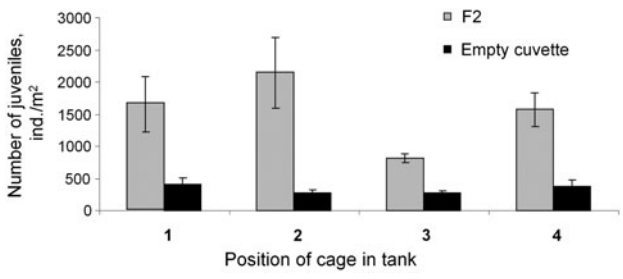

Fig. 3. Density of three-spined stickleback juveniles in empty cages and in cages with fucus (F2) in the experimental tank (means and standard errors are shown).

SERIES 2. TESTING THE HYPOTHESIS `REQUIRED TIME FOR STABILIZATION OF JUVENILE DISTRIBUTION IN SHELTERS´

While determining the minimum sufficient duration of the experiment on different days, we obtained somewhat different results. Thus, if only the results of 12 August (Figure 4) are considered, it can be noted that with an experimental duration from 10 min to 1 h, the number of juveniles in shelters Z was significantly (ANOVA, $F_{6,21} = 7.28$, $P < 0.005$) higher (198 ± 13 ind m^{-2}) than after a lapse of 3 or more hours (73 ± 17 ind m^{-2}). On 11 August, significant differences between experiments of different duration were not observed (ANOVA, $F_{2,9} = 0.71$, $P > 0.05$).

Thus, we concluded that 10 min is enough for juveniles to hide in shelters, and to stabilize their distribution. Subsequently, some of them start to leave the shelter, whereas the pattern has not changed at the 3–9 h experiment mark. Based on this picture and practical convenience of performing experiments, our experiments were carried out for at least 3 h.

SERIES 3. TESTING THE HYPOTHESIS `THE DISTRIBUTION IS DIFFERENT IN LIGHT AND DARK TIMES OF THE DAY´

Analysis of the distribution of juveniles in eelgrass in experiments held on 11–12 and 21–22 August gave differing results. In the first case (11 August), the number of fish in eelgrass was different in the dark (21:00 to 06:00) and light (08:00 to 20:00) hours of the day – 77 ± 11 and 182 ± 8 ind m^{-2}, respectively (the differences were significant, ANOVA, $F_{5,29} = 2.86$, $P < 0.05$). Whereas, 26–32% of the total number of juveniles in the tank occurred in eelgrass at night of 11 August, and around 52–58% in the daytime (Figure 5). In the second series (21 August) the number of juveniles in eelgrass by day and night differed insignificantly (364 ± 55 and 263 ± 8 ind m^{-2}, respectively, ANOVA, $F_{4,25} = 0.5$, $P > 0.05$). In the dark hours in eelgrass there was about 67–69% of the total number of juveniles in the tank, and during daylight hours from 50–80%. The number of juveniles associated with substrates on 21 August was significantly higher than that on 11 August, and the proportion was 71–89% of the total number in the tank. The length of the juveniles that stayed in shelters on 11 and 21 August were similar: 16.5 ± 0.1 and 16.4 ± 0.1 mm, respectively.

Based on the results of this experiment, in the further experiments we decided to stick to the scheme of cage lifting several times a day to consider the possibility of different

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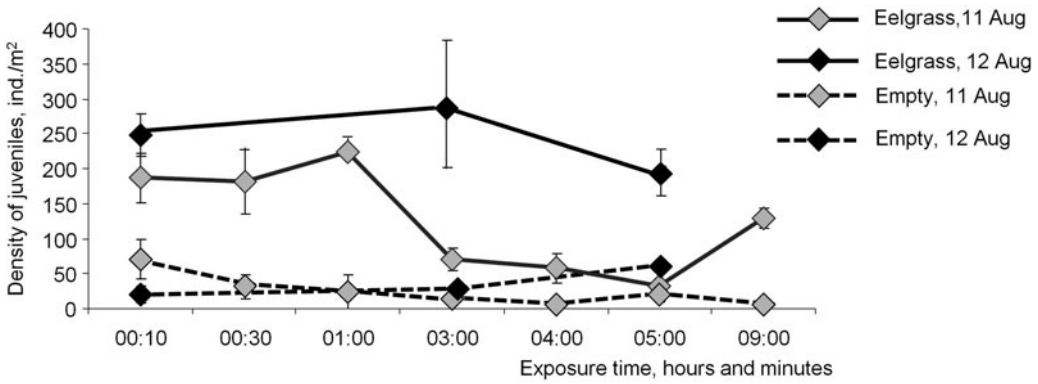

Fig. 4. Density of three-spined stickleback juveniles in eelgrass and empty cuvettes depending on duration of experiments (means and standard errors are shown).

juvenile behaviour in the darkness and daylight. Since the number of juveniles in shelters in different periods of light (i.e. at 08:00 and 20:00) and dark (21:00 to 06:00) did not differ, two experiment options were left, ending at 14:00 and 00:00.

SERIES 4. TESTING THE HYPOTHESIS – `THE JUVENILES OF STICKLEBACK ACTIVELY PREFER EELGRASS OVER FUCOIDS´

One of the goals of this series was to check if the juveniles prefer their 'native' habitat, i.e. if the juveniles caught in fucoids prefer fucoids, and those caught in eelgrass prefer eelgrass. The results showed no difference between number of juveniles from different habitats (ANOVA, $F_{1,90} = 0.11$, $P > 0.05$), i.e. juveniles originating from both fucoids and eelgrass preferred eelgrass (Table 3).

Significant differences between the experiments conducted in the dark and daylight hours were found in only one of the four days. Based on that, we concluded that this was caused by non-controlled factors (such as, for instance, in series 3), and therefore, this factor does not play a significant role in juvenile substrate preference.

Juvenile preference of substrate depended largely on the date of the experiment (which is most likely due to the different ages of juveniles) (ANOVA, $F_{1,90} = 41.02$ $P < 0.001$) and the substrate (ANOVA, $F_{2,90} = 19.04$ $P < 0.001$) (Table 3). Furthermore, ANOVA shows significant interaction of these two factors. In particular, on 14–15 August, the number of juveniles associated with different substrates differed insignificantly: Z – 79 ± 7, F2 – 101 ± 11 and F1 – 48 ± 8 ind m^{-2}, ANOVA, $P > 0.05$, (Figure 6). During this period 12–25% of the total number of juveniles in the tank was associated with vegetation. Later, on 22–23 August, the number of juveniles associated with substrates increased and reached 33–55% of the total number in the tank, and the differences between substrates became larger: Z – 326 ± 35, F2 – 157 ± 18, and F1 – 88 ± 14 ind m^{-2} ($P < 0.05$). Thus, the increase in the number of juveniles associated with substrates was caused by juvenile preference for eelgrass because only here did the number of juveniles, when comparing data obtained on 14–15 and 22–23 August, increase significantly (ANOVA, $P < 0.001$) (Table 3).

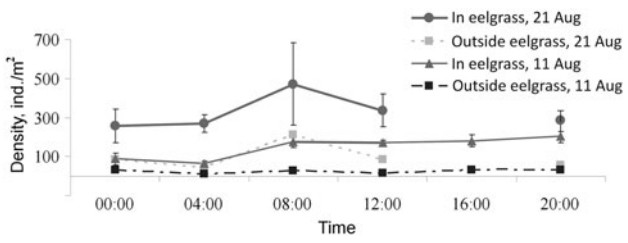

Fig. 5. Density of stickleback juveniles in eelgrass in different time of a day (means and standard errors are shown).

DISCUSSION

The Keret' Archipelago provides very heterogeneous conditions for three-spined stickleback juveniles in terms of types of aquatic vegetation and sediments. Juveniles were distributed very unevenly, with the highest densities in small inlets with dense eelgrass beds. Adults also had higher numbers associated with eelgrass beds, but in general, were distributed more evenly than juveniles, approaching relatively high densities in fucoids (Lajus *et al.*, 2011, Ivanova *et al.*, 2016 in press). Published data, mostly discussing distribution of adult fish, report that they are mainly or exclusively found in areas with aquatic vegetation, particularly eelgrass beds (Lazzari *et al.*, 2003; Polte & Asmus, 2006).

Several concurrent hypotheses can explain higher spatial heterogeneity of juveniles in comparison to adults: (i) actual spawning occurs only in eelgrass, although spawners can also be observed near fucoids; (ii) lower juvenile mortality in eelgrass than in other habitats, which can be caused by both more effective shelter provided by eelgrass, and/or more favourable feeding conditions; (iii) active migration of juveniles to eelgrass beds from other habitats. The likelihood

Table 3. Effect 'habitat of origin' (eelgrass, fucoids), 'date' (14–15 August, 22–23 August 2009) 'time of a day' (dark, light) and 'substrate' (eelgrass, dense fucoids, scarce fucoids) on juvenile distribution in experiments (series 4).

Factor	SS	Df	MS	F	*P*
Habitat of origin	8.17	1	8.17	0.12	0.73
Date	2795.04	1	2795.04	41.02	0.00
Time of a day	1.50	1	1.50	0.02	0.88
Substrate	2595.40	2	1297.70	19.04	0.00
Error	6132.85	90	68.14		

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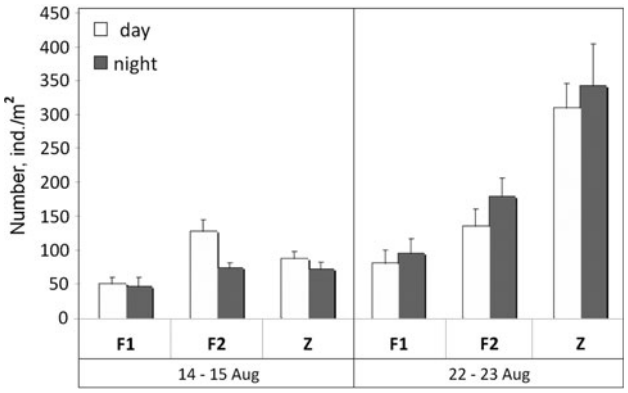

Fig. 6. Density of stickleback juveniles in three substrates (F1 – Fucus with 70% ground vegetation cover, F2 – Fucus with 100% ground vegetation cover, Z – eelgrass) (means and standard errors are shown). Two experiments were performed in 14–15 August and 22–23 August in day and night.

of these hypotheses is considered below based on obtained results and published data.

Spawning nests of the genus *Gasterosteus* are built by the male and located on the bottom, in shallow waters among aquatic vegetation and various shelters (Zyuganov, 1991). For nest building, males usually use dead vegetation, although some authors report nests being built of sand (Zyuganov, 1991]; Smirnov, 1951). It is assumed that the presence of dense vegetation is favourable for spawning stickleback because in such conditions males can build nests in more hidden places. This reduces their energy costs for the guarding of nesting territory and, therefore, leaves more energy to care for their offspring and in particular for the aeration of eggs. Ultimately, this increases the offspring survival rate (Sargent & Gebler, 1980). We observed nests of stickleback not only on the soft-bottom eelgrass beds, but also, less frequently, on gravel and rocky bottoms and in intertidal pools (E. Rybkina, D. Lajus, unpublished data). Thus, the stickleback in the White Sea can use different habitats but the density of nests on soft bottoms with eelgrass beds is higher.

As for the second hypothesis, it is known that more structured habitats have higher productivity and juveniles there find shelter more easily, helping them to avoid predation (Mikheev *et al.*, 2010). Higher patchiness of habitats is usually associated with higher visual heterogeneity, which attracts juveniles. Visual heterogeneity in natural conditions is associated with objects vital to juveniles: zooplankton, bottom invertebrates, demersal organisms in areas overgrown with macrophytes, and due to this, juveniles prefer aquatic vegetation. Patches of aquatic vegetation reduce risk of predation for juveniles. Juvenile sticklebacks were reported to be associated with marine vegetation floating in the open sea, such as *Fucus, Chorda* and *Chordaria*, with a frequency of occurrence of 0.5% (Khalaman & Berger, 2006).

Why do the juveniles tend to prefer eelgrass over fucoids, which seemingly have similar characteristics? One possibility is that during the low tide parts of fucoids dry out, reducing sheltering area, which makes juveniles more exposed to predation. A number of fish species in the area prey on juvenile stickleback (Trofimenko, 2013; Bakhvalova *et al.*, 2016 in press; A. Bakhvalova, T. Ivanova, M. Ivanov, D. Lajus, unpublished data): cod (*Gadus morhua* (Linnaeus, 1758)), saffron cod (*Eleginus nawaga* (Pallas, 1814)), European sculpin (*Myoxocephalus scorpius* (Linnaeus 1758), fourhorn sculpin (*Triglopsis quadricornis* (Linnaeus, 1758). These predatory fish occur both in fucoids and eelgrass sites, but their pressure can be different. As for eelgrass, they mostly live in the sublittoral zone, and the plants there are larger. Also, it can be suggested that eelgrass and sediments contain greater variety and biomass of organisms which are suitable as food for juveniles, than other available habitats such as hard grounds and fucoids (Williams & Heck, 2001). Studies reported that growth and survival rate of three-spined stickleback are higher in eelgrass beds compared with other habitats (Pihl *et al.*, 2006). It has been shown in three species of sticklebacks – *G. aculeatus, Pungitius pungitius* (L.) and *Apeltes quadracus* (Mitchill), that they prefer to stay in eelgrass growths even if such growths were located at a considerable distance from each other (Lazzari *et al.*, 2003). Analysis of feeding of juvenile stickleback in the eelgrass beds in the area of our study shows that the fish prey on both planktonic and benthic forms and change their diet depending on size and availability (Demchuk *et al.*, 2015; Rybkina *et al.*, 2016 in press). Therefore we may conclude that eelgrass may provide more effective shelters and better feeding conditions than other habitats.

The third explanation on the possibility of active migration of juvenile stickleback to other habitats was addressed in our experiments. In addition to the main question, attention was also paid to a number of other smaller questions, focusing on the methodology of the experiments. (i) We studied whether the distribution of juveniles in shelters depends on the position of these shelters in the experimental tank, and did not find such a dependency, which suggests equal conditions in different parts of the tank. (ii) We compared juvenile distribution in day and night time and found that in the dark juveniles were distributed more evenly, which may result from their lower aggregation and agrees with the published data. Decrease of brightness in twilight below the threshold is followed by shoal disintegration and fish spreading. At dawn, following the illumination increase, the opposite takes place (Pavlov & Kasumyan, 2003). Differences in juvenile behaviour in light and dark periods did not affect the results of our experiments, because comparisons were separately made for the light (14:00) and dark (00:00) time of the day. (iii) We analysed the time required to stabilize the distribution of juveniles in shelters. It occurred that 10 min was sufficient for juveniles to hide in shelters. Subsequently, the number of juveniles leaving and entering the shelters was balanced and distribution patterns did not change after 3 or 9 h of the experiment. Leaving of the shelters deals with exploratory behaviour, which is very important for the fish (Mikheev *et al.*, 2010). (iv) The next question addressed whether preferences of the stickleback juveniles depended on the habitat where they were caught in the wild (eelgrass or fucoids). This question has been answered negatively which shows no evident influence of previous juvenile experience on their behaviour in experimental conditions.

Addressing these methodical questions allowed us to shift to the central question of our work – 'What type of substrate do the juveniles prefer?' The study demonstrated that juveniles tend to prefer eelgrass to fucoids even if density of fucoids was higher. This preference was stronger in older juveniles, which were identified by a larger size of juveniles in the

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shelter compared with the rest of the tank, and by comparing preferences of juveniles.

CONCLUSION

Our experiments show that stickleback juveniles tend to prefer eelgrass over fucoids. At the same time, as results of some experiments were mixed, we cannot say this conclusively. Nevertheless, from our point of view, the obtained results can help to explain the higher numbers of stickleback juveniles in eelgrass beds compared with fucoids in the wild and a slower decrease in density with time in eelgrass than in other habitats. These patterns can also be explained by the lower mortality of juveniles in eelgrass, which likely provides better shelter from predators. Such findings confirm the importance of eelgrass for the reproduction of stickleback in the White Sea and changes in eelgrass abundance may result in changes of abundance of stickleback in the White Sea.

It should also be noted that the White Sea relationship between stickleback and eelgrass may not only be unidirectional, when plants provide better conditions for fish, but bilateral, since fish may also enhance the environment for eelgrass bringing a large amount of organic matter to the inshore zone. Stickleback spend most of their life cycle (excluding spawning and early ontogenesis) offshore, where they use the primary production of the open sea by feeding on zooplankton, and then, during inshore spawning migration make this production available for coastal ecosystems via mortality, predation and release of sexual products. Therefore, the process of interaction between stickleback and eelgrass may be described in terms of a positive feedback loop system, when both components enhance each other. Because of that, further research directed towards the analysis of interaction between these two high biomass species is important for understanding the functioning of the entire White Sea ecosystem.

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REFERENCES

Bakhvalova A.E., Ivanova T.S., Ivanov M.V., Demchuk A.S., Movchan E.A. and Lajus D.L. (2016) Long-term changes in the role of three-spine stickleback (*Gasterosteus aculeatus*) in the White Sea: predatory fish consumption reflects fluctuating abundance during the last century. *Evolutionary Ecology Research* 17, In press.

Demchuk A., Ivanov M., Ivanova T., Polaykova N., Mas-Marti E. and Lajus D. (2015) Feeding patterns of three-spined stickleback *Gasterosteus aculeatus* juveniles undergoing growth in seagrass beds. *Journal of the Marine Biological Association of the United Kingdom* 95, 1635–1643.

Gomeliuk V.E. (1976) The influence of environmental factors on the three-spined stickleback behavior of males *Gasterosteus aculeatus* in the White Sea in the period of care of posterity. *Voprosi Ichthyologii* 16, 1943–1053. [In Russian]

Hansson S. (1984) Competition as a factor regulating the geographical distribution of fish species in a Baltic archipelago: a neutral model analysis. *Journal of Biogeography* 11, 367–381.

Harrington R., Woiwod I. and Sparks T. (1999) Climate change and trophic interactions. *Trends in Ecology and Evolution* 14, 146–150.

Hemminga M.A. and Duarte C.M. (2000) *Seagrass ecology*. Cambridge: Cambridge University Press.

Ivanova T.S., Ivanov M.V., Golovin P.V., Polyakova N.V. and Lajus D.L. (2016) The White Sea threespine stickleback population: spawning habitats, mortality, and abundance. *Evolutionary Ecology Research* 17, In press.

Jackson E.L., Rowden A.A., Attrill M.J., Bossey S.J. and Jones M.B. (2001) The importance of seagrass beds as a habitat for fishery species. *Oceanography and Marine Biology* 39, 269–303.

Khalaman V.V. and Berger V.Ya. (2006) Floating seaweeds and associated fauna in the White Sea. *Oceanology* 46, 827–833.

Kikuchi T. and Peres J.M. (1977) Consumer ecology of seagrass beds. In McRoy C.P. and Helffrich C. (eds) *Seagrass ecosystems: a scientific perspective*. New York, NY: Marcel Dekker, pp. 147–193.

Lajus D.L., Ivanova T.S., Ivanov M.V. and Shatskikh E.V. (2011) Waves of life sticklebacks in the White Sea. *Materiali VIII nauchnogo seminara chtenia pamyati K.M. Deryugina*. SPb, Kafedra ikhtiologii i gidrobiologii Sankt Peterburgskogo gosudarstvennogo universiteta, 64–98. [In Russian]

Lajus D.L., Ivanova T.S., Ivanov M.V. and Shatskikh E.V. (2013a) How many stickleback in the White Sea now? In Nemova N.N., Murzina S.A. and Mescheriakova O.V. (eds) *Problemy izuchenia, ratsionalnogo ispolzovania I okhrany prirodnykh resursov Belogo moria. XII Mezhdunarodnaya konferentsia s elementami shkoly dlia molodykh uchenykh i aspirantov. Sbornik materialov*. Petrozavodsk: Karelsky nauchny tsentr RAN, pp. 185–188.

Lajus D.L., Shatskikh E.V., Ivanova T.S. and Ivanov M.V. (2013b) "Waves of life" of White Sea three-spined stickleback. *Priroda* 4, 43–52. [In Russian]

Lazzari M.A., Sherman S. and Kanwit J.K. (2003) Nursery use of shallow habitats by epibenthic fishes in Maine nearshore waters. *Estuarine, Coastal and Shelf Science* 56, 73–84.

Lee S.Y., Fong C.W. and Wu R.S.S. (2001) The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: a study using artificial seagrass units and sampling of natural beds. *Journal of Experimental Marine Biology and Ecology* 259, 23–50.

Mikheev V.N., Afonina M.O. and Pavlov D.S. (2010) Habitat heterogeneity and fish behaviour: units of heterogeneity as a resource and as a source of information. *Journal of Ichthyology* 50, 386–395.

Murphy M.L., Scott W.J. and Csepp D.J. (2000) A comparison of fish assemblages in eelgrass and adjacent subtidal habitats near Craig, Alaska. *Alaska Fishery Research Bulletin* 7, 11–21.

Nakaoka M. (2005) Plant–animal interactions in seagrass beds: ongoing and future challenges for understanding population and community dynamics. *Population Ecology* 47, 167–177.

Pavlov D.S. and Kasumyan A.O. (2003) *Stainoe povedenie ryb [Schooling behavior of fish]*. Moscow: Izdatelstvo Moskovskogo gosudarstvennogo universiteta. [In Russian]

Pihl L., Baden S., Kautsky N., Ronnback P., Soderqvist T., Troell M. and Wennhage H. (2006) Shift in fish assemblage structure due to loss of seagrass *Zostera marina* habitats in Sweden. *Estuarine, Coastal and Shelf Science* 67, 123–132.

Polte P. and Asmus H. (2006) Intertidal seagrass beds (*Zostera noltii*) as spawning grounds for transient fishes in the Wadden Sea. *Marine Ecology Progress Series* 312, 235–243.

Portner H.O. and Farrell A. (2008) Physiology and climate change. *Science* 332, 690–692.

Sargent R.C. and Gebler J.B. (1980) Effects of nest site concealment on hatching success, reproductive success and paternal behaviour of the threespine stickleback, *Gasterosteus aculeatus*. *Behavioural Ecology and Sociobiology* 7, 137–142.

Shatskikh E.V., Lajus D.L. and Ivanova T.S. (2010) Habitat preference of juvenile three spine stickleback *Gasterosteus aculeatus* L. in natural and experimental conditions. *Vestnik SPBGU Seria* 3. 4, 61–70. [In Russian]

Short F.T., Carruthers T.J.R., Waycott M., Kendrick G.A., Fourqurean J.W., Callabine A., Kenworthy W.J. and Dennison W.C. (2010) *Zostera marina*. *The IUCN red list of threatened species. Version 2014.2*. http://www.iucnredlist.org

Smirnov A.I. (1951) On the biology of three-spine stickleback *Gasterosteus aculeatus L. Doklady Akademii Nauk SSSR* 80, 837–840. [In Russian]

Rybkina E.V., Demchuk A.S., Ivanova T.S., Ivanov M.V., Lajus D.L. and Galaktionov K.V. (2016) Parasite infestation of marine three-spined stickleback *Gasterosteus aculeatus* during early ontogenesis. *Evolutionary Ecology Research* 17, In press.

Telegin A.V. (1999) Multi-year changes in the ecosystem of the Babye more (Kandalakshksky Bay of the White See). In Kliuikov E. Yu. (ed.) *3 Mezhdunarodnyi seminar "Ratsionalnoe ispolzovanie pribrezhnoi zony severnykh morei" 17 iulia 1998 g. g. Kandalaksha. Materialy dokladov*. Saint Petersburg: Rossiskii gosudarstvennyi gidrometeorologicheskii institut, pp. 68–76.

Trofimenko E.V. (2013) *Ryby pribrezhia Keretskogo arkhipelaga: rost, pitanie, dinamika chislennosti [Fishes of inshore area of Keret' Archipelago: growth, feeding, population dynamics]*. Bachelor thesis. Sankt Petersburg: Sankt Peterburgskii gosudarstvennyi universitet.

Vecutich J.A. and Waite T.A. (2003) Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conservation Genetics* 4, 639–645.

Vekhov V.N. (1970) *Vosstanovlenie zarosley zostery na Belom more. Trudy Belomorskoi biologicheskoi stantsii MGU*. Volume 3. Moscow: Izdatelstvo Moskovskogo universiteta, pp. 149–153. [In Russian]

Vekhov V.N. (1992) *Zostera morskaya (Zostera marina L.)* Belogo moria *[*Eelgrass *(Zostera marina L.)* of the White Sea*]*. Moscow: Izdatelstvo Moskovskogo universiteta.

Williams S.L. and Heck K.L. (2001) Seagrass community ecology. In Bertness M.D., Gaines S.D. and Hay M.E. (eds) *Marine community ecology*. Sunderland, MA: Sinauer Associates, pp. 317–337.

Wootton R. J. (1984) *A functional biology of sticklebacks*. New York, NY: Springer.

and

Ziuganov V.V. (1991) *Semeistvo koliushkovykh (Gasterosteidae) mirovoi fauny* [Family Gasterosteidae of world fauna.] Leningrad: Nauka. [In Russian]

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