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Dynamics of parasite community during early ontogenesis of marine threespine stickleback, *Gasterosteus aculeatus*

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ABSTRACT

Hypothesis: Parasite infection of juvenile stickleback increases during their early ontogenesis owing to transmission from adults and other juveniles, as well as changes in diet.

Organisms: Threespine stickleback (*Gasterosteus aculeatus*) aged one week to two months, their ecto- and endoparasites, and their stomach contents.

Times and places: July to September 2012 and 2015; two locations in the Kandalaksha Bay of the White Sea – Seldyanaya Inlet with dense seagrass beds and an unnamed lagoon in Sukhaya Salma Inlet.

Methods: Quantitative sampling of stickleback at 10 day intervals, and quantitative analysis of their parasites and stomach contents.

Results: As sticklebacks grew, their parasite load increased. We identified three size groups of stickleback that differ significantly in their parasite species composition and infection indices: hatchlings 7.0–8.5 mm long were infected with three parasite species (prevalence 43%); juveniles 9–11 mm harboured four or five species (100%); and juveniles 12–30 mm were infected by 12 species (100%). As stickleback grew, copepods played an increasing role in their diet, and infection with trematodes and cestodes rose accordingly.

Keywords: feeding, *Gasterosteus aculeatus*, juveniles, parasites, threespine stickleback, White Sea.

INTRODUCTION

Threespine stickleback (*Gasterosteus aculeatus* L.) are common in marine, brackish, and fresh waters of the northern hemisphere (Wootton, 1976). In the White Sea, where our study was conducted, stickleback are now the most abundant fish species and spend the warm season, including the spawning period, near shore in the intertidal and upper subtidal zones (Ivanova *et al.*, 2016). Habitats frequented by stickleback include protected inlets and lagoons with

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seagrass beds, rocky bottoms covered with fucoid seaweeds, littoral pools, reeds, and shallows (Ivanova *et al.*, 2016). During the cold season, by contrast, these fish live offshore in the open sea (Mukhomediarov, 1966). The abundance of the stickleback in this area varied widely during the twentieth century. Following large population sizes in the 1930s, abundance declined in the 1950s and, between the 1960s and 1990s, stickleback had nearly disappeared. In the last decade, the number of stickleback has started to increase again, approaching the historical maximum (Lajus *et al.*, 2013).

Adult stickleback appear near shore from late May to early June and spawn in the last weeks of June. Juveniles hatch in July to August. After the eggs are laid, adult females leave the near shore, whereas males remain to guard the nests and, later, the hatchlings (Ivanova and Lajus, 2008). Newly hatched stickleback are about 7–7.5 mm long. After several days in the nest, they emerge and start feeding (E. Rybkina, unpublished data). Juveniles from the same clutch remain together under the care of their father for several more days. At that time, they reach approximately 8 mm in length. For the next several weeks, juveniles swim freely in the nearshore area. They leave shallow water by mid-September, when they are about 25–30 mm long (Bakhvalova *et al.*, 2016).

The population growth of threespine stickleback necessarily affects other components of the White Sea ecosystems, including parasites. During the time spent near shore, stickleback may facilitate transmission of parasites that have life cycles associated with intertidal and upper-subtidal ecosystems. The scale of this facilitation increases as stickleback numbers increase. One possible indicator of this effect is the recently reported increase in prevalence of the trematode *Podocotyle atomon* in periwinkles (*Littorina saxatilis* and *L. obtusata*) (Levakin *et al.*, 2013). Adults of this trematode parasitize fishes, including stickleback, and it is considered a typical parasite of juvenile fish near shore (Polyansky, 1955; Polyansky and Shulman, 1956). On the other hand, parasites could also influence the abundance of stickleback, especially when the latter reach high densities. Such an effect has been demonstrated for other fish species, such as smelt (*Osmerus mordax*) infected with the microsporidean *Glugea hertwigi* in the Great Lakes (Sindermann, 1987).

Research on the parasites of threespine stickleback in the White Sea began in the 1950s and 1960s (Shulman and Shulman-Albova, 1953; Isakov, 1970). This early work described the species composition of parasites from limited numbers of adult stickleback and provided experimental data on salinity tolerance for specific parasites: monogeneans (*Gyrodactylus* spp.) and ciliophorans (*Trichodina* spp.). Such relatively limited attention to these parasites is hardly surprising given the very low abundance of stickleback at that time and the lack of importance to fisheries. Published data on the parasites of freshwater stickleback are extensive (e.g. Kalbe *et al.*, 2002; Poulin *et al.*, 2011; Barber, 2013). On the other hand, studies devoted to parasites of marine stickleback, especially juveniles, are few (for Barents Sea: Polyansky and Shulman, 1956; for Baltic Sea: Banina and Isakov, 1972; Valtonen *et al.*, 2001; Zander, 2007).

A number of studies of adult freshwater stickleback have been devoted to the heterogeneity of parasite composition among host populations (Wegner *et al.*, 2003; MacColl, 2009; Bell *et al.*, 2016), the link between diet and parasites (see, for example, Jakobsen *et al.*, 1988), and the influence of parasites on feeding strategies (Milinski, 1984; Ranta, 1995). We are unaware of similar studies on marine stickleback. Since the majority of parasites infect their fish hosts through the food web (Marcogliese and Cone, 1997; Marcogliese, 2003; Poulin, 2007), an understanding of the diet of hosts is extremely important in parasitological studies. Abdel-Malek (1968), and later Demchuk *et al.* (2015), examined the feeding patterns of juvenile stickleback from the White Sea. However, simultaneous studies on parasites and feeding have not been published.

Detailed studies of changes in parasite infection during the early stages of threespine stickleback ontogenesis are extremely rare: for instance, most parasitological studies consider young-of-the-year as a single age group (Gusev *et al.*, 1972). Yet during their first year of life, juveniles experience dramatic changes in morphology, physiology, and behaviour (Zyuganov, 1991). These changes should influence the composition and numbers of the parasites infecting a juvenile.

The aim of the present study is to investigate the dynamics of parasite species composition and abundance in juvenile threespine stickleback in the White Sea in connection with ontogenetic changes in the fish diet and patterns of parasite transmission.

METHODS AND MATERIALS

Study sites

Threespine stickleback juveniles were collected from two sites in the Kandalaksha Bay of the White Sea (Fig. 1):

1. Seldyanaya Inlet (66°20′14″N, 33°37′20″E): a triangular bay with a wide entrance (depth of 8 m). The head of the bay is shallow, with limited freshwater drainage. Water exchange during the tidal cycle is intense (tidal amplitude of 2.5 m). The terminal part of the inlet is covered with dense eelgrass (*Zostera marina*) beds.
2. An unnamed lagoon in Sukhaya Salma Inlet (66°18′51″N, 33°38′23″E): with an area of 0.064 km^2, this inlet is almost isolated from the sea except for two channels. The central area of the lagoon is about 4 m deep, whereas the channels are shallower. Water exchange during the tidal cycle is restricted (tidal amplitude of only 30 cm). The bottom of the lagoon supports less dense seagrass beds than in the Seldyanaya Inlet; instead, green filamentous algae are abundant.

Both sites represent typical spawning habitats for threespine stickleback in the White Sea. Water temperature was slightly higher in the lagoon, whereas salinity did not differ between the two study sites (24–25‰ in the Seldyanaya Inlet and 22–23‰ in the lagoon).

Juvenile sampling

We sampled juveniles for density and diet analyses once (1 August) in 2011 and five times (from 27 July to 13 September) in 2012 at both sites (Table 1). In August of both 2011 and 2012, we sampled the juveniles every 10 days. All juveniles collected were counted and had their lengths measured, and about 30 juveniles were selected for the diet study.

We used an equal-winged beach seine with net wings 7.5 m long (mesh size 5 mm), a net pocket entrance of 3 mm, and a gauze cod-end of mesh size 1 mm. When deployed, the seine was 1.5 m high, with a catchment area of about 150 m^2. Fish were collected in a 30 m wide zone near shore. The density of the juveniles was expressed as the number of individuals per square metre within 30 m of the nearshore zone, ignoring any microscale spatial heterogeneity. Catch efficiency (the ratio of fish caught relative to the total number of fish in the catch area) of the seine was estimated at 0.6 using mark–recapture techniques (Lockwood and Schneider, 2000) in the lagoon (T. Ivanova, M. Ivanov and D. Lajus, unpublished). Differences

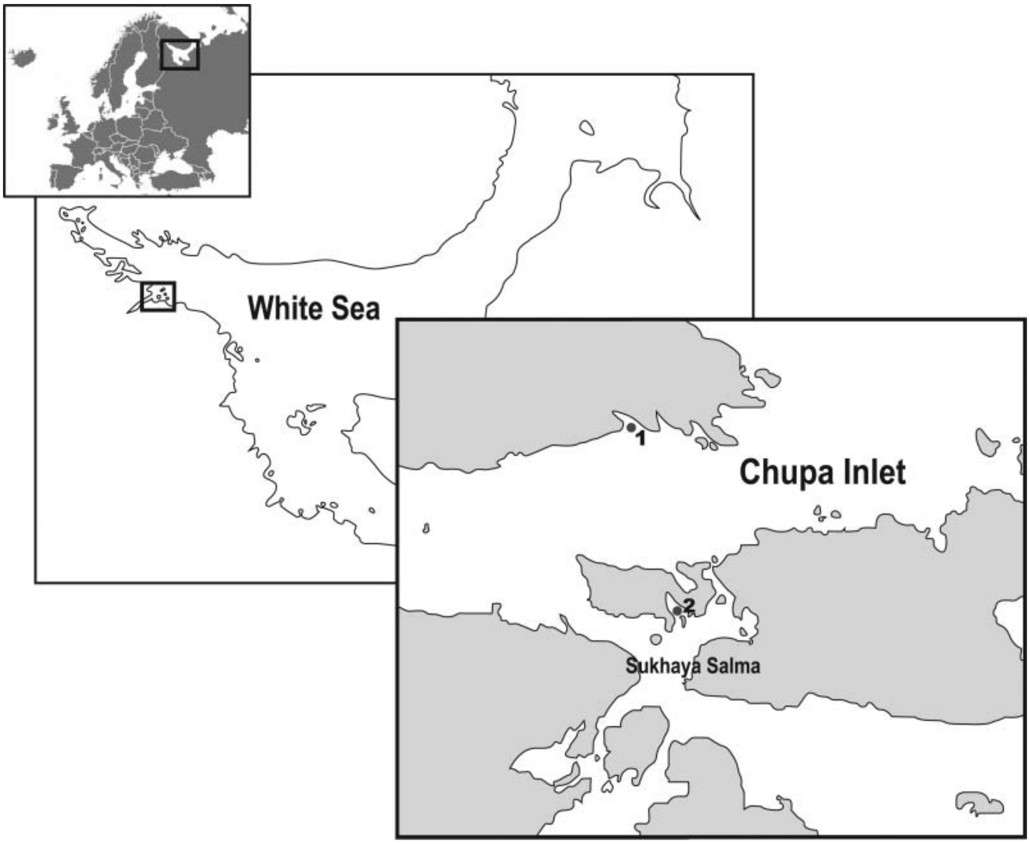

Fig. 1. Sites used to sample juvenile threespine stickleback in the Chupa Inlet (Kandalaksha Bay, White Sea). 1, Seldyanaya Inlet; 2, lagoon in Sukhaya Salma Inlet.

between replicated hauls performed under similar conditions were about 10% (T. Ivanova, M. Ivanov and D. Lajus, unpublished).

All estimates of size and abundance dynamics during the summer were done with the seine samples. The smallest fishes were underrepresented in those samples because of the relatively large mesh. In these samples, the smallest juveniles were 9 mm long, whereas they usually hatch at about 7.5 mm. Therefore, our size and density estimates start with relatively larger juveniles.

We began sampling for the parasitological study on 29–30 July each year. We used both beach seines as described above, and gauze hand nets for collection. In August 2011, we collected juveniles six times (four times in Seldyanaya, twice in the lagoon) at approximately 10 days intervals. In 2012, six samples were collected in August (three at each site) and four in September (three in Seldyanaya and one in the lagoon), with equal intervals of about 10 days. The last sampling was conducted on 24 September. We measured the length of all juveniles from each sample, and then selected 15–20 fish of three different size classes for parasitological analysis. To better represent the smallest size class (hatchlings), an additional 42 stickleback 7–8.5 mm long were collected from Seldyanaya Inlet on 20 July 2015.

Table 1. Samples collected on different dates at the two locations, and the types of analyses performed

	Seldyanaya Inlet			Lagoon		
Date	Density	Diet	Parasites	Density	Diet	Parasites
1 August 2011	+	30	17	+		7
6 August 2011		30	7			
22 August 2011		30	14			
27 August 2011		13	7			5
27 July 2012	+		27	+		
2 August 2012		30	6		30	19
11 August 2012	+	30	19	+	30	6
18 August 2012						12
22 August 2012	+	30	8	+	26	
29 August 2012	+	30		+	30	27
3 September 2012			21			
9 September 2012			15			9
13 September 2012	+	30		+		
24 September 2012			4			
20 July 2015			42			
Total		253	141		120	85

Note: For parasites and feeding, the number of juveniles analysed is indicated, and in the case of density, ‘ + ’ = presence of samples.

Processing of juveniles

Fish body length was measured with a ruler accurate to 0.5 mm.

When analysing stomach contents, we followed protocols described in an earlier study (Demchuk *et al.*, 2015). In brief, samples were preserved in 4% formaldehyde, fish were dissected and their stomachs removed, and food items were identified to the lowest possible taxonomic rank (usually species or genus) and counted. In total, 150 stomachs of fish from the Seldyanaya Inlet and 116 stomachs of fish from the lagoon were examined.

For parasitological analysis, we examined a total of 274 juveniles with a full parasitological dissection procedure (according to Bykhovskaya-Pavlovskaya 1985). Fishes were dissected either on the day of collection or on one of the two following two days. Fish were kept alive in water from their collection site at 10°C. The parasites were fixed and stained following standard protocols (Bykhovskaya-Pavlovskaya, 1985). For trichodine analysis, we used the following scale: 0, 1, 10, 100 by approximate counting of the parasites and rounding the actual figure to the nearest value on the logarithmic scale. For example, figures of 1–3 were recorded as 1, figures of 4–40 were recorded as 10, and so on. Both trichodines and metacercariae (*Cryptocotyle* spp.) were identified to the genus level.

Data analysis

The juveniles were divided into size groups using 3 mm steps from 7 to 27 mm (Table 2). Prevalence, mean abundance, and mean intensity of the parasites were calculated following Bush *et al.* (1997). We report mean values, together with standard errors (SE) in the figures.

Table 2. The number of investigated juveniles of different size groups

Size group (mm)	Food			Parasites				
	Seldyanaya Inlet		Lagoon	Seldyanaya Inlet			Lagoon	
	2011	2012	2012	2011	2012	2015	2011	2012
7.5						45		
10.5	10				18			9
13.5	20	9	4	1	24			13
16.5	20	28	14	13	25		1	10
19.5	30	37	46	20	14		6	29
22.5	23	44	39	8	10		3	12
25.5		31	13	3	9		2	2

All statistical tests were performed using STATISTICA v.2.0. To compare parasite mean abundance across sites, years, and sizes, we employed general linear models (GLM) and principal component analysis (PCA) using PAST v.3. Spearman correlations were calculated to determine the association between fish size, collection date, and abundance of parasites.

RESULTS

Seasonal changes in the size and abundance of stickleback

Overall, the densities of juvenile stickleback in the lagoon and in Seldyanaya Inlet were similar (Table 3); however, their seasonal dynamics displayed different patterns. In Seldyanaya Inlet, the abundance of juveniles decreased through time, whereas in the lagoon no pronounced changes were observed (Table 3). The density of juveniles in Seldyanaya Inlet was higher in 2012 than in 2011 at all times.

The size of juveniles in our beach seine samples increased steadily in Seldyanaya Inlet from 12.2 ± 0.1 mm on 29 July to 21.7 ± 1.5 mm on 15 September. Some small fish were present in all samples, indicating protracted spawning of the stickleback in this area (Fig. 2). In the lagoon, the size of the juveniles fluctuated with no obvious increase over time (Fig. 2).

Stomach contents

In the stomachs of juveniles from Seldyanaya Inlet, we found organisms from eight major taxonomic groups: crustaceans (Copepoda, Cladocera, and Gammarida), Ciliophora, Rotifera, molluscs (Bivalvia and Gastropoda), and insects (Orthocladiinae). All of these groups, except for gammarids, were also present in the diet of the juveniles from the lagoon (Fig. 3).

The dominant taxa – copepods (nauplii and adults of different species) and ciliates (*Helicostomella subulata*) – together accounted for more than 80% of the food items and appeared in more than 95% of stomachs. Other prey organisms were also commonly present in stomachs, but their cumulative contributions never exceeded 10–20% of total food items.

Table 3. Seasonal changes in the density of juvenile stickleback in Seldyanaya Inlet and in the lagoon in 2011 and 2012

	Density (#fish per m^2)			
	Seldyanaya		Lagoon	
Date	2011	2012	2011	2012
25 July	—	538	—	8
30 July	15	—	15	—
11 August	4	42	—	28
23 August	7	28	—	19
29 August	1	7	—	35

Note: The true dates might differ from those indicated here by one or two days, but were adjusted to facilitate comparison between years and sites. — , missing data.

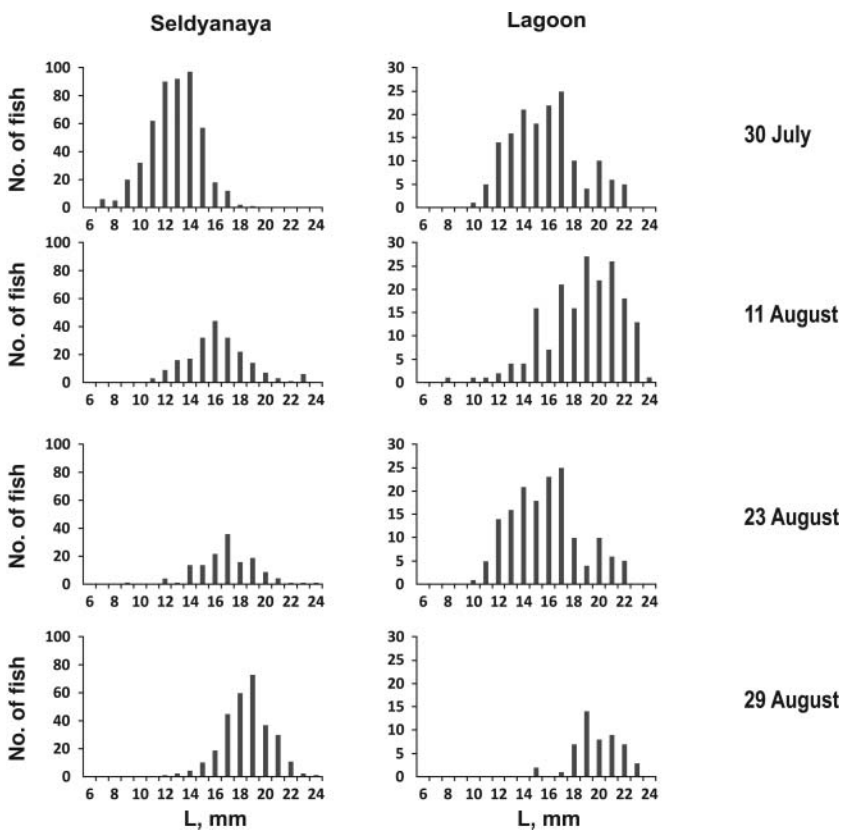

Fig. 2. Size distribution of juvenile stickleback during the summer of 2012 in Seldyanaya Inlet and in the lagoon.

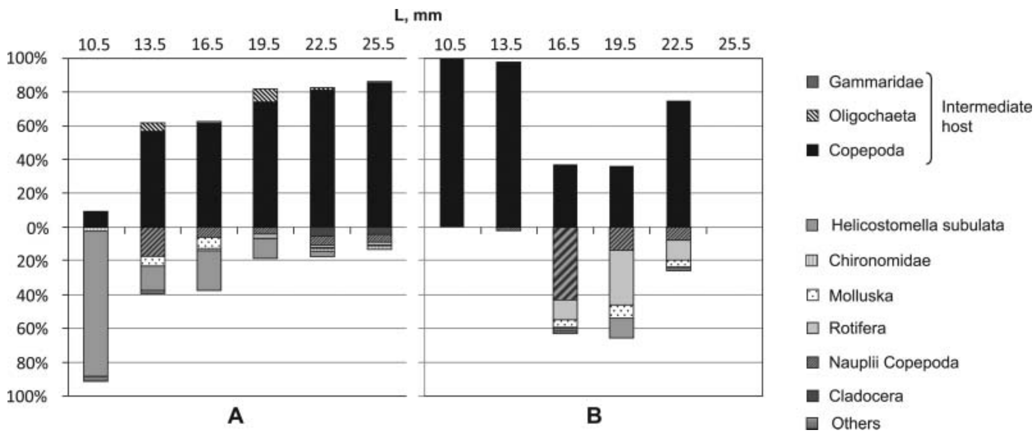

Fig. 3. Food items retrieved from the stomachs of juvenile threespine stickleback of different size groups in the Seldyanaya Inlet (A) and in the lagoon (B).

Copepods, gammarids, and oligochaetes are intermediate hosts of some stickleback parasites (see below). Among these groups, only copepods were numerous, which therefore became the focus of further analysis. The two sites did not differ in the number of copepods in stickleback stomachs (GLM, $F_{1,262} = 0.41$, $P = 0.52$). At both sites, copepods dominated as food organisms, but their species composition differed: in Seldyanaya Inlet, the key species were *Temora longicornis* and *Microsetella norvegica*, whereas in the lagoon the dominant species was *Acartia longiremis*. In Seldyanaya Inlet, copepod consumption increased with the size of the juveniles (GLM, $F_{1,249} = 26.8$, $P < 0.0001$), whereas in the lagoon no such correlation was observed (Fig. 4). In Seldyanaya Inlet, where we had samples for two different years, the abundance of copepods in stomachs was significantly higher in 2011 than in 2012 in all stickleback size groups (GLM, $F_{1,249} = 9.53$, $P = 0.002$). The two sites did not differ in the number of copepods in stickleback stomachs (GLM, $F_{1,262} = 0.41$, $P = 0.52$). Both in the lagoon and in Seldyanaya Inlet, copepods dominated over other food organisms, but their species composition was different: in Seldyanaya Inlet, the key species were *T. longicornis* and *M.norvegica*, whereas in the lagoon it was *A. longiremis*.

Parasite composition

We identified a total of 12 parasite species associated with stickleback juveniles (www.evolutionary-ecology.com/data/2994Appendix.pdf). Ectoparasites included species typical for the family Gasterosteidae such as the monogenean *Gyrodactylus arcuatus*, the copepod *Thersitina gasterostei*, and the ciliate *Trichodina* sp.

The prevalence of *G. arcuatus* was high (up to 100%), whereas the infection intensity reached 14.8–21.8 in the large size classes. Typically, only one copepod *Th. gasterostei* was found on a juvenile, but sometimes we recorded up to three on a single fish. *Trichodina* sp. mostly occupied the body surface, but also appeared on the gills of juveniles from all size groups.

Endoparasites were widespread and included flatworms, nematodes, myxosporeans, and microsporeans. Trematodes were the most common and diverse group of parasites

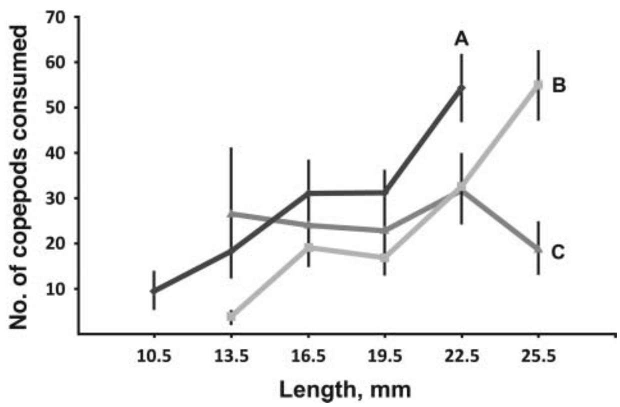

Fig. 4. Correspondence between the mean number of copepods in juvenile stickleback stomachs and juvenile size. (A) Seldyanaya Inlet, 2011; (B) Seldyanaya Inlet, 2012; (C) the lagoon, 2012 (errors bars represent standard errors).

(2994Appendix.pdf). Among the adult worms, the most common species was *Brachyphallus crenatus*, found in juveniles larger than 12 mm – its prevalence from Seldyanaya Inlet was 10–33%, with an infection intensity of 1–4. *Lecithaster gibbosus* and *Derogenes varicus* occurred only sporadically. *Podocotyle atomon* appeared in juveniles larger than 9 mm with a prevalence of 6–25%. The metacercariae of *Cryptocotyle* spp. were extremely widespread and infected stickleback of all size classes. In the lagoon, 100% of the juveniles were infected.

Plerocercoids and young cestode *Bothriocephalus scorpii* were recorded in all size groups of juvenile stickleback. Prevalence varied from 50 to 93% in Seldyanaya Inlet and from 11 to 52% in the lagoon. *Nematoda* gen. sp. were found in the host intestine on only a few occasions. The myxosporea *Myxobilatus* sp. occurred fairly often (6–25%), but only in juveniles from the lagoon. The abundance of this parasite tended to increase with the size of stickleback. Finally, a single juvenile caught in the lagoon was infected with the microsporean *Glugea anomala*. Based on our own data (E.V. Rybkina, unpublished), large numbers of juveniles of the ninespine stickleback (*Pungitius pungitius*) from the lagoon were infected with *G. anomala* at the same time.

Size-related dynamics of juvenile infection

The average number of parasite species and their mean number per juvenile increased with increasing juvenile size (Fig. 5). This trend was evident in Seldyanaya Inlet and in the lagoon. Thus we pooled data from the two sites for further analyses. We found a statistically significant correlation between the number of parasite species and the size of juvenile stickleback (Spearman correlation coefficient = 0.6, d.f. = 273, $P < 0.001$).

Size, site, and inter-annual patterns in juvenile infection

We analysed only the common parasite species. To address variations in parasite abundance, we subdivided them into the following groups: ectoparasites (*Thersitina gasterostei*, *Gyrodactylus arcuatus*, *Trichodina* sp.), actively penetrating parasites (*Cryptocotyle* spp.),

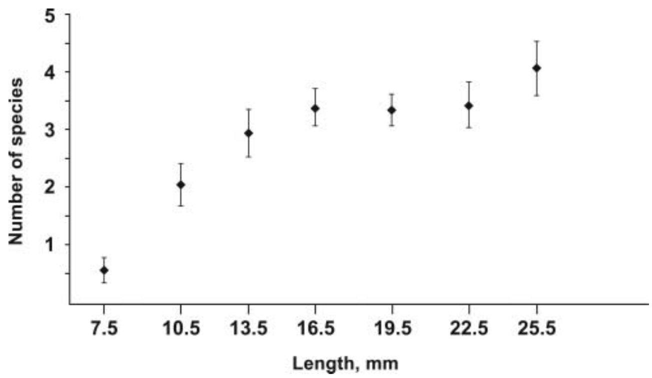

Fig. 5. Mean number of parasite species per individual juvenile stickleback of different sizes from Seldyanaya Inlet and the lagoon pooled in 2012 and 2015 (error bars represent confidence intervals).

and intestinal trophically transmitted parasites (*Bothriocephalus scorpii*, *Brachyphallus crenatus*, *Podocotyle atomon*). Principal component analysis demonstrated that the first and second groups have relatively high loadings on PC1 (explaining 27.5% of the total variance), and the third group high loadings on PC2 (17.3% of the total variance) (2994Appendix.pdf). The size of juveniles was similarly correlated with PC1 and PC2. Abundance of the parasites clearly differed between the two sites (Fig. 6). Stickleback juveniles from the lagoon were spread along PC1, whereas fish from Seldyanaya Inlet were observed to spread more along PC2 (Fig. 6).

Size-related dynamics of juvenile infection

Ectoparasites

The mean abundance of *Gyrodactylus arcuatus* and *Trichodina* sp. depended strongly on juvenile size (GLM, 2994Appendix.pdf) but did not differ between sites or years. The mean abundance of *Thersitina gasterostei* depended on the size of juveniles and on location (GLM: size, $F_{1,227} = 30.65$, $P < 0.001$; locality, $F_{1,227} = 14.84$, $P < 0.001$), but did not depend on year ($P = 0.28$). The mean abundance of this species was relatively high in the lagoon and increased with increasing size of juveniles. In Seldyanaya Inlet, the mean abundance of *Th. gasterostei* was too low to allow us to separate the effects of juvenile size and season: only large juveniles were infected with *Th. gasterostei* at the end of August.

Actively penetrating larvae of Cryptocotyle spp.

The mean abundance of the metacercariae of *Cryptocotyle* spp. depended on juvenile stickleback size and locality (GLM: size, $F_{1,227} = 21.0$, $P < 0.001$; locality, $F_{1,227} = 272$, $P < 0.001$). The abundance of this species was about ten-fold higher in all juvenile size groups in the lagoon than in Seldyanaya Inlet. In Seldyanaya Inlet, abundance in 2011 was significantly lower than in 2012 (GLM, $F_{1,142} = 18.5$, $P < 0.001$) (Fig. 8), but no difference between years was observed in the lagoon (GLM, $F_{1,84} = 0.37$, $P = 0.54$).

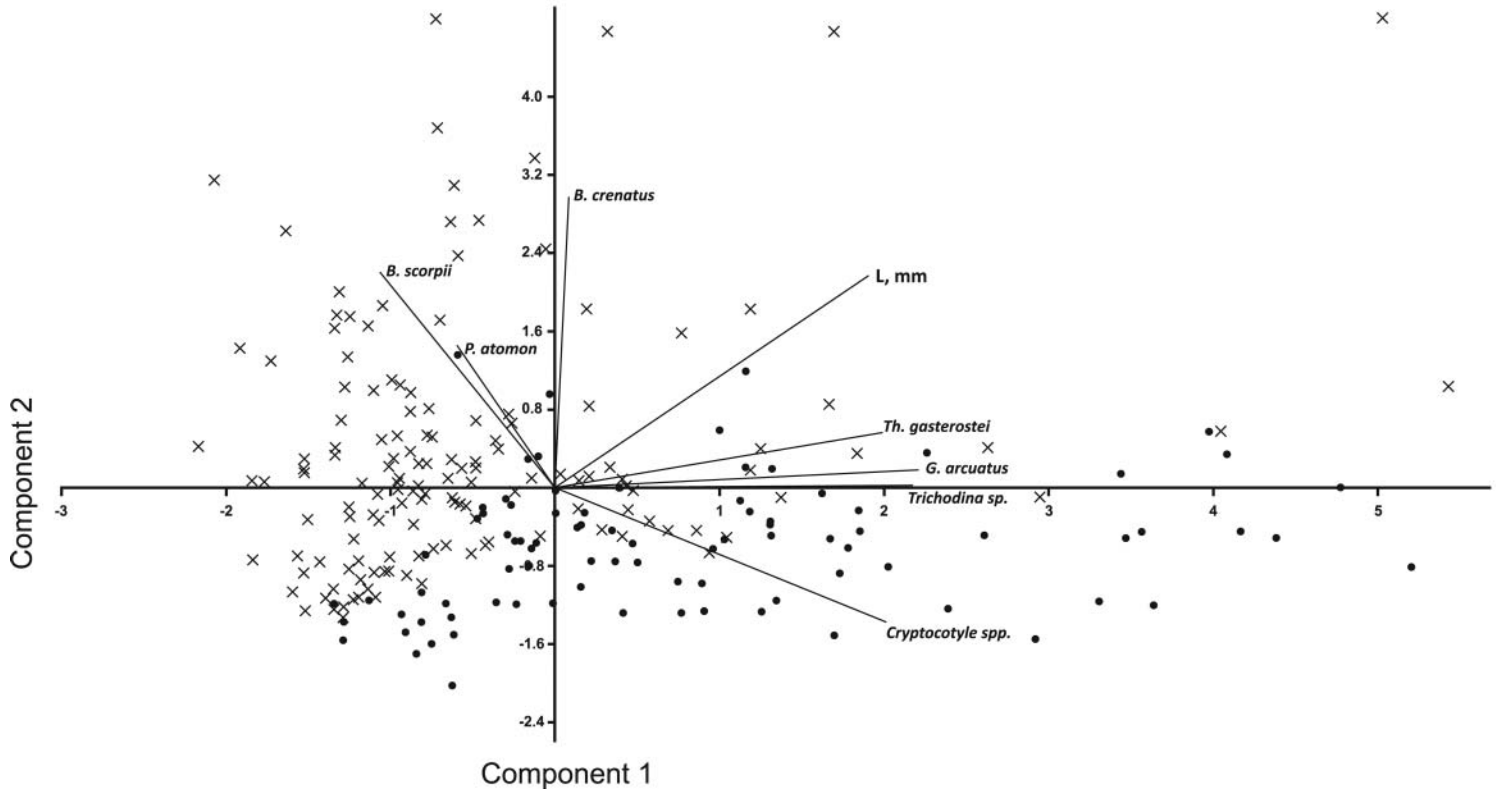

Fig. 6. The positions of stickleback individuals from the lagoon (●) and Seldyanaya Inlet (×) along the PC1 and PC2 axes of parasite composition. The intensities of seven common parasite species (*Podocotyle atomon*, *Brachyphallus crenatus*, *Bothriocephalus scorpii*, *Trichodina* sp., *Cryptocotyle* spp., *Thersitina gasterostei*, *Gyrodactylus arcuatus*) are shown.

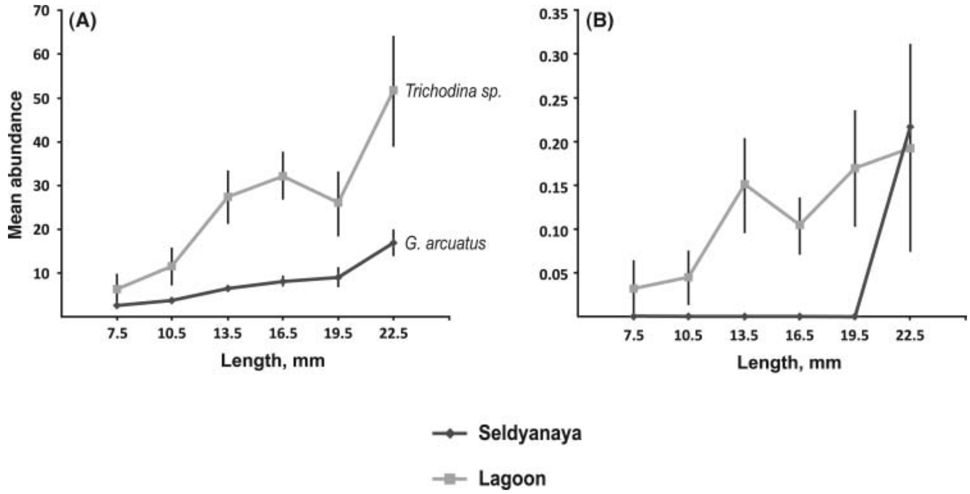

Fig. 7. Dependence of mean abundance of *G. arcuatus* and *Trichodina* sp. (A) and *Th. gasterostei* (B) on the size of juvenile stickleback at the two sites (errors bars represent standard errors).

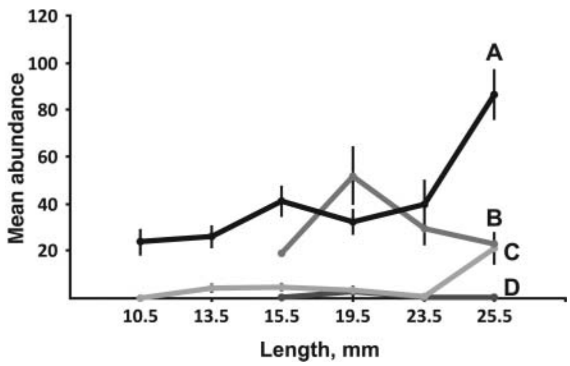

Fig. 8. Dependence of mean abundance of *Cryptocotyle* spp. on the size of juvenile stickleback at the two sites. (A, B) Mean abundance of *Cryptocotyle* spp. in the lagoon in 2012 and 2011; (C, D) mean abundance of *Cryptocotyle* spp. for Seldyanaya Inlet (errors bars represent standard errors).

Endoparasites transmitted by copepods

Juvenile stickleback were more intensively infected with *Bothriocephalus scorpii* in Seldyanaya Inlet than in the lagoon (GLM, $F_{1,227} = 46.6$, $P < 0.001$). The mean abundance of cestodes in Seldyanaya Inlet was 10 times higher than in the lagoon (Fig. 9A). No difference in mean abundance of cestodes was found between years (GLM, $F_{1,227} = 1.5$, $P = 0.22$) when both sites were included in the analysis. The mean abundance of cestodes clearly depended on juvenile size. Small juveniles were infected with only a few plerocercoids, whereas mean abundance approached maximum levels in juveniles of 18–21 mm. The mean abundance of *B. scorpii* then decreased in larger stickleback. This pattern was observed in both Seldyanaya Inlet and in the lagoon (Fig. 9B, C). The mean abundance of plerocercoids showed a weak negative correlation with fish length (Spearman correlation, $R = -0.2$, non-significant).

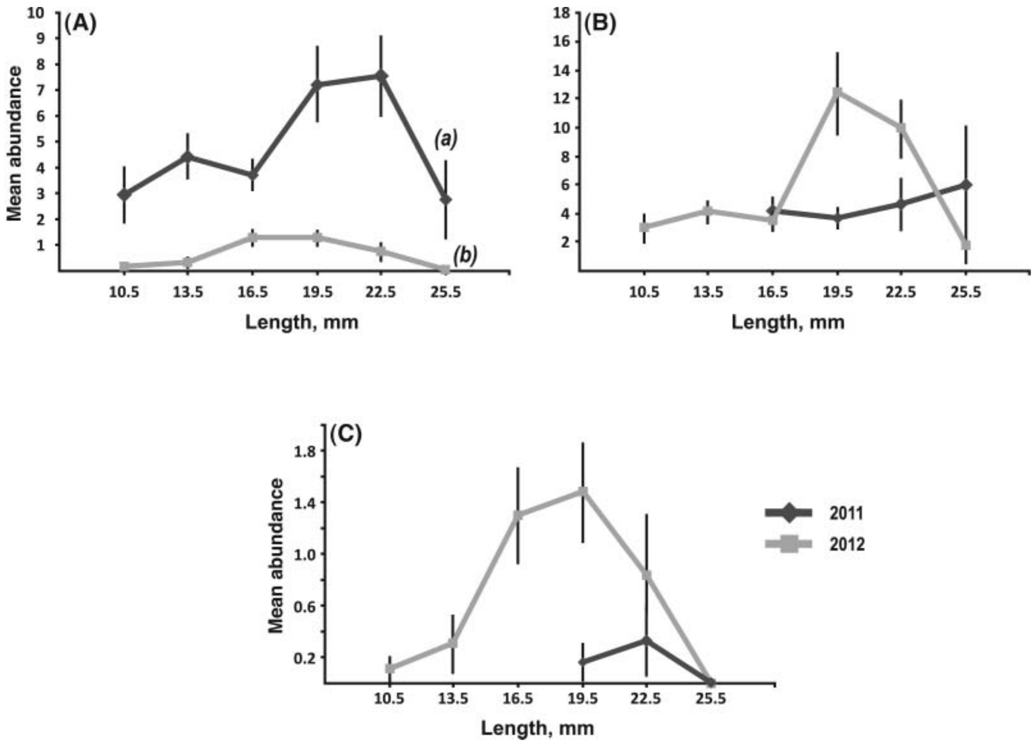

Fig. 9. Correspondence of mean abundance of *B. scorpii* and juvenile stickleback size. (A) Mean abundance of *B. scorpii* at two localities: (a) Seldyanaya Inlet, (b) the lagoon. (B, C) Mean abundance of *B. scorpii* on juvenile stickleback of different sizes in Seldyanaya Inlet and the lagoon (errors bars represent standard errors).

The mean abundance of *Brachyphallus crenatus* was higher in Seldyanaya Inlet than in the lagoon, where this species was rarely observed. The mean abundance of *B. crenatus* differed between juvenile sizes and years (GLM: size, $F_{1,142} = 18.3$, $P < 0.001$; year, $F_{1,142} = 6.6$, $P = 0.01$). In 2011, mean abundance of this parasite was extremely low and, in 2012, it clearly increased with the size of juvenile stickleback (Fig. 10).

Feeding and infection

The most abundant food item in the stomachs of juvenile stickleback was copepods. These crustaceans played the role of intermediate hosts for *Brachyphallus crenatus* and *Bothriocephalus scorpii* (see Discussion). The latter species did not show any association with intensity of copepod consumption by fishes (Spearman correlation, $R = -0.12$, d.f. = 14, $P < 0.05$). However, a positive correlation between the mean number of copepods in stomachs, juvenile size, and the mean abundance of *B. crenatus* was found in Seldyanaya Inlet, where this parasite was abundant (Spearman correlation, $R = 0.8$, d.f. = 5, $P < 0.05$) (Fig. 11).

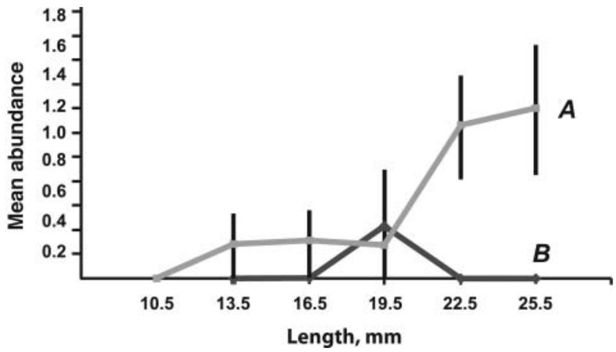

Fig. 10. Dependence of mean abundance of *B. crenatus* on juvenile stickleback size in Seldyanaya Inlet: (A) 2012, (B) 2011 (errors bars represent standard errors).

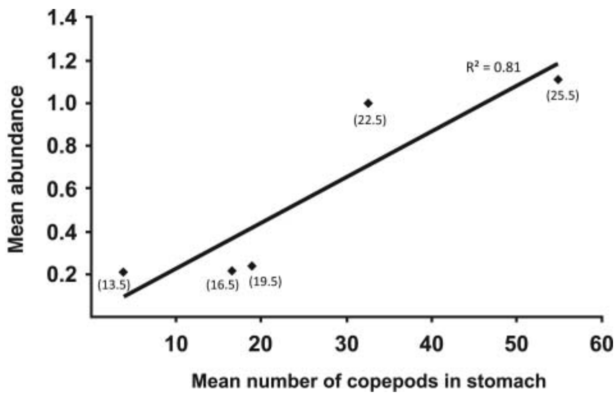

Fig. 11. Association between mean abundance of *B. crenatus* and the mean number of copepods in the stomachs of juvenile stickleback of different size groups in Seldyanaya Inlet. The average length of size groups is indicated in brackets.

DISCUSSION

The present study provides the first description of parasite communities of White Sea threespine stickleback juveniles at various stages of development, and it sheds light on the diet and densities of this species. The study was performed during a period of increasing stickleback population density (Lajus *et al.*, 2013). Polyansky and Shulman (1956) were the first to study stickleback parasites in the White Sea. Since that time, the population of stickleback fell close to zero before recovering. Polyansky and Shulman reported just five parasitic species, whereas the present study revealed a total of 12. The lists overlap except for the monogenean *Gyrodactylus rarus* described in 1956 but not found in our samples. This discrepancy may be due to the overall decrease in the frequency and abundance of this parasite, even in adult hosts, especially compared with *G. arcuatus* (Shulman and Shulman-Albova, 1953; E.V. Rybkina, unpublished data). *Gyrodactylus rarus* inhabits fish gills, which are small and yet underdeveloped in the young. We believe that transmission of this parasite from adults to juveniles is unlikely. Other differences include more limited infection by *Gyrodactylus* spp.

and *Trichodina* sp., and more widespread infection by *Podocotyle atomon* in the 1950s than now.

An overall comparison of the data of Polyansky and Shulman (1956) with our results reveals a notable increase in the number of parasite species. In the main, the parasite fauna of juveniles has been enriched by trophically transmitted parasites. The intensity and prevalence of ectoparasitic infection have also increased, which may be associated with population growth of the hosts (Marcogliese, 2005). However, detailed comparison of our data with those provided by Polyansky and Shulman (1956) is difficult because the latter did not report either the size or the age of the juveniles, and their sample size was limited.

Parasite fauna and abundance on stickleback as reported here for the White Sea is similar to that in the Baltic Sea, but somewhat richer. Zander (2007) reported a total of 10 parasite species at two sites, with smaller juveniles being infected with three species and larger juveniles (21–25 mm) with up to six species. The most common and abundant parasites in both the White and Baltic Seas were *Gyrodactylus* sp., *Trichodina* sp., and *Cryptocotyle concavum.* Juveniles from the Barents Sea hosted fewer parasite species: only *Trichodina* sp., *G. arcuatus*, and *Podocotyle atomon* were identified (Polyansky and Shulman, 1956). Possible reasons for this paucity of parasite fauna in the Barents Sea include: (1) lower abundance of stickleback, and (2) lower summer water temperatures, which may slow transmission of some parasites. Finally, in the delta of the Neva (Gulf of Finland), a nearly freshwater part of the Baltic Sea, larvae that were departing the nests in June (Banina and Isakov, 1972) carried higher numbers of ectoparasites than in our study. The parasite fauna there was enriched with freshwater species, such as *Apiosoma amoebae*, *Trichodina domerguei*, *Epistylis lwoffi*, *Vorticella* sp., *Hemioprys branchianum*, *Ichthyophthirius* sp., *Chilodenella cyprini*, and *Gyrodactylus arcuatus* (Banina and Isakov, 1972). As juveniles began to migrate offshore in late July, they acquired a freshwater trematode *Diplostomum paraspathaceum.* The parasite fauna of juveniles generally has been formed by the early autumn. *Bunodera luciopercae* and *Myxobilatus gasterostei* were added to this list in winter, although they were rarely recorded. By the early spring, most parasites had disappeared, and juveniles harboured only *Apiosoma amoebae*, *Trichodina domerguei*, and *Epistylis lwoffi.*

At present, stickleback are the dominant fish species in the study area of the White Sea in summer (Trofimenko, 2013). Moreover, we found similarly high levels of juvenile abundance at two sites differing in environmental conditions. Differences between the sites in patterns of seasonal dynamics of the density and size of juvenile stickleback are likely caused by their different connection with the open sea. In the open Seldyanaya Inlet, the density of juveniles fell by September because of their immigration and mortality, and size reflected their growth. The average size and density of juveniles near the entrance of the semi-isolated lagoon, where the sampling site was located, might differ from those of the lagoon as a whole, because it seems that only juveniles that are ready to leave the lagoon for offshore migration concentrate here, and their physiological condition can differ from that of other juveniles in the lagoon.

Isakov (1974) reported that protracted spawning dictates that the intensity of infection and seasonal dynamics of parasite species composition (prevalence) may not coincide in juveniles hatching at different times. In the White Sea, however, the spawning period of threespine stickleback is relatively short (Yershov, 2011). The bulk of the fish caught during August belonged to the size class appropriate to the collection date, which means that most juveniles hatched at similar times. Although juveniles of the smallest size class were present in all our samples, their density in late August and September was extremely low.

In general, the parasite fauna of adult and juvenile threespine stickleback is similar: all 12 parasite species found in juveniles are also present in adults (Shulman and Shulman-Albova, 1953; E.V. Rybkina, unpublished data). However, pronounced temporal dynamics were observed in the composition and abundance of parasite species during the juveniles' growth. The youngest juveniles were infected by parasites with direct life cycles (*Gyrodactylus arcuatus*, *Trichodina* sp., *Thersitina gasterostei*) and by parasites that actively penetrated the host (metacercariae of *Cryptocotyle* spp.). Species with trophic transmission appeared later. Our observations conformed to a model of age-related dynamics of the parasite communities of juvenile stickleback first described by Polyansky and Shulman (1956). Since adult males guard the nests and the hatchlings, they maintain close contact with the young, which facilitates the transmission of ectoparasites. The youngest stickleback (7–8.5 mm long) were infected only with ectoparasites and cogeneric trematodes (*Cryptocotyle* spp.). Small juveniles 9–11 mm long typically begin to feed actively and acquire trophically transmitted parasites. Thus, as the juveniles grew, both the prevalence and intensity of parasite infection increased.

Differences in parasite species composition and density match well the data on the diet of juveniles. Hatchlings either did not feed or had a mixed diet. Small juveniles (9–11 mm long) that fed mostly on ciliates never hosted trematodes or cestodes, which are transmitted by copepods. By the end of the season, however, as the juveniles grew, the role of copepods in their diet increased, and cestodes and trematodes began to infect them. As the juveniles developed, two processes occurred in parallel: the fish attracted new parasitic species, while those parasites already present multiplied. At the same time, we observed a decrease in mean abundance of *Bothriocephalus scorpii* in larger juveniles, possibly a result of: (1) increased mortality of the most infected juveniles, (2) increased mortality of plerocercoids due to natural or immunological causes, or (3) increased mortality of large parasites due to intraspecific competition within their host. Further research is required to distinguish among these alternatives.

We found inter-annual differences in mean abundance of the most common intestinal parasites, such as the cestode *Bothriocephalus scorpii* and the trematode *Brachyphallus crenatus*. The higher mean abundance of *B. scorpii* can be explained by changes in the abundance of the adult stickleback. In 2012, the abundance of adult fish was on average 56 individuals per square metre, whereas in 2011 it was only 16 per square metre (T. Ivanova, M. Ivanov and D. Lajus, unpublished data). More adults (final host of cestodes) resulted in more parasite eggs, which had a higher probability of infecting copepods, and thus eventually juvenile stickleback. A similar logic likely explains the results for *B. crenatus*. The first intermediate host of this parasite, benthic molluscs, can be infected at the beginning of the warm season when adult stickleback appear inshore. During the next few weeks, the parthenogenetic generations of *B. crenatus* develop in the molluscan hosts and begin to produce cercariae. The cercariae infect the second intermediate host, copepods, where metacercariae grow and become infective for fish closer to the end of the season. This rationale can explain the observed increase in mean abundance of *B. crenatus* in larger size groups of juveniles observed in late summer. In addition, recent growth of the stickleback population (see above) might have resulted in increased infection of molluscs by *B. crenatus* parthenitae. This change would facilitate growth of copepod infection with *B. crenatus* metacercariae, thus promoting infection of juveniles. We found spatial heterogeneity in parasite infection of juvenile stickleback between the two sites. Such unevenness of infection is often explained by local adaptations or differing immune resistance of the hosts (Zuk and Stoehr, 2002; Kawecki and Ebert, 2004; Gandon and Nuismer, 2009; Eizaguirre *et al.*, 2012; Konijnendijk *et al.*, 2013; Robertson *et al.*, 2016). In the present case, however, we believe that differences in abundance of the intermediate hosts play a major role. Indeed, previous studies indicate that sticklebacks likely spend the winter together in offshore areas (T. Ivanova, M. Ivanov and D. Lajus, unpublished).

The prevalence and mean abundance of hemiurids was lower in juveniles from the lagoon, despite the fact that, in both locations, the contribution of copepods to stickleback diets was high. Copepods are the second intermediate hosts of these parasites (for a review, see Galaktionov, 2001) and different host species may transmit different flatworms. Although juveniles from the two sites preyed on different copepod species, this difference is unlikely to be the reason for the different infection rates. Instead, we believe that juveniles from the lagoon are less exposed to the infected crustaceans. Subtidal buccinids, which are the first intermediate hosts of the hemiurid trematodes, are absent in the shallow lagoon. Therefore, the local population of copepods is probably not infected and the bulk of parasites come from the open sea. Since water exchange is very limited in the lagoon, the number of trematode-infected crustaceans gaining entrance is also likely small. On the other hand, Seldyanaya Inlet, with its wide and relatively deep entrance, provides ample opportunity for infection.

Infection by the cestode *Bothriocephalus scorpii* was also less pronounced in juveniles from the lagoon. The reasons for this difference are unclear, because *Acartia longiremis*, which dominates in the diet of the lagoon juveniles, was described in the White Sea as an intermediate host of *B. scorpii* (Grozdilova and Makrushin, 1985).

Surprisingly, infection by *Podocotyle atomon* was fairly high in juveniles from Seldyanaya Inlet, even though its second intermediate hosts – intertidal gammarids – although highly infected (E.V. Rybkina, unpublished), are rarely eaten by sticklebacks (Fig. 2, Table 1). We suggest that rare cases of gammarids being eaten by stickleback juveniles could be enough to maintain relatively high infection levels of this parasite. Juveniles from the lagoon were infected with *P. atomon* to a lesser extent, probably due to a low density of their first intermediate hosts – periwinkles *Littorina* spp.

In contrast, metacercariae of *Cryptocotyle* spp. were found in all juveniles from the lagoon. This exceptionally high degree of infection might be due to favourable conditions for parasite transmission. Mudsnails [*Hydrobia* spp., the first intermediate hosts of *Cryptocotyle concavum* and *C. jejuna* (Deblock, 1980)] are abundant in the lagoon, and the site is frequently visited by seagulls – the final hosts of these parasites. Differences between the sites were also observed in temporal patterns of parasite infection. The appearance of the largest (25–27 mm) juveniles infected with *Thersitina gasterostei* and *Mixobilatus* sp. in Seldyanaya Inlet in September may be caused by local migration from other biotopes, such as the lagoon, where stickleback of all size classes are infected.

Given the available data, it is difficult to evaluate the possible effect of parasites on the population dynamics of stickleback. Still, a few inviting examples are apparent. The hemiurid trematodes *Brachyphallus crenatus* and *Lecithaster gibbosus* were only seen in the large juveniles, being absent in the younger ones. We believe these two species to be lethal to stickleback below a certain size or age, as they are for herring larvae, when even a single parasite can kill a fish (Ivanchenko and Grozdilova, 1981). Infection of the larger juveniles, on the other hand, probably is not lethal. Population growth of stickleback facilitates transmission of these trematodes and increases their abundance, which, in turn, may depress the abundance of the host species.

CONCLUSION

In this study, we were looking at a system where the host species (threespine stickleback) rapidly increased in abundance, which now is probably close to its historical maximum. The number of parasite species of juvenile stickleback in the inshore zone was about half that of adults. Since the abundance and diversity of the parasites is usually positively correlated with the abundance of the host (Poulin, 2007), we may expect a further increase of parasite diversity and abundance as the White Sea population of the threespine stickleback stabilizes or grows.

The study of parasites of the White Sea stickleback is useful for understanding host–parasite interactions, because the abundance of this fish changed dramatically in a relatively short period of decades. Moreover, stickleback are now an abundant species playing an important role in both coastal and pelagic communities, and providing a bridge between these biotopes in terms of exchange of energy, biogenesis and, it would seem, parasites.

We believe that it is especially important to carry out such studies based on quantitative information. We took special efforts to undertake quantitative analysis of changes in stickleback abundance during the last century (Lajus *et al.*, 2013), their spatial distribution and association with different biotopes (Ivanova *et al.*, 2016; Rybkina *et al.*, submitted), the role of stickleback in food webs as a prey (Bakhvalova *et al.*, 2016) and as a predator (Demchuk *et al.*, 2015). Data obtained in the present study is the first step in quantifying the parasite load on the White Sea stickleback. We hope this will further our understanding of the mechanisms of dynamics of this key species and ecosystems of the entire White Sea.

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