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Effect of tidal level on abundance of symbionts in the White Sea blue mussel

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ABSTRACT: In the White Sea, the blue mussel *Mytilus edulis* occupies a wide range of biotopes and is associated with numerous symbiotic organisms. At some sites, mussel cover spreads continuously from the intertidal to the subtidal zone. We checked whether the patterns of infection by different associated organisms differed among the upper subtidal, zero-depth and lower intertidal zones at 3 sites in the Kandalaksha Gulf and the Onega Bay of the White Sea. Organisms belonging to 13 taxa were found in mantle cavities and tissues of blue mussels. Parasitic green algae, a sporocyst and metacercariae of 5 species of digenean trematodes occupied mussel tissues; commensal ciliates, rhabdocoelans and some free-living invertebrates were found in mantle cavities. Quantitative composition of symbiotic communities of mussels was not the same at different tidal levels: *Urastoma cyprinae* (commensal rhabdocoelans) were more abundant in the subtidal and zero-depth zones, while encysted metacercariae of *Renicola roscovita* and *Himasthla* sp. were more abundant at the zero-depth and intertidal zones. We suggested several hypotheses to explain this heterogeneity.

KEY WORDS: *Mytilus edulis* · Symbionts · Vertical distribution · Blue mussel · Digenea · *Urastoma cyprinae*

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INTRODUCTION

Close interspecific associations between organisms are very common (Surindar & Vernon 2000, Combes 2001, Roberts & Janovy 2009). We use the term 'associated organism' for organisms regularly found on the host's surface or inside its cavities and tissues regardless of the strength of their connection (facultative or obligate), the term 'accidentally associated organism' when the association is not essential, and the term 'symbiont' for an organism for which the association with the host is obligatory, regardless of the resulting effects (e.g. parasitism, commensalism). In the most intimate associations, the host acts as a (semi-)closed microenvironment for the associated organisms (e.g. Crompton 1997). However, even in the closest associations, the host cannot entirely dampen the effects of the external environment (e.g. Möller 1987, MacKenzie et al. 1995, Wolinska & King 2009). This results in spatial heterogeneity of symbiont distribution, driven by either abiotic or biotic environmental factors. Different factors can prevail at different scales (Fredensborg et al. 2006). The role of 'large-scale factors' in controlling the distribution of symbionts has been described by Buck et al. (2005), Thieltges et al. (2009), Wilson et al. (2013), Galaktionov et al. (2015), Rogers et al. (2015) and Leydet & Hellberg (2016). Examples of the role of 'small-scale' factors can be found in Granovitch et al. (2000), Poulin et al. (2000), Smith (2001), and Granovitch & Mikhailova (2004).

An associated organism can be influenced by ambient environmental factors due to (1) direct contact of the symbiotic stage with the environment, (2) impact on the free-living stages, (3) changes in the condition of the host which are caused by the environment and (4) environmental effects on the hosts' distribution. For example, aquatic symbionts

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living on the host's body surface or in its outer cavities (e.g. mantle cavities of molluscs) can be influenced by changes in the properties of water (MacKenzie et al. 1995, Finstad et al. 1995, Soleng & Bakke 1997, Brooks 2005, Lei & Poulin 2011). Free-living stages of symbionts are even more susceptible to environmental factors (Pietrock & Marcogliese 2003, Morley & Lewis 2004, Prinz et al. 2011). For instance, at exposed rocky shores, digenean miracidiae and cercariae are often cast ashore or washed to the subtidal zone (Galaktionov & Dobrovolskij 2003). Harsh environmental conditions can weaken the host organism so that it cannot resist the intrusion of parasites, resulting in increased intensity of infection (Khan & Thulin 1991, Sures 2004). Heterogeneity in the environment can cause heterogeneity in host density, which in turn leads to spatial differences in symbiont distribution. For example, sites with higher densities of cockles had a lower percentage of specimens infected with digeneans (Mouritsen et al. 2003). For symbionts with complex life cycles, e.g. digeneans, the connection between upstream host density and infection patterns is well known (Smith 2001, Hechinger & Lafferty 2005, Fredensborg et al. 2006, Thieltges 2007).

A good opportunity to study the influence of environmental factors on the distribution of associated organisms is provided by hosts that occupy biotopes with contrasting conditions. The blue mussel *Mytilus edulis* L. 1758 is one of the habitat-forming species in the intertidal and upper subtidal zones in North Atlantic; it is also widely used in aquaculture (Lukanin 1985, Sukhotin 1993). *M. edulis* hosts many different symbionts such as rhabdocoelans, digeneans and crustaceans, at least 7 species in the White Sea (Chubrick 1966, Zelikman 1966, Kulatchkova 1985, 1987). Numerous studies have dealt with symbionts of the blue mussel all over the world; most of which have considered symbiont species diversity and factors controlling the intensity and prevalence of digenean infection (Pregenzer 1983, Fateev et al. 2000, Nikolaev et al. 2006, Wilson et al. 2013, Galaktionov et al. 2015). These studies were mostly focused on mussels from intertidal beds and artificial substrates, but very few have considered differences between intertidal and subtidal mussels (Kruczynski 1974, Buck et al. 2005).

In the White Sea, *M. edulis* lives on rocks, brown algae and soft sediments from the middle intertidal zone to the upper subtidal (depth of 3 m) (Naumov 2006), and occasionally occurs in the upper intertidal or at depths down to 17 m (V. A. Krapivin pers. obs.). In some habitats, mussel cover continuously extends from the intertidal to the subtidal zone. This implies a strong environmental gradient. In the White Sea, tides are up to 3 m high; desiccation time in the lower intertidal zone is about 2 h. In summer, sea surface temperature during low tide can rise as high as +19.3°C and salinity drops down to 0.2‰, compared to 0 to 14°C and 28‰ in the subtidal zone (Babkov 1998, Basova et al. 2004). These factors make the White Sea a convenient location to study the effects of environmental gradients on the distribution of mussel-associated organisms.

The distribution of mussel-associated organisms is highly likely to be affected by the tidal level: a considerable complex of abiotic and biotic factors varies between tidal levels. Digeneans that use birds as definitive hosts are usually more abundant in intertidal gastropods, while those that use fishes are more abundant in subtidal gastropods (Galaktionov & Dobrovolskij 2003). This heterogeneity is likely to be caused by differences in density of the upstream hosts: many birds feed at the intertidal zone and most fishes spend much of their lifespan in the subtidal zone. This distribution may also be beneficial for the digeneans, given that the second intermediate host is not highly motile, cercariae emerging from gastropods at the appropriate tidal level will have a greater chance of encountering the second intermediate host, which will be eaten by the final host (a bird at the intertidal zone and a fish at the subtidal zone). That is why we expected that intertidal mussels would also be more infected with digeneans whose life cycle includes intertidal gastropods and marine birds. Moreover, some mussel symbionts might be less tolerant of intertidal conditions (such as high temperature, desiccation and low salinity) than their mussel hosts, so they would be expected to occupy subtidal mussels rather than intertidal ones. That is why we expected symbiotic assemblages from mussels from spatially close subtidal and intertidal habitats to differ in species composition and species abundances.

We compared structures of communities of organisms associated with *M. edulis* as well as abundances of certain groups of symbionts in subtidal, zero-depth and intertidal zones at 3 sites at the White Sea to find out if there is variation between different tidal levels.

MATERIALS AND METHODS

Sampling

Sampling was done from July to September 2013 at 3 sites at the Kandalaksha Gulf and Onega Bay of the White Sea. Sampling sites were spaced from ~2 to

~100 km apart. Two sites were located in Chupa Inlet in the Kandalaksha Gulf: Bolshoi Gorelyi Island (BG) and the Lebiazhya Inlet (LE); and one site was located at the Solovetsky Archipelago in Onega Bay: Bolshoi Solovetsky Island (BS) (Fig. 1).

In Chupa Inlet, the surface water temperature fluctuates throughout the year from −1.4 to 19°C, and the average annual temperature is about 2°C. Salinity fluctuates from 14.5‰ in spring to 26‰ in summer. Near the Solovetsky Archipelago, the surface water temperature varies from 0 to 24°C and salinity ranges from 20 to 27‰. Maximum tidal amplitudes at both regions are about 2 m (Babkov 1998, Basova et al. 2004).

At the studied sites, mussels occupied an area spanning the lower intertidal to the upper subtidal zones (max. depth from 3 to 15 m at different sites). The surface in the studied areas is mainly soft-sediment, and the mussels are aggregated into groups of several specimens attached to uniformly spread hard substrata, predominantly stones and brown algae (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/d130p131_supp.pdf).

At each site, three 2 m wide plots were chosen: (1) the lower plot, at the subtidal level at a depth of 3 m; (2) the middle plot, at the spring low-tide level; and (3) the upper plot, at the intertidal level (0.6 to 0.7 m above mean sea level) (Fig. 1). Distances between the lower and middle plots were approximately 5 m, and between the middle and the upper plots, about 2 m. In each plot at every site, all live mussels were collected and counted from 3 randomly selected 1 m^2 quadrats located 2 m apart. Mussels from the lower plots were collected with the use of

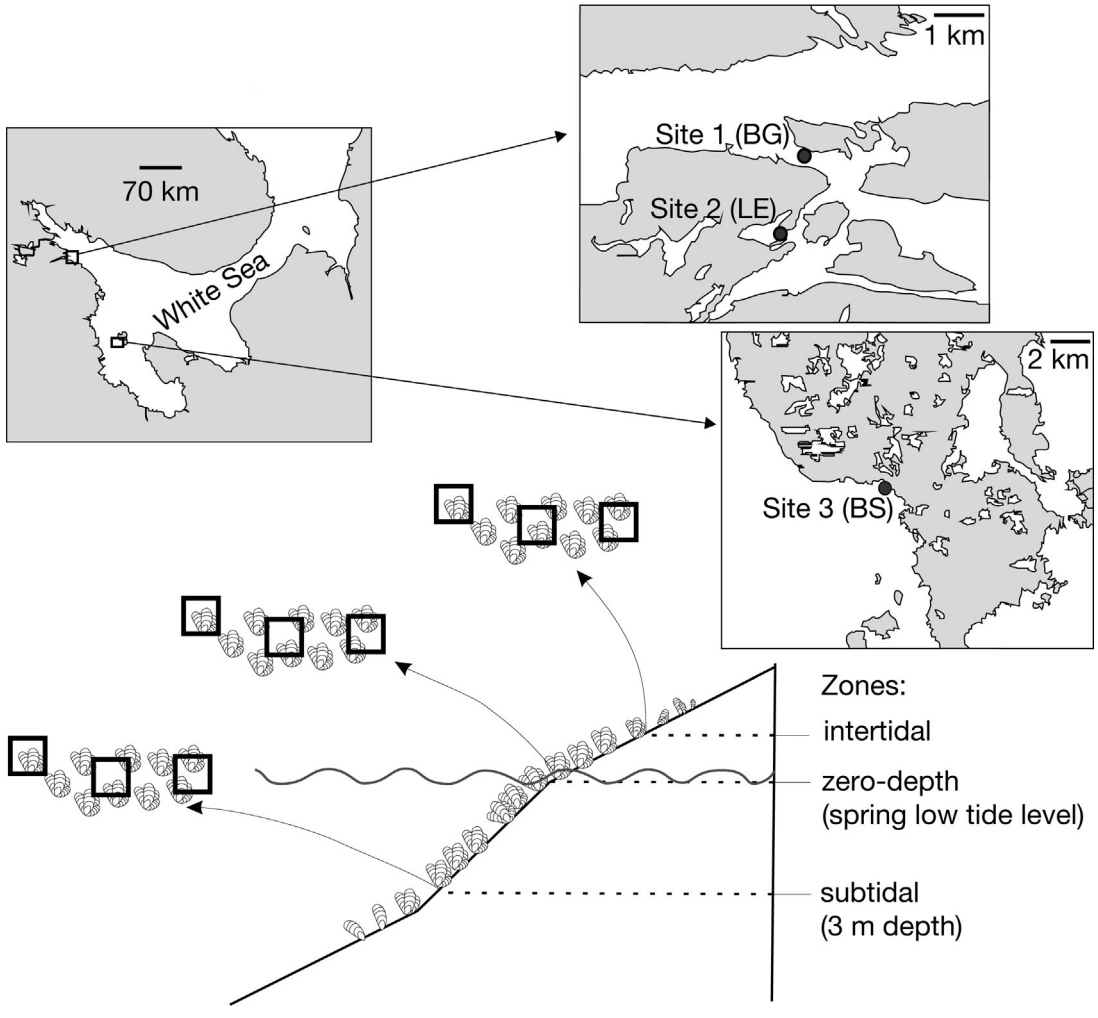

Fig. 1. Sampling sites (based on www.google.com/maps) and sampling scheme. Sampling site coordinates: Site 1 (Bolshoi Gorelyi Island, BG) 66° 18.8′ N, 33° 36.8′ E; Site 2 (Lebiazhya Inlet, E) 66° 17.7′ N, 33° 36.3′ E; Site 3 (Bolshoi Solovetsky Island, BS) 65° 02.0′ N, 35° 41.2′ E. At each of the 3 sites, 3 zones at different tidal levels were chosen, and at each zone, mussels *Mytilus edulis* were collected from three 1 × 1 m quadrats. From each quadrat, 20 mussels with shell length >10 mm were dissected

diving equipment; at the middle and the upper plots sampling was performed during low tides. Differences in average mussel densities were not significant across all the sites and plots ($F_{8,18}$ = 1.34, p = 0.29) (see Table S1 in the Supplement).

Processing

Length of the mussel shells was measured to the nearest 0.1 mm. A total of 20 mussels with shell length >10 mm, randomly chosen from each quadrat, were examined for any associated organisms. Smaller mussels were not examined because associated organisms were extremely rare in such specimens according to preliminary investigations. The approximate age of the mussels was estimated by counting the external growth marks. The mussels were dissected, their mantle fluid was examined, and soft tissues were squeezed between 2 slides and viewed under binocular microscope. All detected associated organisms were counted and preserved in 70% alcohol, 4% formaldehyde or Bouin fluid.

In this study, we did not consider commensal ciliates, because their abundance changes drastically during the few hours after host mollusc collection but before dissection, and therefore is difficult to estimate properly (V. A. Krapivin pers. obs.). We can only mention that the ciliates *Peniculistoma mytili* and *Ancistrum mytili* were common in mussels' mantle cavities at all tidal levels and at all sites explored.

Analyses

For most groups of associated organisms, there were strong positive correlations between parameters of infection: mean infection intensity (number of symbiont specimens vs. number of infected hosts), prevalence of infection (number of infected hosts vs. total number of sampled hosts) and abundance (number of symbiont specimens vs. total number of sampled hosts) (Table S2 in the Supplement). So we found it redundant to use all of them in statistical analyses. Abundance was preferred (where possible) over the other 2 estimations because it accumulates the most quantity of information (it depends on both the percentage of hosts infected and the number of symbionts in each host). We tested the effect of tidal level on the abundance of associated organisms. For multidimensional analyses, we used only mussels infected with at least 1 symbiont (due to restrictions of Bray-Curtis dissimilarity), so these analyses estimate the effect of tidal level on intensity of infection instead of abundance. For unicellular green algae, ranks were used instead of the number of specimens (Table S3 in the Supplement). The data on different species of accidentally associated organisms were pooled.

All calculations were performed using the R statistical environment (R Core Team 2016); graphs were created with the help of the 'ggplot2' package (Wickham 2009), and p-values less than 0.05 were considered significant. The Bonferroni correction was used to adjust the p-values in multiple tests. For mean values, 95% confidence intervals are given. The 'hmisc' package was used for calculations of Wilson confidence intervals for percentages (Harrell 2016).

It is known that the number of organisms associated with a host specimen and the probability of infection can depend on the age of the host (Nikolaev et al. 2006). In our samples there was a significant correlation between host shell length, host age and patterns of infection by most groups of associated organisms. Because of the collinearity of these 2 variables, we decided to include only mussel age as an explanatory variable. Another problem is that the mean ages of infected mussels differed among levels and sites, and some age values were not present in every level–site combination (Table S4 in the Supplement). To avoid unreasonable extrapolations, we fitted our models using only the 3 to 8 yr old mussels, which were present in all samples (so the final sample became unbalanced with sizes varying from 36 to 60 specimens) (see Table S5 in the Supplement). Outliers were detected in the *Renicola roscovita* abundance data, but dropping them out of the analysis did not affect the significance of statistical tests.

To visualize the differences in symbiont community composition between different tidal levels, we performed a non-metric multidimensional scaling (nMDS) (Quinn & Keough 2002) using the 'metaMDS' function from the 'vegan' R package (Oksanen et al. 2016), based on the matrix of Bray-Curtis dissimilarities between individual mussels, infected with at least one symbiont (accidentally associated organisms not included). Correlations between the 2-dimensional nMDS axes and each of the original variables were calculated. Significance of the correlations was tested with randomization tests (1000 permutations). Variables significantly correlated (after adjustment for multiple tests) with the nMDS axes were used for biplots. To test the significance of the effect of tidal level on symbiont community composition, we used multivariate analysis of variance with permutational hypotheses testing (PERMANOVA) (Anderson 2001, Legendre & Legendre 2012) with 1000 permutations

using the 'adonis' procedure provided by the 'vegan' package. For the analysis, a matrix of Bray-Curtis dissimilarities between individual mussels was obtained. Level was the explanatory variable, the number of organisms in infected mussels was the response variable, and site was the grouping factor.

To test the effect of tidal level on abundance of a particular group of associated organisms, we fitted generalized linear models (GLMs) for count data (Zuur et al. 2007). The full models included discrete effects of level, site, mussel age and the site × level interaction. We followed a protocol proposed by Zuur & Ieno (2016). We started with the GLMs with Poisson error distribution and log link function. To validate the models, we computed dispersion statistic, and examined for patterns the plots of residuals versus fitted values and covariates. Since the dispersion statistic for Poisson models indicated overdispersion, we fitted GLMs with negative binomial error distribution and log link function using the 'glm.nb' function from the 'MASS' package (Venables & Ripley 2002). The validation of these models indicated no problems. During model selection, non-significant predictors were removed from the models based on series of likelihood ratio tests. Final models were again checked for overdispersion and residual patterns.

To verify how the final models complied with the observed data, we simulated 10 000 data sets from each of them. For each simulated data set, we calculated the percentage of zeros and the dispersion statistic. The values observed on the raw data corresponded well with the simulated distributions.

The percentage of explained deviance was calculated for each final model. Planned comparisons of abundances of associated organisms among the tidal levels (within each site in case of significant site × level interaction) were done using linear contrasts with the help of the 'multcomp' package (Hothorn et al. 2008). Only 2 sites were analysed for metacercariae of *Gymnophallus bursicola* and symbiotic green algae *Choricystis* sp., because the former were absent from the LE and the latter from BS.

The original data are available online at https://doi.pangaea.de/10.1594/PANGAEA.870539.

RESULTS

Taxonomic composition of fauna associated with *Mytilus edulis*

In total, 14 groups of organisms (not counting 2 ciliate species) were found in the mussels' tissues and mantle cavities (see Tables S5 & S6 in the Supplement). The rhabdocoelan flatworm *Urastoma cyprinae* and metacercariae of the digeneans *Renicola roscovita* and *Himasthla* sp. were most common, and occurred at all 3 sites. Metacercariae of the Gymnophallidae family were present in mussels from BS, a single specimen was recorded from a mussel from BG and no gymnophallid metacercariae were observed at LE. Based on unpublished data kindly provided by Dr. K. V. Galaktionov, the metacercariae was identified as *Gymnophallus bursicola*. An unidentified species of metacercaria was found at all 3 sites. The only mussel infected with a sporocyst of *Prosorhynchus squamatus* was collected at the intertidal zone of BS. Parasitic unicellular green algae were absent at BS, but quite common at the 2 other sites. According to Kvitko & Migunova (2011), the algae were identified as *Choricystis* sp.

Some free-living invertebrates were associated with *Mytilus edulis*. The most abundant were nematodes (predominantly of genus *Enoplus*) and copepods (mostly *Microsetella norvegica*). Halacarid mites (*Rhombognatus* sp. and *Halacarellus floridiarum*), chironomid larvae and isopods of genus *Jaera* were rarely observed. One mussel hosted an ostracod. For the full list of groups of associated organisms, see Table S6).

We did not observe strong differences in taxonomic composition of the mussel-associated organisms among the tidal levels. Most taxa occurred at all 3 levels, at least at some sites (see Table S7 in the Supplement).

Localization in host

Green algae were present in mantle, adductor muscle and (in cases of heavy infection) in gill, palp and foot tissues of mussels. *U. cyprinae* were located on the gill, palp, mantle and foot surfaces, or were freely swimming in the mantle fluid. *R. roscovita* metacercariae often occurred in the digestive gland and palps, and rarely in gill, foot and foot retractor muscle tissues. *Himasthla* sp. metacercariae were found in the foot tissues, and rarely in the foot retractor muscle and mantle tissues. *G. bursicola* larvae occupied space between the mantle and the shell. Unidentified metacercariae occurred in the digestive gland. A sporocyst of *P. squamatus* was located in the mantle tissues. All the accidentally associated organisms were found in the mussels' mantle cavities attached to gills, palps, mantle surface or free in the mantle fluid.

Tidal level effects on symbiont community composition and abundance

According to nMDS based on the infection intensity, the 95% CI for centroids of subtidal, zero-depth and intertidal mussels were separated from each other (Fig. 2). However, the 3 groups significantly overlapped. Randomization tests showed that infection intensities of the 6 symbiont taxa significantly changed in the ordination space. Subtidal and intertidal mussels diverged from each other mainly along the horizontal axis, along which varied the intensity of infection by *U. cyprinae* and *Himasthla* sp. Differences in symbiont community composition of subtidal and intertidal mussels were confirmed by PERMANOVA ($F_{2,384}$ = 62.147, p = 0.001) (Table S8 in the Supplement).

We tested the tidal level effect on abundances of the above-mentioned groups of associated organisms using GLMs (Table 1). For *U. cyprinae*, *R. roscovita*, *Himasthla* sp., unidentified metacercariae, *Choricystis* sp. and the group of accidentally associated organisms, the interaction of site and level was significant. For *R. roscovita,* unidentified metacercariae, *Choricystis* sp. and accidentally associated organisms we also could not exclude the effect of mussel age. For *G. bursicola*, only the tidal level effect was significant. For a closer examination of infection patterns, we concentrated on the planned comparisons of levels within sites (Table 2).

Table 1. Model selection for the abundance of organisms associated with mussels *Mytilus edulis*. Generalized linear models with negative binomial error distribution were used. (×) denotes an interaction term. The best model in each set is shown in **bold**

Full and reduced models	df	Log-likelihood ratio	p-value (χ^2-test)	Explained deviance (%)
Urastoma cyprinae				
~ Level + site + site × level + age				61.9
~ Level + site + site × level	5	9.453	0.09	61.0
~ Level + Site	4	41.445	<0.01	56.5
Renicola roscovita				
~ Level + site + site × level + age				43.0
~ Level + site + site × level	5	30.417	<0.01	35.6
~ Level + site + age	4	39.632	<0.01	33.3
Himasthla sp.				
~ Level + site + site × level + age				42.2
~ Level + site + site × level	5	5.055	0.41	41.1
~ Level + site	4	28.310	<0.01	34.6
Gymnophallus bursicola				
~ Level + site + site × level + age				42.5
~ Level + site + age	2	8.746	0.647	41.9
~ Level + site	5	8.650	0.124	37.2
~ Site	2	13.935	<0.01	26.9
~ Level	1	25.562	<0.01	15.2
Choricystis sp.				
~ Level + site + site × level + age				37.3
~ Level + site + site × level	5	20.510	0.001	32.1
~ Level + site + age	2	30.245	<0.001	29.0
Accidentally associated organisms				
~ Level + site + site × level + age				22.4
~ Level + site + site × level	5	19.779	0.01	17.0
~ Level + site + age	4	43.880	<0.01	10.5

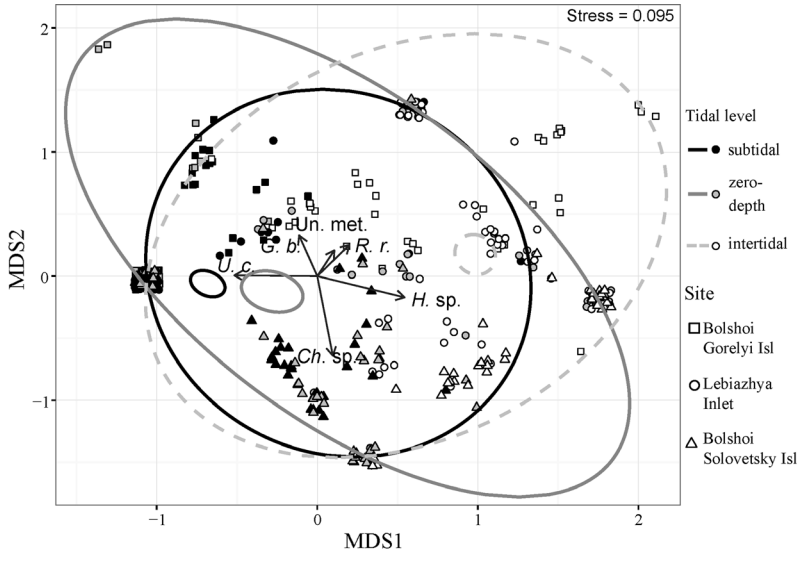

Fig. 2. Non-metric multidimensional scaling (nMDS) ordination plot of the infected *Mytilus edulis* from 3 sites based on a matrix of Bray-Curtis dissimilarities of infection intensity of individual mussels. (To avoid overplotting, random noise was added to the point positions). Small ellipses: 95% confidence intervals of tidal level centroids; large ellipses outline all points characterized by the specified tidal level. Original variables, significantly correlated with the nMDS axes, are presented as arrows on the plot to demonstrate their contribution to the observed patterns (*Ch.* sp.: *Choricystis* sp.; *U. c.: Urastoma cyprinae*; *R. r.: Renicola roscovita*; *H.* sp.: *Himasthla* sp.; Un. met.: unidentified encysted metacercariae; *G. b.*: *Gymnophallus bursicola*)

Table 2. Differences of mean abundances of organisms associated with mussels *Mytilus edulis* from different tidal levels. Comparisons were based on results of negative binomial generalized linear models (GLMs). Within each site, the first 2 comparisons were made using linear contrasts; the third is a regression coefficient that codes corresponding comparison in a given GLM

Location	Comparison	Estimate	SE	*z*-value	Adjusted p (>|z|)
Urastoma cyprinae					
Bolshoi Gorelyi Island (BG)	Intertidal vs. zero-depth	−4.3	1.03	−4.17	<0.001
	Subtidal vs. zero-depth	1.7	0.25	6.79	<0.001
Lebiazhya Inlet (LE)	Intertidal vs. zero-depth	−19.9	1608.74	−0.01	1.000
	Subtidal vs. zero-depth	1.6	0.2327	7.47	<0.001
	Intertidal vs. subtidal	−21.7	1608.74	−0.01	1.000
Bolshoi Solovetsky Island (BS)	Intertidal vs. zero-depth	−1.2	0.34	−3.65	0.002
	Subtidal vs. zero-depth	1.4	0.36	3.86	<0.001
	Intertidal vs. subtidal	−2.1	0.27	−7.93	<0.001
Renicola roscovita					
BG	Intertidal vs. zero-depth	2.0	0.47	4.26	<0.001
	Subtidal vs. zero-depth	−0.4	0.56	−0.72	0.98
LE	Intertidal vs. zero-depth	−1.7	0.97	−1.77	0.393
	Subtidal vs. zero-depth	−0.5	1.02	−0.52	0.996
	Intertidal vs. subtidal	−1.8	0.73	−2.50	0.083
BS	Intertidal vs. zero-depth	6.8	1.19	5.75	<0.001
	Subtidal vs. zero-depth	5.7	2.27	4.46	<0.001
	Intertidal vs. subtidal	2.7	0.51	5.27	<0.001
Himasthla sp.					
BG	Intertidal vs. zero-depth	0.20	0.31	0.64	0.978
	Subtidal vs. zero-depth	−2.41	0.58	−4.17	<0.001
LE	Intertidal vs. zero-depth	2.41	0.80	3.04	0.014
	Subtidal vs. zero-depth	−0.24	1.08	−0.22	1.000
	Intertidal vs. subtidal	2.93	0.56	5.21	<0.001
BS	Intertidal vs. zero-depth	1.65	4116.61	<0.001	1.000
	Subtidal vs. zero-depth	−18.31	6487.90	−0.003	1.000
	Intertidal vs. subtidal	1.65	0.60	2.73	0.038
Gymnophallus sp.					
All locations	Intertidal vs. zero-depth	−1.22	0.83	−1.47	0.295
	Subtidal vs. zero-depth	1.04	0.48	2.15	0.077
Choricystis sp.					
BG	Intertidal vs. zero-depth	0.42	0.48	0.86	0.875
	Subtidal vs. zero-depth	−34.68	6560285	0.00	1.000
LE	Intertidal vs. zero-depth	−0.90	0.29	−3.08	0.010
	Subtidal vs. zero-depth	0.14	0.25	0.58	0.968
	Intertidal vs. subtidal	−1.04	0.27	−3.94	<0.001
Unidentified metacercariae					
BG	Intertidal vs. zero-depth	0.31	1.24	0.25	1.000
	Subtidal vs. zero-depth	−0.28	1.42	−0.19	1.000
LE	Intertidal vs. zero-depth	−24.34	66720	0.01	1.000
	Subtidal vs. zero-depth	−24.28	67230	0.01	1.000
	Intertidal vs. subtidal	−0.57	94710	0.01	1.000
BS	Intertidal vs. zero-depth	26.85	74380	0.01	1.000
	Subtidal vs. zero-depth	−0.12	104400	0.01	1.000
	Intertidal vs. subtidal	26.96	73250	0.01	1.000
Accidentally associated organisms					
BG	Intertidal vs. zero-depth	−1.03	0.41	−2.50	0.074
	Subtidal vs. zero-depth	−1.76	0.54	−3.25	0.008
LE	Intertidal vs. zero-depth	2.33	0.71	3.28	0.007
	Subtidal vs. zero-depth	3.67	0.73	5.04	<0.001
	Intertidal vs. subtidal	0.74	0.56	1.32	0.653
BS	Intertidal vs. zero-depth	−1.44	0.79	−1.81	0.325
	Subtidal vs. zero-depth	−1.01	0.93	−1.18	0.753
	Intertidal vs. subtidal	0.12	0.64	0.18	0.999

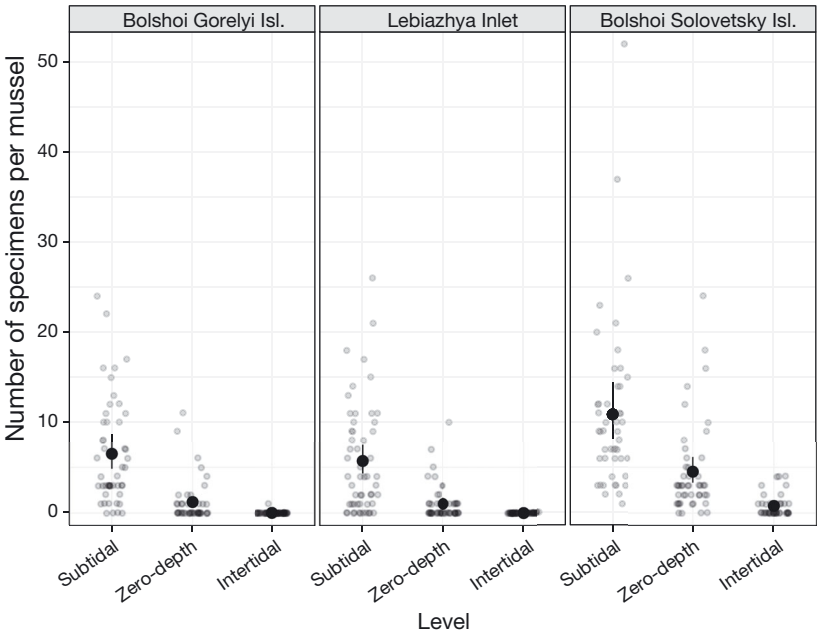

Fig. 3. Abundances of *Urastoma cyprinae* associated with *Mytilus edulis* at different tidal levels. Raw data and predicted values from negative binomial generalized linear model (GLM) with 95% confidence intervals are presented

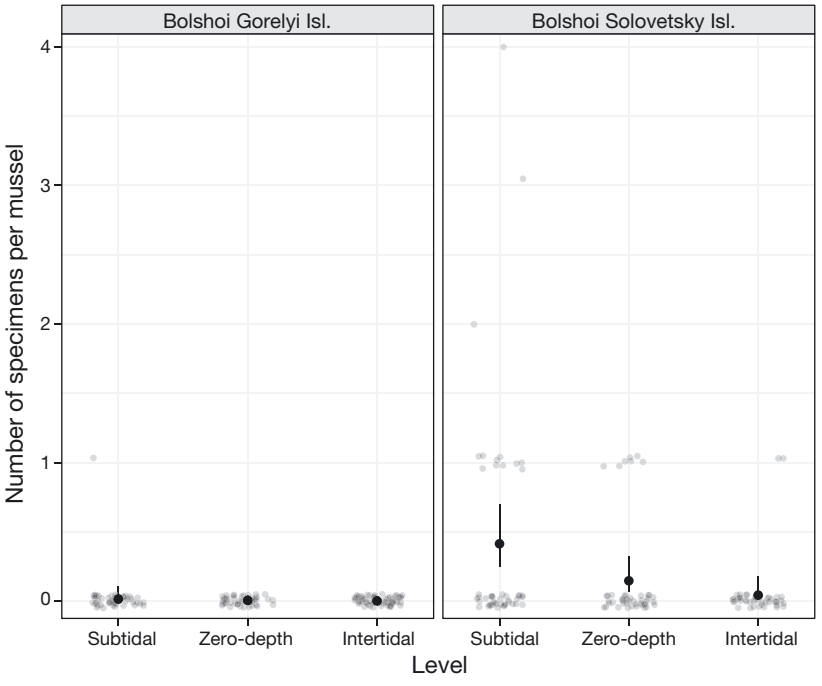

Fig. 4. Abundances of *Gymnophallus bursicola* associated with *Mytilus edulis* at different tidal levels of the 2 sites where it occurred. Raw data and predicted values from negative binomial generalized linear model (GLM) with 95% confidence intervals are presented

Two taxa — *U. cyprinae* and *G. bursicola* — were more abundant at the lower levels (Table 2). The abundance of *U. cyprinae* was higher at the lower levels of all 3 sites (Fig. 3). At LE, *U. cyprinae* abundance significantly differed only between subtidal and zero-depth levels, while at the other sites it differed among all 3 levels. At both sites where *G. bursicola* metacercariae occurred, they were significantly more abundant at the subtidal than at the intertidal level (Fig. 4).

The abundance of metacercariae of 2 digenean species — *R. roscovita* and *Himasthla* sp. — was higher at the upper levels (Table 2). *R. roscovita* was more abundant at the intertidal level than at the zero-depth and subtidal levels (Fig. 5); however, these differences were significant only at BG and BS. At the latter site, the abundance of *R. roscovita* was also higher at the subtidal level than at the zero-depth level due to presence of several highly infected specimens. At LE, no significant differences in abundance of *R. roscovita* between the levels were detected due to low infection prevalence. Abundance of *Himasthla* sp. was also higher at the upper levels: at BG it was significantly higher at zero-depth and the intertidal level compared to subtidal, at LE it was higher at the intertidal compared to subtidal and zero-depth levels, while at BS only the differences between intertidal and zero-depth levels were significant (Fig. 6).

Abundance of *Choricystis* sp. differed among the levels only in 1 of the 2 sites where it was present (Table 2). At LE, this parasitic green algae was significantly less abundant in the intertidal compared to zero-depth and subtidal levels.

Abundance of unidentified metacercariae did not differ significantly among levels (Table 2).

Abundance of accidentally associated organisms differed among levels at 2 sites, but with no definite direction (Table 2). Analyses based on separate groups of free-living organisms (Nematoda and Copepoda) also did not reveal any trends (Tables S9 & S10, Figs. S2 & S3 in the Supplement).

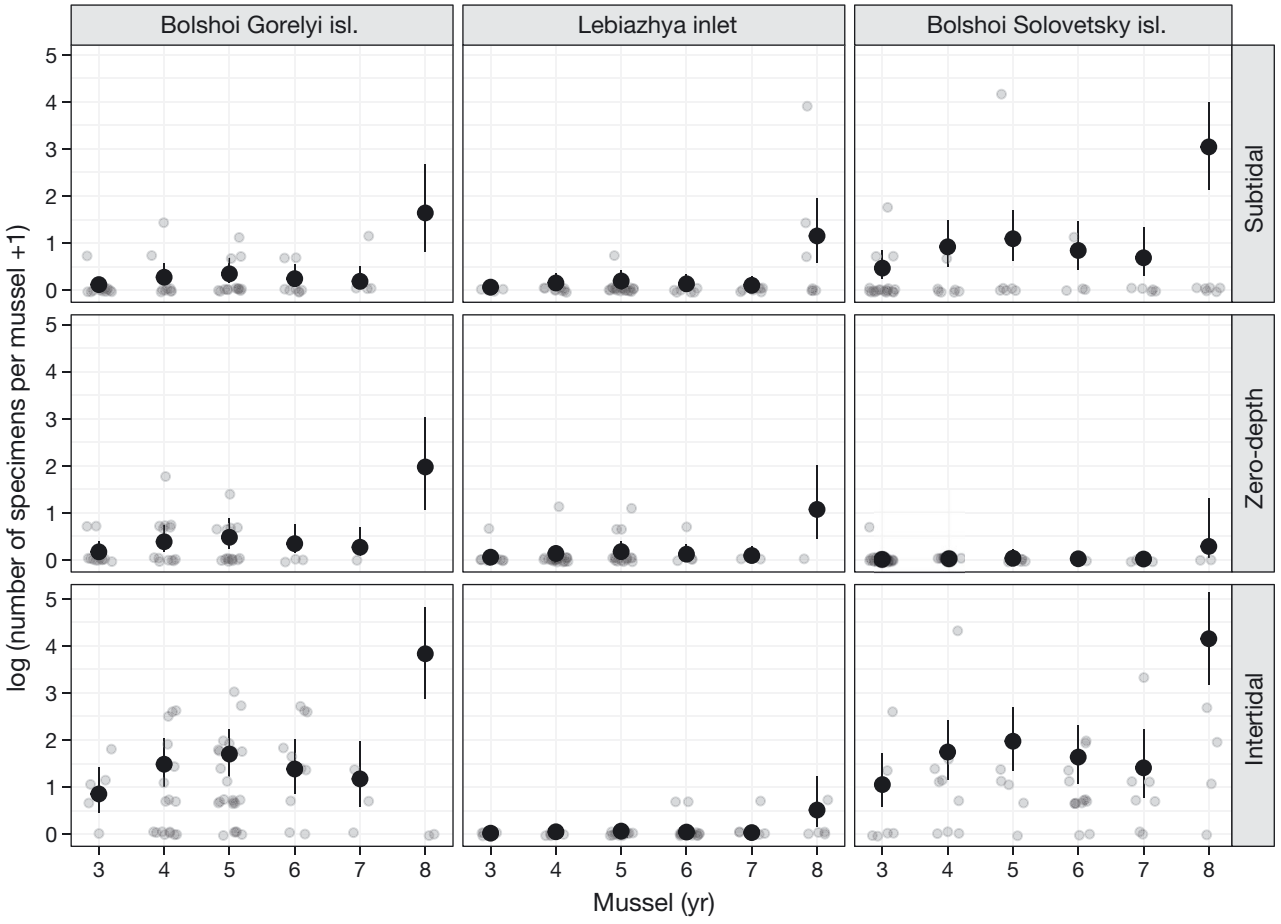

Fig. 5. Abundances of *Renicola roscovita* associated with *Mytilus edulis* at different tidal levels. Raw data and predicted values from negative binomial generalized linear model (GLM) with 95% confidence intervals are presented. Logarithmic scale is used to improve the appearance

DISCUSSION

Community composition of organisms associated with White Sea *Mytilus edulis*

The community of mussel-associated organisms at examined sites was quite diverse. It consisted of 2 ecological groups: symbionts and free-living organisms accidentally found in the mussels' mantle cavities. All the symbiotic species we observed have already been recorded in the White Sea blue mussel (Chubrick 1966, Zelikman 1966, Kulatchkova 1985, Fateev et al. 2000, Krapivin 2012).

We did not observe commensal harpacticoids *Tisbe* sp., previously mentioned as a common associate of *Mytilus edulis* in an area very close to BG and LE by Fateev et al. (2000). The only commensal Tisbidae described from the blue mussel is *Tisbe celata* (Humes 1954). In our mussels, we observed only free-living harpacticoids (see Table S6). Along with the other harpacticoids, we found some Tisbidae, but none of them resembled *T. celata* described by Humes (1954). There is a possibility that in the article mentioned, free-living harpacticoids, such as *Microsetella norvegica* or free-living *Tisbe*, found in the unusual habitat (mussels' mantle cavity) were considered commensals.

The finding of a *Prosorhynchus squamatus* sporocyst in the intertidal zone seems quite surprising. The second intermediate and final hosts of *P. squamatus* are liparid and cottid fishes (Chubrick 1966, Lauckner 1983). Although it is known that near the Solovetsky Archipelago these fishes visit the intertidal zone from time to time (in contrast to Kandalaksha Bay, where they prefer deeper sites due to low salinity), they apparently spend most of their life in the subtidal zone. So for the digenean, the possibility of contact with the upstream as well as the downstream host is higher in the subtidal. Considering this, it seems natural for *P. squamatus* to use subtidal molluscs as first intermediate hosts. For example, in Kan-

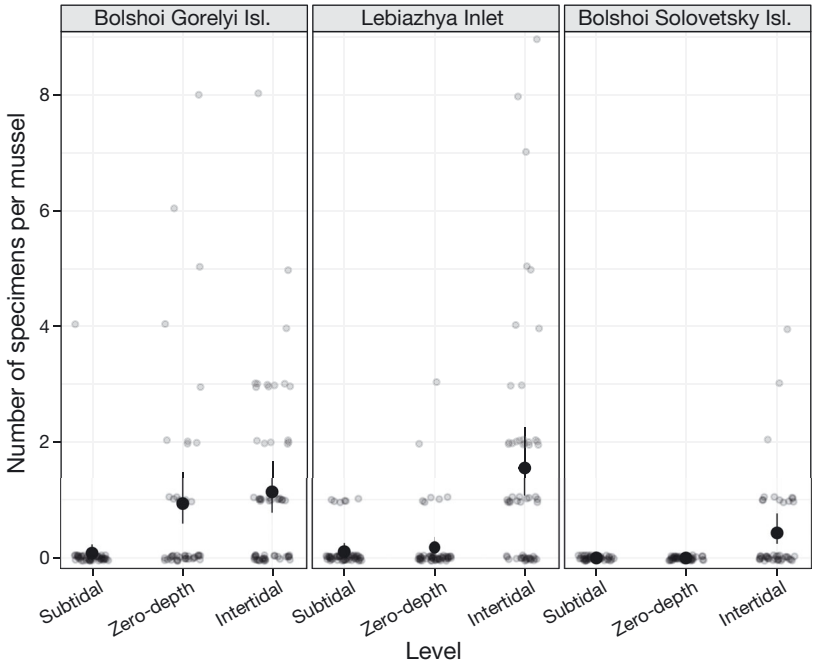

Fig. 6. Abundances of *Himasthla* sp. associated with *Mytilus edulis* at different tidal levels. Raw data and predicted values from negative binomial generalized linear model (GLM) with 95% confidence intervals are presented

dalaksha Bay the sporocyst was found in subtidal mytilid bivalves *M. edulis* and *Musculus laevigatus* (Zelikman 1966), and near BS, bucephalid sporocysts often occurred in *Musculus discors*, also in the subtidal zone (V. A. Krapivin pers. obs.).

Some non-symbiotic animals were found inside *M. edulis* mantle cavities. This is not uncommon for bivalves. For example, some free-living nematode species have been reported in oysters and soft-shell clams *Mya arenaria* (Schuurmans Stekhoven 1942, Anderson & Bourne 1960). Nematodes in mantle cavities of the White Sea mussels were previously mentioned by Konstantinova & Maximovich (1985). Two species of marine mites usually inhabiting mussel beds were reported from *Mytilus galloprovincialis* (Cáceres-Martínez et al. 2000). Since all of these groups were described as free-living benthic (nematodes, most harpacticoids, halacarid mites) or planktonic (harpacticoid *M. norvegica*) organisms, we suppose these animals were accidentally trapped inside the mussels' mantle cavities and managed to survive in these new conditions.

Spatial distribution of mussel-associated organisms

Connection between tidal level and infection pattern has previously been reported for a number of parasites of molluscs. Most of the papers considered intertidal molluscs and digeneans. For instance, it was shown that at higher shore levels, the prevalence of sporocysts and metacercariae of digeneans in snails that use birds as final hosts is higher than at the lower levels (e.g. Granovitch & Johannesson 2000). Data on differences between subtidal and intertidal infection patterns is scarce. Chubrick (1966) reported considerable differences in the composition of digenean communities between subtidal and intertidal bivalves in the White Sea and Barents Sea: 15 of 16 species of digeneans that use birds as final hosts infected only intertidal molluscs, and of 21 species of 'fish' digeneans, only 5 were found at the intertidal zone (Chubrick 1966). Buck et al. (2005) compared parasitic loads in mussels from inshore intertidal and subtidal sites: intertidal mussels were more infected with larvae of digeneans that use birds as final hosts (predominantly *Renicola roscovita* and 2 *Himasthla* species) than subtidal ones, for 1-host symbionts (boring polychaetes and copepods), no differences in infection patterns have been shown (Buck et al. 2005). The description of differences in infection patterns of 1-host symbionts between intertidal and subtidal mussels was made by Kruczynski (1974): symbiotic pea-crabs were abundant in mussels from subtidal sites and almost absent in intertidal mussels (Kruczynski 1974).

In our case, the taxa forming the symbiotic communities were almost the same at all tidal levels, while quantitative composition of the communities differed significantly. At different sites, the patterns were not exactly the same (presumably due to the influence of large-scale factors), but some tendencies remained quite stable. *Urastoma cyprinae* were more abundant at the lower levels (Fig. 3), while *R. roscovita* and *Himasthla* sp. larvae were found at higher levels (Figs. 5 & 6). The spatial distribution of *Gymnophallus bursicola* larvae was different from the distribution of the other 2 metacercariae. There were no significant differences between tidal levels, and in the subtidal zone its abundance was even higher than in the intertidal (Fig. 4). Several mechanisms could be suggested to explain these patterns.

U. cyprinae, being a mantle cavity dweller, is surrounded by the seawater that passes through the cav-

ity, and can be affected by its characteristics (e.g. temperature, salinity and chemical composition). The rhabdocoellan also has free-living breeding and infective stages, which are also susceptible to direct environmental effects (Crespo González et al. 2005). These features it shares with the symbiotic crab mentioned by Kruczynski (1974), and like the crab, the rhabdocoelan is more abundant at lower levels. The highest abundance of *U. cyprinae* in the intertidal zone was at BS. At BG and LE, intertidal mussels were almost free of the symbiont. The surface water at these 2 sites is much less saline than at BS because of the near proximity of the Keret River estuary (Babkov 1998). We assume that *U. cyprinae* is affected by water salinity. It is possible that its free-living or even symbiotic stages are less tolerant of low salinity than the host mussels, which might explain why *U. cyprinae* was less abundant at the intertidal level, where salinity can drop significantly during low tides, and why at the site with highest surface water salinity these symbionts occurred at higher levels.

Digenean metacercariae, unlike *U. cyprinae*, inhabit mussel tissues and do not come into direct contact with the seawater. However, the digeneans have free-swimming stages, which can be affected by harsh intertidal conditions (Pietrock & Marcogliese 2003, Studer et al. 2012). Nevertheless, abundance of these metacercariae was much higher at intertidal and zero-depth levels than at the subtidal, except for sites with a very low percentage of infected mussels (LE for *R. roscovita* and BS for *Himasthla* sp.). This pattern may be caused by (1) among-level differences in mussel density, (2) migration of emerging cercariae towards the intertidal zone, (3) redistribution of infected mussels, (4) among-level differences in density of the upstream hosts (*Littorina* snails) or (5) among-level differences in infection rate of the upstream hosts.

We can reject the first hypothesis because the density of mussels among sites and levels varied insignificantly.

The second mechanism seems more plausible. Positive phototaxis has been described for cercariae of some bird-parasitizing digeneans (Prokofiev 2001, Prokofiev & Galaktionov 2009). Such migration of cercariae to the intertidal level is likely to be adaptive since the intertidal mussels are obviously more available for birds.

Active landward migration of infected mussels seems unlikely because adult mussels do not usually move long distances.

The fourth mechanism that can shape the infection pattern of these metacercariae is the distribution of the upstream hosts — the snails of genus *Littorina*. We do not have data on snail densities on the 3 sites but we have observed potential upstream hosts at all 3 levels: *L. saxatilis* and *L. obtusata* at the intertidal and zero-depth zones and *L. littorea* at the zero-depths and subtidal zones. More data is needed to make certain conclusions.

Finally, differences in infection levels of snails may be present due to heterogeneity in definitive hosts' (birds) availability or active migration of miracidiae and/or infected snails. The connection between bird availability and patterns of digenean infection in snails is well described on large scales (Robson & Williams 1970, Bustnes & Galaktionov 1999, Hechinger & Lafferty 2005, Fredensborg et al. 2006). This effect could be less important at smaller scales (Fredensborg et al. 2006, Byers et al. 2015) except the cases of very aggregated bird distribution (Smith 2001). Either way, we cannot exclude the possibility that the intertidal snails had higher rates of infection with digeneans than the subtidal snails.

Thus, we conclude that the observed distribution of digenean larvae in mussels can be the result of migration of free-swimming stages to the intertidal zone (increasing the chance of meeting the final host) or the consequence of heterogeneity in upstream host distribution and infection rate.

Some details remain unclear, for example, the origin of the few mussels heavily infected with Renicolidae at the subtidal levels (see Fig. 5). It is possible that mussels were infected by cercariae that emerged from subtidal littorinids or migrated from the upper levels (however this does not explain the highly aggregated pattern). Another possibility is that these mussels actually became infected at the intertidal and than slipped down to the lower levels with digenean larvae preserved in their tissues (metacercariae of *R. roscovita* [ex. *Cercaria*] *parvicaudata* can survive in a mussel for more than 2 yr; Nikolaev et al. 2006). Manipulative experiments may be useful in testing these (at this time purely speculative) hypotheses.

Gymnophallus sp. was mentioned by Chubrick (1966) to be the only 'bird' digenean in the White Sea whose metacercariae can often be found in subtidal molluscs (Chubrick 1966), which is consistent with our results. For this digenean, no increase in number at higher tidal levels has been shown. This can be linked to the feeding behavior of *G. bursicola*'s final hosts — eiders — that dive down to 40 m to get molluscs (Madsen 1954, Brun 1971). Moreover, it is possible that the first intermediate host of *G. bursicola* is a subtidal bivalve. For example, in Onega Bay,

unidentified gymnophallid sporocysts were discovered in *Serripes groenlandicus* (V. A. Krapivin pers. obs.). In Kandalaksha and Onega bays, many subtidal bivalves are infected with metacercariae of gymnophallid digeneans (Chubrick 1966, authors' pers. obs.). It is possible that the upstream host inhabits the subtidal zone, and we assume that *G. bursicola* cercariae do not need to migrate to the intertidal for their second host (bivalve molluscs) to meet the final host (eiders).

Choricystis sp. was absent at BS, at BG it occurred only at the zero-depth and intertidal levels, and at the LE it occurred at all the 3 levels. The between-site distribution of the parasitic green algae *Choricystis* sp. corresponds well with earlier observations of this symbiont occurring more often in regions with lower salinity (Petrova et al. 2006, Kvitko & Migunova 2011). BS is characterized by the highest average salinity out of the 3 sites (26 to 28‰), at BG salinity is intermediate (23 to 25‰) and the lowest salinity is observed at LE (18 to 23‰) (Babkov 1998, Basova et al. 2004). However, within-site distribution of the parasitic green algae may also be governed by factors other than salinity (e.g. desiccation time): at LE, *Choricystis* sp. was significantly less abundant in the intertidal than at the lower levels.

Accidentally associated organisms had different patterns of spatial distribution at different sites. Assuming that these animals were entrapped in mussels' mantle cavities by pure chance, it is logical to suppose that their distribution depends on the ambient fauna; those animals more plentiful at a given site, and small enough to pass though the mussel's inhalant siphon, have a greater chance of being 'entrapped' by mussels.

CONCLUSIONS

The distribution patterns of mussel symbionts are determined by a combination of large-scale and local factors. Our research focused on small-scale heterogeneity among different tidal levels. We showed that the quantitative composition of symbiofauna of mussels at lower levels differs from that at the upper levels: the abundance of rhabdocoelans was higher at the subtidal and zero-depth zones than in the intertidal, while encysted metacercariae — *R. roscovita* and *Himasthla* sp. — were more abundant at the zero-depth and intertidal zones.

In the real world it is difficult to separate the factors that govern distribution patterns across the vertical shore gradient. We proposed several mechanisms to explain the distribution patterns of some symbiont species. Further investigations and field experiments could help to determine which factors are crucial in creating differences in occurrence of mussel symbionts between tidal levels.

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