



## Clumping behavior and byssus production as strategies for substrate competition in *Mytilus edulis*

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**Abstract.** Laboratory experiments showed that the mussel *Mytilus edulis* aggregated more intensely around living organisms (the bivalve *Hiatella arctica* and the solitary ascidian *Styela rustica*, which commonly co-occur with mussels in fouling communities) than around inanimate objects. When exposed to an inanimate object, mussels attached their byssal threads primarily to the substrate, close to the object, but when exposed to a living organism, they attached their byssal threads directly to the organism. The ascidian was more intensely covered with byssal threads than was the bivalve. Mussel attachment to the ascidians was apparently determined by the physical characteristics of the tunic and to a lesser extent by the excretion-secretion products released by *S. rustica*. This study indicates that mussels can use byssus threads as a means of entrapment of potential competitors for space. It remains unclear why mussels preferentially attached to ascidians compared to the bivalve. This can be explained either by competitive interactions, or by attractiveness of the ascidian tunic as an attachment substratum.

*Additional key words:* *Hiatella arctica*, *Styela rustica*

Competition for space is one of the most important processes that shapes the structure and succession of epibenthic communities (Connell 1970; Dayton 1971; Buss 1986; Underwood 1992; Fairfull & Harriott 1999). This phenomenon has been explored mainly in modular organisms (bryozoans, sponges, and corals), because the competitive success of these animals is relatively easy to quantify by measuring colony growth and overgrowth of other organisms (Buss 1979; Suchanek & Green 1981; Russ 1982; Nandakumar & Tanaka 1994).

Among sedentary solitary animals, recruitment is an important indicator of success in colonizing the substratum, as is the somatic growth of individual organisms (Suchanek 1978; Bushek 1988; Petraitis 1995; Khalaman 2005a; McQuaid & Lindsay 2007). Some of these animals retain some ability to move throughout their adult life. Among these, various species of mussels are important components of epibenthic communities. Behavioral responses of adult individuals may have a great significance for these bivalves in competition for space with other types of sedentary and sessile organisms (Khalaman & Lezin 2004; Schneider

et al. 2005). When different mussel species compete with one another, behavioral characteristics and differences in their resistance to environmental factors are of paramount importance (Harger 1972; Kennedy 1984; Schneider et al. 2005; Nicastro et al. 2008).

A certain degree of mobility and continuous production of byssal threads could provide mussels with advantages over sessile organisms that lack these abilities. Behavioral responses have been shown to play a crucial part in protecting mussels from predators. These responses include throwing drilling whelks off the shell (Wayne 1987), intensive clumping (Dolmer 1998; Côté & Jelnicar 1999), increase in byssus production and provision of a more secure attachment to the substratum (Côté 1995; Kulakowski & Lezin 1999; Cheung et al. 2009; Brown et al. 2011), and entrapment of predatory snails with byssal threads (Petraitis 1987; Wayne 1987; Day et al. 1991; Davenport et al. 1996; Ishida & Iwasaki 1999). However, it is unknown whether behavior can also provide advantages in competitive interactions of mussels with other sessile organisms. To address this question, we performed experiments focused on two species that compete in the fouling communities in the White Sea, the blue mussel *Mytilus edulis* LINNAEUS 1758 and the solitary ascidian *Styela rustica* LINNAEUS 1767.

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Competitive interactions between mussels and various ascidian species in fouling communities are well known. These interactions have recently acquired increasing importance because of the problem of biological invasions by species such as *Ciona intestinalis* LINNAEUS 1767 and *Styela clava* HERDMAN 1882, and because of the damage that ascidians cause to mussel farms (Lesser et al. 1992; LeBlanc et al. 2007; Rajbanshi & Pederson 2007; Arsenault et al. 2009). In the White Sea, native *S. rustica* and *M. edulis* form long-lasting fouling communities in the terminal stages of succession. Blue mussels dominate in the top, sun-warmed, layer of water, not deeper than 1.5 m, while *S. rustica* is better adapted to cold waters and dominates at depths over 5 m (Oshurkov 1985, 2000; Khalaman 2001b, 2005a). Within the narrow depth range from 1 to 5 m, mussels and ascidians co-occur and compete for space. More or less regular fluctuations in dominance are observed, where populations of *S. rustica* replace those of *M. edulis*, and vice versa. The duration of each stage varies from 3 to 15 years (Oshurkov 1992, 2000; Khalaman 2001b, 2005a, 2010, 2013). The alternation of dominant taxa is explained by the fact that both *M. edulis* and *S. rustica* lack in-advance mass recruitment that might replace members of the aging population. At the same time, juveniles of the competing species are successfully introduced as the aging population of its competitor is gradually degraded (Khalaman 2005a). These changes can be described by inhibition model of succession (Connell & Slatyer 1977; Dean & Hurd 1980).

Earlier studies showed that substances released into the water by *S. rustica* cause an increase in the number of byssal plaques deposited by blue mussels before they find a permanent attachment site (Lezin & Khalaman 2007; Khalaman et al. 2009). Blue mussels respond in a similar way to the excretory-secretory products (ESP) of the predatory starfish *Asterias rubens* LINNAEUS 1758 and the toxic sponge *Halichondria panicea* PALLAS 1766 (Khalaman et al. 2009). In contrast, substances released by conspecifics and other fouling organisms, such as the bivalve mollusk *Hiatella arctica* LINNAEUS 1767 and the ascidian *Molgula citrina* ALDER & HANCOCK 1848, do not cause an increase in byssus production (Lezin & Khalaman 2007; Khalaman et al. 2009). Neither *H. arctica* nor *M. citrina* successfully compete with *M. edulis* for substratum, nor do they dominate in long-standing fouling communities in depths from 1 to 5 m (Oshurkov 1985; Maximovich & Morozova 2000; Khalaman 2001a). In a field experiment, ascidians (*S. rustica*) placed into a cage with mussels became densely covered by byssal

threads and died within a month; this was not seen when blue mussels were held together with *H. arctica*, or when *S. rustica* was kept with *H. arctica* (Khalaman & Komendantov 2007).

The proximate causes and the biological role of the increased byssus production of mussels in the presence of *S. rustica* are not well understood and require further investigation. Specifically, it remains unclear whether this response is a behavioral adaptation to suppress competitors, or a by-product of the increased search time for an appropriate permanent attachment site by mussels in the presence of *S. rustica*. To address this question, we examined whether clumping behavior and patterns of byssus attachment differ when mussels aggregate around living organisms (the ascidian *S. rustica* and the bivalve *H. arctica*) or around inanimate objects (plastic molds of *S. rustica* and *H. arctica*). The bivalve *H. arctica* was used because it commonly co-occurs in fouling communities with *M. edulis* and *S. rustica* but is not considered a strong competitor of either species (Khalaman 2001a, 2005b, 2010). We also determined whether the physical properties of animal surfaces or chemical substances released by a competitor affect the attachment of the mussels, and whether mussels demonstrate taxis toward the strong competitor *S. rustica*.

## Methods

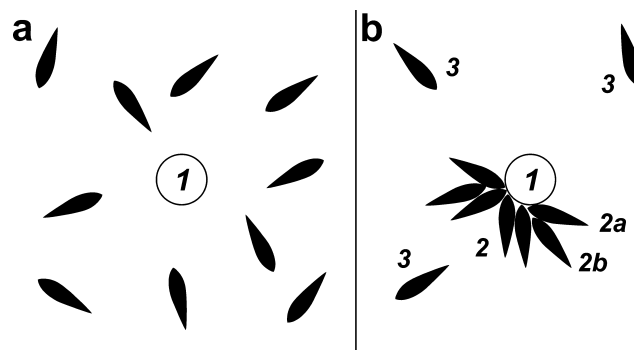
The study was conducted at the White Sea Biological Station, Zoological Institute, Russian Academy of Sciences (Chupa Inlet, Kandalaksha Bay: 66°20.230' N; 33°38.972' E). The experiments were performed using the solitary ascidian *Styela rustica* and the bivalves *Mytilus edulis* and *Hiatella arctica*. The study species were collected from fouling communities on artificial substrata submersed at a depth of 2–3 m. Only epibiont-free individuals were sampled. Mussels were 2–3 years old and 25–30 mm long. *Styela rustica* and *H. arctica* were comparable in size: average body lengths were  $24.5 \pm 1.8$  mm (SE) and  $25.4 \pm 0.9$  mm, respectively. Animals were maintained and experiments were carried out in a temperature controlled room. Prior to experimentation, the animals were allowed to acclimate to the laboratory conditions (temperature 10°C, salinity 25 ppt, constant illumination and active aeration, no food) for 3 d, but experiments were performed in the tanks with still water, without active aeration. Laboratory conditions were equivalent for all experiments and resemble those from the abiotic environment (temperature 12–13°C, salinity 25 ppt, Arctic day). Three series of experiments were conducted.

### Mussel clumping and attachment to organisms and inanimate objects

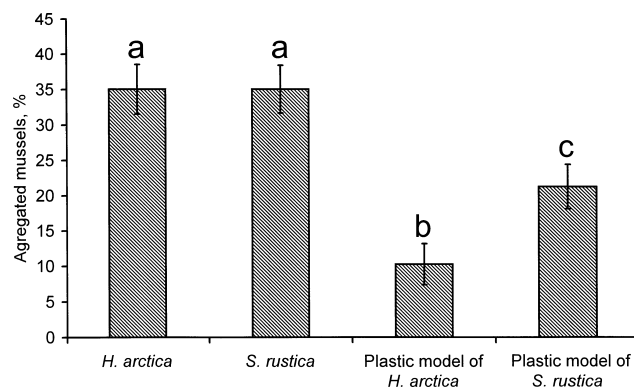
Ten mussels were placed in plexiglas tanks (10×10 cm bottom area) and randomly distributed across the bottom. Prior to the experiment, a test object was fixed using cyanoacrylate adhesive in the center of each tank as a potential aggregation center for mussels. The following test objects were used: (1) a living specimen of *H. arctica*; (2) a living specimen of *S. rustica*; (3) a plastic dummy of *H. arctica*; and (4) a plastic dummy of *S. rustica*. The animal dummies were prepared by casting prosthodontic polymer Protacryl-M (manufactured by STOMA, Kharkov, Ukraine) into plaster molds made from living animals. Both the Protacryl-M and plexiglas are polymethylmethacrylate. Prior to casting, ascidians were anesthetized fully outstretched with lidocaine (produced by Moskhimpharmpreparaty, Moscow, Russia), until completely unresponsive to mechanical stimuli. Before the experiment, the dummies were immersed for 5 d in seawater to remove residual polymerization components from their surface, and to allow the establishment of a biofilm. The length of dummies corresponded to sizes of living test objects: 26.7 mm for the copies of *H. arctica*, and 27.9 mm for the copies of *S. rustica*.

The mussels were kept in the tanks for 24 h, after which the number of mussels aggregated around the test object was recorded in each tank. Mussels were considered to be aggregated around the object if they formed a patch around this object and had either a direct contact with it (physical contact between mussel shell and test object) or an indirect contact through other individuals (Fig. 1). Each experimental treatment listed above was replicated 40 times. Thus, 160 tanks were used in total. All replicates of all treatments of the experiment were run simultaneously.

The numbers of byssal threads by which a mussel was attached to each of the following sites (the test object, nearby mussels and the tank bottom) were determined. To take stock of all threads of the examined mussel, we cut its threads one after another and visually determined the attachment site of each successively removed thread. Since it was impossible to process all aggregated mussels, the number of examined individuals was reduced to 25 individuals in each treatment. The mussels were selected as follows. Twenty-five of 40 replicate tanks were randomly selected, and in each selected tank, the mussels that had a direct contact with the test object were numbered and the numbers used to randomly choose a mussel for the byssus count. Thus,



**Fig. 1.** Design of the experiment aimed at identifying mussel clumping behavior and patterns of byssus attachment in response to living organisms or inanimate objects. **a.** Representate positions of mussels in the tank before the experiment. **b.** Positions of mussels in the tank at the end of the experiment. 1, test object (aggregation center); 2, mussels aggregated around the object; 2a, mussel having direct contact with the test object; 2b, mussel having indirect contact with the test object; 3, solitary mussels.



**Fig. 2.** Percentage of mussels aggregated around living organisms and inanimate objects. Bars represent standard error. Bars labeled with the same letters do not differ statistically.

100 mussels were examined in total (one per selected tank and 25 tanks per treatment).

### Factors affecting mussel attachment to ascidians

Factors affecting mussel attachment to the ascidians were tested. The type of tanks, age and size of mussels, number of mussels per aquarium, and exposure time were the same as in the previous experiment, but different test objects were used, namely: (1) a living specimen of *S. rustica*; (2) a dead, stuffed *S. rustica* (which had the same surface physical characteristics as a living ascidian, but did not release metabolites into the environment); (3) a living specimen of *S. rustica* enclosed in a thin-walled perforated cylinder (an object that has

different surface physical characteristics than a living ascidian, but releases excretory/secretory products into the environment); (4) an empty perforated plastic cylinder as control (an object that does not release metabolites into the environment and has different surface physical characteristics than a living ascidian). The cylinders used in the experiment were 35 mm in height and 17 mm in diameter. The cylinders, as well as the bottom and sides of the tanks, were made of Plexiglas. Thirty replicates of each experimental treatment were carried out. The attachment sites of byssal threads were determined in 69, 51, 46 and 43 mussels in treatments (1), (2), (3) and (4) of the experiment, respectively, utilizing the same methods as described in the previous experiment. The number of evaluated individuals corresponds to the number of mussels that had direct contact with the test object in each treatment.

The dead, stuffed ascidians were prepared as follows. Living ascidians were anesthetized and relaxed by immersion in a weak solution of  $\text{CoCl}_2$ , and then killed by gradually adding ethanol. The tunic was stripped away and soaked in 70% ethanol for 5 d with twice-daily changes of the solution, until any visible traces of extracts were absent. The tunic was then carefully rinsed with fresh and seawater, and stuffed with foam plastics. This process provided a good imitation of the mechanical properties of the body and tunic surface texture of a living ascidian.

### Mussel taxis in relation to ascidians

Taxis of mussels in relation to ascidians was tested. The experiment was carried out in a rectangular plastic tank (10×32 cm), with an individual *S. rustica* placed near a short side of the tank prior to the experiment. Test mussels (one per tank; 2 years old, 20–25 mm shell length) were introduced to the center of the tank (~16 cm from the ascidian) in random orientation. After 24 h, the position of the mussels (the distance from the center of the tank to the test animal) was measured. Displacement toward the ascidian was scored as positive, and displacement away from the ascidian as negative. Water temperature was constant (10°C), and illumination was constant and spatially uniform throughout the experiment. A total of 85 mussels were tested in these “long” tanks.

The experiment was also performed in a shortened tank, with the working distance (long side of the tank) reduced to 10 cm by installing a water-permeable transverse partition. In this experiment, the distance between the mussel and ascidian was reduced to 5 cm. Seventy-five mussels were tested in these “short” tanks.

To ensure that there was no preferential direction for the mussels' movements, we tested mussels in control tanks containing no ascidians. A short side of the control tanks was marked before the experiment. The displacement toward the marked side was scored as positive, and the displacement away from it as negative. We tested 80 and 59 mussels in control tanks using long and short tanks, respectively.

### Statistical analysis

All percentages were subject to Fisher's  $\phi$ -transformation. The effect of the type of the object (living organisms and inanimate objects) on clumping behavior and attachment of mussels was analyzed using a two-way ANOVA with the following factors: species (*S. rustica* or *H. arctica*) and life status of the object (living organisms or inanimate copies of organisms). Factors affecting mussel attachment to ascidians were analyzed by two-way ANOVA with the following factors: the surface quality of the object (ascidian tunic or plastic) and the presence of excretory/secretory products (ESPs) released by living ascidians (ESPs present or absent). Comparisons of the means were made using post-hoc LSD tests that included all possible comparisons, ignoring interactions. Mean values are given with their standard errors. Fisher's  $\phi$ -transformation resulted in asymmetrical standard errors.

Data concerning taxis of mussels in relation to ascidians were analyzed using Student's *t*-test; the average position of mussels at the end of the experiment was compared with their initial position. The critical significance level was set at  $\alpha=0.05$ .

## Results

### Mussel clumping and attachment to organisms and inanimate objects

Both life status and species significantly affected the clumping behavior of mussels with a significant interaction among these factors (Table 1a), as living *Hiatella arctica* and *Styela rustica* were surrounded by a similar number of aggregated mussels ( $p=0.999$ ), while plastic copies attracted different numbers of mussels ( $p=0.001$ ). Approximately, 35% of mussels aggregated around living *H. arctica* and *S. rustica*, while significantly fewer ( $p<0.005$ ) mussels aggregated in the vicinity of plastic copies of *H. arctica* ( $10.3\pm 2.9\%$ ) or *S. rustica* ( $21.3\pm 3.2\%$ ) (Fig. 2).

There was a significant interaction between life status and species for the byssal attachment of mussels to a test object, with both factors being also

**Table 1.** Results of two-way ANOVA on factors affecting mussel behavior in the mussel clumping experiment.

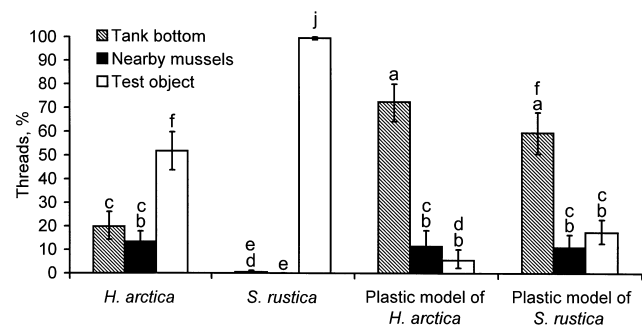
Comparison	df	SS	MS	F	p
a. Clumping around test object.					
Species	1	1.764	1.764	5.445	0.021
Living/inanimate object	1	13.856	13.856	42.681	<0.001
Species×Living/inanimate object	1	1.770	1.770	5.463	0.021
Residual	86	50.534	0.324		
Total	89	67.893	17.683		
b. Attachment to test object.					
Species	1	16.662	16.662	39.652	<0.001
Living/inanimate object	1	56.620	56.620	134.746	<0.001
Species×Living/inanimate object	1	5.276	5.276	12.557	<0.001
Residual	86	36.137	0.420		
Total	89	115	79		
c. Attachment to nearby conspecific mollusks for mussels aggregated around test object.					
Species	1	2.838	2.838	7.512	0.007
Living/inanimate object	1	1.845	1.845	4.885	0.029
Species×Living/inanimate object	1	2.649	2.649	7.013	0.009
Residual	86	32.488	0.378		
Total	89	40	8		
d. Attachment to the tank bottom for mussels aggregated around test object.					
Species	1	5.943	5.943	12.189	<0.001
Living/inanimate object	1	39.944	39.944	81.923	<0.001
Species×Living/inanimate object	1	1.372	1.372	2.815	0.097
Residual	86	41.931	0.488		
Total	89	89	48		

highly significant independently (Table 1b). Similar byssal attachment of mussels to plastic copies of *H. arctica* and *S. rustica* ( $p=0.07$ ) was observed, while around living specimens of *S. rustica* and *H. arctica* the numbers of byssal threads attached to test objects differed significantly ( $p<0.001$ ).

The same result was obtained when byssal attachments to nearby mussels were examined (Table 1c). In this case, differences were not found between mussels aggregated around the copy of *H. arctica* and the copy of *S. rustica* ( $p=0.95$ ), or between mussels concentrated around *H. arctica* and its plastic copy ( $p=0.77$ ).

For attachment of mussels to the tank bottom, both life status and species were significant factors (Table 1d).

Most byssal threads ( $52\pm 8\%$ ) of the mussels aggregated around living specimens of *H. arctica* were attached to shells of *H. arctica* (Fig. 3). A significantly lower percentage was attached to the tank bottom and to surrounding *Mytilus edulis*:  $20\pm 6\%$  ( $p=0.001$ ) and  $13\pm 4\%$  ( $p<0.001$ ), respectively. The mussels aggregated around *S. rustica* attached almost all of their byssal threads ( $99.4\pm\frac{0.5}{0.7}\%$ ) to the tunics of these animals, a significantly higher percentage than those attached to the shells of *H. arctica* ( $51.7\pm\frac{7.9}{8.0}\%$ ) ( $p<0.001$ ). The percentages of



**Fig. 3.** Localization of byssal attachment points for mussels aggregated around living organisms and inanimate objects. Bars represent standard error. Bars labeled with the same letters do not differ statistically.

threads anchored to the tank bottom and to the nearby mussels were negligibly low ( $0.5\pm\frac{0.61}{0.38}\%$  and  $0.02\pm\frac{0.072}{0.024}\%$ , respectively).

The mussels aggregated around the plastic copies imitating both shape and surface texture of the animals were attached primarily to the tank bottom (Fig. 3):  $72\pm\frac{7.5}{8.2}\%$  of the threads for the copies of *H. arctica*, and  $60\pm\frac{8.6}{8.9}\%$  of the threads for the copies of *S. rustica*, respectively; these means were not statistically different ( $p=0.23$ ). The percentage of byssal threads attached to the test objects and to

**Table 2.** Results of two-way ANOVA on factors affecting mussel behavior in the mussel attachment to ascidians experiment.

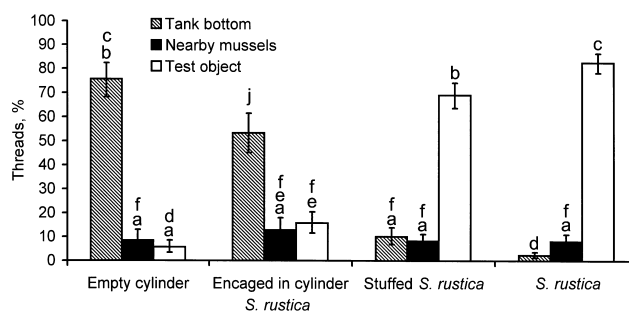
Comparison	df	SS	MS	F	p
a. Attachment to test object.					
Surface quality	1	109.440	109.440	161.227	<0.001
Excretory-secretory products	1	5.443	5.4430	8.019	0.005
Surface quality×Excretory-secretory products	1	0.003	0.003	0.004	0.948
Residual	205	139.152	0.679		
Total	208	254	116		
b. Attachment to tank bottom for mussels aggregated around test object.					
Surface quality	1	98.603	98.603	121.731	<0.001
Excretory-secretory products	1	8.31	8.31	10.259	0.002
Surface quality×Excretory-secretory products	1	0.213	0.213	0.263	0.609
Residual	205	166.05	0.81		
Total	208	273	108		

nearby mussels varied from 6 to 17%; no statistically significant differences were found in attachment to the test objects or nearby mussels (Fig. 3).

A total of  $5.8 \pm 0.5$  and  $5.8 \pm 0.6$  threads per mussel were produced when mussels aggregated around *H. arctica* and *S. rustica*, respectively. The number of threads used by mussels to anchor near the plastic copies of *H. arctica* and of *S. rustica* averaged  $5.9 \pm 0.8$  and  $5.0 \pm 0.5$  threads per mussel, respectively. No statistically significant differences were found in the number of threads per mussel in any of the experimental treatments ( $p > 0.32$ ).

#### Factors affecting mussel attachment to ascidians

Both physical properties of the attachment surface and the substances released by ascidians had effects on mussel attachment (Table 2). The percentage of threads attached to the test object increased in the following order (Fig. 4): empty cylinder ( $6 \pm \frac{2.8}{2.3}$ %)—“caged” ascidian ( $16 \pm \frac{4.7}{4.2}$ %)—stuffed ascidian ( $69 \pm \frac{5.1}{5.4}$ %)—living ascidian ( $83 \pm \frac{3.9}{4.2}$ %). The percentage of byssal threads attached to the tank bottom mirrored this trend, decreasing in the following order: empty cylinder ( $75 \pm \frac{6.7}{7.4}$ %)—“caged” ascidian ( $53 \pm \frac{8.1}{8.2}$ %)—stuffed ascidian ( $10 \pm \frac{3.8}{3.2}$ %)—living ascidian ( $2 \pm \frac{1.3}{1.0}$ %) (Fig. 4). Mussels attached more threads to living ascidians than to stuffed ascidians ( $p = 0.03$ ). The same (albeit only marginally significant) trend was found for the cylinders containing living ascidians vs. the empty plastic cylinders ( $p = 0.05$ ). In both cases an increase in the number of threads due to the presence of the excretory/secretory products of the ascidian was similar (10%;  $t = 0.047$ ;  $p = 0.96$ ). The difference in the percentage of the byssal threads attached to the tank bottom was significant between the empty cylinders ( $75 \pm \frac{6.7}{7.4}$ %)



**Fig. 4.** Localization of byssal attachment points for mussels aggregated around living ascidians and around objects with some ascidian characteristics. Bars represent standard error. Bars labeled with the same letters do not differ statistically.

and the living ascidians caged in cylinders ( $53 \pm \frac{8.1}{8.2}$ %) ( $p = 0.01$ ), as well as between the living ( $2 \pm \frac{1.3}{1.0}$ %) and stuffed ascidians ( $10 \pm \frac{3.8}{3.2}$ %) ( $p = 0.04$ ). The percentage of the byssal threads attached to the nearby mussels varied from 8 to 13%, and was not significantly affected by the experimental treatment (Fig. 4).

#### Mussel taxis in relation to ascidians

In the long experimental containers, mussels moved toward ascidians by an average distance of  $1.8 \pm 0.95$  cm in 24 h, which was statistically indistinguishable from zero ( $t = 1.84$ ;  $p = 0.069$ ). In the short containers, the average position of mussels at the end of the experiment was essentially the same as the initial position ( $0.3 \pm 0.39$  cm,  $t = 0.64$ ;  $p = 0.52$ ). The average shift of mussels in ascidian-free (control) containers was not significantly different from zero ( $0.8 \pm 1$  cm,  $t = 0.76$ ;  $p = 0.45$  and  $0.3 \pm 0.46$  cm,  $t = 0.63$ ;  $p = 0.53$  in the long and short experimental containers, respectively).

## Discussion

Mussels showed significantly higher clumping activity around living organisms compared to artificial models, although this effect was complicated by an interaction between life status and species. This result refutes the hypothesis that the covering of ascidians with byssus detected in field experiments (Khalaman & Komendantov 2007) is a by-product of a mussel escape response provoked by ascidians in close proximity, consisting of an extended search by mussels for a permanent attachment site. At the same time, <40% of all mussels formed clumps around the objects presented to them as potential aggregation centers. One possible explanation for this low activity is that aggregations form as a result of random movements (Côté & Jelnicar 1999). Moreover, the strategies used by mussels to form aggregations (number and spatial distribution of clumps, number of involved individuals, and formation rates) depend on the population density. At low population densities, such as those used in this study, some individuals tend to remain solitary (Khalaman & Lezin 2004), a finding that agrees with our current results.

The numbers of byssal threads produced per mussel was the same in mussels exposed to living organisms and those exposed to inanimate objects. An increase in production of byssal threads is generally believed to be a response to environmental stressors such as intense hydrodynamics or the presence of predators (Lin 1991; Voronkov 1995; Clarke & McMahon 1998; Dolmer 1998; Kulakowski & Lezin 1999; Leonard et al. 1999; Kulakowski et al. 2001; Selin & Vekhova 2004; Brown et al. 2011). However, this trait appears unaffected in mussels exposed to animals that are not predators and do not pose an immediate threat (Lezin & Khalaman 2007; this study). The most reliable marker of the mussels' reaction to the presence of both predators and non-predatory animals is the number of the byssus plaques that mussels leave behind in search of the place of permanent attachment (Lezin & Khalaman 2007; Khalaman et al. 2009). This may increase the cost of total byssus produced in search of a suitable attachment in response to the presence of competitors, while the number of byssal threads per mussel used in the final attachment remains the same (Lezin & Khalaman 2007; Khalaman et al. 2009; this study).

Mussel attachment patterns were different in mussels aggregating around living *Hiatella arctica* and *Styela rustica* or their plastic dummies. When mussels were exposed to living organisms, most

byssal threads were attached directly to the organism, but when an artificial (Plexiglas) object was presented, most threads were anchored to the bottom near the test object. The tank bottom was made of the same material (Plexiglas) as the test object. The shape and the crude surface texture of aggregation centers is probably not an important determinant of attachment, because artificial models were casts of the living animals. Similarly, preferential attachment to animal surfaces vs. plastic cylinders was found in the experiments with free and encaged ascidians. This indicates that mussels could discriminate living organisms from inanimate objects.

Mussel clumping behavior is considered an adaptation that protect mussels against environmental stress (such as desiccation) and predators (Buss 1981; Okamura 1986; Dolmer & Svane 1994; Reush & Chapman 1997; Dolmer 1998; Lezin 2001). At the same time, mussels, like other epibenthic organisms, compete for space. A common mechanism for this competition is the overgrowth of individuals of other species (Buss 1986). The increased aggregation of mussels around living objects and preferential byssal attachment directly to the living organisms found in our present study suggests that clumping around individuals of other species may be part of competitive behavior. In our experiment, mussels attached almost all their byssal threads to the ascidian *S. rustica*, but only half as many to the bivalve *H. arctica*. It is possible that these differences were caused by the mobility of the ascidian body, which is capable of sudden contraction in response to mechanical stimuli. Such a moving substratum could require a greater number of threads for attachment (or for immobilization) than firm and essentially immobile shell valves of the mollusc *H. arctica*. Nonetheless, it is unlikely that the differences in mussel response to *S. rustica* and *H. arctica* can be explained only by the differences in substrate mobility. Both *Mytilus edulis* and *S. rustica* are dominant competitors in fouling communities characterized by intense interspecific competition (Oshurkov 1992; Khalaman 2005a, 2010, 2013). In contrast, *H. arctica* is a subdominant species in both mussel- and ascidian-dominated fouling communities (Khalaman 2001b, 2005b, 2010). Therefore, it is reasonable to expect that competition between *M. edulis* and *S. rustica* is more intense than between *M. edulis* and *H. arctica*, which may explain differences in the aggregation and attachment responses of the mussels towards *S. rustica* and *H. arctica*. The mechanisms behind how the mussels can differentiate between the two potential competitors are not known, but may involve chemical cues as has been

shown in predator recognition by mussels and other bivalves (Smith & Jennings 2000; Fässler & Kaiser 2008; Freeman et al. 2009; Kobak et al. 2010). Moreover, mussels differentiate between the chemical cues from physiologically different individuals of the same predator species and respond according to the apparent threat (Smith & Jennings 2000; Fässler & Kaiser 2008; Freeman et al. 2009), indicating a well-developed ability to discern chemical signals.

Our study indicates that physical characteristics of the surface of *S. rustica* may be crucial for the blue mussels in choosing this ascidian as a substratum for attachment. The substances released by ascidians also appear to contribute to this choice, but the attractive effect of excretory/secretory products of *S. rustica* is weak. The attractiveness of the ascidian tunic as an attachment substrate for the mussels agrees with earlier reports of ascidians covered by the byssus of the blue mussels in the field experiments (Khalaman & Komendantov 2007). This occurs despite the fact that the tunic of *S. rustica* is an unstable substratum for attachment capable of sudden contractions. The mussels can also attach their byssal threads to other moving substrates. Thus, mussels can immobilize predatory snails with their byssal threads (Petraitis 1987; Wayne 1987; Day et al. 1991; Ishida & Iwasaki 1999), although it is unclear whether this response is specific to predatory gastropods (Davenport et al. 1996). In habitats where hard substrates are rare (such as the silty sands in the intertidal zone of the White Sea), blue mussels can attach to the small herbivorous snail *Hydrobia ulvae* (PENNANT 1777), which results in the death of the latter (Khaltov & Artemieva 2004). Taken together, these data indicate that blue mussels exhibit a non-specific response, which involves the entrapment of any disturbing live organisms with byssus. This response is polyfunctional and may be used as protection against predatory snails, and as a method to fight competitors and prepare the substratum for attachment.

*Styela rustica* is a known competitor with mussels for space, and it may also (directly or indirectly) affect physiology and fitness of the mussels. Thus, the blue mussels living among these ascidians have a low growth rate (Maximovich & Morozova 2000). The excretory/secretory products of *S. rustica* can induce changes in the activity of lysosomal enzymes within mussel tissues similar to those caused by the chemical cues of the starfish *Asterias rubens*, the primary predator of blue mussels (Skidchenko et al. 2011). Blue mussels typically cannot settle on substrate that previously was occupied by dense populations of *S. rustica* (Khalaman 2005a,b). How-

ever, mussels can attach to the solitary ascidians, killing the latter (Khalaman & Komendantov 2007). Even siphons of ascidians may be sealed by byssal plaques (Khalaman & Lezin, unpubl. data). Such behaviors of blue mussels may prevent the development of dense ascidian populations, and may explain the byssal activity of *M. edulis* with respect to *S. rustica*. Alternatively, the preferential attachment of mussels to ascidians may provide increased defense and camouflage from predators by the close proximity of *S. rustica*. Laudien & Wahl (1999, 2004) showed that some epibiotic species reduce starfish (*A. rubens*) preference for *M. edulis*. However, this is unlikely to be the case for *S. rustica*, which rarely settles on shells of living blue mussels. Moreover, blue mussels also showed attachment preference for *H. arctica*, which never occurs as an epibiont of *M. edulis* and cannot mask the mussels from the predators such as the starfish *A. rubens*, which consumes *H. arctica* as well as *M. edulis*.

The mussels showed no directional movement either towards or away from *S. rustica*, when the initial distance between the mussel and the ascidian was 5 or 16 cm. This might be explained by the haphazard nature of mussel movements in search of attachment sites, by the low percentage of mussels that demonstrate clumping activity (about 35%), and by the lack of any apparent influence of ascidians on this aspect of mussel behavior. Some researchers consider that chemotaxis in mussels plays an important role only at the final aggregation stages during the formation of clumps, immediately before the individuals come into direct contact (Geesteranus 1942; Côté & Jelnicar 1999).

Overall, our data indicate that blue mussels may recognize the presence of *S. rustica* (their major competitor in fouling communities) and may use byssal attachment as a means to neutralize the competitor. The primary characteristics used by blue mussels to identify individuals of *S. rustica* are physical properties of the ascidian tunic; chemical substances released by *S. rustica* play a relatively minor role in the ability of mussels to recognize ascidians.

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