Patches of barnacles and ascidians in soft bottoms: Associated motile fauna in relation to the surrounding assemblage

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Abstract

Epibenthic patches dominated by barnacles Balanus crenatus Bruguiere and solitary ascidians Styela spp., Bolthenia echinata (L.) and Molgula spp. in the White Sea shallow subtidal develop on bivalve shells and small stones surrounded with muddy sand. The space between barnacles and ascidians is filled with muddy sediment inhabited by motile taxa. We hypothesized that (i) epibenthic patches and unstructured sediment would attract different motile fauna and (ii) motile fauna of the patches would be affected by local abundances of epibenthic foundation species. Most dominant motile species demonstrated a significant difference in abundance between the two microhabitats. In contrast to the fauna of the sediment, species composition observed in aggregations of barnacles and ascidians was stable across different locations. In the field experiment initially clear bivalve shells after 5 years of exposure developed barnacle clusters with motile fauna similar to that observed in natural aggregations. Amphipods, isopods and bivalves, capitellid polychaets, Cirratulus cirratus (Müller) and Pholoe minuta Fabricius (Polychaeta) dominated in the sediment inside epibenthic patches. The proportion of capitellids, known to be sensitive to organic enrichment, was much higher within the patches than outside. The abundances of motile taxa found in aggregations were mostly determined by the number of barnacles of different size and of their empty shells, the biomass of ascidians, and the effect of location. Different dominant species demonstrated sensitivity to different parameters.

Physical structure of the habitat, provided by barnacles and ascidians, as well as their biodeposition activity are regarded as the main factors structuring the motile fauna in the community studied. The spatial pattern observed seems to imply a range of pattern-generating biogenic processes, similar to those previously revealed in patches of filter-feeding bivalves, tube-building worms and seagrass.

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For creatures your size I offer
a free choice of habitat
W. H. Auden, “A New Year Greeting”

1. Introduction

Discrete regular spatial patterns, observed in ter-
restrial or marine benthic communities, are usually
described in terms of “patches”, areas of a certain
size with species composition differing from that out-
side (Paine and Levin, 1981; Connell and Keough,
1985; Sousa, 1984; Wu and Levin, 1994). These
“islands” originate from spatially localised processes
and events that result in substantial alteration of one or
more of the ecological resources available. Often such
a resource is space itself. Patches that develop where
empty space is added to an existing habitat may utilise
areas cleared by natural or artificial disturbance (Type
I patches) or those provided by settling and growing
environment-modifiers (Type II patches) (Connell and
Keough, 1985; Sousa, 1984). Relatively large sessile
organisms, as habitat engineers (Jones et al., 1994),
usually harbor many smaller ones (e.g. Monteiro et
al., 2002; Tsuchiya, 2002). Discreteness of their
bodies or aggregations (or colonies) results in corre-
sponding discrete spatial pattern of the assemblage.

Quantification of spatial structure in sea bottom
communities is recognised as a necessary step towards
understanding underlying processes (Underwood et al.,
2000; Bergström et al., 2002). Due to their remarkable
spatial effect, patches of suspension-feeding bivalves
were studied most extensively. Aggregated mussels
were proved to modify species diversity and composi-
tion, the latter being mainly affected by age structure of
the molluscs and their patch size (Tsuchiya and Nishi-
hira, 1985, 1986; Tsuchiya, 2002). Biodeposition and
specific alteration of physical structure of habitat have
been regarded as the main processes affecting the
spatial structure (Crooks and Khim, 1999; Tsuchiya,
2002). Other benthic keystone species studied in
respect to community structure associated with their
patches are ascidians (Monteiro et al., 2002), tube-
building worms (Woodin, 1974; Khaitov et al., 1999;
Zühlke, 2001; Bolam and Fernandes, 2002) and sea-
grass (Bell and Westoby, 1986; Edgar and Robinson,
1992; Bowden et al., 2001; Lee et al., 2001). The
spatial effects observed were mainly explained by spe-
cific physical properties of the patches formed by these
organisms. Thus, patchy structure in benthic commu-
nities has been studied on a limited number of objects.

Though barnacles and ascidians are known as key-
stone species in different subtidal and intertidal habi-
tats (McDougall, 1943; Sutherland, 1978; Dean and
Hurd, 1980; Dean, 1981; Bros, 1987; Monteiro et al.,
2002; Yakovis et al., 2004), the structure of their joint
patches surrounded with soft sediment has never been
investigated. The main reason is that most barnacle
and ascidian species inhabit hard substrates and are
generally infrequent in soft bottoms. However, in the
White Sea subtidal barnacles Balanus crenatus are
often found on relatively small shells or stones, with
up to several tens of individuals per substrate. The
clusters described usually bear also solitary ascidians
from the genera Styela, Molgula and Dendrodoa
attached to barnacle shells and to each other. The
communities associated with coexisting barnacle clus-
ters and ascidian clumps form an important part of the
Onega Bay (the White Sea) ecosystems (Grishankov,
1995; Yakovis, 2002). Numerous empty barnacle
shells also appear to be an essential component of
these aggregations, providing surface for live cirriped-
ians, ascidians and other empty barnacle shells. The
space between barnacles and ascidians is filled with
muddy sediment inhabited by motile taxa; the sur-
rounding bottom is also covered with muddy sand. As
a result, these islands are Type II patches in relation to
the surrounding soft bottom assemblage. Our previous
communication reported the density of such patches at
study sites near the Solovetsky Islands ranging from
10 to 40 per m$^2$. The macroinvertebrate assemblage
associated with the sediment between the aggrega-
tions is spatially structured itself, species abundances
depending on the distance to the nearest epibenthic
patch and on their density (Yakovis et al., 2004).

In the present study we describe the fauna asso-
ciated with aggregations (clusters) of barnacles and
ascidians in relation to that of the surrounding sedi-
ment. The hypothesis tested was that species compo-
sition of motile taxa found inside the patches studied
would be affected by physical parameters of the
aggregation (e.g. the proportion of barnacles and ascidi-
ans, their size structure) rather than by the sample’s
location and characteristics of the surrounding fauna.
Aggregations sampled at different sites were com-
pared to cores of bare sediment from the same sites.
The effects of location and patch type on species abundance were assessed. Clusters with different abundance of barnacles (living and dead) and ascidians were also compared to estimate the contribution of different epibenthic keystone taxa to the variation of the associated fauna. In addition, we examined the motile fauna of aggregations that developed on empty shells artificially added to the habitat (hereafter “experimentally reared” or “experimental”). We used *Serripes groenlandicus* (Bruguiere) (Lamellibranchia) shells, since they had been recorded as substrates for most natural clusters (Yakovis et al., 2004). Taking into account their probable origin from local *Serripes* population, it was assumed that natural aggregations generally develop on initially clear shells. This may happen only after the death of molluscs that normally reside under the sediment surface. Experimentally reared clusters were expected to attract a motile fauna similar to that found in natural ones, which should prove the causal nature of the effects revealed in observations. Another objective was to trace the development of the epibenthic patches under study (at least at initial stages, since the whole “life term” was unknown). Regular and consistent temporal pattern, if any, should be a further evidence of a relatively high integration level of the system under study as well as other similar marine benthic assemblages.

2. Methods

2.1. Study site

Sampling was carried out in July 2000–2004 near Solovetskiy Island (Onega Bay, White Sea) at two sites separated by 1820 m, 100 m off the shore (65°01.2’N, 35°39.7’E for Site 1 and 65°00.7’N, 35°41.7’E for Site 2; Fig. 1). Though the whole region of the Solovetskiy archipelago demonstrates an extreme variety of sea bottom conditions, the area selected for this particular study was a flat muddy bottom at a depth of 11.2–12.5 m (Site 1) and 14.5–15.0 m (Site 2). Sea bottom temperature in July was about 8°C at both sites. The salinity was not measured, but previous investigations (Grishankov et al., 1997) had reported the range of 24.4‰ to 27.6‰. On both sites the sediment was muddy sand, more mud being observed at Site 2. The experiment on the development of aggregations on
empty shells was conducted for 6 years from July 1998
to July 2004 at Site 1.

2.2. Sampling and laboratory techniques

Samples of two types, sediment cores and aggregations
of barnacles and ascidians, were collected by
SCUBA divers in 18 square frames (replicates)
1.2 × 1.2 m each. Any substrate found on the sediment
surface was examined, regardless of the presence of
barnacles and ascidians. We recorded the type of
primary substrate and total weight and number of
solitary ascidians (Styela rustica (L.), Styela coriacea
(Alder et Hancock), Dendrodoa grossularia (Beneden),
Bolthenia echinata (L.), Molgula spp.—referred
to below as “ascidians”) and barnacles (B. crenatus)
for each aggregation. Barnacles as well as their empty
shells were individually measured (aperture carino-
rostral length accurate to 1 mm) and ascidians were
individually weighed accurate to 0.01 g. All the sedi-
ment found in the aggregation was extracted (to do
that, the latter usually had to be carefully destroyed).
This sediment and its inhabitants together with the rest
of the motile macrofauna found in the aggregation (on
the shell and ascidian surfaces, in crevices, etc.) was
examined as one sample (hereafter “A-samples”). In
addition, 55 cm² cores of bare sediment (hereafter “S-
samples”) were obtained among the epibenthic
patches (2–8 samples per replicate, depending on the
amount of aggregations and technical conditions). In
total, 12 replicates (containing 295 aggregations and
60 cores) were sampled at Site 1 and 6 replicates
(containing 47 aggregations and 38 cores) were
sampled at Site 2. The disproportion of the data
available for the two sites is associated with low
visibility and density of aggregations, which, together
with sediment properties, made sampling at Site 2
much more difficult than at Site 1.

Sediment from each sample was sieved (0.5 mm
mesh diameter) and sorted. All benthic macrofaunal
invertebrates were removed, identified to the lowest
taxonomic category possible (generally to the species
level), counted and weighed.

2.3. Experiments

We used clear S. groenlandicus shells attached to
small concrete blocks as substrates for experimentally
reared aggregations, these shells being the most com-
mon substrate for the aggregations collected during
the sampling. Blocks were positioned on the bottom at
Site 1, buried in sediment so that their upper sides
with substrates were left open. Substrates were added
every July since 1998 and partially removed for inves-
tigation every July since 1999, so that 61 shells were
totally exposed for 1–5 years. Experiments were con-
ducted until 2004 and are still being continued. The
number of shells examined in relation to the exposure
term is given in Table 1. The substrates collected were
examined following the procedure similar to that
applied to natural aggregations (see above). To com-
pare the faunal changes on experimental substrates to
those in the surrounding habitat, the sediment near
substrates was sampled with a 0.025 m² Petersen grab,
4–10 samples each year. Faunal states in natural
aggregations of barnacles and ascidians sampled at
Site 1 in 1998–2004 were also used as a reference for
the experiment.

2.4. Statistical analysis

2.4.1. General notes

Unless stated otherwise, the abundance measure
used was the number of individuals per sample.
Since the sampling area representing aggregations
could not be fixed, the abundance of each species
was standardised by the total abundance of all species
in a sample (hereafter referred to as “relative
abundance”). The resulting proportions were subjected
to angular transformation (Sokal and Rohlf, 1995) in
order to normalise their distribution for further use in
parametric analyses.

The significance criterion for all tests was ɑ=0.05.
Means, where given, are ± 1 S.E.

2.4.2. Aggregations and bare sediment

A-samples and S-samples were subjected together
to type III sum of squares two-factor multivariate
ANOVA with Site as a random effect, and Microha-

Table 1
Number of substrates (Serripes shells) exposed during the field

<table>
<thead>
<tr>
<th>Exposure term (years)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of substrates examined</td>
<td>10</td>
<td>13</td>
<td>10</td>
<td>12</td>
<td>16</td>
</tr>
</tbody>
</table>
bitat (A-sample or S-sample) as a fixed one. The variables were the transformed abundances of 20 taxa with the largest average standardised densities. Variances were checked for homogeneity using Cochran C statistics, and for those species where they were found to be heterogeneous regardless of the transformations performed, Pearson correlations between variances and means were tested for the significant difference from zero. In case of the absence of a significant correlation the data were assumed to meet assumptions of ANOVA (Statsoft, Inc., 1995). Since in many cases this correlation was present, a non-parametric ANOVA (Kruskall–Wallis) was additionally performed. The same variables were analysed with PCA [principal component analysis, see Gauch, 1982] to examine whether the grouping pattern observed for samples would separate those from different sites and microhabitats. To test the effect of microhabitat on the whole assemblage, ANOSIM based on Bray–Curtis dissimilarity measure was applied to relative abundances of motile taxa using PAST software package (Ryan et al., 1995; Hammer et al., 2001). The ANOSIM R statistic was used as a dissimilarity measure to compare the effect of location on A- and S-samples (Clarke, 1993; Anderson and Underwood, 1997).

### 2.4.3. Aggregations with different characteristics

According to the effect on their shape, size and physical structure, the main quantitative characters of the epibenthic patches were considered to be the abundances of ascidians, living barnacles and their empty shells (hereafter “dead barnacles”). We used total numbers of living and dead barnacles (LB and DB) and numbers of those with the aperture length exceeding 9 mm (LB_{>9\,mm} and DB_{>9\,mm} correspondingly). The threshold of 9 mm was selected to separate “large” individuals, since this size had been the median one for the *B. crenatus* measured in this study. Total weight of ascidians (A) was used as their abundance measure. The same measure was not used for barnacles because it is not applicable to their empty shells. Pearson correlation matrix was calculated for the five parameters selected.

Standardised (as described above) angular transformed abundances of motile fauna found in aggregations were analysed for 10 dominant species using type III sum of squares GLM. Site was a random discrete effect, whereas characteristics of the sessile fauna of aggregations (A, LB, DB, LB_{>9\,mm} and DB_{>9\,mm}) were gradual effects. Assumptions of the analyses were checked as described above for ANOVA.

### 2.4.4. Experimentally reared aggregations

Student’s *t*-test was used to compare parameters of the sessile fauna for natural and artificial epibenthic clusters. Experimental aggregations were compared with natural ones and with sediment cores using the ANOSIM R statistic as a dissimilarity measure. The hypothesis tested was that initially empty *Serripes* shells exposed for 4–5 years would develop a motile fauna more similar to that of natural epibenthic patches rather than to the fauna of the surrounding sediment. ANOSIM on Bray–Curtis dissimilarity measure was applied to relative abundance data for all motile taxa. Mean relative densities of motile species found in natural microhabitats and on experimental substrates were compared using Mann–Whitney *U*-test. Pearson correlation coefficients with year or exposition term were used to compare long-term changes in species abundances on experimental substrates and in natural microhabitats.

### 3. Results

#### 3.1. Aggregations and bare sediment

Out of 168 motile species totally recorded in A- and S-samples, 152 were found in aggregations and 89 in bare sediment, so that 44.6% of the species list was common for both microhabitats. Shannon–Wiener species diversity index was 2.19 for A-samples and 1.97 for S-samples. Average number of motile species per sample was significantly lower (Student’s *t*-test, *p* < 0.001) in A- than in S-samples (12.1 ± 0.4 and 18.6 ± 0.5, correspondingly). Numerous polychaete worms, undetermined isopods and amphipods were the most abundant taxa.

It can be clearly seen on PCA plot (Fig. 2) that the assemblage of motile taxa associated with aggregations of barnacles and ascidians was entirely different from that sampled around the patches. Aggregations grouped together regardless of their experimental or natural origin and of their location. Conversely, cores
of bare sediment from different sites grouped separately. The first of the first two principal components, being responsible for 24.3% of total variance, separated samples according to the microhabitat type. Along the second one (8.7% of total variance) S-samples were separated according to the Site where

**Site 1**
- *Aristoidea*
- *Aricidea nolani*
- *Micronephthys minuta*
- *Sclibregma inflatum*
- *Cossura longicirrata*
- *Praxillella praetermissa*
- *Terebellides stroemi*

**Site 2**
- *Aristoidea*
- *Aricidea nolani*
- *Micronephthys minuta*
- *Sclibregma inflatum*
- *Cossura longicirrata*
- *Praxillella praetermissa*
- *Terebellides stroemi*

Fig. 3. Species composition of motile benthic fauna found at two sites and different microhabitats (in bare sediment [S] and in association with aggregations of barnacles and ascidians [A]). Fauna of the aggregations was similar at both sites (see text for details). Species are listed in order of their decreasing abundance.
Table 2
Summary of analyses comparing angular transformed relative densities of the 20 dominant motile taxa at different sites and microhabitats (aggregations of barnacles and ascidians vs. cores of bare sediment)

<table>
<thead>
<tr>
<th>Species name</th>
<th>Site (random, 2 levels, df=1)</th>
<th>Microhabitat (fixed, aggregation/bare sediment, 2 levels, df=1)</th>
<th>Site × Microhabitat (random, df=335)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Means</td>
<td>ANOVA</td>
<td>Kruskal–Wallis</td>
</tr>
<tr>
<td></td>
<td>S1</td>
<td>S2</td>
<td>MS</td>
</tr>
<tr>
<td>Pholoe minuta Fabricius</td>
<td>0.47</td>
<td>0.22</td>
<td>0.86</td>
</tr>
<tr>
<td>Sphaerosyllis erinaceus Claparede</td>
<td>0.03</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>Micronephthys minuta (Theel)</td>
<td>0.02</td>
<td>0.27</td>
<td>2.19</td>
</tr>
<tr>
<td>Polycirrus medusa Grube</td>
<td>0.03</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Apistobranchus bulleri (Theel)</td>
<td>0.07</td>
<td>0.07</td>
<td>0.24</td>
</tr>
<tr>
<td>Pygospio elegans Claparede</td>
<td>0.08</td>
<td>0.00</td>
<td>0.23</td>
</tr>
<tr>
<td>Scoloplos armiger (Müller)</td>
<td>0.08</td>
<td>0.13</td>
<td>0.01</td>
</tr>
<tr>
<td>Aricidea nolani (Eliason)</td>
<td>0.03</td>
<td>0.20</td>
<td>0.94</td>
</tr>
<tr>
<td>Chaetozone setosa Malmgren</td>
<td>0.03</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>Cirratulus cirratus (Müller)</td>
<td>0.16</td>
<td>0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>Heteromastus filiformis Zachs</td>
<td>0.07</td>
<td>0.11</td>
<td>0.00</td>
</tr>
<tr>
<td>Capitella capitata (Fabricius)</td>
<td>0.10</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>Praxillea praetermissa Malmgren</td>
<td>0.06</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Rhodine loveni Malmgren</td>
<td>0.11</td>
<td>0.03</td>
<td>1.83</td>
</tr>
<tr>
<td>Scalibregma inflatum Rathke</td>
<td>0.03</td>
<td>0.11</td>
<td>0.20</td>
</tr>
<tr>
<td>Isopoda</td>
<td>0.09</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>Gammaroidea</td>
<td>0.18</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>Crenella decussata (Montagu)</td>
<td>0.05</td>
<td>0.14</td>
<td>0.31</td>
</tr>
<tr>
<td>Musculus cf. discors (L.)</td>
<td>0.43</td>
<td>0.29</td>
<td>0.02</td>
</tr>
<tr>
<td>Hiattella arctica (L.)</td>
<td>0.05</td>
<td>0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Average angular transformed standardised numbers of individuals per sample (core or aggregation): S1—at Site 1, S2—at Site 2, A—in aggregations, BS—in bare sediment. Higher means are highlighted in bold where both the effect and Tukey HSD post-hoc test for means are significant (p < 0.05). Since in most cases variances were not homogeneous, non-parametric ANOVA (Kruskall–Wallis) results were added. ns: not significantly different; *p < 0.05; **p < 0.01; ***p < 0.001.
they had been obtained. Each of the resulting three groups of samples (A-samples, S-samples from Site 1 and S-samples from Site 2) was characterised by a specific composition of dominant taxa (Fig. 3).

Different sources of the abundance variation were analysed in multivariate ANOVA. The results are summarised in Table 2. Microhabitat type had a significant effect on the abundance of 19 out of 20 dominant species, 9 and 10 being more abundant, correspondingly, in aggregations and in bare sediment. For 6 species this effect was different depending on the location (a significant interaction Site × Microhabitat was found). Seven out of the 10 dominant species that demonstrated a significant variation of their abundance depending on location were associated with bare sediment, only 2 were aggregation-specific. The results of non-parametric ANOVA were the same as above for the effect of microhabitat type and very similar for the effect of location (Table 2). The dissimilarity between the two sites as indicated by ANOSIM R statistic based on relative abundance data for all species was higher in S-samples ($R=0.52$) than in A-samples ($R=0.19$). Larger difference ($R=0.85$) was observed between A- and S-samples from both sites. In all cases the dissimilarity was significant ($p<0.001$).

### 3.2. Aggregations with different characteristics

The main noticeable variation in aggregations studied was that of their size (and weight) and composition of the key sessile taxa, barnacles and ascidians. Average total weight of clusters without primary substrates was $44.9 ± 4.0$ g, ranging from 1 to 490 g. Barnacles and ascidians dominated in biomass. There was high positive correlation between the number of large dead barnacles and the weight of ascidians (Table 3).

The results of GLM analysis with Site and parameters of the aggregations (A, LB, DB, LB$_{>9}$ mm and DB$_{>9}$ mm) as effects are summarised in Table 4. Effect of location was significant for all the dominant aggregation-specific motile species. For all but one of them one or more parameters of aggregations also had significant effect on the abundance. Amphipods, isopods and *Musculus* (Lamellibranchia) were associated with high abundances of ascidians, the amount of dead barnacles, although correlated with ascidian biomass (see above), had either no effect on them or a negative one. Most other species demonstrated association with

### Table 3
Correlations between the main characters of epibenthic fauna in natural aggregations of barnacles and ascidians

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>LB</th>
<th>DB</th>
<th>LB$_{&gt;9}$ mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB</td>
<td>-0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DB</td>
<td>-0.03</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB$_{&gt;9}$ mm</td>
<td>0.02</td>
<td>0.55</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>DB$_{&gt;9}$ mm</td>
<td>0.45</td>
<td>-0.01</td>
<td>0.22</td>
<td>0.23</td>
</tr>
</tbody>
</table>

A—biomass of solitary ascidians; LB—number of living *Balanus crenatus*; DB—number of *B. crenatus* empty shells; LB$_{>9}$ mm—number of living *B. crenatus* with the aperture length exceeding 9 mm; DB$_{>9}$ mm—number of empty *B. crenatus* shells with the aperture length exceeding 9 mm. Data in cells are highlighted in bold if the correlation is significant at $p<0.05$ level.

### 3.3. Experimentally reared aggregations

Changes in the epibenthic flora and fauna found on experimental substrates in relation to the exposure term are summarised in Fig. 4. *Serripes* shells exposed for 5 years had developed *B. crenatus* clusters, with total covers and abundances demonstrating no significant difference from those observed in natural aggregations based on the same substrate type. Ascidians found in experimental clusters, though, were infrequent and small. The numbers of empty barnacle shells as well as the maximal barnacle sizes were also low (Table 5).

As mentioned above, experimental and natural aggregations grouped together on PCA plot based on densities of 20 dominant motile species (Fig. 2). ANOSIM R statistic based on relative abundance data for all species indicated low similarity between sediment cores and aggregations, both experimental and natural ($R=0.93$ and 0.85, correspondingly), in relation to the similarity observed between aggregations of different origin ($R=0.32$). The difference was significant in all the three cases ($p<0.001$). The longer the exposure term was, the less difference was found in motile 1 fauna between experimental and natural aggregations: the number of common dominant species out of the first 10 increased and ANOSIM $R$ decreased (Table 6). Though several species of those abundant on experimental substrates significantly
changed their mean density in the surrounding sediment and in natural aggregations during 1998–2004, only *Pholoe minuta* (Polychaeta) and isopods increased their abundance both on experimental substrates and in the surrounding sediment (Table 6). During the 5 years of exposure all dominant motile taxa increased their abundance on *Serripes* shells, with the only exception of amphipods (Fig. 5). Substrates exposed for 2 years were inhabited mostly by amphipods (61% of all individuals) and *Musculus cf.*

Table 4

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>A</th>
<th>LB</th>
<th>DB</th>
<th>LB-9 mm</th>
<th>DB-9 mm</th>
<th>Site</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradual (G)/discrete (D)</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>Fixed (F)/random (R)</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Species name</td>
<td>df</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

**Pholoe minuta**

- R: 0.07
- LB: 0.09
- DB: 0.03
- LB-9 mm: 0.03
- DB-9 mm: 0.03
- Site: 11.11
- Residual: 0.07

**Capitella capitata**

- R: 0.10
- LB: 0.21
- DB: 0.07
- LB-9 mm: 0.30
- DB-9 mm: 0.13
- Site: 0.39
- Residual: 0.02

**Cirratulus cirratus**

- R: 0.05
- LB: 0.12
- DB: 0.10
- LB-9 mm: 0.24
- DB-9 mm: 0.25
- Site: 0.15
- Residual: 0.01

**Polycirrus medusa**

- R: 0.06
- LB: 0.17
- DB: 0.18
- LB-9 mm: 0.12
- DB-9 mm: 0.17
- Site: 0.15
- Residual: 0.01

**Pygospio elegans**

- R: 0.07
- LB: 0.02
- DB: 0.01
- LB-9 mm: 0.12
- DB-9 mm: 0.03
- Site: 0.21
- Residual: 0.02

**Isopoda**

- R: 0.12
- LB: 0.06
- DB: 0.08
- LB-9 mm: 0.08
- DB-9 mm: 0.03
- Site: 0.29
- Residual: 0.02

**Gammaroidea**

- R: 0.33
- LB: 0.04
- DB: 0.00
- LB-9 mm: 0.10
- DB-9 mm: 0.00
- Site: 0.00
- Residual: 0.00

**Hiatella arctica**

- R: 0.02
- LB: 0.06
- DB: 0.05
- LB-9 mm: 0.15
- DB-9 mm: 0.02
- Site: 0.67
- Residual: 0.03

**Musculus cf. discors**

- R: 0.20
- LB: 0.03
- DB: 0.12
- LB-9 mm: 0.12
- DB-9 mm: 0.08
- Site: 0.21
- Residual: 0.03

**Pearson correlation coefficients** ($R$) are added to the results for gradual effects.

Codes for effects: A—biomass of solitary ascidians; LB—number of living *Balanus crenatus*; DB—number of *B. crenatus* empty shells; LB-9 mm—number of living *B. crenatus* with the aperture length exceeding 9 mm; DB-9 mm—number of empty *B. crenatus* shells with the aperture length exceeding 9 mm; Site—the location of the sampling site (2 levels). Data in cells are highlighted in bold if the effect is significant. Variables are angular transformed standardised numbers of individuals per aggregation.

ns: not significantly different; *$p<0.05$; **$p<0.01$; ***$p<0.001$.

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discors (17%); the latter (24%) dominated on substrates exposed for 5 years together with amphipods (12%) and *Capitella capitata* (Fabricius) (9%).

### 4. Discussion

Barnacles and solitary ascidians are known as keystone species in various rocky shore communities (McDougall, 1943; Sutherland, 1978; Dean and Hurd, 1980; Dean, 1981; Bros, 1987; Monteiro et al., 2002). When found in muddy habitats on relatively small substrates like stones or shells, they act as Type II patch providers (Connell and Keough, 1985; Sousa, 1984) in relation to the surrounding sediment.

We observed a strong difference in motile fauna between epibenthic patches and patches of bare sediment, which is in accordance with the hypothesis that aggregations of barnacles and ascidians would attract specific taxa, as it had been previously proved for patches of aggregated bivalves (Tsuchiya and Nishihira, 1985; 1986; Tsuchiya, 2002), tube-building worms (Woodin, 1974; Khaitov et al., 1999; Zühlke, 2001; Bolam and Fernandes, 2002) and seagrass (Bell and Westoby, 1986; Edgar and Robinson, 1992; Bowden et al., 2001; Lee et al., 2001). The effect of microhabitat determines the distribution of all but one dominant motile species. Spatial variation is high in samples of bare sediment, the fauna found
Table 6
Motile fauna and its temporal changes compared in experimental aggregations (EA) and natural microhabitats (S and NA) at Site 1

<table>
<thead>
<tr>
<th>Species name</th>
<th>Bare sediment (S)</th>
<th>Natural aggregations (NA)</th>
<th>Experimental aggregations (EA)</th>
<th>U-tests: experimental aggregations and natural microhabitats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean, R with year</td>
<td>Mean, R with year</td>
<td>Mean, %</td>
<td>Changes 1998 –2004 and 1+–5+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1+ 2+ 3+ 4+ 5+</td>
<td>5+ vs. S 5+ vs. NA 5+ vs. NA_B</td>
</tr>
<tr>
<td>Pholoe minuta</td>
<td>1</td>
<td>0.63***</td>
<td>17</td>
<td>0.42*** 0 3 8 7 6 0.36*</td>
</tr>
<tr>
<td></td>
<td>−0.11 ns</td>
<td>1 −0.09*</td>
<td>0 0 2 0 0</td>
<td>0.05 ns 453 ns 250***</td>
</tr>
<tr>
<td>Sphaerosyllis erinaceus</td>
<td>0</td>
<td>−0.10*</td>
<td>0 0 0 0 0</td>
<td>0.15 ns 473 ns 804***</td>
</tr>
<tr>
<td>Micronephthys minuta</td>
<td>2</td>
<td>0.07 ns</td>
<td>0</td>
<td>0.11 ns 140***</td>
</tr>
<tr>
<td>Polycirrus medusa</td>
<td>−0.15 ns</td>
<td>−0.24***</td>
<td>0 0 3 1 1</td>
<td>0.19 ns 442 ns 981***</td>
</tr>
<tr>
<td>Apistobranchus tullbergi</td>
<td>7</td>
<td>0.20*</td>
<td>2</td>
<td>0.09* 62***</td>
</tr>
<tr>
<td>Pygospio elegans</td>
<td>3</td>
<td>0.04 ns</td>
<td>3</td>
<td>0.09* 334*</td>
</tr>
<tr>
<td>Scoloplos armiger</td>
<td>9</td>
<td>−0.25**</td>
<td>1</td>
<td>0.10* 24***</td>
</tr>
<tr>
<td>Aricidea nolani</td>
<td>4</td>
<td>−0.05 ns</td>
<td>0</td>
<td>−0.13*** 112***</td>
</tr>
<tr>
<td>Chaetozone setosa</td>
<td>9</td>
<td>−0.40***</td>
<td>1</td>
<td>−0.23*** 56***</td>
</tr>
<tr>
<td>Cirratulus cirratus</td>
<td>0</td>
<td>−0.23**</td>
<td>6</td>
<td>−0.02 ns −0.42*</td>
</tr>
<tr>
<td>Heteromastus filiformis</td>
<td>5</td>
<td>0.10 ns</td>
<td>2</td>
<td>0.06 ns 256***</td>
</tr>
<tr>
<td>Capitella capitata</td>
<td>0</td>
<td>−0.27***</td>
<td>3</td>
<td>−0.08 ns 215***</td>
</tr>
<tr>
<td>Praxillella praetemissa</td>
<td>5</td>
<td>0.17*</td>
<td>1</td>
<td>0.13*** 225***</td>
</tr>
<tr>
<td>Rhodine loveni</td>
<td>11</td>
<td>0.63***</td>
<td>2</td>
<td>−0.12** 8***</td>
</tr>
<tr>
<td>Scalibregma inflatum</td>
<td>1</td>
<td>0.16 ns</td>
<td>0</td>
<td>−0.12*** 294***</td>
</tr>
<tr>
<td>Isopoda</td>
<td>0</td>
<td>0.38***</td>
<td>3</td>
<td>0.06 ns 294***</td>
</tr>
<tr>
<td>Gammaroidea</td>
<td>1</td>
<td>0.40***</td>
<td>6</td>
<td>0.09* 246***</td>
</tr>
<tr>
<td>Crenella decussata</td>
<td>1</td>
<td>0.14 ns</td>
<td>1</td>
<td>0.08 ns 295***</td>
</tr>
<tr>
<td>Musculus cf discors</td>
<td>0</td>
<td>0.07 ns</td>
<td>26</td>
<td>0.27*** 148***</td>
</tr>
<tr>
<td>Hiattella arctica</td>
<td>0</td>
<td>−0.20***</td>
<td>3</td>
<td>−0.19*** 221***</td>
</tr>
<tr>
<td>Number of common dominant</td>
<td></td>
<td></td>
<td>0</td>
<td>0.40 ns 210 ns 600***</td>
</tr>
<tr>
<td>species with NA</td>
<td></td>
<td></td>
<td>5</td>
<td>0.33 ns 157 ns</td>
</tr>
<tr>
<td>ANOSIM R with NA</td>
<td>0</td>
<td>0.67***</td>
<td>1</td>
<td>0.42* 8***</td>
</tr>
</tbody>
</table>

Species included were 20 dominant motile ones, taxa specific for natural aggregations of barnacles and ascidians (see Table 2) highlighted in bold.

Bare sediment: Mean relative densities of motile taxa in 0.025 m² Petersen grab samples of the unstructured sediment at Site 1. R with year: Pearson correlation between the relative density and sampling year (R, p).

Natural aggregations: Mean relative densities of motile taxa in all patches of barnacles and ascidians sampled at Site 1. R with year: Pearson correlation between the relative density and sampling year (R, p).

Experimental aggregations: Mean relative densities of motile taxa on initially clear Serripes groenlandicus shells exposed for 1–5 years (1+–5+). ANOSIM R dissimilarity measure with natural aggregations: samples for exposure terms 2–3 and 4–5 years were merged to increase the sample size. Number of common dominant species indicates the number of species, out of the 10 most abundant ones, common for experimental (1+–5+) and natural aggregations.

U-tests: Mean relative densities of motile taxa on experimental substrates (5+) were compared to those in sediment cores (2003–2004, Site 1), in all natural aggregations (NA) and in natural aggregations based on Serripes shells dominated by barnacles (NA_B) from the same site (U, p).

S ↑ (S↓), NA ↑ (NA↓), EA ↑ (EA↓)—linear trends summarised for relative abundance changes in bare sediment, natural aggregations and experimental aggregations, correspondingly, based on the sign and significant difference from zero of Pearson R. ns: not significantly different; *p<0.05; **p<0.01; ***p<0.001.
in them being to a great extent site-specific. In contrast, species composition and relative abundance of different motile taxa observed in aggregations were much less spatially variable. This is indicated both by the results of ANOSIM, the effect of location being higher for S-samples, and by PCA ordination, where aggregations grouped together whereas cores of bare sediment grouped separately by sampling site.

As shown by GLM results (Table 4), species composition of the aggregation-specific motile fauna, though dependent on location, is almost equally affected by parameters of sessile fauna. Previous research on dense assemblages of benthic keystone species suggests that there are two main processes leading to attraction or avoidance of these patches by motile taxa. They are (1) feeding mediated interactions (including biodeposition and predation on larvae) and (2) the effect of modified physical structure of habitat (Woodin, 1974; Rosenberg and Loo, 1983; Hunt et al., 1987; Hines et al., 1989; Crooks and Khim, 1999; Norkko et al., 2001; Hewitt et al., 2002). The latter primarily implies increasing the habitat complexity, known as direct and indirect modifier of species diversity and composition (see Raffaelli and Hawkins, 1996; Langellotto and Denno, 2004). In our case, any of those processes might have been expected for both barnacles and ascidians. However, we suggest that shells remaining from dead barnacles are directly contributing only to the second one. Comparison of the effects of the abundance of living and dead barnacles on motile fauna may help assessing the relative importance of the two processes.

The observed effect of barnacle abundance on motile fauna depended on the size of the cirripedians. Total numbers of living barnacles (LB) and of empty shells (DB), in contrast to the numbers of those larger than 9 mm (LB >9 mm and DB >9 mm), generally reflect the amount of small cirripeds in aggregations. No correlation found between the first two parameters and the abundance of the motile fauna seems to indicate none or little influence of young barnacles on aggregations’ inhabitants.

Effects of large living barnacles and of their empty shells are different, the latter only being significant for one abundant species. This species, the polychaete C. cirratus, is often found in subtidal muddy habitats with cavity-loaded matrix, such as algal holdfasts (Tzetlin et al., 1997; Norderhaug et al., 2002; our unpublished

Fig. 5. Changes in abundance of dominant motile taxa found on experimental substrates in relation to the exposure term. Means ±1 S.E. are plotted and −1 S.E. marks are omitted for clarity.
data). Modification of the habitat physical structure seems to be of less importance for associated mobile organisms in barnacle clusters than it has been observed in aggregations of other benthic habitat engineers (Woodin, 1974; Crooks and Khim, 1999). Furthermore, sessile fauna, particularly bivalves and ascidians, unlike the motile one studied, had previously demonstrated similar dependence both on barnacle shell mimics and on living barnacles (Dean, 1981). Most of aggregation-specific motile taxa are positively sensitive to the abundance of either ascidians or large living barnacles.

Being opportunistic, capitellid polychaets are mostly found in organically enriched habitats (Hily, 1987; Rossi, 2003) including those where the source of the enrichment is excretion by larger suspension-feeders (Mattison and Linden, 1983). Positive correlation between their abundance and that of living barnacles appears to reflect the effect of biodeposition. The presence of this effect is also supported by the results of motile fauna comparison close to and between the patches of barnacles and ascidians (Yakovis et al., 2004): capitellids were significantly more abundant and had a greater individual mean weight close to the patches. Though the same mechanism may account for the spatial pattern revealed in several other aggregation-specific polychaete species, it is probably inapplicable to the filter-feeding bivalve Hiatella arctica. Known as an inhabitant of cavities in hard substrate (e.g., Cocito et al., 2000; Gaymer and Himmelman, 2002), this species could be expected to prefer empty shells because they provide an appropriate space for adult molluscs. In fact, according to our non-quantified observations, adult Hiatella occupy large empty shells quite often. Thus, the effect of living barnacles observed may reflect the distribution of prevailing smaller bivalve individuals that fit into crevices between the shells of living cirripedians.

Association between different nest-building mytilids (including Musculus) and solitary ascidians was reported from various habitats and locations (Bertrand, 1971). Younger individuals are usually harbored by ascidian tunic’s texture depressions. Older ones often lose mobility, being overgrown by the tunic and embedded in its matrix with only siphonal end left outside. The nature of this association (mutualistic, commensal or parasitic) is unknown.

Barnacles (both living and dead) in aggregations are much less fouled by red algae than ascidians (Yakovis, 2002). We suggest that epibenthic crustaceans (amphipods and isopods) are attracted to ascidians by their extensive macroalgal cover (see Gunnill, 1982; Norderhaug et al., 2002; Norderhaug, 2004). According to our preliminary results (Yakovis, 2002), clumps of solitary ascidians seem to develop in barnacle aggregations under study only after the latter exceed a certain age and size. At least, ascidians were almost never found either on primary substrates of the clusters or on the shells of young barnacles (they were associated with the shells of living or dead large barnacles and with conspecific individuals). Thus, it may be expected that experimentally reared aggregations would lack the ascidian population for several years. Similar sequence of keystone species replacement on hard substrata was documented by Dean (1981), who observed ascidians’ expansion only at the patches previously occupied by barnacles and hydroids. It was suggested that the settlement and population growth in ascidians had been facilitated by physical structure of the habitat provided by earlier arrivals. The results of our experiments may therefore reflect temporal changes during the “pre-ascidian” period of aggregations’ development. Data of observations support the hypothesis that the motile fauna of epibenthic patches is specific and stable because of the influence of both barnacles and ascidians. Similarity found in motile species composition and proportional abundance of dominant taxa between the substrates initially lacking motile fauna after 5 years of exposure and natural aggregations gives a strong evidence for the structuring role of at least the barnacle population. The main difference observed between natural aggregations and 5-year-old experimental ones was that there was more of C. capitata and less of P. minuta in the experiment (Table 6). Increased abundance of Capitella is not unexpected, given that experimental aggregations were dominated by barnacles, indicating their positive effect on this worm (Table 4). Spatially variable (Table 4) abundance of Pholoe within aggregations cannot be explained in terms of effects revealed in the present study.

It is probable that, beginning with an empty shell of dead Serripes or a stone left by the melting spring ice, the epibenthic patch dominated by barnacles and ascidians passes a succession of specific consequent
changes. The primary goal of our further research is to determine the order of its stages and to reveal the temporal pattern of the community under study. We expect the succession of both motile and sessile components of the assemblage constituted by barnacles and ascidians to be traced in several years, when the long-term experiment started in 1998 has been completed. The present data support the conclusion that the motile fauna is strongly dependent on the sessile one and differs from that of the surrounding sediment. Taking into account the previously confirmed distant spatial effect of the aggregations of barnacles and ascidians (Yakovis et al., 2004), we regard the system investigated as an important example of regularly structured (both in space and in time) marine benthic community, which expands the range of potential objects for the research of pattern-generating biogenic processes.

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