



1 Low salinity as a biosecurity tool for minimizing biofouling in 2 ships sea-chests

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15 **Abstract.** Biofouling is a major vector in the transfer of non-native species around the world. Species can be
16 transported on virtually all submerged areas on ships (e.g. hulls, sea-chests, propellers) and so antifouling systems
17 are used to reduce fouling. However, with increased regulation of biocides used in antifoulants (e.g. the International
18 Maritime Organization tributyltin ban in 2008), there is a need to find efficient and sustainable alternatives. Here,
19 we tested the hypothesis that short doses of low salinity water could be used to kill fouling species in sea-chests.
20 Settlement panels were suspended at 1.5 m depth in a Plymouth marina for 24 months by which time they had
21 developed mature biofouling assemblages. We exposed these panels to three different salinities (7 psu, 20 psu and
22 33 psu) for two hours using a model sea chest placed in the marina and flushed with freshwater. Fouling organism
23 diversity and abundance was assessed before panels were treated, immediately after treatment, and then one week
24 and one month later. Some native ascidian *Dendrodoa grossularia* survived, but all other macrobenthos were killed
25 by the 7 PSU treatment after one week. The 20 PSU treatment was not effective at killing the majority of fouling
26 organisms. On the basis of these results we propose that sea-chests be flushed with freshwater for at least two hours
27 before ships leave port. This would not cause unnecessary delays or costs and could be a major step forwards in
28 improving biosecurity.

29

30 1 Introduction

31 Biofouling is a major vector in the transfer of non-native species around the world (Carlton et al., 1995; Ruiz et al.,
32 1997; Gollasch, 2002; Coutts & Taylor, 2004; Castro et al., 2017). Species can be transported on virtually all
33 submerged areas of ships so anti-fouling systems are used. However, some areas on ships hulls, such as sea chests
34 and chain lockers, are difficult to access and coat with anti-foulants. Consequently, these areas often get heavily
35 fouled by a wide variety of marine organisms such as hydroids, serpulid polychaetes, barnacles, mussels, bryozoans
36 and tunicates (Coutts & Taylor, 2004; Murray et al., 2011).



37 Non-native species introduction and spread is increasing, e.g. due to the opening of new trade routes, climate change
38 and the increasing speed of vessels. The International Maritime Organization (IMO) decided to tackle this problem
39 initially by adopting a set of voluntary regulations. In 2011, the IMO Marine Environment Protection Committee
40 issued Resolution MEPC.207(62) outlining measures to minimize the risk associated with ship biofouling. These
41 regulations are directed at many stakeholders (e.g. States, shipmasters, operators and owners, shipbuilders, port
42 authorities, ship repair, dry-docking and recycling facilities, anti-fouling paint manufacturers / suppliers). Two
43 subsequent sets of guidance on biofouling have since been released: one for recreational craft less than 24 meters in
44 length (MEPC.1/Circ.792, 2012), and the second evaluating the 2011 Guidelines for the control and management of
45 ship biofouling to minimize the transfer of invasive aquatic species (MEPC.1/Circ.811, 2013) (Castro, 2014).
46 Following the entry into force of the Ballast Water Convention in 2017, it seems probable that ship biofouling may
47 soon become the subject of a new international treaty. In May, 2017, a programme called “Building Partnerships to
48 Assist Developing Countries to Minimize the Impacts from Aquatic Biofouling” (or “GloFouling Partnerships”)
49 was approved by the Global Environment Facility to be implemented by the United Nations Development
50 Programme and executed by the IMO. An implementation phase will start in the second half of 2018 and last five
51 years (IMO Circular Letter No 3768). In some countries, biofouling management plans and record books are
52 already in place as part of national regulations (e.g. in the United States of America, Australia and New Zealand).
53 For instance, in the State of California (USA), ship owner/operators of vessels of 300 gross tons or larger need to
54 answer eleven questions about hull husbandry every year (Scianni et al., 2013).
55 Biofouling increases shipping operational costs; even microbial fouling, which is a pre-cursor to macro-fouling,
56 increases fuel consumption due to frictional drag. There are also the costs of hull cleaning and painting (Schultz et
57 al., 2011; Dobretsov et al., 2013; Davidson et al., 2016). Some organisms (e.g. bryozoans) are tolerant to antifouling
58 compounds and can grow on freshly applied antifouling paint, and are subsequently used as a substratum for other
59 species (Murray et al., 2011). With the ban of tributyltin in 2008, other anti-fouling systems started to be used.
60 Antifouling compounds have been developed from marine bacteria, cyanobacteria, fungi as well as eukaryotic
61 organisms (Dobretsov et al., 2013). Glycerophospholipids from soybeans are also effective booster biocides in
62 antifouling paint (Batista et al, 2015). In terms of mechanical tools to remove biofouling, Hearin et al. (2016)
63 showed that mechanical grooming is helpful in reducing fouling on submerged surfaces coated with fouling-release
64 coatings.
65 Niche areas on vessel hulls (e.g. gratings and propellers) represent a great challenge to minimising biofouling. On
66 larger vessels, sea chests maximize seawater inflow (e.g. for internal cooling systems and ballast water). These box-
67 shaped structures are difficult to access and coat, they have edges and welds that provide sheltered areas for
68 organisms to settle and recruit (Coutts & Dodgshun 2007). In Canada, a study of 82 sea-chests from commercial
69 ships showed that 80% of them had fouling organisms and that almost half had non-native species (Frey et al.,
70 2014).
71 Setting biosecurity goals and implementing measures for controlling non-indigenous species helps to avoid their
72 spread (Collin et al., 2015). In order to control biofouling in niche areas on ships, a simple efficient treatment
73 method is needed. Numerous methods are available, for example ultraviolet light (Titus & Ryskiewich, 1994),



74 heated water and steam, (Leach, 2011; Piola & Hopkins, 2012; Growcott *et al.*, 2016) or soaking areas in acids (e.g.
75 acetic acid) or alkalines, such as hydrated lime (Rolheiser *et al.* (2012). In Alaska, the invasive colonial ascidian
76 *Didemnum vexillum* was exposed to various treatments using acetic acid, bleach, freshwater or brine with 100%
77 mortality when exposed to freshwater for four hours (McCann *et al.*, 2013). In Brazil, Moreira *et al.* (2014) tested
78 the use of freshwater to combat the spread of invasive corals *Tubastraea tagusensis* and *T. coccinea*. For both these
79 species, two hour exposure to fresh water killed all the corals and this treatment is now routinely used for combat the
80 spread of *Tubastraea* spp. on oil industry infrastructure. In New Zealand, Jute and Dunphy (2016) showed that two
81 hour exposure to fresh water killed the invasive Mediterranean fan worm *Sabella spallanzanii*, while in hypersaline
82 conditions (50 psu) 100% mortality was reached after 24 hours. Finally two studies conducted in Plymouth, UK,
83 showed that low saline treatments can be highly effective at reducing biofouling and can be used in conjunction with
84 anti-fouling coating systems (Minto, 2014; Quinton, 2014). Although chemical treatments, the use of heat, or the use
85 of UV light all work they can be costly, or pose health and safety risks and also increase corrosion of hulls. On the
86 other hand, freshwater is not dangerous, and it is cheap and widely available.
87 Given the importance of biofouling as a vector in the world transfer and spread of non-native species, this study
88 tested the hypothesis that a low saline environment can kill fouling species and offers a simple and efficient
89 biosecurity management tool to minimize biofouling in ship sea-chests.

90

91 **2 Methods**

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93 **2.1 Study area**

94 An experiment was conducted in two phases, the first in November 2016 and the second in July/August 2017 in
95 Millbay Marina (50°21'47''N; 004°09'02''W), Plymouth, UK. The marina is tidal and open to Plymouth Sound, a
96 large bay on the south coast of Devon (SW England) that is sheltered by an artificial breakwater (Bremekamp,
97 2012).

98

99 **2.2 Research design**

100 A model sea-chest was built to find out the lowest steady salinity that could be achieved when the chest was flushed
101 with freshwater whilst submerged and open to surrounding seawater. The sea-chest was a polypropylene 80 l
102 container (external dimensions: 600 x 400 x 420 mm); 12 panels were fixed inside with stainless threaded rods to
103 simulate gratings. A YSI 556 Multiparameter meter, complete with conductivity probe, was hooked inside the box to
104 measure salinity. The box was deployed so that the panels were vertical and about 1.5 m from the seawater surface;
105 measurements of temperature and salinity started immediately after the deployment and were recorded every 10
106 seconds. To create a hyposaline environment inside our immersed sea-chest, freshwater was flushed into the box
107 through a hose connected to a tap on the pontoon. A flow rate of approximately 8 l/min was kept during the
108 experiment bearing in mind the necessity of preventing excess turbulence inside the box. Flow was suspended after
109 86 minutes when the salinity stabilised and the probe stopped recording five hours later.



110 Polyvinyl chloride (PVC) settlement panels (each 12 x 12 x 0.5 cm) were deployed in the same marina > two years
111 before the experiment, in June, 2014. Initially they were fixed in grids horizontally orientated with the roughened
112 side facing outwards, in a depth of approximately 1.5 m, avoiding sedimentation and algae growth (Quinton, 2014).
113 Five months before the low salinity experiment, panels were rearranged in a vertical position tied to a rope and
114 attached to the pontoon. At this stage, panels were less exposed to the light, almost under the pontoon which also
115 helped to preclude macroalgae. Fifteen of these panels were selected based on the existence of a well-developed
116 fouling community, including the native ascidian *Dendrodoa grossularia* on all panels and the non-native encrusting
117 bryozoan *Watersipora subatra* on most of the panels. The objective was to examine the effects of low salinity water
118 treatments on the whole community assemblage on each panel.

119 Panels were subjected to one of the following treatments: 7 psu, 20 psu and control (33 psu) for two hours (five
120 panels per treatment). The lowest salinity (7 psu) was chosen as it was the lowest steady value achieved inside our
121 simulated sea-chest. The exposure time was chosen based on the studies conducted by Moreira *et al.* (2014) and Jute
122 & Dunphy (2016). On the day before the experiment started, water from the marina was collected and stored in a
123 constant temperature room at Plymouth Marine Laboratory. The water used to prepare the different salinity
124 treatments during the experiment was a mix of local sea water and pure fresh water (Milli-Q water), stored in the
125 same room.

126

127 **2.3 Analysis**

128 An acrylic 12 x 12 cm quadrat divided into a 1 cm² square grid was used to enumerate organisms on the settlement
129 panels. The apparatus (settlement panel & quadrat) were submerged in seawater in a Pyrex dish for analysis. At each
130 intersection point on the grid, organisms were identified, where possible to species level. Each taxon was
131 enumerated, with colonial invertebrates counted as one maximum per square. Analysis times were set to a maximum
132 of 25 minutes in order to minimise stress to the organisms. Panels were evaluated regarding the abundance and
133 mortality of fouling organisms before the exposure to fresh water, immediately afterwards, and on two more
134 occasions: one week and one month after. Mortality was assessed e.g. through detachment of the organisms from the
135 panels, a lack of response (e.g. tunicates with no reaction when siphons were touched), absence of zooids in erect
136 bryozoans, alterations in the texture / colour of the organisms.

137 Data from fouling communities were entered into PRIMER-E for abundance analysis and were square root
138 transformed prior to clustering analysis according to Clarke *et al.*, 2016. Dendrogram plots were used to determine
139 similarity of fouling communities before, immediately after, one week and one month after the exposure to one of
140 three salinities targeted by this experiment.

141

142 **3 Results**

143 The first phase of the experiment was to ascertain the lowest salinity that could be maintained inside our simulated
144 sea-chest. The salinity was initially 32 psu, decreasing to 24 psu after 25 mins, to 9 psu after 60 mins before
145 stabilizing at 7 PSU from 86 mins onwards. Once the freshwater supply was switched off the salinity inside the sea-



146 chest increased slowly over a 5 h 20 min period to 27.3 PSU, when the recordings ended. During this time the water
147 temperature varied between 13 and 13.6°C.

148 Biofouling communities were similar on panels before and immediately after treatment but thereafter there were
149 marked differences since low salinity treatments killed most of the organisms present. Cluster analysis of the
150 biofouling community composition one week after the treatment (Fig. 1, and one month after, not shown) showed
151 that panels submitted to the same treatment were clustered together, as they had similar communities present. Tight
152 clustering was found for panels exposed to 7 psu; few mortality effects were found at 20 psu and no effects were
153 found on control panels (33 psu).

154 Figure. 1. Dendrogram showing significant separation between biofouling communities grown on settlement panels
155 treated with 7 psu and all the others treated with 20 psu and 33 psu (n=5 for each treatment).

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157 On panels treated with 7 psu terebellid worms quickly disintegrated and the erect native bryozoan *Bugula neritina*
158 leached a purple/brown colour into the water. The native ascidian *Ciona intestinalis* was less reactive when touched
159 with forceps than before the exposure. Neither *Dendrodoa grossularia*, the most frequent organisms on all panels,
160 nor *Watersipora subatra* colonies showed immediate visual responses to the treatments. After one week levels of
161 mortality were much more noticeable: for example 142 *D. grossularia* were counted on the five panels submitted to
162 7 psu - after a week 52 of these disintegrated when touched and were clearly dead. Erect bryozoans fell apart when
163 touched with forceps and all of the *Ciona intestinalis* had fallen off the panels. All of the native ascidian *Asciidiella*
164 *mentula*, were killed by the 7 psu treatment and had lost colour with flaccid tests filled with a dark liquid of rotting
165 tissue. Most organisms exposed to the 33 or 20 psu treatments survived (Fig. 2). More grid squares with bare panel
166 or biofilm were counted on all panels treated with 7 PSU (Table 1). All *W. subatra* individuals were dead after a
167 week with dark slime covering the panels and the distinct odour of rotting organisms.

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169 Figure. 2. A) Settlement panel one week after exposure to a 33 psu treatment showing the high biomass and diverse
170 biofouling community that had developed over two years at 1.5 m depth in a marina off Plymouth, UK. B) Example
171 of a panel one week after exposure to a 20 psu treatment with many members of the biofouling community still
172 alive. C) Panel one week after a 7 psu treatment showing black sulphurous rotting tissues. D) Typical panel
173 appearance one month after exposure to 7 psu showing a much reduced fouling community.

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175 In the 20 psu exposures *C. intestinalis* were less responsive immediately after treatment. After one week, 50% of *W.*
176 *subatra* colonies were dead, of a total of 60 *D. grossularia* only two (3.3%) had died. Many *D. grossularia*
177 individuals were covered with *Diplosoma listerianum*, not previously observed. This colonial tunicate is widespread
178 in the United Kingdom and shows rapid reproduction and growth rates (Bullard *et al.*, 2004, 2007; Vance *et al.*,
179 2009).

180 One month after exposure to the three salinity treatments there were still very clear differences among the treatment
181 groups although some recolonisation had begun on the 7 psu panels (Table 1). Numbers of species and Shannon-
182 Wiener diversity index show a decrease in diversity after one week and a small increase after one month for panels
183 exposed to 7 psu (Fig. 3).

184



185 Table 1: Average number of biofouling individuals per panel subjected to treatment with 7 psu, 20 psu and 33 psu
186 (control) water, showing % change in abundance after one week and after one month.

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188 Figure 3. A) Average number of species and B) Shannon-Wiener diversity index (H') of two year old biofouling
189 communities developed on PVC panels at 1.5 m depth in a marina off Plymouth, UK. Panels exposed to 7, 20 and
190 33 psu (Control) before treatment (ST), immediately after exposure (AF), one week after (IW) and after one month
191 of exposure (IM). Error bars are \pm SD, $n=15$.

192

193 4 Discussion

194 We obtained a steady value of 7 psu inside our model sea-chest when immersed at Millbay marina while flushed
195 with fresh water. This was the minimum salinity we used in an experiment to assess the mortality of fouling
196 organisms attached to PVC panels when exposed to three different salinities (7, 20 and 33 psu (Control)). The 7 psu
197 treatment was highly effective at killing most of the macrobenthos on the panels, whereas communities exposed to
198 20 and 33 psu were largely unaffected. There was some recolonization of bare substrata on the panels after one
199 month, thus this treatment would be best carried out on sea chests before a vessel leaves port, if she is destined for
200 another biogeographic region.

201 Freshwater exposure is an efficient way of controlling sublittoral marine fouling organisms as most suffer osmotic
202 stress (Moreira et al., 2014; Quinton, 2014; Minto, 2014; Jude & Dunphy, 2016). Most organisms were killed by our
203 two hour treatment with 7 psu water. For example, although *D. grossularia* had only 38% of mortality all the non-
204 native *W. subatra* were all killed after one week. After one week many dead rotting organisms were seen, which
205 then fell off the panels leaving bare space and revealing an understorey of organisms that were previously obscured,
206 such as *Pomatoceros* sp. (Table 1).

207 Of the two commonest species found in this study, *D. grossularia* and *C. intestinalis*, the first is a small, robust
208 tunicate, while the second is large, soft and highly contractile tunicate. Their bauplan possibly contributed to their
209 differing vulnerability to the treatment. After one month, new *Clavelina lepadiformis* had colonized along with
210 small erect bryozoans and *W. subatra* colonies (Table 1; Fig. 3). Thus flushing sea chests with seawater would be an
211 effective treatment for removing biofouling but will be time-dependent, with new recruitment occurring within a
212 month. For vessels which stay for long periods in berth we suggest low salinity flushing of sea chests is applied
213 shortly before vessels depart for the next port of call.

214

215 5 Conclusion

216 Very high levels of mortality occurred in mature biofouling communities subjected to two hour treatment with 7 psu
217 water, although some *Dendrodoa grossularia* were resilient. Low salinity treatments can be an efficient way of
218 minimizing biofouling from ship sea-chests, and offer a promising tool to be incorporated in vessel operation. This
219 would be an environmentally friendly biosecurity tool for minimizing and controlling ships sea-chest biofouling that
220 is simple and would not cause undue delay or costs.

221

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228 **Disclosure statement**

229 The authors declare that they have no conflict of interest.

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231 **References**

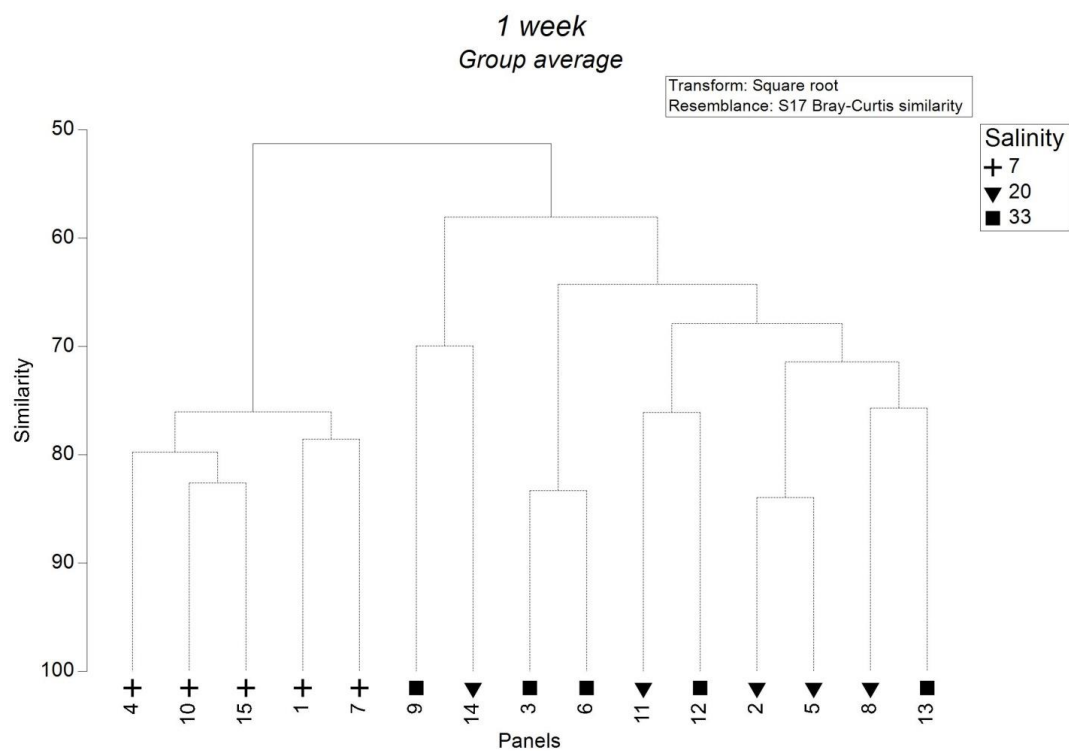
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Figure. 1. Dendrogram showing significant separation between biofouling communities grown on settlement panels treated with 7 psu and all the others treated with 20 psu and 33 psu (n=5 for each treatment).



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Figure 2. A) Settlement panel one week after exposure to a 33 psu treatment showing the high biomass and diverse biofouling community that had developed over two years at 1.5 m depth in a marina off Plymouth, UK. B) Example of a panel one week after exposure to a 20 psu treatment with many members of the biofouling community still alive. C) Panel one week after a 7 psu treatment showing black sulphurous rotting tissues. D) Typical panel appearance one month after exposure to 7 psu showing a much reduced fouling community.

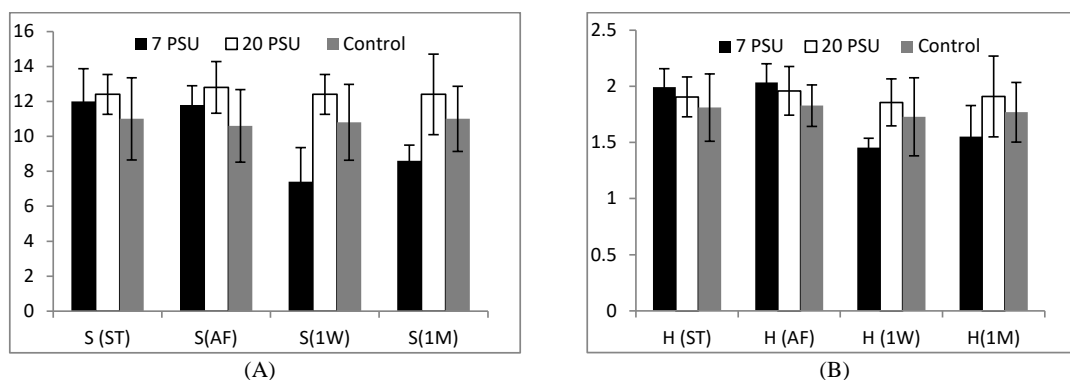


Figure 3. A) Average number of species and B) Shannon-Wiener diversity index (H') of two year old biofouling communities developed on PVC panels at 1.5 m depth in a marina off Plymouth, UK. Panels exposed to 7, 20 and 33 psu (Control) before treatment (ST), immediately after exposure (AF), one week after (1W) and after one month of exposure (1M). Error bars are \pm SD, n=15.



Table 1: Average number of biofouling individuals per panel subjected to treatment with 7 psu, 20 psu and 33 psu (control) water, showing % change in abundance after one week and after one month.

Taxa	Abundance data (average number of individuals/panel ± SD n=5) Pre treatment			% change after 1 week			% change after 1 month		
	7 psu	20 psu	Control	7 psu	20 psu	Control	7 psu	20 psu	Control
Bare substratum	8.2 ±3.0	4.2 ±5.3	5 ±3.3	404.9	142.9	40.0	385.4	109.5	48.0
Biofilm	27.2±11.7	28.6±14	23±10.4	21.3	-2.8	3.5	83.8	-15.4	13.9
<i>Sycon ciliatum</i>	0.4	3±1.7	4.6±6.2	0.0	-33.3	-17.4	-100.0	-80.0	-78.3
<i>Halichondria panicea</i>	3.8±3.5	2±1.7	7.2±8	5.3	170.0	-30.6	21.1	70.0	-27.8
<i>Corynactis viridis</i>	1.6±4.2	0.0	0.0	-100.0			-75.0		
Sabellaridae	2±1.5	1.2±0.6	0.2±0.7	0.0	0.0	100.0	-100.0	-16.7	0.0
<i>Pomatoceros</i> sp.	1±1.2	0.8	0.0	860.0	200.0		1060.0	325.0	
Terebellidae	0.0	0.2	0.8±2.8		-100.0	-50.0		-100.0	-100.0
<i>Watersipora subatra</i>	1±1.2	0.8±1.4	0.4±0.6	-100.0	75.0	-100.0	-20.0	225.0	-100.0
<i>Bugula neritina</i>	7.8±4.6	8.6±9.7	8±11	-100.0	-67.4	57.5	-46.2	-51.2	42.5
erect bryozoans	12.6±8.6	10.6±8.3	12.6±10.7	-100.0	-32.1	-23.8	-58.7	-30.2	-33.3
<i>Aplidium glabrum</i>	1.6	0.0	0.0	-100.0			-25.0		
<i>Diplosoma listerianum</i>	1±0.7	2.6±2.1	0.6±1	-20.0	-53.8	366.7	-100.0	38.5	200.0
<i>Botryllus schlosseri</i>	0.8±1.4	0.0	0.0	-100.0			-100.0		
<i>Asterocarpa humilis</i>	1.0	0.6	0.6±1	-100.0	66.7	-33.3	-100.0	166.7	-100.0
<i>Styela clava</i>	0.0	0.2	0.2±0.7		0.0	-100.0		-100.0	-100.0
<i>Corella eumyota</i>	0.0	0.0	0.4±0.6			-100.0			-100.0
<i>Clavelina lepadiformis</i>	4.6±5.3	6.2±6.9	8.6±15.7	-100.0	-38.7	-67.4	65.2	19.4	-18.6
<i>Asciidiella aspersa</i>	7.2±8.7	7.8±7.3	3.8±5.1	-88.9	-46.2	10.5	-100.0	-23.1	-63.2
<i>Ascidia conchilega</i>	0.0	0.0	0.2±0.7			-100.0			0.0
<i>Ascidia mentula</i>	8.4	2.8±5.7	12.4±43.8	-100.0	-21.4	12.9	-100.0	-100.0	8.1
<i>Ciona intestinalis</i>	18.6±14.1	14.2±6.9	10.4±4.7	-100.0	-33.8	-15.4	-100.0	-43.7	32.7
<i>Dendrodoa grossularia</i>	29±17.9	44.6±17.6	43.2±30.1	-37.9	19.3	9.3	-52.4	26.0	1.9