

Research Article

Ascidians in the succession of marine fouling communities

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Editor's note:

This paper is a contribution to the proceedings of the 3rd International Invasive Sea Squirt Conference held in Woods Hole, Massachusetts, USA, on 26–28 April 2010. The conference provided a venue for the exchange of information on the biogeography, ecology, genetics, impacts, risk assessment and management of invasive tunicates worldwide.

Abstract

Approximately 200 grey PVC settlement plates were hung along at sites along the Dutch coast at a depth of 1 meter. The cover of each species that could be identified on the plates was recorded every three months from March 2009 to March 2010 to study the role of native versus non-native ascidians in the succession of marine fouling communities. Three native ascidian species, *Ciona intestinalis*, *Ascidella aspersa* and *Botryllus schlosseri*, and five species of cryptogenic and/or non-native origin, *Molgula socialis*, *Styela clava*, *Diplosoma listerianum*, *Botrylloides violaceus* and *Didemnum vexillum*, were recorded. After the Cnidaria, Ascidiacea were found to be the group that explained most of the differences found in species communities on settlement plates at different stages in the succession. Regardless of the fact that non-native ascidian species were very abundant at times, their role in the succession of the studied marine fouling communities appears to be limited. After the polyp stage of the moon jelly *Aurelia aurita* and hydroid species of the genus *Obelia*, the two native ascidian species *Ciona intestinalis* and *Ascidella aspersa* explained most of the differences that were found between the successive stages in the fouling communities. In general, the three native ascidian species appeared to determine the succession of the fouling communities more than the five cryptogenic and/or non-native ascidian species. Whether this is linked to species being either native or non-native is questionable however. The various ascidian species differed strongly in the period of settlement, the ability to settle on prior settlers and the ability to survive the winter. The non-native ascidians settled mostly in June to December, while the native ones settled from March to December. Although the native ascidians *M. socialis* and *B. schlosseri* were found to be restricted in their settlement by the presence of prior settlers, the non-native species *B. violaceus* and *D. vexillum* were not. Native and non-native ascidian species survived the winter equally well. Native *C. intestinalis* and *A. aspersa* and non-native *B. botrylloides* and *D. vexillum* were most successful in surviving winter water temperatures of just below 0°C. Thus the results of this study, based on one year of data, suggest that the roles of ascidian species in the succession of fouling communities vary, and that being native or non-native is not necessarily linked to the degree to which species influence the development of marine fouling communities. Their individual seasonality, abilities to overgrow prior settlers and survival of winter temperatures appear to be more important.

Key words: Tunicata, succession, non-native species, community, winter

Introduction

The long-term dynamics of marine communities can be described by ecological succession, which is defined as directional changes in a community composition over time (MacMahon 1979). Succession has mostly been described for terrestrial communities, but more recently many

studies also focus on biofouling communities, describing community development in marine environments (Qvarfordt 2006; Greene and Grizzle 2007). In these studies, succession is thought to be mainly dependent on the unique life-history characteristics of particular species in a specific habitat and physical factors such as salinity, temperature, light and flow rate.

Because these factors tend to differ strongly among seasons, seasonality is thought to be an important factor in the development of marine fouling communities in temperate regions (Scheer 1945; Breitburg 1985; Whomersley and Picken 2003; Dziubińska and Janas 2007). In the present study we focus on the role of ascidians in the succession of marine fouling communities. Especially in temperate areas, non-native sea-squirts are rapidly spreading and influencing ecosystems worldwide (Lambert 2009; Stefaniak et al. 2009). However, the impact of these invaders in the succession of species communities can differ greatly between regions. A comparative study of fouling communities of ascidians on mussel ropes in The Netherlands and in Prince Edward Island, Canada, indicated that the species communities formed by the same ascidian species may differ greatly in seemingly similar environments (Gittenberger 2009). In the Dutch province of Zeeland, fouling species on mussel lines form diverse assemblages of ascidian and other species. In Canada, the same ascidian species form monocultures dominating the mussel lines. In this study we focus on the role of native versus non-native ascidians in the seasonal development of fouling communities.

Materials and methods

Study area

Marine fouling communities were monitored at 13 sites located along the Dutch coast. Several sites were located in the sea-arms Oosterschelde and Westerschelde and in the saltwater lake Grevelingen. Others were located along the North Sea coast and in the estuary of the Dutch Wadden Sea (Figure 1, Appendix 1). Although the Grevelingen can be considered to be a lake without tidal influence, it is connected to the North Sea by two sluices and therefore maintains a saline environment (Figure 1).

Plate deployment

About 200 14×14×0.5 cm grey PVC plates were deployed over periods of three, six, nine and twelve months from March 2009 until March 2010, for three, six and nine months from June 2009 to March 2010, for three and six months from September 2009 to March 2010, and for three months from December 2009 to March 2010. Each individual plate was attached to a brick for weight and stability and hung

horizontally from either floating or fixed structures. The side of the plate facing down in the water was sanded to optimize conditions for attachment of organisms. Only organisms on this side of the plate were evaluated during the monitoring. Plates hanging from floating docks were submersed one meter below the water surface, while those deployed off piers in tidal waters hung one meter below the lowest low water level (LLWL). The minimum distance between the plates was one meter. They were hung in open water, avoiding contact with the bottom and other structures. The number of plates per location varied between 10 and 16, depending on the available space.

Photographic protocol

Every three months, the plates were retrieved from the water, photographed and redeployed. The underside of the PVC plate was photographed in a small tub with just enough sea water to submerge the plate. Taking the plates out of the water and into the tub was done as quickly as possible ensuring that they were not out of the water for more than 10 seconds. Care was taken not to damage organisms attached to the plate during this procedure. A black piece of plastic or cloth was used to prevent reflection from the sun and a scuba-diving flashlight was used to light the plates for photographing. An overview photograph and multiple close-up (macro) photographs were taken from each plate with a digital camera (10 megapixel or greater).

Analyses

All detectable species per plate were recorded from the overview and the macro photographs. To get an estimate of species abundance per plate, the overview photographs were digitally subdivided in 25 (5×5) equal grids and the species presence/absence per grid were recorded. On each plate, the abundance of a species was determined by the number of grids it was present on, ranging from 0 to 25. On the basis of these values the average species abundance of each species was determined per location.

Multivariate analyses to investigate community structure were conducted using the PRIMER 6.1.10 computer program (Plymouth Routines in Multivariate Research) (Clarke 1993; Clarke and Gorley 2006). On the basis of the average species abundance measures, Bray-Curtis similarities (Bray and Curtis 1957) were

Figure 1. Study locations along the Dutch coast. Locations in red lie relatively exposed close to the North Sea and Wadden Sea; purple represents the Westerschelde; blue represents the Grevelingen; green represents the Oosterschelde.



calculated and plotted using Multi-Dimensional Scaling plots to investigate similarities between the plates that were first submerged in March 2009, after 3, 6, 9 and 12 months, and those that were first submerged in June and September after equal periods of time up to 9 and 6 months of submersion respectively. PERMANOVA was used to investigate the multiple factors involved (location, region, submersion period and submersion month), including interactions between periods and regions, and periods and location. Based on the results of the main test, pair-wise tests were done to investigate significant results in more detail.

Multiple Analysis of Similarities (ANOSIMs) were performed to test for significant differences in fouling communities between locations and submersion periods (Clarke 1993). The output shows a Sample Statistic (R-value) and a Significance level of sample statistic (%). The R value, which can take a value between 0 and 1, is a measure of the degree of separation between the samples. An R-value close to zero indicates

no significant differences. The statistical level % assesses the chance of observing the R-value (Clarke and Gorley 2006). Pairs of samples with an $R \geq 0.75$ are well separated from each other. R-values between 0.5 and 0.75 are overlapping but clearly different and pairs with $R \leq 0.5$ are barely separated (Clarke and Gorley 2006). The similarity percentage procedure (SIMPER) was used to identify the phyla and more specifically the species responsible for the differences found in the ANOSIM (Flemer et al. 1997). It was also used to calculate similarities of species communities on plates within regions and dissimilarities between regions.

Results

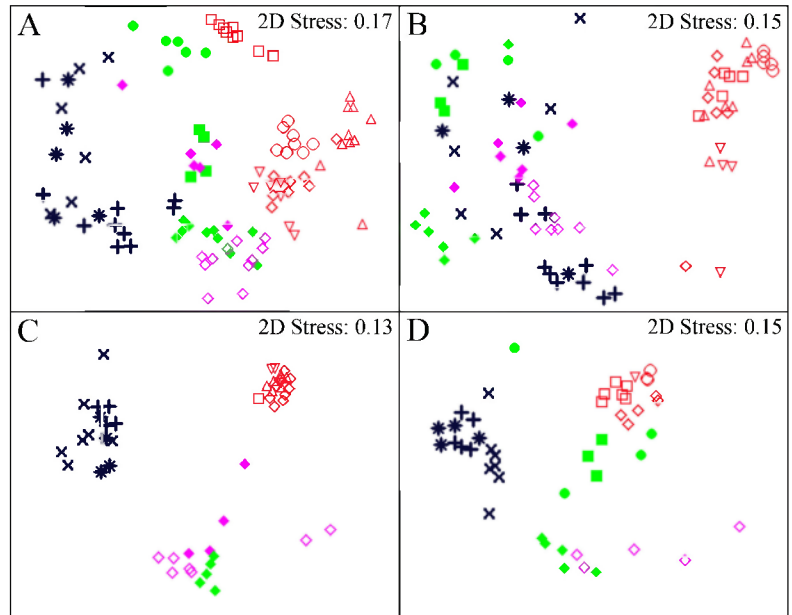
General succession of fouling communities

A general analysis of the data showed that the factors location, region and period all had a significant effect on the species communities (Table 1). Also, there was a significant

Table 1. PERMANOVA analysis of the effects on regions (fixed), locations (nested in regions) and periods (submersion time; nested in submersion month) on SETL-plate communities; p-values were obtained using 999 permutations; an asterisk indicates a significant difference.

Source of variation	df	P
Regions	3	0.022*
Locations (regions)	9	0.001*
Periods (subm month)	9	0.001*
Periods \times Regions	21	0.001*
Periods \times Location (Regions)	41	0.001*

Figure 2. Multi-Dimensional Scaling plots of Bray-Curtis similarities between species communities found on settlement plates that were submerged in March 2009 for a period of (A) 3 months, (B) 6 months, (C) 9 months, and (D) 12 months. Study locations are indicated in Figure 1. Red dots, North Sea and Wadden Sea; Purple dots, Westerschelde; Blue dots, the Grevelingen; Green dots, the Oosterschelde.



interaction when submersion periods and regions, and submersion periods and locations were combined (Table 1). For plates that were submerged in March 2009 (Tables 2–6), we found that after three months most locations had their own distinct species composition with hardly any overlap with other locations ($R = 0.875$; $p = 0.001$) (Figure 2A; Table 2).

After six months, a division became apparent between sites more exposed to the open sea (North Sea / Wadden Sea) versus the more inland locations in the Grevelingen, Oosterschelde and Westerschelde (Figures 1, 2B). The species compositions per location were not clearly different anymore, and especially the open sea locations formed a single, distinct group (red locations in Figure 2B).

After nine months, the variation among the open sea locations had decreased even more, whereas the more inland locations clustered in two groups: one with the locations from the Grevelingen and the other one with the Oosterschelde/Westerschelde records (Figure 2C). At that time dissimilarities between Oosterschelde and Westerschelde were 64%, while between all other regions they were more than 90% (Table 5). When tested for significance, the differences between the North Sea and more inland locations were still significant. The difference between the Grevelingen and Oosterschelde/Westerschelde locations was found not to be significant however (Table 3; $p = 0.056$). The variation in species composition after being deployed for

Table 2. Analysis of similarity (ANOSIM) of species composition compared between locations, submerged for periods of 3, 6, 9 and 12 months in March and June. An asterisk indicates a significant difference.

Periods from March		
<i>Submersion periods</i>	<i>R-statistic</i>	<i>P-value</i>
March - June	0.875	0.001*
March - September	0.769	0.001*
March - December	0.755	0.001*
March 2009 - March 2010	0.784	0.001*
Periods from June		
<i>Submersion periods</i>	<i>R-statistic</i>	<i>P-value</i>
June - September	0.715	0.001*
June - December	0.921	0.001*
June 2009 - March 2010	0.924	0.001*

Table 3. PERMANOVA analysis of the effects of period (submersion time of 3, 6, 9 and 12 months) on SETL-plate communities between regions, with plates deployed in March 2009, and with plates deployed in June 2009. P-values were obtained using 999 permutations; an asterisk indicates a significant difference.

Deployed in March, checked after:	3 months	6 months	9 months	12 months
In the month:	Jun	Sep	Dec	Mar
North Sea vs. Grevelingen	0.019*	0.006*	0.004*	0.024*
North Sea vs. Oosterschelde	0.123	0.013*	0.028*	0.031*
North Sea vs. Westerschelde	0.089	0.007*	0.009*	0.117
Grevelingen vs. Oosterschelde	0.075	0.316	0.163	0.056
Grevelingen vs. Westerschelde	0.091	0.449	0.077	0.246
Oosterschelde vs. Westerschelde	0.512	0.388	0.338	0.380
Deployed in June, checked after:	3 months	6 months	9 months	
Checked for species in the month:	Sep	Dec	Mar	
North Sea vs. Grevelingen	0.013*	0.008*	0.013*	
North Sea vs. Oosterschelde	0.002*	0.163	0.105	
North Sea vs. Westerschelde	0.064	0.031*	0.321	
Grevelingen vs. Oosterschelde	0.441	0.218	0.177	
Grevelingen vs. Westerschelde	0.001*	0.046*	0.161	
Oosterschelde vs. Westerschelde	0.0141	0.218	0.848	

nine months had clearly decreased within all three groups after nine months in comparison to the situation after six months (Table 4).

After 12 months of submersion, the three groups were still separated, but the variation significance, only differences between the North Sea and Grevelingen and between North Sea and Oosterschelde locations were significant (Table 3).

When plates were submerged in March, the differences in species composition between the locations decreased with time. After three months, 74 out of 78 pairwise comparisons between all locations (95%) differed significantly in species composition from each other ($R = 0.875$; $P = 0.001$). After six months, 67% still had a location specific species composition ($R = 0.769$; $P = 0.001$). After nine months this had decreased to 42% ($R = 0.755$; $P = 0.001$) and after 12 months only 25% of the locations still had a significantly different, i.e.

location specific, species composition. After 12 months, within group variation increased slightly ($R = 0.784$; $P = 0.001$) (Table 2). When looking at regions, the communities became more similar with time until nine months of submersion (57.61%; Table 4). After 12 months, the variation increased again (49.25%) (Table 4).

For the plates that were submerged in June 2009 (Tables 2–6), we found that after three months the species compositions per location differed less obviously (Figure 3A) as compared to the plates deployed in March 2009, after three months (Figure 2A). Between regions, the community compositions on the plates deployed in June already showed significant differences after three months of deployment, between the North Sea and Grevelingen/Oosterschelde locations ($p = 0.013$ and $p = 0.002$; Table 3). Additionally a significant difference was found between the Grevelingen and Westerschelde locations ($p = 0.001$) (Table 3).

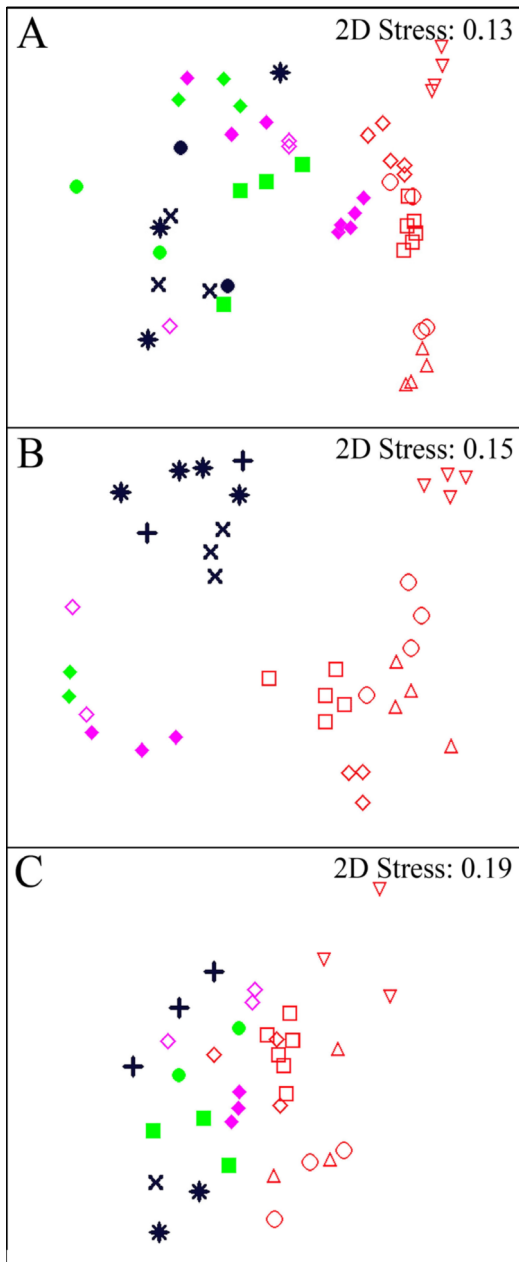


Figure 3. Multi-Dimensional Scaling plots of Bray-Curtis similarities between species communities found on settlement plates that were submerged in June 2009 for a period of (A) 3 months, (B) 6 months, and (C) 9 months. Study locations are indicated in Figure 1. Red dots, North Sea and Wadden Sea; Purple dots, Westerschelde; Blue dots, the Grevelingen; Green dots, the Oosterschelde.

After six months (Figure 3B), three groups of locations clustered in a similar way to what was observed for the plates submerged in March after nine months: a group for the Grevelingen, one for the Westerschelde and Oosterschelde, and a group with North Sea and Wadden Sea locations. The variation between the species communities within these areas was higher on the plates that were deployed in June (Figure 3B) as compared to those that were started earlier in March (Figure 2C; Table 4). Significant differences were found between the North Sea and the Grevelingen ($p = 0.008$), the North Sea and the Westerschelde ($p = 0.031$) and the Grevelingen and the Westerschelde ($p = 0.046$). Again, differences between the locations in the Oosterschelde and the Westerschelde were smaller than between the other regions (46%) (Table 5), but also in this case no significant difference was found between these two regions ($p = 0.218$). At that time the Oosterschelde locations did not significantly differ from the other regions (Table 3).

After 9 months of deployment (Figure 3C) the variation among the species communities increased again so that these groups became less distinct (Table 4).

For plates that were submerged in June, the locations differed significantly in species composition after three ($R = 0.715$; $P = 0.001$), six ($R = 0.921$; $P = 0.001$) and nine months ($R = 0.924$; $P = 0.001$). These differences between locations increased with time (Table 2). For submersion in June, the regions were most similar after six months submersion (50.22%). After nine months the variation increased again (35.48%) (Table 4).

Significant differences were found between submersion periods of three versus nine months, and three versus twelve months for both the plates that were deployed in March and the ones deployed in June (Table 6). Also for plates that were submerged in September, significant differences were found ($R = 0.308$; $P = 0.003$) (Table 6).

The differences between two submersion periods in the present study were mainly caused by cnidarians, followed by tunicates, when the plates are submerged in March (Table 7). Cnidarians explain 43% and 44% of the differences between submersion periods, whereas tunicates explain 27% and 34%, respectively

Table 4. SIMPER similarities (%) of period (submersion time of 3, 6, 9 and 12 months) on SETL- plate communities between regions, with plates deployed in March 2009, and with plates deployed in June 2009; 0 indicates maximum difference, 100 indicates no difference.

Deployed in March, checked after: Checked for species in the month:	3 months Jun 2009	6 months Sep 2009	9 months Dec 2009	12 months Mar 2010
North Sea	45.8	66.6	75.8	75.0
Grevelingen	44.2	40.6	46.6	57.1
Oosterschelde	42.7	44.5	73.4	35.5
Westerschelde	51.5	57.2	34.7	32.5
Average Similarity	46.0	52.2	57.6	49.3
Deployed in June, checked after: Checked for species in the month:	3 months Sep 2009	6 months Dec 2009	9 months Mar 2010	
North Sea	42.4	32.5	31.6	
Grevelingen	45.3	46.0	27.9	
Oosterschelde	38.9	77.8	39.2	
Westerschelde	41.0	44.6	43.3	
Average Similarity	41.9	50.2	35.5	

Table 5. SIMPER dissimilarities (%) of period (submersion time of 3, 6, 9 and 12 months) on SETL- plate communities between regions, with plates deployed in March 2009, and with plates deployed in June 2009; 0 indicates no difference, 100 indicates maximum difference.

Deployed in March, checked after: In the month:	3 months Jun 2009	6 months Sep 2009	9 months Dec 2009	12 months Mar 2010
North Sea vs. Grevelingen	92.0	88.6	96.0	91.3
North Sea vs. Oosterschelde	73.0	94.9	97.3	78.8
North Sea vs. Westerschelde	75.8	85.9	91.9	94.4
Grevelingen vs. Oosterschelde	76.0	70.2	91.8	84.6
Grevelingen vs. Westerschelde	83.9	59.2	90.7	92.7
Oosterschelde vs. Westerschelde	63.6	62.2	64.0	79.3
Deployed in June, checked after: Checked for species in the month:	3 months Sep 2009	6 months Dec 2009	9 months Mar 2010	
North Sea vs. Grevelingen	94.4	90.9	89.6	
North Sea vs. Oosterschelde	87.6	98.2	81.6	
North Sea vs. Westerschelde	75.0	93.9	71.3	
Grevelingen vs. Oosterschelde	63.4	83.3	82.6	
Grevelingen vs. Westerschelde	72.8	85.0	84.4	
Oosterschelde vs. Westerschelde	69.9	46.5	57.1	

(Table 7). For the cnidarians, the polyp stage of the moon jelly *Aurelia aurita* (L., 1758) (21–25%) and hydroid species of the genus *Obelia* (16–24%) are mostly responsible for the differences (Table 7). For the tunicates, the species which are mostly responsible for the differences are *Asciidiella aspersa* (16%) and *Ciona intestinalis* (12–14%) (Table 7).

Ascidians in succession

In total, eight species of ascidians were found during this study on the settlement plates: *Ciona intestinalis* (Linnaeus, 1758), *Asciidiella aspersa* (Müller, 1776), *Molgula socialis* (Alder, 1848), *Styela clava* Herdman, 1881, *Diplosoma listeria-num* (Edwards, 1841), *Botryllus schlosseri* (Pallas, 1766), *Botrylloides violaceus* Oka, 1927 and *Didemnum vexillum* Kott, 2002. Of these

species, *A. aspersa*, *C. intestinalis*, *B. schlosseri* and *B. violaceus* were abundant during at least one of the submersion periods studied. *Didemnum vexillum* was the least abundant. Long-term submersion compared to seasonal submersion (control periods of three months submersion) showed that there were species specific differences in the ability to settle in the initial or following succession stages. This was found while comparing the fouling communities that developed over a study period of 12 months on plates that were deployed in March (black columns in Figure 4), to the ‘pioneer’ fouling communities that were recorded on plates that hung submerged for only three months (white columns in Figure 4) and were deployed in June, September and December, respectively. Some species, such as *C. intestinalis*, were present throughout the year. Other ones were only

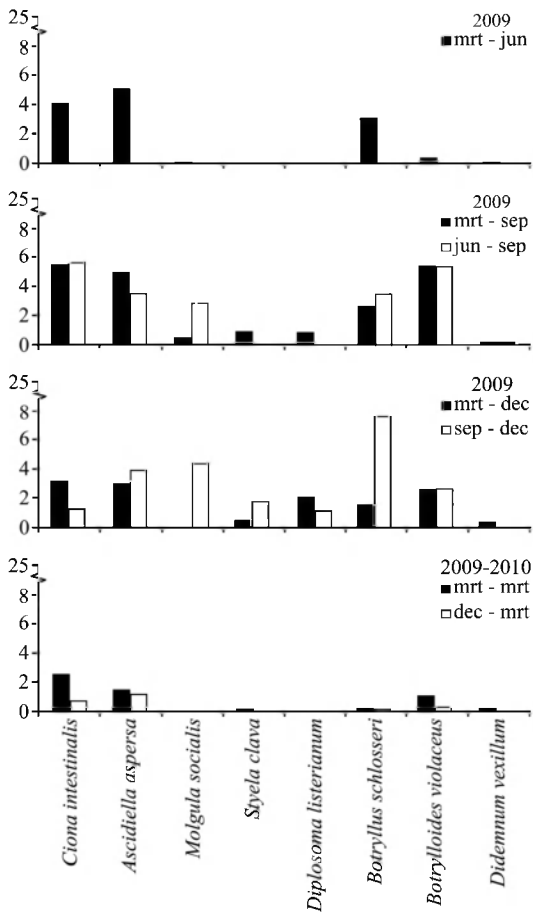


Figure 4. Average cover of ascidian species (in total # of grids) on settlement plates that have been deployed from (A) March-June 2009 (B) March-September 2009, (C) March-December 2009, and (D) March 2009-March 2010, including control periods from (A) March-June (B) June-September 2009, (C) September-December 2009, and (D) December 2009-March 2010.

present during the warmer months, such as *D. listerianum*. During the study period, some species, like *Botrylloides violaceus*, could settle equally well on empty plates and those with settled species already present (Figure 4).

Other species, like *Molgula socialis* and *Botryllus schlosseri*, settled at high abundances on relatively empty plates (white columns in Figure 4) and hardly or not at all on plates that were already overgrown (black columns in Figure 4). Finally, a selection of species, like

Ciona intestinalis, survived the winter (December-March) while others, like *Botryllus schlosseri* and *Diplosoma listerianum*, disappeared or decreased in abundance in the colder months of the year during the study period (Figure 4).

A typical example of the succession over a period of 12 months of a fouling community on a plate deployed in March 2009 is shown in Figure 5. Regardless of the relatively long and cold Dutch winter of 2009–2010, with water temperatures of just below 0°C for several months, the ascidians *Ciona intestinalis* and *Didemnum vexillum* survived. Although some regression was seen in *Didemnum vexillum* colonies because of these temperatures, some colonies, like the one in Figure 5, quickly overgrew the plate in spring 2010 and remained the dominant species on this plate until June 2011 when the plates were last checked (data not presented here).

Discussion

General succession of fouling communities

Differences between locations

The fouling communities that were recorded in this one year study were found to be location specific, especially three months after submersion in March 2009 (Figure 2A). During the following months the variation among the communities on these settlement plates decreased in time (Table 2). The species communities that were formed after the initial three months on the plates that were deployed in June differed less (Figure 3A). When looking at regions, plates deployed in June differed more regionally than those deployed in March (Table 3). Species communities were most similar in December (after nine months of submersion with deployment in March and after six months when deployed in June) (Table 4). These results resemble those of the fouling community study by Cifuentes et al. (2010) in Bahía La Herradura, Chile, where the species communities differed significantly at first in the spring/summer, but became more similar later in the year. In Chile this convergence of species communities at the study sites later in the year was linked to a dominant ascidian species, *Pyura chilensis*. No dominant ascidian species was encountered in the present study that can completely explain the decrease in community variation found on the plates in The Netherlands. Although the recruits of the native ascidian species *Molgula socialis*

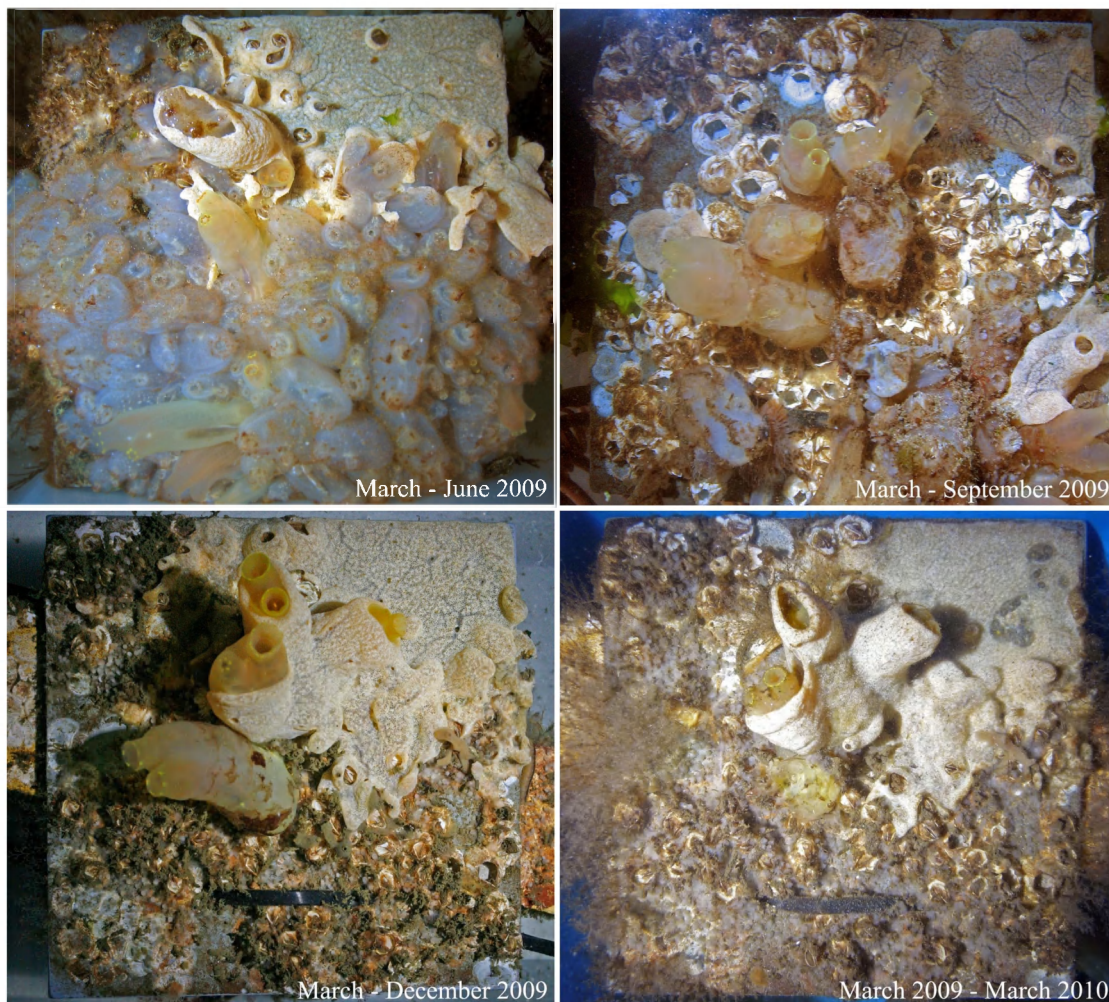


Figure 5. The succession that took place on a settlement plate that was deployed in March 2009 off the island Hompelvoet in the Grevelingen (Photographs by the GiMaRIS research team).

and *Botryllus schlosseri* were abundant between September–December, *B. schlosseri* settled about four times as much on the “clean” plates deployed in September, as on the already fouled plates that were deployed in either March or June (Figure 4), while *M. socialis* was virtually absent on the plates deployed in March and June. It appears therefore that these species are not dominant and that their settlement was in fact hampered by species that had already settled on the plates deployed in March and June. The settlement of the recruits of the non-native colonial ascidian *Botrylloides violaceus* did not seem to be hampered in their settlement

however, i.e., they were found in more or less equal numbers on both the newly deployed, “clean” plates and on the already fouled plates that were deployed three to six months earlier (Figure 4). *B. violaceus*, which was mainly found to settle between June and December (Figure 4) in The Netherlands, may therefore have had a similar effect as *Pyura chilensis* in Chile (Cifuentes et al. 2010), i.e., reducing the amount of variation between the species communities on the plates later in the year. Like *B. violaceus*, the settlement of the invasive colonial ascidian species *Didemnum vexillum* also did not appear to be hampered by the presence of other fouling

Table 6. Analysis of similarity (ANOSIM) between plates submerged for periods of 3, 6, 9 and 12 months, deployed in March, June, September and December. An asterisk indicates a significant difference.

Periods from March		
<i>Submersion periods</i>	<i>R-statistic</i>	<i>P-value</i>
March-June vs. March-September	0.204	0.048*
March-June vs. March-December	0.779	0.001*
March-June vs. March 2009-March 2010	0.631	0.001*
March-September vs. March-December	0.081	0.083
March-September vs. March-March 2010	0.064	0.005*
March-December vs. March-March 2010	0.088	0.014*
Periods from June		
<i>Submersion period</i>	<i>R-statistic</i>	<i>P-value</i>
June-September vs. June-December	0.222	0.001*
June-September vs. June-March	0.269	0.002*
June-December vs. June-March	0.144	0.015*
Periods from September		
<i>Submersion period</i>	<i>R-statistic</i>	<i>P-value</i>
September-December vs. September-March	0.308	0.003*

Table 7. Contribution of the two phyla that explained most of the significant differences in species composition between submersion periods of 3 versus 9 and 3 versus 12 months, and of these two phyla the two taxa that explained most of the differences; plates deployed in March. n represents the total number of SETL plates analyzed.

Periods	Phyla	
March 2009 - June 2009 vs. March 2009 - December 2009 (n = 162)	Cnidaria	43.5%
	<i>Aurelia aurita</i> polyps	24.5%
	<i>Obelia</i> sp.	15.6%
	Tunicata	27.3%
	<i>Asciidiella aspersa</i>	16.1%
	<i>Ciona intestinalis</i>	11.8%
March 2009 - June 2009 vs. March 2009 - March 2010 (n = 155)	Cnidaria	44.1%
	<i>Aurelia aurita</i> polyps	21.1%
	<i>Obelia</i> sp.	24.4%
	Tunicata	33.5%
	<i>Asciidiella aspersa</i>	16.1%
	<i>Ciona intestinalis</i>	14.4%

species on the plates in the present study. On the few plates where it was recorded, it was of strong influence on the development of the species communities. The colonies regressed somewhat in the winter, as is also found in other studies (Gittenberger 2007; Valentine et al. 2009), but not much (Figure 5), and in spring the colonies regenerated again and remained the most dominant species on the plates concerned until at least June 2011.

The pioneer species that settled on the plates between March and June 2009 (Figure 2A) formed more location-specific communities than the species that settled between June and September (Figure 3A). On a larger regional scale, the species that settled between June and September and between September and December also appeared to differ at the various locations, however (Figures 2B–C, 3B). On the

basis of these observations, three groups of fouling communities were distinguished: a group found in the Grevelingen, one for the Westerschelde and Oosterschelde, and a third group with sites that are situated close to the North Sea and Wadden Sea (Figure 1; Table 3). More research is needed to pinpoint the exact abiotic or biotic factors that distinguish the habitats in these three groups. The most obvious differences that may have been of influence concern (1) the absence of tidal currents in the Grevelingen, (2) the sheltered, more inland, position of the locations in the Westerschelde and Oosterschelde and (3) the greater exposure of the open-water locations along the North Sea and Wadden Sea (Figure 1). On the basis of one year of data it cannot be stated whether these three groups of fouling communities represent more or less stable communities over the years.

The main reason for an increasing variation on the settlement plates, 12 months after submersion in March and nine months after submersion in June, was probably the winter period during which individuals of some species died, resulting in open space, and enabling the settlement of early-successional species in early spring. These were the species that most differentiated between locations, as was concluded on the basis of the March–June plates (Figure 2A). They explained the increase of variation between the fouling communities that were recorded directly after the winter.

Differences between submersion periods

Seasonality plays an important role in determining species composition, as was found in several other studies (Scheer 1945; Dziubińska and Janas 2007; Greene and Grizzle 2007). When plates are submerged in March, significant differences in species composition between March–June versus March–December and between March–June versus March–March were found; no significant differences were found between the other submersion periods. Approximately the same species settled in the period from June to September as from September to December, while larger differences were found between these two periods versus the periods March–June and December–March (Figure 4). This may have been caused mainly by the water temperature, which in The Netherlands remains more or less stable in summer and fall, i.e., from June to December. From December to March, water temperatures decrease from about 14–16°C to temperatures to just below 0°C. In the months from March to June, water temperatures rapidly increase again.

Between September 2009 and March 2010, independent of the month of deployment, the community composition changed less than between March 2009 and September 2009 (Figures 2–4; Tables 2, 6). This could be explained by the fact that species settle mostly in the warmer months of the year until around October, depending on how fast the water temperatures decrease. There is less settlement after October due to decreased biodiversity in the colder months. Additionally, the high settlement of tunicates between June–September (Figure 4) could inhibit settlement later in the year, preventing the community from changing. The absence of significant differences between the communities in December and in March suggests

that at least in our one year study, species that occurred abundantly in December, like *Ciona intestinalis* and *Didemnum vexillum* on the plate in Figure 5, survived the winter period.

Of the ascidian species recorded, the non-native *B. violaceus* and *Didemnum vexillum*, and the native ascidian *Ciona intestinalis* were most likely to survive the winter (Figures 4, 5). By surviving the winter, these three species may inhibit to some degree the settlement of early-succession species in spring. They may therefore play an important role in the development of fouling communities, decreasing seasonal influences in the long term. These potential effects could not be studied, however, because the present project only included only one year of data. It does illustrate how ascidian species can play a significant role in fouling community development.

Native and non-native ascidians in the succession of fouling communities

The four taxa that explained most of the differences in the species compositions are all native: *Aurelia aurita*, *Obelia* sp., *Ascidella aspersa* and *Ciona intestinalis*. This result is congruent with a recent study off the coast of Massachusetts, USA, in which the recruitment and growth of native species influenced the long-term species composition on settlement plates more than the pre-seeding of these plates that was done by Agius (2007) with colonies of the non-native ascidians *Botrylloides violaceus* and *Diplosoma listerianum*. Whether these results can be linked to species being native or non-native is questionable, however. In the present study differences in species composition related to submersion periods could be explained initially by the settlement of season-bound species. Hydrozoa settle early in the year (unpublished data), explaining why differences between March–June and March–December and between March–June and March–March have been documented to be mainly caused by Cnidaria (Table 7). Polyps of the moon jelly *Aurelia aurita* have been documented to be common in the colder months of the year (Berrill 1949). During the warmer months, this jellyfish species is rare in its sedentary form as the species has by then developed into its free living form. This explains the significant differences in composition caused by this species between March–June and March–December (Table 7).

Next to the polyps of *A. aurita*, the cnidarian *Obelia* sp. was also found to explain much of the differences between the various submersion periods (Table 7). The hydroid *Obelia* sp. was especially common in the start of the year. It may at least partly be responsible for the fact that *C. intestinalis* and *A. aspersa* were the two ascidian species that explained most of the differences between the submersion periods (Table 7). Schmidt (1983) already described how hydroids can form ideal settling substrates, and that their presence can therefore be directly linked to the abundant occurrences of the solitary ascidian species *C. intestinalis* and *A. aspersa*. Schmidt (1983) furthermore described how, when hydroids were absent, colonial ascidians became more abundant on settlement plates instead, regardless of the number of larvae of *C. intestinalis* and *A. aspersa* that were present in the water column. Further research is necessary to test this hypothesis, but the results of Schmidt (1983) indicate that one should take interspecific interactions into account. Another reason why the native species *Ascidella aspersa* and *Ciona intestinalis* were found to be mainly responsible for the differences in the species communities (Table 7), is that these two species, together with the third native ascidian included in this study, *Botryllus schlosseri*, showed relatively high settlement starting in spring, from March to December (Figure 4). The five non-native / cryptogenic ascidian species that were recorded, *Molgula socialis*, *Styela clava*, *Diplosoma listerianum*, *Botrylloides violaceus* and *Didemnum vexillum*, started to settle later in the year and were most abundant from June to December (Figure 4). Therefore, the role of non-native ascidians in the succession of fouling communities in The Netherlands in the present one year study remained limited in comparison to the importance of the native species. This finding cannot simply be generalized because *C. intestinalis* and *A. aspersa* are considered invasive in many other areas in the world (Blum et al. 2007) and their abundances in the present study may at least partly be explained by the dominant presence of the hydroid *Obelia* sp. Furthermore the recruitment patterns of the ascidian species *C. intestinalis* are similar in its native and its non-native range in the northern hemisphere. As in The Netherlands, *C. intestinalis* is most common in the period June to September in its non-native range, i.e., PEI (Prince Edward Island), Canada (Figures 4–5; Ramsay et al. 2009). Recruits do start to settle

earlier in The Netherlands, in March–June (Figures 4–5), than in PEI (Ramsay et al. 2009). This is probably because in general the water temperatures in The Netherlands are warmer.

The settlement plate methodology was chosen because it has successfully been used in similar studies along the coastline of New Zealand, America and Europe (De Riviera et al. 2005; Ruiz et al. 2006; Templado et al. 2010). As a consequence of our methods, i.e., the use of artificial settlement plates, the results presented here were confined to the succession of fouling communities in relatively shallow, one meter deep, water. This may explain why *Styela clava*, *Diplosoma listerianum* and *Didemnum vexillum*, were not found in high abundances even though they occur commonly in Dutch waters (Gittenberger 2007; Gittenberger et al. 2010).

Conclusion

In general, the patterns of species settlement on the plates deployed in March and checked in June resulted in location-specific fouling communities of early-succession species. Later in succession, the differences decreased, so that eventually three categories of fouling communities remained. We believe that these are more habitat- than location-related. The first category of fouling communities was found in areas without tidal influences (Grevelingen locations), the second one includes more inland habitats which had strong tidal influences (Westerschelde and Oosterschelde locations), but less sedimentation than the third category, which had strong tidal influences and is more exposed to the open sea (North Sea and Wadden Sea locations). As the present study only includes one year of data, additional research is necessary to pinpoint the exact (a) biotic characters that play a role here. Second to cnidarians, the ascidians explained most of the differences among the species communities on settlement plates in the different stages of community development. Regardless of the fact that non-native ascidian species were very abundant at times, their role in the present study appeared to be limited. The two native species *Ciona intestinalis* and *Ascidella aspersa* explained most of the differences. This difference may be linked to the fact that the various ascidian species, native and non-native, differed strongly among each other in the period of settlement, the ability to settle on prior settlers and the ability to survive the winter. The three native ascidian species started settling at

relatively high abundances during the months March–June, whereas the main settlement season of the five non-native and cryptogenic ascidians started several months later in June–September. Although the recruits of the native ascidians *M. socialis* and *B. schlosseri* appeared to be hampered in their settlement by the presence of prior settlers, the non-native species *B. violaceus* and *D. vexillum* were not. Both native, *C. intestinalis* and *A. aspersa*, and non-native ascidian species, *B. botrylloides* and *D. vexillum*, survived the winter about equally well. Thus the results of this study suggest that the roles of ascidian species in the succession of fouling communities vary, and that being native or non-native is not necessarily linked to the degree in which they influence the development of marine fouling communities. Their individual seasonality, abilities to overgrow prior settlers and winter temperature survival skills appear to be more important.

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Supplementary material

The following supplementary material is available for this article.

Appendix 1. Distribution records of tunicates collected on settlement plates in The Netherlands.

This material is available as part of online article from:

http://www.aquaticinvasions.net/2011/AI_2011_6_4_Lindeyer_Gittenberger_Supplement.pdf