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Research Article

Artificial structures in harbors and their associated ascidian fauna

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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

We tested the hypothesis that marine non-native fouling species are more abundant than native species on artificial structures, focusing on ascidians and using fouling plates deployed in various Dutch harbors. A more detailed study was conducted in the pleasure craft harbor of Breskens, in the south of The Netherlands, where a species assessment was done of iron harbor walls, wooden pilings, diagonal stone rip-rap dikes, fouling plates, and the inside, outside and underside of the floats of floating docks. Ascidians were found only on the floating structures. Non-native ascidians were not found to be significantly more abundant than native ascidians, however. Solitary and colonial tunicates were compared, assuming that they fundamentally differ in their abilities to occupy space. In general, colonial species like the native *Botryllus schlosseri* and the non-native *Botrylloides violaceus* were more abundant on fouling community plates, while solitary ascidians like the native species *Ciona intestinalis* and *Ascidiella aspersa* appeared to be more successful later in succession. The inside, outside and underside of a floating dock harboured significantly different species communities. *Ascidiella* aspersa for example was found significantly more often on the underside than on the inside and outside of the dock floats. When assessing the ascidian species diversity in a harbor it is therefore advised to search several different habitats on floating structures.

Key words: harbor, non-native ascidians, floating dock, fouling community

Introduction

When artificial structures are placed in coastal waters, new habitats are introduced into the marine environment. These artificial habitats, especially ones with hard substrata, attract a higher number of non-native fouling species than natural habitats. It was hypothesized that native fouling species were less well adapted to such new habitats and, therefore, are more easily outcompeted by non-native fouling species on artificial surfaces (Byers 2002; Alpert 2006). However, not all artificial structures attract the same number of non-native fouling species (Gittenberger et al. 2010). The densest communities of fouling species form on floating structures like mussel rope cultures (Gittenberger 2009; Locke et al. 2007) and floating docks (Connell 2000; Minchin 2007; Pederson et al. 2005). For some of these structures, micro-habitats can be distinguished which may vary in the amount of fouling and diversity of species that they attract. Grey (2009) investigated whether one can study the dispersal of alien fouling communities by looking at only the outside of the sides of floating docks, instead of getting in the water to study the underside of docks by snorkel or SCUBA. Our study concentrated on native and non-native ascidians, as they play a prominent role in the alien fouling communities of artificial structures (Minchin 2007; Reinhardt et al. 2010). We questioned which artificial structures and associated habitats are fouled by ascidians and whether this can be

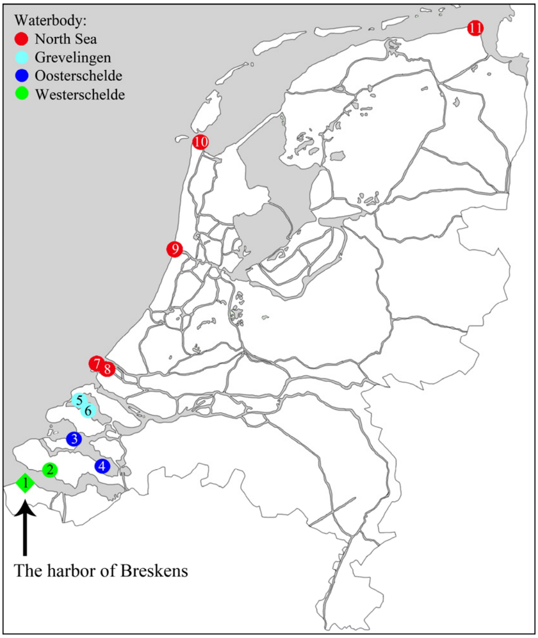

Figure 1. Research localities along the Dutch coast. The present study focuses mainly on the southernmost locality, the harbor of Breskens.

linked to ascidians being either native or non-native, and/or to other characters like ascidians being either solitary or colonial. This was hypothesized because solitary and colonial animals inhabiting marine hard substrata differ fundamentally in their growth patterns and other life-history attributes and thus in their ability to use space (Jackson 1977). In some environments colonial ascidian species therefore tend to dominate the community (Jackson 1977), while in other environments the solitary ones do (Greene et al. 1983). The focus of the present study was at the pleasure craft harbor of Breskens, situated close to the North Sea, and comparisons were made with other harbors along the Dutch coast. All non-native ascidians that were found were introduced to The Netherlands more than ten years ago (Gittenberger 2007). Pleasure crafts may have been one of the main vectors through which they were introduced in The Netherlands, as pleasure craft harbors are hotspots of marine non-native species (Gittenberger et al. 2010). The boats travel long distances along the European coast and some even go to the American coast and back.

Methods

Study areas and habitats

To study fouling communities in harbors, a series of field studies was conducted in 2008 and 2009, with a special focus on hard substratum habitats in the pleasure craft harbor of Breskens in the south of The Netherlands (Figure 1). In that harbor, an inventory was made of all the artificial hard structures and the various habitats that could be distinguished there: [1] diagonal stone rip-rap dikes, [2] vertical iron harbor walls, [3] wooden pilings, [4] the outside, underside and inside of the floats of several floating docks (Figure 2). In the sublittoral zone the harbor bottom was composed of sand and mud and no tunicates were found. Therefore, the hard surfaces of these artificial structures (with the exception of the floating docks) were only searched in the littoral zone. In addition to the habitats described above, 14 × 14 cm PVC fouling plates were examined, as was also done in similar studies (Ruiz et al. 2006; Templado et al. 2010). The grey PVC plates were horizontally attached below bricks, about 2 kg in weight each, with tie-wraps (Figure 3). They were deployed hanging on a rope at a depth of 1 m (Figure 3), from a floating dock, always in the shadows, in about half a meter of space between the dock and vertical iron harbor walls. Ten plates were deployed in the harbor of Breskens in March 2009 and checked for species after three, six and nine months, in June, September and December.

Species assessment

The fouling community plates were taken out of the seawater and placed upside down in a container with seawater, being out of the water for no more than 10 seconds, and examined for fouling ascidians. The plates were photographed in overview and a close-up photograph of each species was taken with a digital camera with at least 10 megapixel resolution. An LED scuba-diving torch was used as an extra light source. After photographing, the plates were returned to their original position.

In the laboratory, the overview photos were digitally subdivided in 25 grids and all species that were visible were scored (presence/absence only) for each grid.

Because the fouling plates were 14 × 14 cm in size, the other habitats (diagonal stone rip-rap dikes, vertical iron harbor walls, wooden pilings,

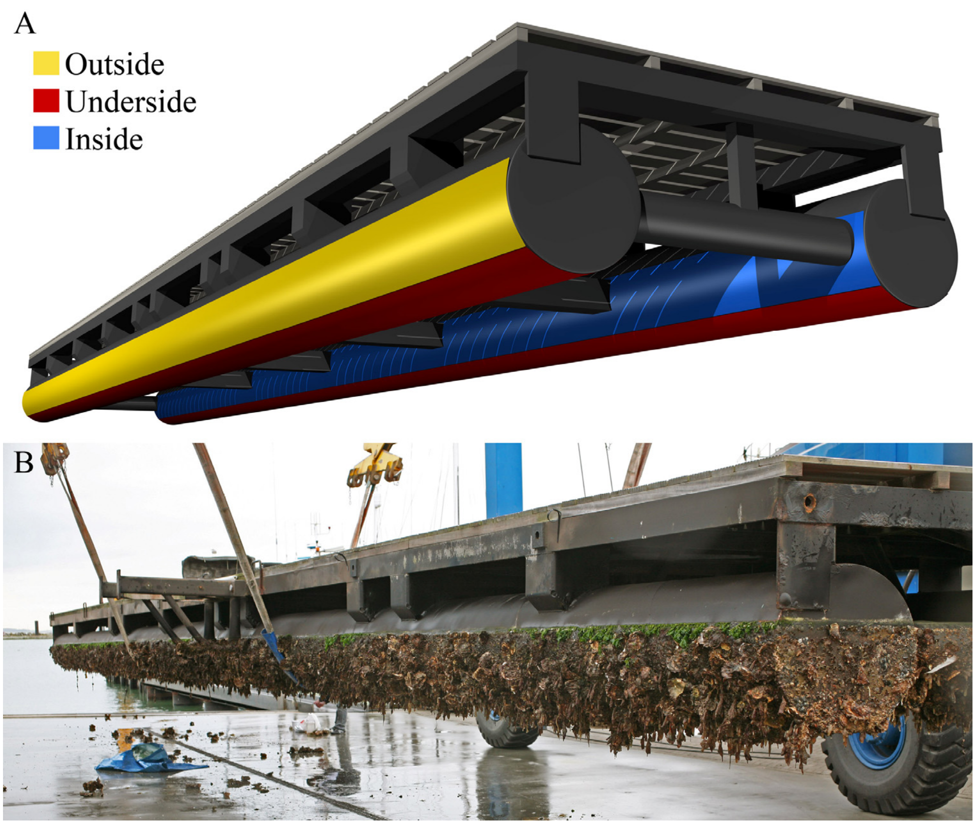

Figure 2. Floating dock in the harbor of Breskens, schematic view (**A**) and overview photo (**B**). For maintenance purposes these docks are taken out of the water about every ten years. Directly after they were lifted from the water, rapid assessments were done of the fouling species on the outside (yellow area), inside (blue area) and underside (red area) of these docks. Photograph by the GiMaRIS research team.

Figure 3. Fouling community plate. The PVC plate measures 14×14 cm and is roughened on the underside. It is deployed at a depth of 1 m.

and the outside, underside and inside of the floats of several floating docks) were analyzed by photographing them through an iron frame subdivided into 14 × 14 cm grid lines (Figure 4). Evenly divided over the littoral zone from the high water to low water line, 321 quadrats (14×14cm) were photographed on the diagonal stone rip-rap, 84 quadrats were photographed on vertical iron harbor walls, and 40 quadrats were photographed on the wooden pilings. In December 2008 two floating docks were lifted out of the harbor of Breskens for maintenance after being in the water for about ten years, and were immediately photographed. Because they had to be cleaned for maintenance purposes immediately, the time available only allowed for photographing two habitats per dock. On one of the docks the inside (20 quadrats) and outside of the dock side (26 quadrats) was analyzed, on the

Figure 4. To estimate the surface cover the floating dock was photographed in overview and in detail through a frame that was subdivided by lines in 14 × 14 cm grid. The detail photos of these grids were digitally subdivided in 25 grids of 2.8 × 2.8 cm in which the presence / absence of each visible species was scored. Photograph by the GiMaRIS research team.

other one the outside (20 quadrats) and underside (20 quadrats) of the dock was analyzed (Figure 2). Analyses of the photographs were done as described above.

Statistical analysis

Differences between species occurrences, e.g., the relative number of plates on which they were found, and the number of grids per plate on which they were found, were tested for significance with a Chi-squared contingency table analysis ($p<0.05$). Similar analyses were conducted to compare the occurrence of native vs. non-native species, and colonial vs. solitary species.

Comparisons of fouling communities were done on the basis of Bray-Curtis similarities and 2D Multi-dimensional scaling (MDS) analyses of similarity levels between the communities, with the program Primer 6.1.10 (Clarke and Gorley 2006). These analyses compared the entire communities found on the rip-rap dikes, iron harbor walls and wooden pilings in Breskens, the species communities on the fouling plates that were deployed in Breskens harbor and the communities on the plates in ten other harbors in The Netherlands (Figure 1). We compared square-root, fourth-root and log-transformed data to untransformed data as the basis of the Bray-Curtis similarity matrix for the MDS. The lowest 2D stress value occurred with no transformation (2D stress value= 0.09), which is what was used for MDS.

Results

Seven ascidian species were found in the harbor of Breskens (Figure 5): the four solitary species *Ascidiella aspersa*, *Ciona intestinalis*, *Molgula socialis* and *Styela clava*, and the three colonial species *Botryllus schlosseri*, *Botrylloides violaceus* and *Diplosoma listerianum*. The non-native (or cryptogenic) ascidians *Molgula socialis*, *Styela clava* and *Diplosoma listerianum*, were relatively rare (Figure 5). They occurred on significantly fewer plates than the other four ascidian species on the plates that were deployed for three, six and nine months (*M. socialis*: χ^2 = 9.7, 7.8, 13.4, 17.1; *S. clava*: χ^2 = 10.7, 9.1, 15.6, 19.1; *D. listerianum*: χ^2 = 6.5, 6.1, 11.8, 13.2 in comparison to *A. aspersa*, *C. intestinalis*, *B. schlosseri* and B. *violaceus* respectively; df = 2; $p < 0.05$). *Diplosoma listerianum* did extensively cover some of the fouling plates that were deployed from March to December 2009, however (Figure 5).

The four more common ascidian species (the native species *A. aspersa*, *C. intestinalis* and *B. schlosseri*, and the non-native *Botrylloides violaceus*) differed in their abundances in the various habitats in Breskens (Figure 5). The native *Botryllus schlosseri* and the non-native *Botrylloides violaceus* were both common and in similar abundances on the fouling plates after three, six and nine months, and relatively rarely on the floating dock (Figure 5). They did not differ significantly from each other in the number of plates on which they were found (χ^2 = 0.9; df = 2; $p > 0.05$). Colonial ascidians were

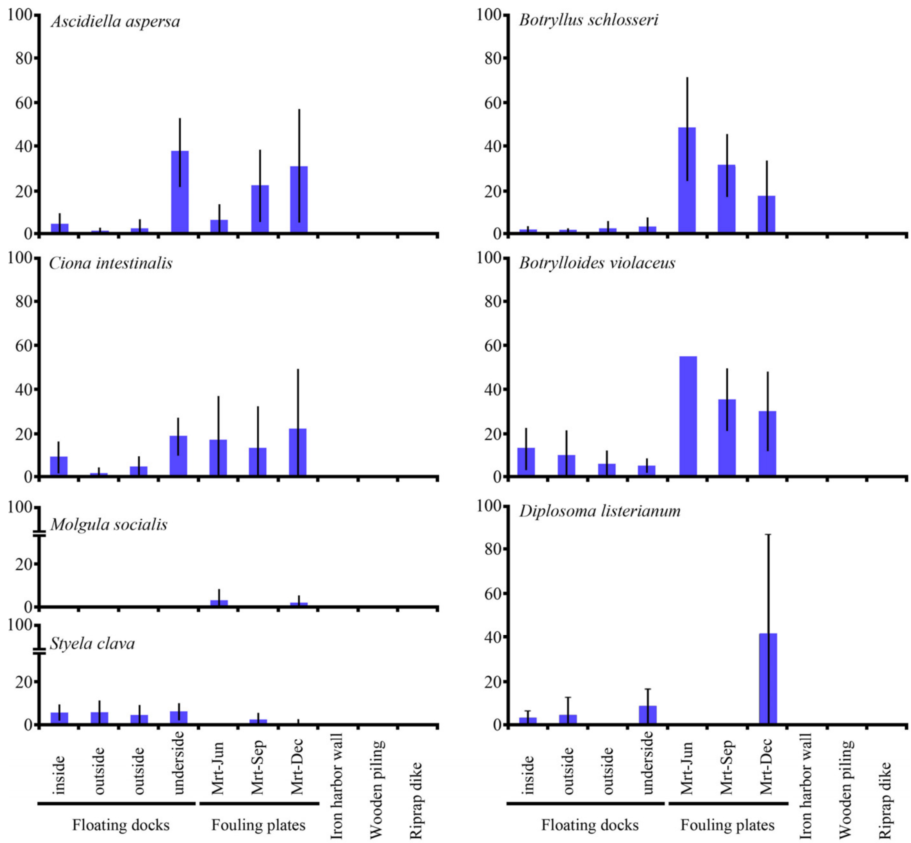

Figure 5. Average percentage of surface cover on floating docks and fouling community plates in the harbor of Breskens, of the solitary ascidians *Ascidiella aspersa* (A), *Ciona intestinalis* (B), *Molgula socialis* (C) and *Styela clava* (D), and the colonial ascidians *Botryllus schlosseri* (E), *Botrylloides violaceus* (F), and *Diplosoma listerianum* (G). No ascidians were detected on the iron harbor wall, wooden piling, and riprap dike. Standard deviation is indicated in the columns.

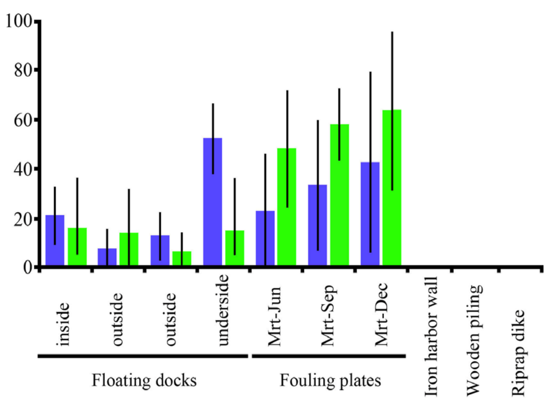

Figure 6. Average percentage of surface cover of solitary ascidians (blue columns) and colonial ascidians (green columns) on floating docks and fouling community plates in the harbor of Breskens. Standard deviation is indicated in the columns.

significantly more abundant on the fouling plates than solitary ascidians (χ^2= 15.7; df = 2; $p < 0.05$) (Figure 6). On the underside of the floating dock the native species *Ascidiella aspersa* was recorded at significantly higher abundances than the other five ascidian species (χ^2 = 93.5, 57.8, 45.9, 17.4, 74.5, 63.0; df = 1; $p < 0.05$). *Molgula socialis* was not found on the floating docks. *Ascidiella aspersa* and *Ciona intestinalis* were found significantly less (χ^2= 73.1, 20.9 respectively; df = 2; $p < 0.05$), on the inside and outside of the floating docks than on the underside (Figure 5). Solitary ascidians were found to be significantly more abundant on the floating docks than colonial forms (χ^2= 23.0; df = 1; $p < 0.05$). The abundance of solitary and colonial ascidians on the sides of the dock (inside and outside) was more or less similar, i.e. did not

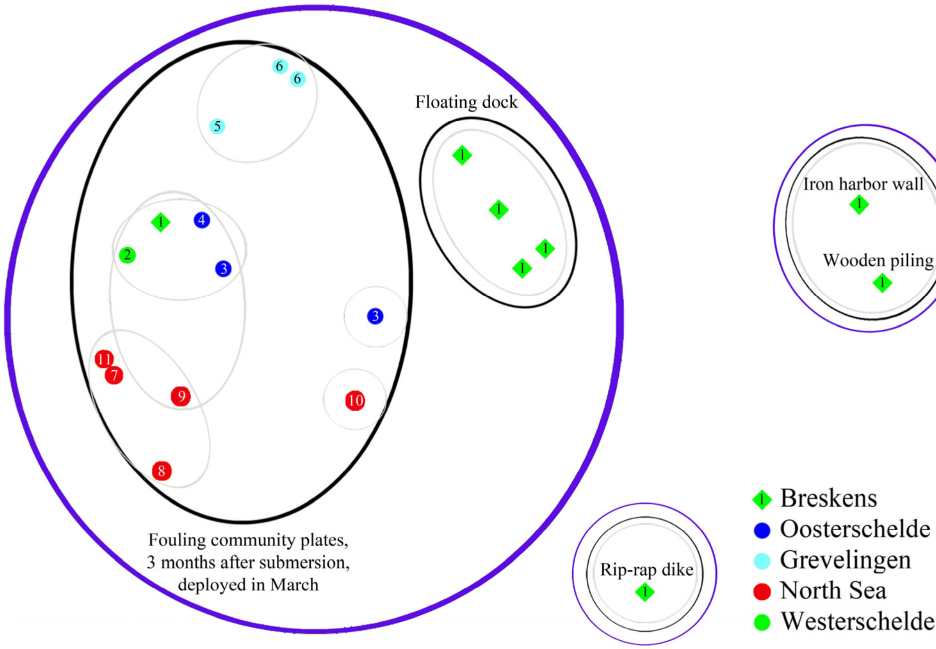

Figure 7. Multi-dimensional scaling plot of Bray-Curtis similarities between species communities, calculated within the program Primer 6.1.10 (Primer-E) on the basis of the average percentage of surface cover found for 81 species that were recorded in the various habitats in the harbor of Breskens and on fouling community plates that were checked for species and replaced by new ones every three months from March 2006 until 2009 at ten localities along the Dutch coast (colors correspond with those used in Figure 1). The contours indicate the level of similarity between the communities (blue = 25%; black = 35%; grey =55%).

differ significantly (χ^2= 2.8; df = 1; p > 0.05), but on the bottom of the dock (underside), solitary species were found significantly more abundant (χ^2= 76.1; df = 1; p < 0.05) (Figure 6).

Examining the entire fouling community composition (81 species) in all sampled habitats and water bodies, the communities that were recorded on floating substrates (fouling plates and the floating docks) grouped together at a level of 25% similarity (blue contours in the MDS plot in Figure 7) as compared to non-floating substrates (rip-rap dikes, iron harbor walls and wooden pilings) where no ascidians were recorded. The species communities on the fouling plates in Breskens and those on the fouling plates in other areas of The Netherland were more similar to each other than to the species communities on the floating docks in Breskens harbor, grouping together at a 35% similarity level (black contours). Within the fouling community plates, the plates from Grevelingen were grouped together, those from the North Sea were mainly grouped together, and those from Oosterschelde were mainly grouped with the Westerschelde (including Breskens), each grouping (grey contours) occurring at a 55% similarity level.

Discussion

Only two floating docks could be included in the present study. Using more would yield a more reliable result, but the data acquired during the present study were sufficiently distinct to enable some conclusions. Four of the seven ascidian species recorded were considered to be non-native or cryptogenic to The Netherlands (Gittenberger 2007, 2010; Wolff 2005), i.e., *Molgula socialis*, *Styela clava*, *Botrylloides violaceus* and *Diplosoma listerianum*, illustrating the importance of artificial structures in harbors in the introduction of such species as is already well documented in the literature (Connell 2000; Minchin 2007; Pederson et al. 2005). Not all artificial habitats in harbors were equally important however. No ascidians were found on the non-floating structures in the littoral zone, while ascidians were abundantly found on the floating structures (Figures 5, 6). In the harbor of Breskens, the sub-littoral zone was muddy and without any hard substrata. The floating structures probably presented the most suitable habitat in the harbor because ascidians need hard substratum to settle on and they do not survive well in the littoral zone because of the influence of wind and temperature during air exposure periods, the effect of waves and the relatively high speed of the water flow (Gama et al. 2006).

Of the non-native species, only *B. violaceus* was common (Figure 5). The three native ascidians species recorded, *Ascidiella aspersa*, *Ciona intestinalis* and *Botryllus schlosseri*, were all common (Figure 5). The results of the present study did not support the hypothesis that non-native species tend to be more successful than native species on artificial structures (Byers 2002; Alpert 2006).

Botryllus schlosseri and *Botrylloides violaceus* appeared to be mainly pioneer (primary settler) species, i.e., the first colonizers of bare substrate as was for example found on newly deployed settlement plates (Gittenberger and Moons, this volume). *Ascidiella aspersa* and *Ciona intestinalis* were also found as pioneers on the plates. Additionally they were found in similar to slightly higher abundances on the floating docks compared to the plates, while *Botryllus schlosseri* and *Botrylloides violaceus* were much rarer on the docks than on the plates (Figure 5). This may indicate that *Ascidiella aspersa* and *Ciona intestinalis* were more successful later on in succession, which is defined as directional changes in a community composition over time (MacMahon 1979). This supported the results found by Greene et al. (1983) in Puget Sound where solitary species were found to dominate communities later in succession. As we only included one year of data, aspects like inter-specific relationships with other species in the communities, and reproduction cycles, were not taken into consideration and alternative hypotheses may therefore apply. One such alternative hypothesis would be that a species like *Ascidiella aspersa*, although it was already found to settle in spring, i.e., on the plates that were deployed for three months (Figure 5), settled in higher numbers later in the year. As the plates that were included in the present study were deployed in March, no 'bare substrate' was available for species settling later in the year, for example in September. This hypothesis does not explain however why *Ascidiella aspersa* occurred significantly more commonly on the docks than on the plates (after nine months of deployment) in December.

Grey (2009) concluded that surveys of the sides of floating docks are as effective for the rapid assessment of exotic species presence/absence and relative abundance as inspecting the underside of floating docks. Our studies in the harbor of Breskens did not support this view. We found that there are major differences in the abundances of species on the inside, the underside and the outside of floating docks (Figure 5). For example, *Ascidiella aspersa* was found to be significantly more common on the underside, in comparison to the inside and outside of the dock.

Conclusion

In the harbor of Breskens, no support was found for the hypothesis that marine non-native ascidians are more abundant than native ascidians on artificial structures. The ascidian species that were recorded differed significantly from each other in their relative occurrences in the various hard substratum habitats that were studied. No ascidians were found in the intertidal habitats, while ascidians were found in all habitats studied on floating structures. The floats of a floating dock could be divided in three habitats, i.e. inside, underside and outside, which differed from each other in the number of ascidians found. *Ascidiella aspersa* was for example found to occur significantly more often on the underside than on the inside and outside of the floats. During rapid assessment surveys we therefore recommend that the inside, underside, and outside of floating docks be examined and that newly deployed surfaces also be examined for ascidians. Replicate studies should be conducted to confirm the trends observed in our study.

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