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Research Article

Settlement and possible competition for space between the invasive violet tunicate *Botrylloides violaceus* and the native star tunicate *Botryllus schlosseri* in The Netherlands

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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

Settlement and competition for space of two colonial sea squirts, the non-native violet tunicate *Botrylloides violaceus* and the native golden star tunicate *Botryllus schlosseri*, were compared in The Netherlands. In each year, from March 2006 to March 2010, 125-150 grey, 14 × 14 cm, PVC plates were deployed along the Dutch coast at 13 localities, at a depth of 1 m, and checked for species after three and six months. New plates were deployed every three months. While comparing plates with only one species represented to plates with both species represented, it appeared that *Botrylloides violaceus* outcompeted *Botryllus schlosseri* for space. *Botryllus schlosseri* is nevertheless expected to remain abundant along the Dutch coast because it can inhabit places with low or fluctuating salinities where *Botrylloides violaceus* is at a disadvantage. Settlement and the interactions between these species in The Netherlands resembled the situation in North America where both of them are considered non-native. The interactions between the two species seemed to be independent of their being native or introduced in a particular area.

Key words: Tunicata, competition, non-native species, salinity, syntopic, allotopic

Introduction

Invasions of non-indigenous species are a growing problem. While the number of invasive species is increasing (Wolff 2005; Gollasch 2006), simple, effective solutions for their extermination are not available (Hulme 2006). When an invasive species becomes established within an area, it will not always interact conspicuously with one or more of the native species that are present already. This depends upon the various specific, more or less different ecological niches. To investigate the impact of an alien species on the native species, situations where both species co-occur should be compared with those where only one of the species is present; following Rivas (1964), this represents a comparison between syntopic (inhabiting the same macrohabitat) vs. allotopic (inhabiting different macrohabitats) populations that exist sympatrically.

Invasive species have been characterized as having a high growth rate, a short generation time, a high number of offspring (r-strategists) and outstanding dispersal abilities (Sakai et al. 2001). The relative success of an invasive species depends on these traits in combination with the absence of specialized predators, parasites and pathogens.

In marine systems, fouling communities are relatively rich in invasive organisms (Gollasch 2006). This may partly be due to the fact that

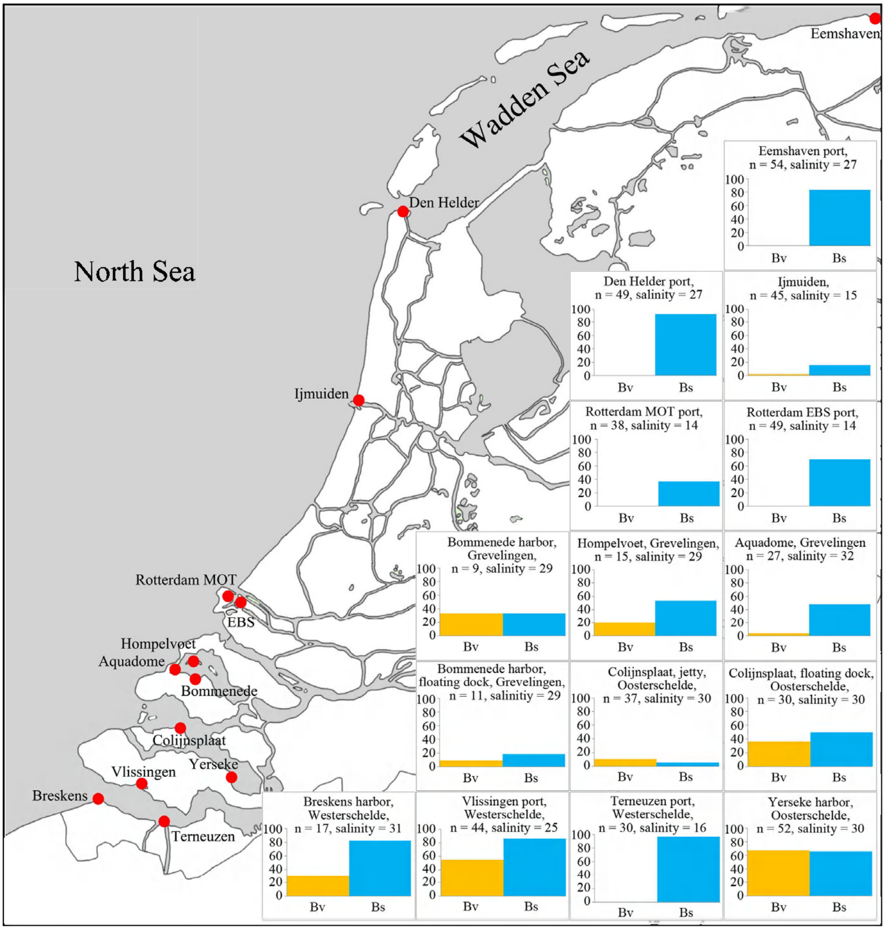

Figure 1. Percentage of fouling plates on which *Botrylloides violaceus* (Bv, orange columns) and *Botryllus schlosseri* (Bs, blue columns) colonies were found. These percentages are calculated on the basis of plates that were deployed from 2006 to 2009, from March to June, June to September, and from September to December. n indicates the total number of fouling plates containing the target species. Salinity was measured in March 2009.

these communities are mostly found on artificial structures. The 'selection regime modification' (SRM) hypothesis states that an intensely altered or disturbed habitat can create a mismatch between traits of the native species and the environmental conditions to which they have long adapted (Byers 2002; Alpert 2006). This mechanism predicts that alien species may dominate artificial habitats (for example, PVC fouling plates), because the native species are not adapted to that kind of habitat. To test this hypothesis and to investigate the population dynamics of similar, sympatric, native versus introduced fouling species in both allotopic and syntopic populations, we have focused on two common, colonial, ascidian species along the Dutch coast, the introduced violet tunicate *Botrylloides violaceus* Oka, 1927 and the native golden star tunicate *Botryllus schlosseri* (Pallas, 1774). The settlement, spread, seasonality, and the role of such species during succession were studied from 2006 to 2010, treating PVC fouling plates as macrohabitats.

Methods

At 13 localities (Figure 1) along the Dutch coast, grey, 14 × 14 cm, PVC plates were deployed horizontally at a depth of 1 m. The fouling communities that grew on the underside of these plates were followed over time. This methodology, including the plate material, size, and

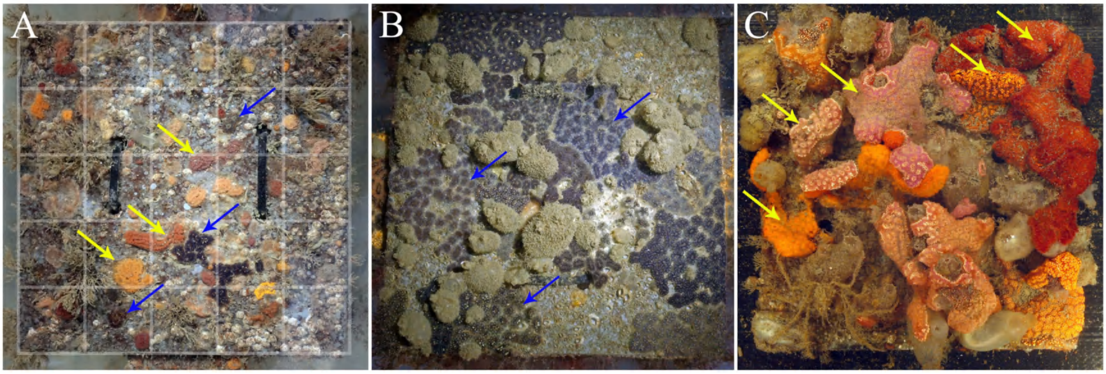

Figure 2. Examples of settlement plates with syntopic (Bv+Bs) and allotopic (Bv-only and Bs-only) populations of *Botrylloides violaceus* (yellow arrows) and *Botryllus schlosseri* (blue arrows). (**A**) Bv+Bs; white lines indicate the 25 grids in which the presence/absence of the species was scored; (**B**) Bs-only; (**C**) Bv-only. Photographs by the GiMaRIS research team.

deployment depth was derived from studies on marine fouling communities in New Zealand, America and Europe (De Rivera et al. 2005; Ruiz et al. 2006; Templado et al. 2010). In The Netherlands, about 125 to 150 plates were checked for species and replaced by new plates every three months from March 2006 until March 2009. From March 2009 until March 2010, the plates were checked every three and six months after placement. Each time the plates were checked, they were taken out of the water for a maximum of 10 seconds, and transferred into a water tank for analysis and photography. Afterwards the plates were returned to their original positions. The entire plate was digitally photographed while barely submersed in seawater, and a close-up photograph was taken of every species. The photos of the plates were digitally subdivided in 25 equal grids (Figure 2A), and the presence of *Botryllus schlosseri* (Bs) and *Botrylloides violaceus* (Bv) on a grid was scored on a monitoring form as a primary settler (directly settling on the PVC plate) and/or as a secondary one (using other species for substrate).

Salinity at the surface was measured at all localities in March 2009, using a portable water quality meter (HI 9828, HANNA Instruments®, 3401 MX IJsselstein, The Netherlands).

The differences in the average surface cover (number of grids/plate occupied) of Bs and Bv on the plates and in the relative numbers of plates on which these species were found, were tested for significance by contingency analysis (Chi-squared) and analysis of variance (ANOVA). All localities were pooled for the analysis. Comparisons were carried out for all plates combined, and for subsets of plates with either syntopic (Bv+Bs) (Figure 2A) or allotopic populations (Bs-only or Bv-only)(Figures 2B,C).

Results

Distribution

Bs was present at all localities, but Bv was absent from six of the thirteen localities (Figure 1). The localities where Bv was absent were all located near fresh water channels or rivers, and may be subject to salinity fluctuations, e.g., after heavy rains. On the single date that salinity was sampled, four of those localities had salinities in the range of 14 to 16 (Figure 1). All other localities had salinities between 25 and 32.

Surface cover

An analysis of Bv vs. Bs cover on only the plates with Bv+Bs (Figure 2A) showed that Bv covered significantly ($p < 0.0001$, ANOVA) larger surfaces than Bs (Figure 3). Analyses of all plates combined, of Bs-only, and of Bv-only (Figure 2B-C) indicated that the surface covers of Bs vs. Bv did not differ significantly ($p>0.05$, ANOVA) (Figure 3). Bv covered significantly larger surfaces in Bv+Bs plates than in Bv-only plates (Figure 3, $p < 0.05$, ANOVA). Bs instead covered significantly smaller surfaces where it co-occurred with Bv (Figure 3, $p < 0.001$, ANOVA).

Primary and secondary settlement

Significant differences in settlement among the two ascidian species were found when plates with

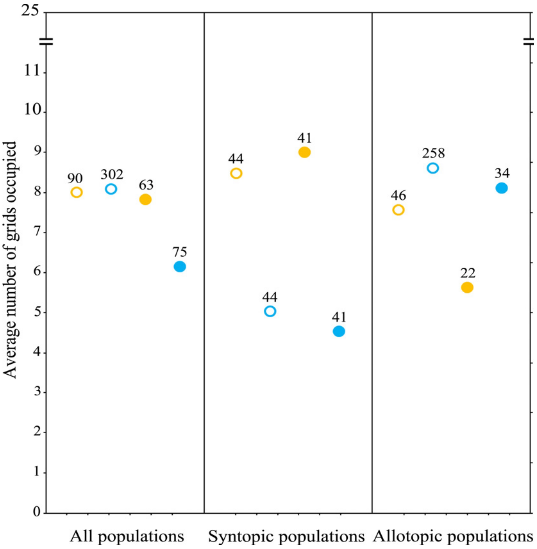

Figure 3. Average fouling plate surface cover (number of grids occupied, out of 25 grids per plate) by *Botrylloides violaceus* (orange) and *Botryllus schlosseri* (blue) in syntopic (Bv+Bs) and allotopic (Bv-only, Bs-only) populations. The results are plotted for plates checked 3 months (open symbols) and 6 months (solid symbols) after deployment. The number of plates in each category, used to calculate the average numbers of grids occupied, is noted in the graph.

syntopic and/or plates with allotopic populations were analyzed separately. When all plates were analyzed together, no significance was detected.

Bv and Bs each occurred more often as secondary settlers than as primary ones in Bv+Bs populations after three months (Figure 4) (respectively χ^2 =4.08 and χ^2 =4.25, df = 1, p < 0.05) and six months of submersion (Figure 5) (respectively χ^2 =21.33 and χ^2 =23.27, df = 1, p < 0.05).

After three months of submersion the occurrence of primary settlers of Bv did not differ significantly from secondary settlers on the Bv-only plates (Figure 4; χ^2 =0.02, df = 1, p>0.05), whereas primary settlement was found significantly more often than secondary settlement (Figure 4; χ^2 =8.50, df = 1, p>0.01) for Bs on the Bs-only plates.

Primary and secondary settlement of each species after three months did not differ between Bv-only and Bs-only populations, and the combined Bv+Bs population (χ^2 =0.82, df = 1, p>0.05 and χ^2 =0.63, df = 1, p>0.05, respectively) (Figure 4).

In Bv+Bs populations, primary and secondary settlement in Bv were equal to the percentages in Bs after three months (χ^2 =0.58 and χ^2 =0.06, df = 1, p>0.05, respectively). In Bv-only and Bs-only populations, secondary settlement occurred significantly more often in Bv than in Bs (χ^2 =4.26, df = 1, p<0.05), but the values for primary settlement were equal (χ^2 =0.63, df = 1, p>0.05) (Figure 4).

Primary settlement of Bv was found much less often after six months (Figure 5) as compared to three months (Figure 4) in Bv+Bs populations (χ^2 =21.33, df = 1, p<0.01). This was also found in analyses of all plates combined (Bv-only and Bv+Bs) (χ^2 =17.78, df = 1, p<0.01), but it was not found in the analysis of only Bv+Bs plates (χ^2 =0.86, df = 1, p>0.05). After six months, primary settlement of Bv was found significantly more often in Bv-only populations than in Bv+Bs populations (χ^2 =5.22, df = 1, p<0.025) (Figure 5). The amount of secondary settlement was equal (χ^2 =0.74, df = 1, p>0.05).

Primary settlement of Bs was found significantly less often than secondary settlement in Bv+Bs populations (χ^2 =23.27, df = 1, p<0.05) after six months (Figure 5). This was also found in 'all populations' (χ^2 =21.04, df = 1, p<0.05), but it was not seen in Bs-only populations alone (χ^2 =3.00, df = 1, p>0.05). Primary settlement of Bs was found significantly more often in Bs-only populations than in Bv+Bs after six months (χ^2 =8.14, df = 1, p<0.05) (Figure 5). The amount of secondary settlement was equal (χ^2 =0.04, df = 1, p>0.05).

Primary and secondary settlement of Bv in both Bv-only (χ^2 =0.01 and χ^2 =0.20, df = 1, p>0.05) and Bv+Bs (χ^2 =0.29 and χ^2 =0.05, df = 1, p>0.05) populations were equal to the values for Bs after six months (Figure 5).

Primary settlement of Bv in Bv+Bs populations occurred significantly more often after three months than after six months (χ^2 =7.04, df = 1, p<0.05). This was also found for 'all populations' (χ^2 =7.56, df = 1, p<0.05), but was not observed in Bv-only (χ^2 =0.62, df = 1, p>0.05). The primary settlement of Bs was found significantly less often in Bv+Bs populations after three months than after six months (χ^2 =5.99, df = 1, p<0.05).

The primary and secondary settlement of Bs differed when comparing Bs-only populations after three months (Figure 4) to the six months settlement (Figure 5). After three months more primary settlement (χ^2 =4.12, df = 1, p<0.05) and less secondary (χ^2 =14.06, df = 1, p<0.01) settlement was found than after six months. The amount of primary vs. secondary settlement of Bs did not differ when 'all populations' were analyzed after three months, but much less primary settlement (χ^2 =23.93, df = 1, p<0.01) and much more secondary settlement (χ^2 =16.15, df = 1, p<0.01) was found after six months (Figure 5).

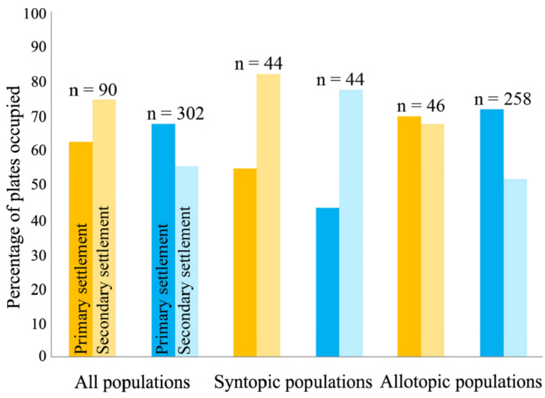

Figure 4. The percentage of the total number of fouling plates, on which either *Botrylloides violaceus* (orange columns) or *Botryllus schlosseri* (blue columns) was recorded as either a primary or a secondary settler, three months after deployment, in syntopic (Bv+Bs) and allotopic (Bv-only, Bs-only) populations.

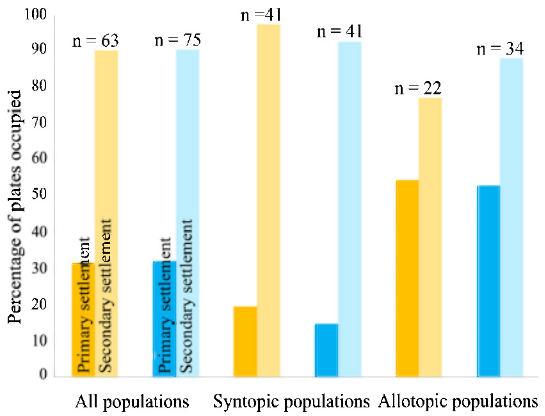

Figure 5. The percentage of the total number of fouling plates, on which either *Botrylloides violaceus* (orange columns) or *Botryllus schlosseri* (blue columns) was recorded as either a primary or a secondary settler, six months after deployment, in syntopic (Bv+Bs) and allotopic (Bv-only, Bs-only) populations.

Discussion

When they occurred separately (Figures 2B-C, 3), *Botryllus schlosseri* and *Botrylloides violaceus* did not clearly differ in their average surface coverage of fouling plates. However, where the species co-occurred (Figure 2A) there was a conspicuous difference in surface cover (Figure 3) and the non-native *Botrylloides violaceus* most likely outcompeted the native *Botryllus schlosseri* for space. However, the negative impact that *Botrylloides violaceus* may have had on *Botryllus schlosseri* was only evident in areas where both species occur sympatrically in syntopic populations. *Botryllus schlosseri* occurred in many habitats along the Dutch coast where it did not encounter *Botrylloides violaceus*, apparently because the salinity in those areas was too low or fluctuating (Figure 1). Under laboratory conditions both species could grow well at high salinities, but the lower limits of salinity differed; 14 for *Botryllus schlosseri* but >20 for *Botrylloides violaceus* (Epelbaum et al. 2009). At some localities along the Dutch coast, like Den Helder and Eemshaven, the salinity measured in March 2009 was relatively high, but *Botrylloides violaceus* was not found. These localities were all situated close to a river or another source of fresh water and might be subject to salinity decreases, in case of heavy rainfall, which might explain the absence of *Botrylloides violaceus*. A recent survey of the Wadden Sea gave a similar result; *Botrylloides violaceus* was found only at high salinities in the harbours of the islands of the Wadden Sea, while *Botryllus schlosseri* was also present in harbours along the mainland shore of the Wadden Sea, with fresh water lakes, rivers and streams nearby (Gittenberger et al. 2010).

Many of the significant differences found in the present study were only found when allotopic and syntopic populations were analyzed separately. Analyzing all populations together could lead to the incorrect conclusion that species like *Botrylloides violaceus* and *Botryllus schlosseri* did not interact and that their average surface cover is equal (Figure 3). It should furthermore be taken into consideration that although our results may seem to indicate that the non-native *B. violaceus* outcompeted the native *B. schlosseri* for space, this may not be so. Differences in the seasonality of these two species, the salinity of the water at the various localities, other fouling species on the plates, and predatory fishes swimming around the plates, may also explain at least part of the results found. A more detailed analysis of these aspects is beyond the scope of the present article, however.

Both species showed less primary settlement after six months of submersion than after three months (Figures 4–5). This was likely the case because the available surface space was declining and competition for space was increasing. This is a normal element in succession and inter- and intra-specific competition for space (Connell et al. 1977; Jackson 1979). The results of the present study were similar to those from other studies in The Netherlands (Gittenberger 2007, 2009) and along the Atlantic coast of the USA, where areas like Portsmouth Harbor are dominated by *Botrylloides violaceus* at present, while they used to be dominated by *Botryllus schlosseri* before the introduction of *B. violaceus* (Dijkstra et al. 2007). A study in British Columbia also indicated that *B. schlosseri* is able to survive in lower salinities than *B. violaceus* (Epelbaum et al. 2009).

Conclusion

Botrylloides violaceus is an invasive species in the Netherlands. It originates from the north-western Pacific Ocean. In the more saline areas it has spread rapidly, and seemed to be more successful as a fouling species on settlement plates than the native ascidian *Botryllus schlosseri*. There, the non-native *B. violaceus* appeared to outcompete the native *B. schlosseri* for space, although other aspects, like seasonality and the presence of predators and other fouling species, may also explain the results. These aspects were not included in the present study, however. Because *Botryllus schlosseri* tolerates lower salinities, environments with low salinities or large fluctuations in salinity may form refugia where *Botryllus schlosseri* is not threatened by *Botrylloides violaceus*. This study showed that in order to study competition for space between fouling species accurately, both syntopic and allotopic populations should be examined.

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References

Alpert P (2006) The advantages and disadvantages of being introduced. *Biological Invasions* 8: 1523–1534, http://dx.doi.org/10.1007/s10530-005-5844-z

Byers JE (2002) Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. *Oikos* 97: 449–458, http://dx.doi.org/10.1034/j.1600-0706.2002.970316.x

Connell JH, Slatyer RO (1977) Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist* 111: 1119–1144, http://dx.doi.org/10.1086/283241

De Rivera CE, Ruiz G, Crooks J, Wasson K, Lonhart S, Fofonoff P, Steves BP, Rumrill SS, Brancato MS, Pegau WS, Bulthuis DA, Preisler RK, Schoch C, Bowlby E, DeVogelaere A, Crawford M, Gittings S, Hines A, Takata L, Larson K, Huber T, Leyman AM, Collinetti E, Pascot T, Shull S, Anderson M, Powel S (2005) Broad-scale non-indigenous species monitoring along the west coast in National Marine Sanctuaries and National Estuarine Research Reserves. Report to United States National Fish and Wildlife Foundation, 126 pp

Dijkstra J, Harris LG, Westerman E (2007) Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. *Journal of Experimental Marine Biology and Ecology* 342: 61–68, http://dx.doi.org/10.1016/j.jembe.2006.10.015

Epelbaum A, Herborg LM, Therriault TW, Pearce CM (2009) Temperature and salinity effects on growth, survival, reproduction, and potential distribution of two non-indigenous botryllid ascidians in British Columbia. *Journal of Experimental Marine Biology and Ecology* 369: 43–52, http://dx.doi.org/10.1016/j.jembe.2008.10.028

Gittenberger A (2007) Recent population expansions of non-native ascidians in The Netherlands. *Journal of Experimental Marine Biology and Ecology* 342: 122–126, http://dx.doi.org/10.1016/j.jembe.2006.10.022

Gittenberger A (2009) Invasive tunicates on Zeeland and Prince Edward Island mussels, and management practices in The Netherlands. *Aquatic Invasions* 4: 279–281, http://dx.doi.org/10.3391/ai.2009.4.1.28

Gittenberger A, Rensing M, Stegenga H, Hoeksema B (2010) Native and non-native species of hard substrata in the Dutch Wadden Sea. *Nederlandse Faunistische Mededelingen* 33: 21–75

Gollasch S (2006) Overview on introduced aquatic species in European navigational and adjacent waters. *Helgoland Marine Research* 60: 84–89, http://dx.doi.org/10.1007/s10152-006-0022-y

Hulme PE (2006) Beyond control: wider implications for the management of biological invasions. *Journal of Applied Ecology* 43: 835–847, http://dx.doi.org/10.1111/j.1365-2664.2006.01227.x

Jackson JBC (1979) Overgrowth competition between encrusting ectoprocts in a Jamaican cryptic reef environment. *Journal of Animal Ecology* 48: 805–823, http://dx.doi.org/10.2307/4196

Rivas LR (1964) A reinterpretation of the concepts "sympatric" and "allopatric" with proposal of the additional terms "syntopic" and "allotopic". *Systematic Zoology* 13: 42–43, http://dx.doi.org/10.2307/2411436

Ruiz GM, Huber T, Larson K, McCann L, Steves B, Fofonoff P, Hines AH (2006) Biological invasions in Alaska's coastal marine ecosystems: establishing a baseline. United States Fish and Wildlife Service report, 112 pp

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics* 32: 305–332, http://dx.doi.org/10.1146/annurev.ecolsys.32.081501.114037

Templado J, Paulay G, Gittenberger A, Meyer C (2010) Chapter 11 - Sampling the Marine Realm. In: Eymann J, Degreef J, Häuser C, Monje JC, Samyn Y, VandenSpiegel D (eds), Manual on field recording techniques and protocols for All Taxa Biodiversity Inventories and monitoring. *ABC Taxa* 8(1): 273–307

Wolff WJ (2005) Non-indigenous marine and estuarine species in The Netherlands. *Zoologische Mededelingen* 79: 1–116