Risk analysis of the colonial sea-squirt *Didemnum vexillum* Kott, 2002 in the Dutch Wadden Sea, a UNESCO World Heritage Site

Issued by The Dutch Ministry of Agriculture, Nature & Food Quality

A. Gittenberger

GiMaRIS

Gittenberger Marine Research Inventory Strategy

GiMaRIS report 2010.08

Date:
June 2010

Report nr.:
GiMaRIS 2010.08

Title:
Risk analysis of the colonial sea-squirt *Didemnum vexillum* Kott, 2002 in the Dutch Wadden Sea, a UNESCO World Heritage Site

Cover:
Didemnum vexillum, an invasive colonial sea squirt in The Netherlands

Author:
dr. A. Gittenberger

Address / Contractor:
GiMaRIS, Leiden BioScience Park
Niels Bohrweg 11-13
2333 CA Leiden

Issued by:
The Invasive Alien Species Team of the Dutch Ministry of Agriculture, Nature & Food Quality

Projectleader:
dr. T.M. van der Have

Address:
Geertjesweg 15
6706 EA Wageningen
Postbus 9102

Layout: drs. M. Rensing
Photos: dr. A. Gittenberger

Index

1. Introduction

Invasive sea squirts are considered to be a growing global problem (Lambert, 2007). In 1991 a colony of a white encrusting colonial sea squirt, i.e. *Didemnum* sp., was discovered by de Kluijver in the salt water lake Oosterschelde in the province Zeeland, The Netherlands (de Kluijver *et al*., 1999). After that the species was not recorded there until 1996, when it was locally found in large numbers throughout the Oosterschelde and in the neighbouring lake Grevelingen. Since then the populations grew exponentially and within a year *Didemnum* sp. became by far the most abundant sea-squirt in the province of Zeeland. Its sudden population growth was recorded in detail by the MOO-project of the Dutch ANEMOON Foundation, an ongoing monitoring project with volunteer divers that score every species they see on a standardized MOO-form since 1994 (Gittenberger, 2007; www.anemoon.org). From 1997 to at least 2010, this *Didemnum* species has been recorded in more than 60 % of the dives per year in the Oosterschelde en Grevelingen.

In the 1990s, at about the same time that a white ascidian was expanding its populations in The Netherlands (Fig. 1), similar white, fast growing didemnids appeared and quickly expanded their populations in New Zealand, along both coasts of Northern America (Fig. 2) and along the Atlantic coast of France. At virtually every location where these ascidians were found, they became extremely successful competitors for space, outcompeting many other species in the benthic communities. Next to having a distinct ecological impact in the invaded ecosystems, they also caused significant economical damage to especially the shellfish industry by overgrowing, smothering and suffocating mussels, oysters, clams and other shellfish.

In general, species in the relatively speciose genus *Didemnum* show much intra-specific morphological variation. Many species can therefore only be identified when fully grown larvae are present (Lambert, 2009). As a consequence,

Fig. 1. *Didemnum vexillum* colonies covering the bottom in the Grevelingen, The Netherlands.

Fig. 2. *Didemnum vexillum* colonies form large colonies in Buzzards Bay, Massachusetts, USA, which are very similar to those in The Netherlands (Fig. 1).

there has been a 15 year long discussion in the literature about the identity of the rapidly expanding, white didemnid colonies world-wide (Lambert, 2009). Initially the question was also raised whether these colonies represented one or several species. Over the years they have been identified as *Didemnum carnulentum* on the US Pacific coast, *D. lutarium* and *D. vestum* in New England, *D. lahillei* and *D. helgolandicum* in France and The Netherlands, *D. vexillum* in New Zealand, and *D. pardum* and *D. moseleyi* in Japan (Lambert, 2009). The discussion came to an end in 2009 with publications on the population genetics (Stefaniak *et al*., 2009) and morphology (Lambert, 2009) of the didemnid. These studies included white didemnids that were collected in Japan, New Zealand, N America and Europe. It turned out that all these populations represent a species that was described as a native for New Zealand in 2002 (Kott, 2002) as *D. vexillum*. Whether *D. vexillum* is native indeed to New Zealand is heavily disputed however. It seems unlikely on the basis of subsequent studies (Coutts, 2002; Couts & Forrest, 2007; Pannell & Coutts, 2007). It has been hypothesized that the most likely native area of this invader is situated in the NW Pacific (Lambert, 2009; Stefaniak *et al*., 2009).

In The Netherlands *Didemnum vexillum* remained abundant in the Grevelingen and the Oosterschelde since 1996, but it did not seem to spread into other areas along the coast (Gittenberger, 2007). In 2008 and 2009 the first colonies of *D. vexillum* were found in the Dutch Wadden Sea however (Gittenberger *et al*. 2009; in press.). The colonies in the Wadden Sea, a region that was designated as a World Heritage Site in 2009 (Common Wadden Sea Secretariat, 2008), were small, relatively rare and only found in the proximity of the island of Terschelling. It remains unclear whether or not *D. vexillum* may expand its populations into the Dutch Waddensea. This assessment will focus on the risk that *D. vexillum* will be able to settle and eventually become harmful in The Wadden Sea. Furthermore will be discussed how this threat may be minimized from a benefit cost point of view, by managing the potential import vectors and eradicating established populations. An inventory is made of the potential ecological, economical and social damage that *D. vexillum* might cause

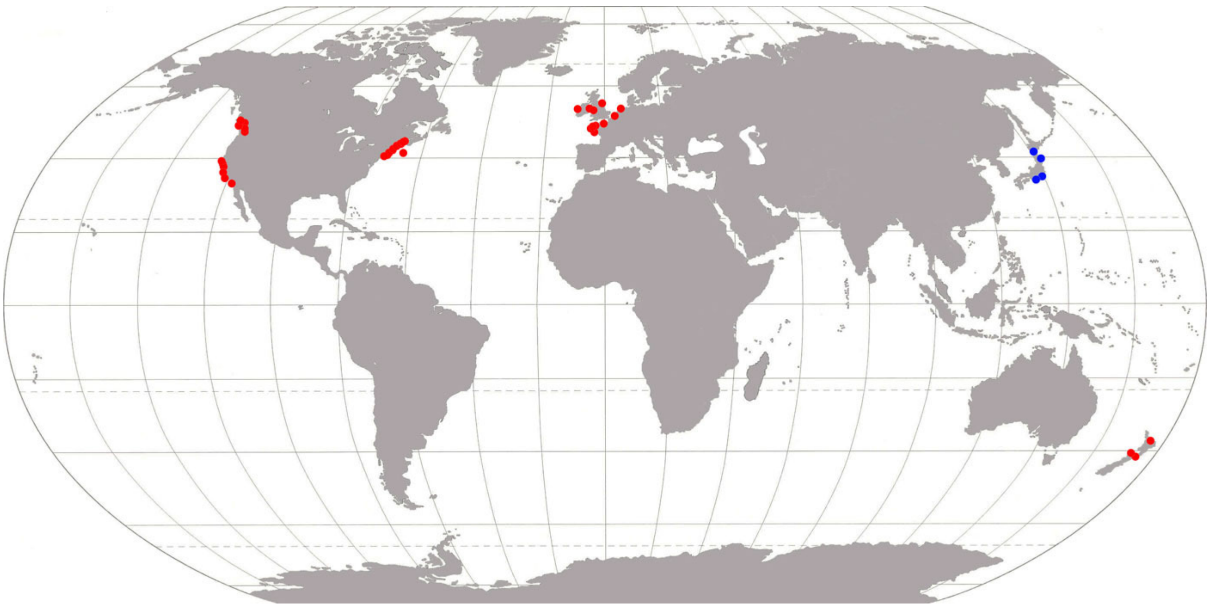


Fig. 3. World-wide distribution records of *Didemnum vexillum*, after Daley & Scavia (2008), Gittenberger (2007), Gittenberger *et al*. (2009; in press.), Griffith *et al*. (2009), Hess *et al*. (2009), Pannell & Coutts (2007), Sewell *et al*. (2008), Stefaniak *et al*. (2009). Red dots: non-native range. Blue dots: hypothesized as the native area.

in the Wadden Sea system. In addition, management options are discussed, focusing on the "prevention of the import of new colonies", the "eradication of imported colonies" and the "control of the already settled colonies".

2. Probability of introduction

2.1 Distribution world-wide

Since the 1990s dense populations of *Didemnum vexillum* have been found world-wide in temperate areas that have a climate with seasons that are roughly comparable to those in The Netherlands (Fig. 3). The species has not (yet) been found in Australia, South America and South Africa, but is expected to be introduced there as well. In fact, it may already be in one or more of those regions, because *D. vexillum* is easily misidentified as a native white didemnid species. Actually, research and marine monitoring efforts along at least the coasts of S Africa and S America have been much less intense than along the coasts of N America and Europe.

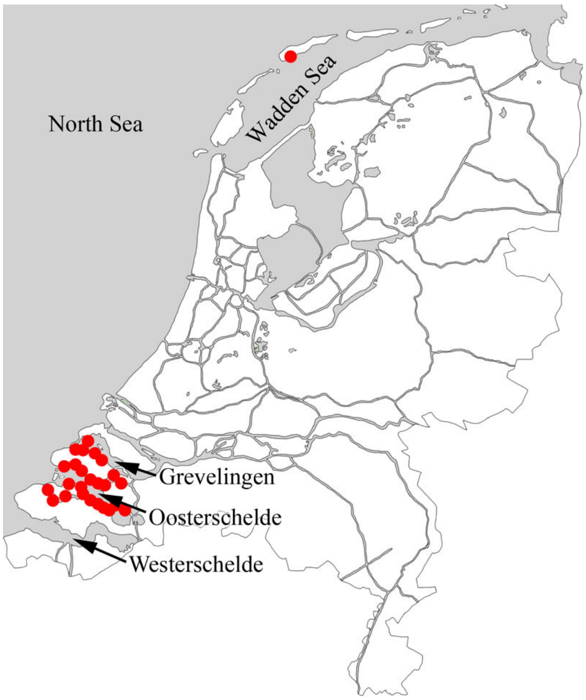


Fig. 4. Records of *Didemnum vexillum* in The Netherlands, based on records of the scuba-diver's marine monitoring project MOO of the ANEMOON Foundation (www.ANEMOON.org) and Gittenberger *et al*. (2009; in press.).

2.2 Distribution in The Netherlands

Since 1996 *Didemnum vexillum* is especially abundant in the southern saline inland lakes Oosterschelde and Grevelingen (Fig. 4). In the Westerschelde, an inland waterinlet south of the Oosterschelde, *D. vexillum* had not been found yet. The Westerschelde differs from the completely saline Oosterschelde and Grevelingen by its estuarine nature with salinities varying from 0 ppt in the east to 32 ppt at the North Sea entrance in the west.

Didemnum vexillum was also found in the north of The Netherlands, in the Wadden Sea (Figs. 4-5). In contrast to the Oosterschelde and Grevelingen, its range in the Wadden Sea still seems to be very limited however. During an inventory focusing on hard substratum related species like *D. vexillum* in the Dutch Wadden Sea, it was only found at two localities in the proximity of the harbour of Terschelling and nowhere else (Fig. 5; Gittenberger *et al*., 2009; in

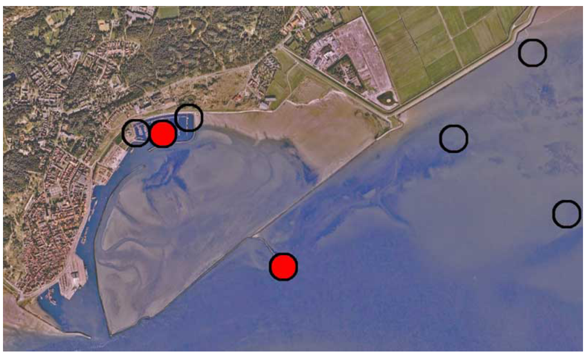


Fig. 6. Records of *Didemnum vexillum* in and near the harbour of Terschelling (red dots). Open circles refer to areas that have been searched in July/ August 2009, without recording *D. vexillum.*

Fig. 5. Records of *Didemnum vexillum* in the Dutch Wadden Sea (red dots). Open circles refer to areas that have been searched in July/August 2009, without recording *D. vexillum*.

press.). A third sighting in the Wadden Sea also concerns a locality off Terschelling, at the mussel beds south of the harbour (R. Dekker, NIOZ, pers. comm.). In the proximity of the harbour its presence is very local. It was only found at two localities from the seven that were searched there (Fig. 6). Within the harbour it was found at the underside of wooden floating docks, close to the entrance (Fig. 7), but not at two localities that were searched in the intertidal zone of the harbour (Fig. 6). It was also not found at three mussel-bed localities just to the southeast of Terschelling, where bottom samples were taken with a mussel dredge from the LNV boat the Stormvogel (Fig. 6). The second sighting concerns a colony in a tide pool on the exposed side of the jetty that protects the harbour (Fig. 8). This intertidal habitat is rather unusual. At least in Europe and along the coasts of N America, *D. vexillum* is usually restricted to the subtidal (Gittenberger, 2007; Lengyel *et al*., 2009; Mercer *et al*., 2009; Pederson *et al*., 2005). It is also found in tide pools throughout its range however, for example in Massachusetts, USA (Valentine *et al*., 2007). These sightings are rare. The relatively local occurrence in the harbour of Terschelling recalls the occurrence of *D. vexillum* recorded in 2008, in the Holyhead marina, N Wales, the first record for Great Britain (Griffith *et al*., 2009; Kleeman, 2009). There, like in Terschelling, it was only found very locally on the floating docks. Floating docks appear to be a preferred habitat in harbours for *D. vexillum* more in general, as this species was also commonly found on marina pontoons in other countries, like Ireland (Minchin & Sides, 2006) and along the US east coast (Pederson *et al*., 2005).

2.3 Introduction into The Netherlands

In 1991 *Didemnum vexillum* was first found in Europe in The Netherlands, in the Oosterschelde (Kluivert *et al*., 1999). After a lag time of at least five years it suddenly rapidly started to expand its range in 1996 and became one of the most abundant sea-squirts in the Oosterschelde and the neighbouring watersystem the Grevelingen from 1997 on. Gittenberger (2007) hypoth-

Fig. 7. *Didemnum vexillum* growing on the wooden floating docks in the harbour of Terschelling (Fig. 6). A, floating docks; B, collected colony of about 50 cm in diameter; C, in situ growing on the dock just beneath the water line in between other fouling organisms.

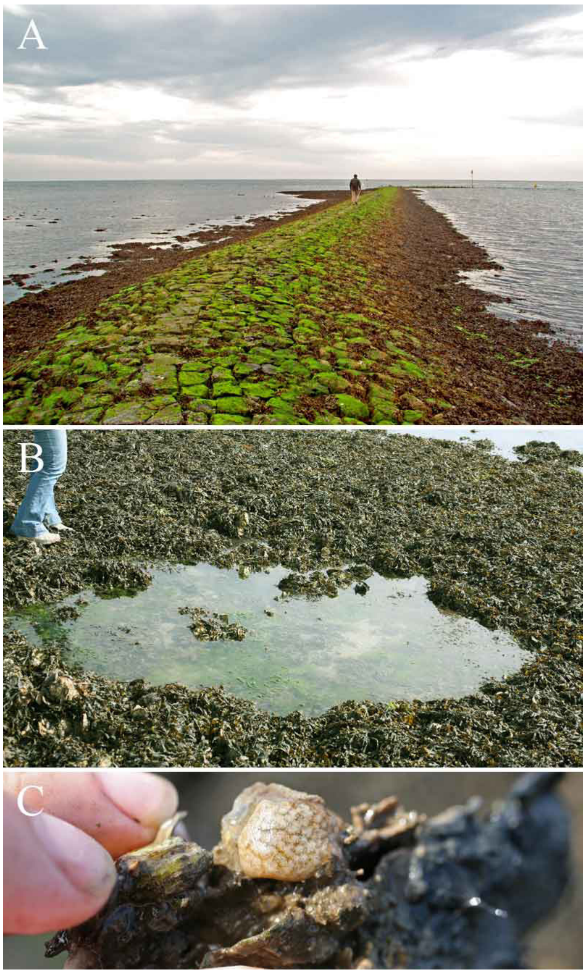


Fig. 8. *Didemnum vexillum* in a tide pool on the exposed side of the jetty protecting the harbour of Terschelling (Fig. 6). A, the jetty, where *D. vexillum* was found at the left side, which is exposed to the Wadden Sea; B, the tide pool; C, *D. vexillum* on the underside of one of the rocks in the tide pool.

esizes that its sudden success may have occurred because of the large amounts of hard substratum that came available after the cold winter of 1995–1996. This winter caused a strong decrease in the population densities of many marine animals, like the sea urchin *Psammechinus miliaris* (Gmelin, 1778) and the brittle star *Ophiotrix fragilis* (Abildgaard, 1789) with population densities that locally went from hundreds of specimens / m² to virtually none. The amounts of space that came available and the relatively low number of grazers like sea-urchins promoted the success of fast growing colonial ascidians in general in 2007. Next to the colonial ascidian *Didemnum vexillum*, the colonial sea-squirts *Aplidium glabrum*, *Diplosoma listerianum* and *Botryllus schlosseri* also rapidly expanded their populations (Gittenberger, 2007). The latter three species differed from *D. vexillum* however, in that they showed a decline in their populations in 1998, while *D. vexillum* has not shown a distinct population decline since its expansion in 1996 (Gittenberger, 2007).

In 2008 Dr. R. Dekker of the Royal Netherlands Institute of Sea Research (NIOZ) found the first colony of *D. vexillum* in the Dutch Wadden Sea on a mussel bed south of Terschelling (Gittenberger *et al*., 2009). During a survey in July/August 2009 it was found at two additional localities, also in the proximity of Terschelling (Figs 6-8). The species was found nowhere else in the Wadden Sea however, although 83 localities were searched specifically for hard substratum related species (Fig. 5; Gittenberger *et al*., 2009). This may be an indication that the population in the Wadden Sea is still in the lag time period of the invasion. In the Oosterschelde *D. vexillum* remained rare in the lag time, i.e. did not expand its populations for the first five years after its discovery in 1991.

2.4 Natural transport vectors

Didemnum vexillum has the potential to reproduce both sexually and asexually and spread rapidly (Bullard *et al*., 2007a, 2007b). Because colonial ascidian larvae remain viable for only 12-24 hours, the long distance spread of *D. vexillum* is unlikely to occur by sexual reproduction, i.e. naturally dispersing larvae however (Daley & Scavia, 2008; Lengyel *et al*., 2009). The species may expand its range around the island Terschelling by naturally dispersing larvae, but it is unlikely that these larvae can reach hard substrata surrounding the neighbouring islands or the main-land (Fig. 5).

Didemnum vexillum may expand its range from Terschelling to other Wadden Sea areas by asexual reproduction however. Its Dutch vernacular name is “druipzakpijp”, i.e. the dripping sea-squirt, because it is known for its ability to form long ‘dripping’ lobes that break loose and travel along with the currents until they settle again (Fig. 9). Fragmentation is generally seen as a way for *D. vexillum* to rapidly expand its populations to new areas (Bullard at al., 2007). The drifting lobes may indeed enable the natural spread of the species from Terschelling to other areas in the Wadden Sea. Osman & Whitlatch (2007) have shown that *D. vexillum* colonies fragment significantly more at some localities, as compared to other, seemingly similar localities. In their studies it remained unclear however, which environmental conditions may have caused this difference. Gittenberger *et al*. (2009) did not specify whether fragmentation in the *D. vexillum* population at the harbour of Terschelling was at a relatively high level, so that the risk that the didemnid will spread to other places in the Wadden Sea by making use of that mechanism also remains uncertain.

Fig. 9. In The Netherlands *Didemnum vexillum* is commonly named Druipzakpijp, i.e. the dripping sea-squirt, because its colonies form lobes which naturally break loose. They drift along with the currents and probably form the most common way of range expansion for this species.

A third natural transport vector are colonies that grow on mobile marine animals like spider crabs of the genus *Macropodia*, which are common along the Dutch coast (Fig. 10). As hitchhikers *Didemnum vexillum* colonies can travel along wherever these animals go.

Taking the distance and currents into account, it is very unlikely that *Didemnum vexillum* was introduced in the Wadden Sea from the Oosterschelde or Grevelingen by natural transport vectors, i.e. drifting larvae, loose colony lobes, or as hitchhikers. As was also concluded by Daley & Scavia (2008), the spread along such large distances is assumed to be predominantly human mediated.

2.5 Anthropogenic transport vectors

As a long distance transport vector the import of Japanese oysters and spat prior to the 1960s from the NW Pacific is often discussed. Lambert (2009) concludes however that this vector is unlikely to be responsible for the range expansion of *Didemnum vexillum* worldwide because there are no reports of sudden didemnid appearances

Fig. 10. Mobile marine animals like this spider crab (*Macropodia* sp.) also form a suitable substrate for *Didemnum vexillum* to settle on. The white spots on its legs and body are didemnid colonies.

prior to the 1970s. A much more likely vector for long distance dispersal is considered to be shipping, either via hull or via sea chest fouling. Ballast water is less likely to be of importance as a long distance vector because colonial ascidian larvae are relatively short lived, i.e. viable for 12-24 hours (Daley & Scavia, 2008).

Therefore, *D. vexillum* was most probably introduced to Europe through hull fouling or sea chest fouling. Fouled recreational crafts, fishing vessels and barges are seen as a medium distance transport vector. Several introduction routes within for example New Zealand (Coutts, 2002; Coutts & Forrest, 2007), Ireland (Minchin & Sides, 2006; Minchin, 2007) and Great Britain (Griffith *et al*., 2009; Kleeman, 2009) could be traced back and linked to one or several barges or vessels with didemnid colonies on their hulls. Although the transport of shellfish is recognized as a potential vector, fouling is generally seen as the main vector of transport. Subsequent local spread is assumed to be mainly by fragmentation of the colonies.

Considering introductions in other areas there are three most likely scenarios of introduction into the Wadden Sea:

[1] *D. vexillum* was introduced in the harbour of Terschelling through hull-fouling on a recreational vessel from the Oosterschelde. From there it spread to the mussel bed south of the island by fragmentation.

[2] *D. vexillum* was introduced on a mussel bed south of Terschelling by a shellfish transport from the Oosterschelde. From there it spread to the harbour of Terschelling by fragmentation.

[3] *D. vexillum* was introduced in the harbour of Terschelling through hull-fouling on a recreational vessel from the Oosterschelde. Independent of the introduction in the harbour, it was introduced on a mussel bed south of Terschelling by a shellfish transport from the Oosterschelde.

The most likely of these three scenarios is the first one. The second is less likely, because this scenario assumes that *Didemnum* forms fragmenting colonies on a mussel bed. Dripping, fragmenting colonies are more often seen on erect or floating structures like docks or rocks from which these colonies hang down (Fig. 9). Furthermore, taking currents into account, it is more likely that fragments float from the harbour of Terschelling out to the mussel bed, than vice versa. The third scenario is even less likely because two independent introductions are assumed there, both in the proximity of Terschelling.

An final anthropogenic transport vector that was found to be responsible for much of the local spread of *Didemnum vexillum* along the American E coast does concern shellfish transports. There, certain areas are heavily dredged for scallops and while the ships are returning to shore or to other fishing areas these scallops are cleaned. Didemnid colonies and other organisms that grew on these scallops were subsequently discarded overboard in previously uninfected areas (Lengyel *et al*., 2009). Spreading through this method could be reduced by the adoption of better fishing practices (Lengyel *et al*., 2009).

2.6 Molecular analyses to trace the origin and invasion route

To develop methods that inhibit the further spread of *Didemnum vexillum* and similar marine invasive species, molecular analyses can be a valuable tool. Misidentifications often form an important reason that invasions remain undiscovered until a stage in which eradication and/or management to reduce ecological and economical damage has become much more difficult than before. *D. vexillum* is one of those species that is very hard to identify on the basis of only its morphology because many morphologically similar *Didemnum* species exist world-wide. Most of these species can only be reliably distinguished from their closest relatives on the basis of the larvae and minute spicules. As is explained in more detail in the introduction of this report, there has been a 15 year long dispute about the identity of the white didemnid colonies that were invading temperate areas world-wide. Price *et al*. (2005) did not reveal the species name but confirmed on the basis of 18S ribosomal RNA sequencing that the white didemnids that were invading the American W Atlantic coast are genetically the same as the ones that were invading the Oosterschelde and Grevelingen in The Netherlands at that time. Stefaniak *et al*. (2009) and Gretchen (2009) finally ended the discussion by concluding, on the basis of both nuclear and mitochondrial DNA markers, that the white fast-growing didemnids invading temperate areas in New Zealand, N America and Europe should all be considered conspecific, i.e. *D. vexillum*. Lengyel *et al*. (2009) and Hess *et al*. (2009) confirmed these findings on the basis of additional DNA-data and didemnid samples.

Although these DNA-studies convincingly showed the identity of *Didemnum vexillum*, they were insufficient to track its invasion route and further population dynamics around the world. The relatively low number of colonies that were included from Japan, also made it impossible to reliably confirm whether Japan is the native area of the species, as is often suggested in the literature (Lambert, 2009). Therefore many more DNA-analyses are necessary. This is ongoing at the University of Connecticut on the basis of hundreds of samples collected from Japan, New Zealand, America and Europe. To get the best

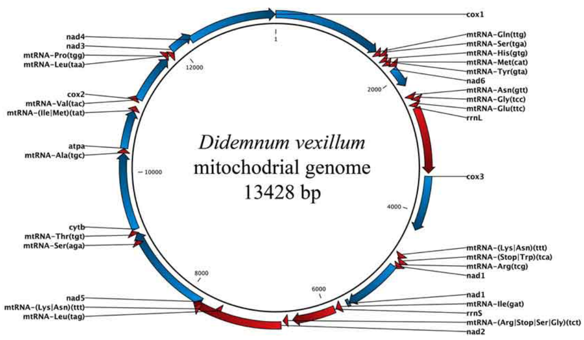


Fig. 11. Total mitochondrial genome of *Didemnum vexillum*. A preliminary result of the ongoing total genome and transcriptome analysis.

possible resolution in such a study, it is necessary to find out which parts of the DNA are most informative for the purpose of comparing the relatively recently dispersed colonies of *D. vexillum*.

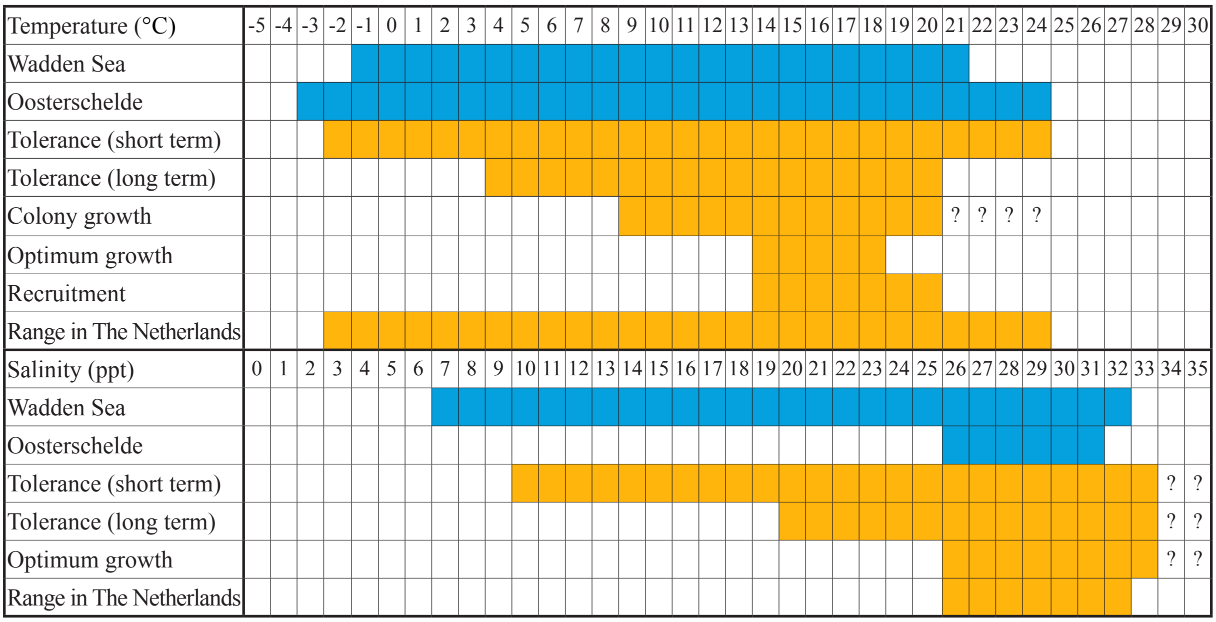
To investigate which parts are most informative, and more in general to get a better idea of the origin of its highly successful invasive behavior, of its evolutionary history, and of its development as a colonial chordate, we sequenced the total nuclear genome, total mitochondrial genome and the total transcriptome of a *Didemnum vexillum* colony collected in the Grevelingen off the island Hompelvoet. This was done in February 2010 with the next generation sequencing apparatus Illumina GAIIx at ZF-Screens (www.ZF-screens.com). From the resulting ~10 Gigabases of sequence (~10,000,000,000 bases), the total mitochondrial genome of 13,428 bases has already been assembled and annotated (Fig. 11). The draft assembly and gene annotation of the total nuclear genome, which is about 135 Mbases, and of the transcriptome will be completed in the coming year. Simultaneously, the *D. vexillum* genome is compared with the total genomes of the solitary ascidians *Ciona intestinalis* and *Ciona savignyi* (Dehal *et al*., 2002; Small *et al*., 2007) in order to find the nuclear and mitochondrial genes that are most suitable for population genetic studies in ascidians in general. In the subsequent population genetic studies, which are also planned for the coming year, didemnid samples from all the areas within The Netherlands, i.e. Grevelingen, Oosterschelde and Wadden Sea, will be included.

3. Probability of settlement

3.1 Environmental cues

The probability that *Didemnum vexillum* settles in an area where it is introduced is strongly dependent on environmental conditions. Salinity, temperature and depth are considered to be important parameters that are linked to the settlement success of *D. vexillum* colonies.

Table 1. Salinity and temperature tolerances and optima of *Didemnum vexillum* (see paragraphs 3.1.1 and 3.1.2). Temperature and salinity characteristics of the Wadden Sea and Oosterschelde are those used by Fey *et al*. (2010) with the exception of the water temperature range in the Oosterschelde, which was deducted from WaterStat, provided by the Dutch ministery of Transport, Public works and Water Management.



3.1.1 Salinity

In general ascidians do not grow well in low salinity areas (Vazques & Young, 2000), making higher salinity areas more vulnerable for invasions. Lab experiments with *Didemnum vexillum* in Massachusetts, USA, show that the colonies grow best in high salinity water of 26-30 ppt (Bullard & Whitlach, 2009) and possibly higher, up to 33 ppt (Herborg *et al*., 2009). They die within a week in water salinities of about 20 ppt (Bullard & Whitlach, 2009), but they can tolerate salinities down to 10 ppt for a short period of time (Herborg *et al*., 2009). Known salinity tolerances and optima of *D. vexillum* are summarised in table 1 together with the didemnid's range in The Netherlands.

3.1.2 Temperature

Regardless of the temperature *Didemnum. vexillum* will remain a competitor for space with many native species in the areas where it manages to settle. It grows faster than other colonial sea-squirts, like *Botryllus schlosseri* and *Botrylloides violaceus*, as was tested at a series of temperatures by McCarthy *et al.* (2007). At temperatures below 8-10° Celsius colony growth stops.

Didemnum vexillum colonies can tolerate water temperatures of -2 to 24° Celsius and daily changes of up to 11° Celsius (Gittenberger, 2007; Valentine et al. 2007a). In the Oosterschelde *D. vexillum* colonies grow best at 14-18° Celsius, and start to degenerate when the temperature drops below ~4 °Celsius (Figs 12-13; Gittenberger, 2007). At high summer temperatures, especially above 20° Celsius, colonies decline and growth speed decreases (Fig. 12: column August; Daley & Scavia, 2008; Gittenberger, 2007; McCarthy *et al*., 2007). It is therefore unlikely that climate change resulting from global warming will automatically increase the invasion potential of this ascidian. If the water temperatures continue to rise, the winter temperature in the future may not become low enough to induce

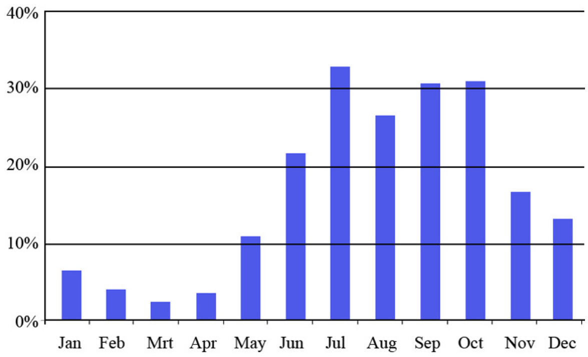


Fig. 12. Average percentage of dives per month during which *Didemnum vexillum* was seen in high densities (>100 colonies per dive) in the Oosterschelde, in the period 1994-2003 (Gittenberger, 2007; MOO-project data, ANEMOON Foundation)

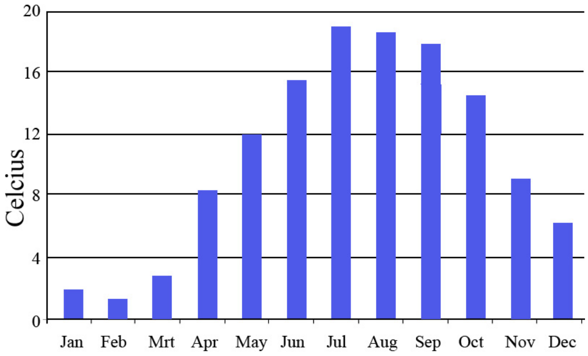


Fig. 13. Average water temperature per month in the Oosterschelde (Waterstat Environmental Data Software of the Dutch Ministry of Transport, Public Works & Water Management)

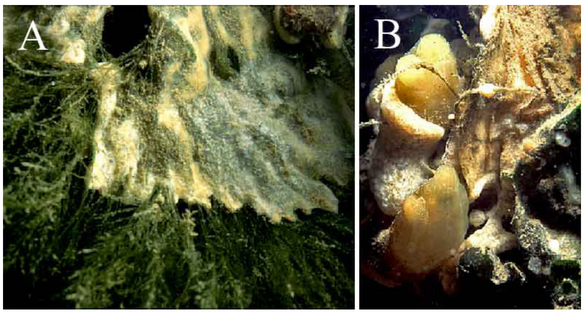


Fig. 14. When water temperatures drop below 4° Celsius most *Didemnum vexillum* colonies in The Netherlands start to degenerate. A, degenerating colony on algae. B, a colony that still looks healthy is overgrowing the solitary tunicate *Ciona intestinalis* on the left, while colonies that overgrow the algal species *Ulva* sp. on the right are degenerating.

Fig. 15. Post-winter regenerating *Didemnum vexillum* colonies in April 2009, when temperatures had just reached about 8° Celsius (Fig. 13) in the Oosterschelde.

colonies to die, and the season with the optimal growth temperature may lengthen. Although this may raise the colonisation success, the period that the water temperature remains above 20° Celsius, killing of didemnid colonies, will probably also lengthen. It is therefore uncertain whether higher temperatures are beneficial to this invader.

Recruitment of *Didemnum vexillum* occurs in the W Atlantic at 14-20° Celsius and persists for 3.5-5 months, dependent on local conditions (Valentine *et al*., 2009).

In winter time, when temperatures drop below 4° Celsius, some colonies are able to survive temperatures as low as -2° Celsius. Although colonies that grow on rocks, stones and plants degenerate and disappear at these temperatures (Fig. 14A), colonies growing on other animals like the solitary ascidians *Styela clava* and *Ciona intestinalis* do survive as seemingly healthy colonies (Fig. 14B; Gittenberger, 2007).

Furthermore, some colonies that appear to have degenerated and died, unexpectedly survive cold winters in an unidentified hibernation form (Daley & Scavia, 2008). In spring, when temperatures rise above ~8° Celsius, these colonies start growing again (Fig. 15).

The known temperature tolerances and optima of *Didemnum vexillum* are summarised in table 1 together with the didemnid's range in The Netherlands.

3.1.3 Depth

Didemnum vexillum colonies can be found from the intertidal (Fig. 8) to at least 65 meters of depth with water current speeds ranging from 0 to about 2 kt, i.e. 100 cm/s (Valentine *et al*., 2007b). Even though most marine invaders are restricted to shallow-water embayments, *D. vexillum* seems to be most successful in deeper (>30 m) water (McCarthy *et al*., 2007). This may be due to the fact that minimum temperatures are higher at deeper water sites compared to shallow sites. In deeper water this may reduce the period of degeneration in winter, and lengthen the recruitment period in summer (Valentine *et al*., 2009).

3.2 Human influences

Invasive tunicates, including *Didemnum vexillum*, are mainly found on anthropogenic surfaces and on natural substrates near these man-made structures (Carman *et al*., 2009b). This is especially the case in the early invasion stages. These structures include floating docks and buoys, but also objects that lie on the bottom, like lobsters cages, lost fishing gear, ropes and chains. On these artificial structures invasive species expand their populations much faster than on natural substrate types in the same environment (Tyrrell & Byers, 2007). Reducing the number of artificial structures in an area will therefore reduce the success of invasive tunicates.

Improving the water quality may also reduce the success of invasive sea-squirts. In an assessment of a series of good water quality sites and fair quality sites off Massachusetts, *Didemnum vexillum* was only found in the watersystems with a fair water quality and not in any of the systems with a good water quality (Carman *et al*., 2007). *D. vexillum* may therefore not only be more tolerant of nitrogen polluted water, but may even prefer it. The definitions for good and fair water quality used in the study of Carman *et al*. (2007) were the following: good = low levels of excess nitrogen, good water clarity and infrequent algal blooms; fair = moderate levels of excess nitrogen, reduced water clarity, low oxygen levels and periodic algal blooms.

3.3 Natural enemies

Even though some seastars and sea urchins (Bullard *et al*., 2007) and some gastropods have been recorded to feed on *Didemnum vexillum* (Lambert, 2009), no natural enemy has been found that eat enough of the colonies to have any significant effect on its population growth. This was confirmed by a study focusing on the potential value of the common periwinkle *Littorina littorea* as a biological control species (Carman *et al*., 2009a) along the American coast. Even though these snails, which are also common in The Netherlands, do feed on especially unhealthy didemnid colonies, they form no threat to their populations.

In The Netherlands two species, both gastropods, were found to consume the colonies (Gittenberger, 2007). Both species are rare however, and eat only extremely small amounts of the sea-squirts tissue. The first species is the cowrie *Trivia arctica*, which is native to NW Europe and became more common in The Netherlands (Holsteijn, 2004) since *Didemnum vexillum* was introduced. The second predatory gastropod is a *Lamellaria* species (Goud & Gittenberger, 2004). It mimics the surface of the didemnid perfectly well, both in colour and pattern, and is therefore thought to be introduced together with *D. vexillum* from the didemnid's native area, i.e. probably NW Pacific (Gittenberger, 2007). This hypothesis is further supported by the fact that this *Lamellaria* sp. could not be identified as a NW European species . Several small greenish copepods that were living on the *Lamellaria* snails, could also not be identified with the literature on the NW European marine fauna and may also be native to the NW Pacific. These species have probably traveled along with the didemnid to The Netherlands using the same transport vectors, i.e. mainly hull fouling and aquaculture transfers.

4. Probability of spread

Herborg *et al*. (2009) could provide a reasonably reliable prediction on the occurrence and spread of *Didemnum vexillum* along the Pacific coast of N America on the basis of a high vector density study. This was partly based on the expert opinions of scientists about the relative importance of transport vectors (Table 2).

Throughout the Wadden Sea there is a high density of the transport vectors "hull fouling with

Table 2. Importance of transport vectors of *Didemnum vexillum* based on expert opinions (Herborg *et al*., 2009)

Transport vector	Importance
Natural larvae dispersal	Moderate
Natural adult dispersal by drift	Moderate
Ballast water release	Low
Hull fouling large vessels (>50 m)	Moderate
Hull fouling small vessels (<50 m)	High
Hull fouling slow moving vessels	High
Aquaculture transfers	High
Commercial fishing	High
Aquarium releases	Very low
Intentional releases to establish a food source	Very low

vessels" and "aquaculture transfers", which were both found to be very important for the spread of *Didemnum vexillum*. It is very likely therefore, that *D. vexillum* will expand its range in the Wadden Sea. Not all areas in the Wadden Sea are suitable for settlement however, as is explained in the next paragraph.

5. High risk areas

5.1 Probability of introduction, settlement and spread

Not all areas in the Dutch Wadden Sea are equally at risk of being infested by *Didemnum vexillum*. Because it has a preference for high salinity waters (Bullard & Whitlach, 2009) the areas in the proximity of the red dots (salinities ≥ 26 ppt) in figure 16 are most prone to infestation. In addition, taking into account that fouled recreational vessels are considered the most important transport vector (paragraph 2.5), one can assume that *D. vexillum* will most probably be spread from its population at Terschelling harbour to other harbours with high salinities (Fig. 17: high risk infestation areas). Following the same considerations, harbours with no or only a few recreational vessels are less at risk, as are intertidal mussel beds close to Terschelling (Fig. 17: medium risk areas). The high risk map presented here (Fig. 17) does not show all areas that are at risk, however. Most of the intertid-

Fig. 16. Salinities in the Dutch Wadden Sea measured in July/August 2009 (Gittenberger *et al*., 2009). Red dots: High salinities ≥ 26 ppt; Orange dots: Mediate salinities: ≥ 20 ppt and < 26 ppt; Yellow dots < 20 ppt.

al mussel beds and other hard substrata in the intertidal are not indicated. To do so, detailed analyses of the bottom structure, depth, salinity ranges and species communities that are present should be conducted. It is furthermore uncertain to what degree hull fouling on fishing vessels also concerns an important transport vector. There is heavy traffic of fishing vessels between the Oosterschelde and the Wadden Sea. Most of the areas within the Wadden Sea are not at risk because they are intertidal, consisting completely of soft substrata, and/or show relatively low salinities, which make those areas unsuitable for *D. vexillum* (Fig.16).

Although most of the surface of the Dutch Wadden Sea may not be at risk, a wide-spread population of *Didemnum vexillum* should be considered a large ecological threat because the harbours form ideal stepping stones that will increase the risk of spreading this species to uninfested areas like the German and Danish Wadden Sea and further north along the European coasts, where hard substrata and seemingly suitable habitas are more common. Another region in the proximity of the Wadden Sea that may be at risk are the "Texelse stenen", an area with rocks and pebbles in the North Sea, just west of the Wadden Sea island Texel. The environmental conditions there seem to be similar to those at Georges Bank, a high salinity area off the east coast of the USA. Within a few years after its introduction *D. vexillum* colonized at least 230 km^2 of pebble gravel habitat there (Valentine *et al*., 2007b).

Fig. 17. Potential high risk areas for *Didemnum vexillum* infestation. 1, high risk of infestation; 2, medium risk of infestation.

5.2 Vulnerability analyses of watertypes

The vulnerability of Dutch water bodies for *Didemnum vexillum* infestation is here calculated with a methodology developed for The Netherlands Ministry of Transport, Public Works & Water Management (Gittenberger & Leewis, 2008; Leewis & Gittenberger, 2008).

The method starts from the assumption that the vulnerability of an area depends on: [1] the severity of the threats to which the area is exposed as a consequence of its properties and the way it is used by men; [2] the elements in the area that may be threatened.

On top of that, the vulnerability of an area for an invading exotic species depends on the potential effect that exotic species may exert onto the water body, which is calculated on the basis of the habitat suitability, the invasion history of the species (did it already cause significant damage in other areas), and its potential to do ecological and economical damage (Leewis & Gittenberger, 2009).

V = (T+ E) / 2, in which

V = the vulnerability of a water body for non-native species, giving an indication of how easily exotic species are introduced into the area, in combination with the severity of the problems that they can cause.

T = the total of threats of a water body (or water type), split into on the one hand "what can threaten the water body" (e.g. pollution, import vectors, number of non-native species already present, etc.), and on the other hand " what can be threatened in the area" (e.g. tourists, drinking water extraction plants, endemic species, number of native species present, etc.).

E = the effect a non-native species or non-native species group can have. E is split into habitat-suitability **(H)**, damage done in other areas **(D)**, and potential damage **(P)**.

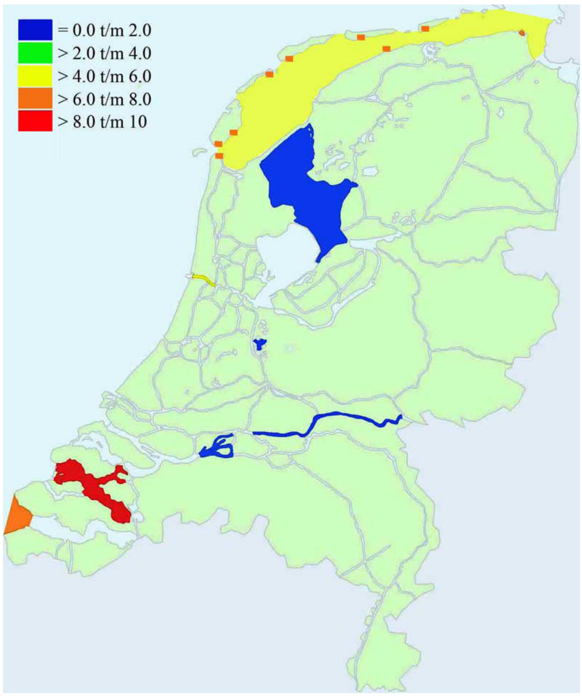


Fig. 18. Relative vulnerability of watersystems for the invasive sea-squirt *Didemnum vexillum* according to the 'RWS vulnerability of watertypes for invasive species' methodology (Gittenberger & Leewis, 2008; Leewis & Gittenberger, 2008). Vulnerability=0.0-2.0, not vulnerable; >2.0-4.0, somewhat vulnerable; >4.0-6.0, moderately vulnerably; >6.0-8.0, very vulnerably; >8.0-10, highly vulnerable.
N.B. 'Uncoloured' water systems (light blue) were not included in the vulnerability analysis.

The values of the parameters V, T and E lie between 0 and 10, indicating e.g. the optimal (0) or worst case scenario (10), e.g. V=0, not vulnerable; V=10, highly vulnerable; T=0, not threatened; T=10, severely threatened; E=0, no effect; E=10, extremely negative effect. The values of the parameters T and E are determined on the basis of a series of yes/no questions that are assumed to give an indication of the threats of a water body and the potential effect of a non-native species on it. Standardized factsheets made by specialists for both the water bodies and the invasive species are used to answer these questions (Leewis & Gittenberger, 2008).

The results of this analysis, focusing on *Didemnum vexillum* in The Netherlands, are presented in figure 18. For a more detailed description of the parameters see Leewis & Gittenberger (2008). The most vulnerable of the tested water bodies in The Netherlands is the Oosterschelde (Fig. 18). The Dutch Wadden Sea, with the exception of the harbours (orange dots in Fig. 18), comes out as much less vulnerable than the Oosterschelde here, because [1] much less of the total surface is suitable for the settlement of *D. vexillum*, i.e. sandy bottom, intertidal, and brackish areas are much more common in the Wadden Sea, and [2] the total diversity of marine species, especially in the subtidal, is much higher in the Oosterschelde.

Even though this method of calculating the vulnerabilities of water bodies for exotic species is unsuitable for fine scale conclusions, it does give water managers a rather quick tool to get an indication of the relative vulnerability of a water body for non-native species like *Didemnum vexillum.*

A somewhat similar method was used by Locke (2009). From a worldwide list of 57 tunicate species with a known history of being invasive, a "watch list" of 17 tunicate species was constructed for which the water bodies along in Atlantic Canada are most vulnerable. This was done on the basis of several filters, including climate zone and shipping route filters (Locke, 2009).

5.3 UNESCO World Heritage and Natura 2000 status

In June 2009 the Dutch and German Wadden Sea were officially added to the UNESCO list of World Heritage sites (Common Wadden Sea Secretariat, 2008). Before that time the complete Dutch Wadden Sea was already a Natura2000 area (Leeuwen *et al.*, 2008). Although some studies, including the ones described in the previous paragraphs, indicate that the Wadden Sea may not be the most vulnerable water body for an invasion by *Didemnum vexillum*, the UNESCO World Heritage site status and the Natura2000 status do indicate the presence of a unique biodiversity, occurring in a series of relatively rare and vulnerable habitats. The management of the Wadden Sea is faced with strict obligations focusing on protecting this area against anything that can threaten the Natura2000 habitats and Natura2000 species, next to the biodiversity and the ecosystem as a whole according to the UNESCO World Heritage regulations (Common Wadden Sea Secretariat, 2008; Leeuwen *et al*., 2008). Regardless of the question whether it is likely that *D. vexillum* will invade the entire area, it certainly does threaten parts of the Wadden Sea as is described in more detail in the next chapter about its potential impact.

6. Impact

6.1 Ecological damage

Didemnum vexillum has a large potential to become invasive and subsequently cause significant ecological damage to at least parts of the Dutch Wadden Sea, as it shares several ecological traits with notorious, damaging, invasive species. It is characterized by a high reproduction and population growth rate in combination with the ability to overgrow benthic organisms, rapid dispersal by fragmentation and tolerance for a wide range of environmental conditions (Daley & Scavia, 2008). At least in its non-native range no predator or disease has been found that can significantly limit its expansion and invasive success.

Didemnum vexillum hinders the native species where it is introduced by inhibiting the settlement of their larvae. These larvae are deterred by the low pH of the tunicate's surface tissue (Morris *et al*., 2009) or cannot tolerate some of the allelophatic chemical compounds that *D. vexillum* produces (Dijkstra *et al*. 2007). A surface covered by *D. vexillum* colonies can also significantly alter the settlement success of local species like mussels. Mussel spat tends to settle at higher densities on structurally more complex surfaces with for example solitary ascidians, hydroids and erect bryozoans (Dijkstra *et al*., 2007).

In areas that are already densely occupied by other species, *Didemnum vexillum* still manages to settle (Fig. 19). Whenever it is settled, colonies can grow rapidly and eventually cover large surfaces then (Fig. 20). Within a few years after its introduction it colonized at least 230 km^2 of pebble gravel habitat in two adjacent areas along the east coast of the USA (Valentine *et al*., 2007b). More local, but similar behavior of *D. vexillum* is encountered in the Oosterschelde and Grevelingen. There it was found to be able to grow over rocks and boulders, almost all plants and algae (Fig. 21), and most benthic animal species (Gittenberger, 2007), like sponges, hydroids, the bivalves *Mytilus edulis*, *Crassostrea gigas* and *Ostrea edulis* and ascidians like *Aplidium glabrum*, *Diplosoma listerianum*, *Ciona intestinalis*, *Styela clava* and *Ascidiella aspersa*. Most benthic species are readily overgrown by *D. vexillum*, but some sea-anemones are able to resist being overgrown somewhat longer than other organisms (fig. 22). Possibly because of a growth inhibitor, the sandy tubes of the peacock worm *Sabella pavonina* are also not overgrown (Fig. 23). Rarely the non-native colonial ascidians *Botrylloides violaceus* and *Diplosoma listerianum* are able to overgrow *D. vexillum* colonies in The Netherlands (Fig. 24; Gittenberger, 2007). These species are exceptions however, to the more general rule that *D. vexillum* colonies will overgrow virtually everything that comes on their way.

Didemnum vexillum differs from most other fouling species in its ability to grow also over sandy substrata (Fig. 25), although it settles mainly on hard substrata. This substantially increases the risk that this fouling species forms for the Wadden Sea environment. Most fouling species are known to overgrow solely hard substrata.

Fig. 19. *Didemnum vexillum* colonies are able to settle and grow in micro-habitats there are already densely overgrown by native species.

Fig. 20. *Didemnum vexillum* colonies overgrow and dominate large areas of the bottom.

Fig. 21. *Didemnum vexillum* overgrowing algae.

Fig. 22. The sea-anemone *Metridium senile* can prevent being overgrown by *Didemnum vexillum*.

Fig. 23. The tube of the peacock worm *Sabella pavonia* does not get overgrown by *Didemnum vexillum*.

Fig. 24. Colonies of the bluish grey ascidian *Diplosoma listerianum* can overgrow *Didemnum vexillum* colonies.

A number of studies on the Atlantic coast of America, focusing on the effects of *Didemnum vexillum* on species communities, indicate that marine communities are significantly changed (Bullard *et al*., 2007; Lengyel *et al*., 2009; Mercer *et al*., 2009). The invasion did not reduce the overall species richness and abundance, however. Apparently *D. vexillum* causes a shift in the benthic community structure and functional group dominance with greater numbers of infauna and deposit-feeders residing inside the didemnid mats, compared to samples collected adjacent to the mats (Mercer *et al*., 2009). In support of these results Lengyel *et al*.. (2009) found that the species composition in the benthic community changed and that the abundance of two polychaete species, viz. *Nereis zonata* Malmgren, 1867 and *Harmothoe extenuate* Grube, 1840, increased significantly in infested areas in comparison to uninfested areas.

In general, Daniel & Therriault (2007) concluded that due to the aggressive colonizing abilities of *Didemnum vexillum*, plant, invertebrate and fish communities may be altered, and native populations can be displaced or lost. Its potential ecological impact on the Dutch Wadden Sea is uncertain but could be severe if colonies start to spread and cover large areas of the bottom. At least in the subtidal this may reduce the normal settlement of native species like bivalves, which form an important food source for the various bird species that reside in this UNESCO world heritage area.

6.2 Economical damage

The effects of invasive tunicates have been near catastrophic for the economic viability of the mussel aquaculture industry of Prince Edward Island, Canada (Gittenberger, 2009; Lock & Carman, 2009). The same invasive species that are fouling the mussels in Canada, are fouling mussels in The Netherlands. The species assemblages that are found on the mussel ropes in both countries differ however. In Canada the mussel lines are overgrown by monocultures of ascidians, while in The Netherlands mussels are fouled by a diversity of assemblages of many different ascidians (Gittenberger, 2009). The latter assemblages cause much less economical damage than the monocultures of ascidians (Gittenberger, 2009). Monocultures can increase the weight of mussel ropes to more than tenfold and smother mussels more than diverse assemblages do. Therefore, the fouling related loss in mus-

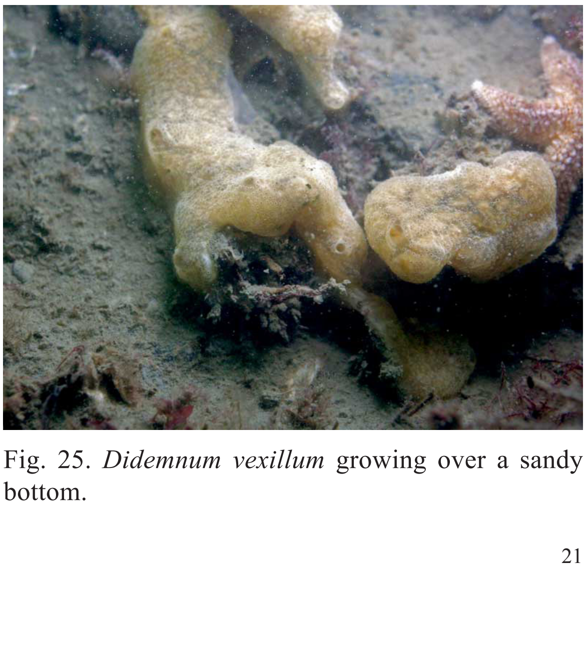


Fig. 25. *Didemnum vexillum* growing over a sandy bottom.

sels, yield during harvesting, is more severe in Canada than in The Netherlands. This illustrates that economical damage caused by invasive species in other areas is not always a good predictor of damage that those species will cause in The Netherlands. It does give an indication of the potential economical damage however.

Although exact economical losses caused by *Didemnum vexillum* have not been assessed and published yet, it is generally assumed that this species threatens shellfish aquacultures and may alter habitats that are essential to various fisheries (Bullard *et al*., 2007; Valentine *et al*., 2007a). These may be affected when high densities of this invader alter the access to commercially important fish species, to critical spawning grounds, prey items, and refugia (Daley & Scavia, 2008). This may also apply to the Dutch Wadden Sea. The aquaculture industry is assumed to be impacted most. Bivalve larvae may not be able to settle in areas dominated by *D. vexillum* (Morris *et al*. 2009), settled shellfish will be impacted by smothering and spatial competition (Sewell *et al*., 2008), and the maintenance costs for cleaning the fouling of aquaculture gear will increase (Morris *at al*., 2009). Kleeman (2009) even predicts that the infestation in 2008 of Holyhead marina, N Wales, in Great Britain (Griffith *et al*., 2009) may cause 90% of subtidal production to be affected within 10 years if eradication is not achieved, and that, where mussel areas are affected, production will fall by 5 up to 25%.

Whether similar economical damages will occur in The Netherlands and more specifically in the Dutch Wadden Sea remains uncertain. In the Oosterschelde *D. vexillum* is smothering many Japanese oysters, *Crassostrea gigas* (Fig. 26), and blue mussels, *Mytilus edulis* (Fig. 27). There are no specific records yet of economical damage caused by this species to the Dutch aquaculture industry however.

6.3 Social damage

Although *Didemnum vexillum* can cause significant ecological and economical damage, there are no records of *D. vexillum* doing any social damage.

6.4 Invasive Species Environmental Impact Assessment (ISEIA)

Non-native invasive species in Belgium are included in the information system Harmonia, which aims at collecting standardized information on exotic species which are assumed to be detrimental to native biodiversity in Belgium (Branquart, 2009). As an simplified environmental impact assessment protocol Harmonia includes the Invasive Species Environmental Impact Assessment (ISEIA) index, which can help to prioritize actions to prevent introductions and mitigate the impact of invasive spe-

Fig. 26. *Didemnum vexillum* overgrows and smothers oysters (Crassostrea gigas).

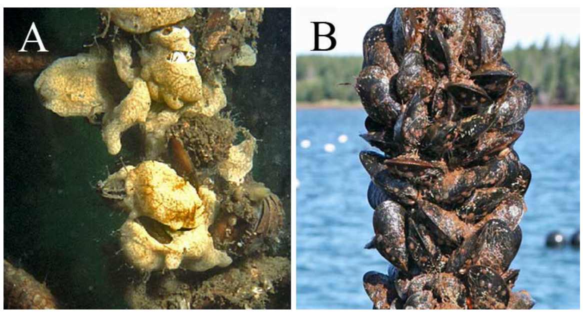


Fig. 27. *Didemnum vexillum* overgrows mussel ropes. A, overgrown mussel rope; B, mussel rope without fouling organisms.

cies, including the improvement of the legislative framework at the federal and regional levels (Branquart, 2009).

The ISEIA index for an invasive species is calculated by appointing a score of 1 to 3 to the potential to disperse, the potential to colonize natural habitats, the potential to have an impact on native species, and the potential to have an impact on ecosystems. Adding up these four scores will result in an ISEIA index value of 4-8 for species with a low environmental risk, 9-10 for species with a moderate environmental risk to be put on a 'Watch list' and 11-12 for species with a high environmental risk to be put on a 'Black list'.

The ISEIA index that was calculated for *Didemnum vexillum* on the basis of a more detailed description of the method in Branquart (2009) is 11 (Table 3). Therefore, according to the Invasive Species Environmental Impact Assessment (ISEIA) index, *D. vexillum* is considered to be a 'Black list' species with high environmental risk.

6.5 Leung & Dudgeon invasion risk analysis

As is indicated by Leung & Dudgeon (2008) the management of an invasion can be performed at several stages and parts of the invasion pathway, i.e. focusing either on preventing an alien species to be introduced, on eradicating it when it has already established a population and/or on controlling its expansion after it has settled. The various approaches that are possible in the case of *Didemnum vexillum* are described in the next chapter. To select which type of management, if any, is most appropriate to minimize the damage that may be caused by an invader from a benefit-cost point of view, an organisms risk analysis can be executed (Leung & Dudgeon, 2008).

In table 2 the organism invasion risk of *Didemnum vexillum* is calculated according to the equation below, which is described in more detail by Leung & Dudgeon (2008) .

Table 3. Calculation of the Invasive Species Environmental Impact Assessment (ISEIA) index for *Didemnum vexillum*. The score of the 'Potential to disperse' is based on paragraphs 2.5 and 2.6. The score of the 'Potential to colonize natural habitats' is based on paragraph 5. The scores of the 'Potential impact on native species' and the 'Potential impact on ecosystems' are based on paragraph 6.1.

Didemnum vexillum	Score (1-3)
Potential to disperse	2
Score = 2: Medium risk: Except when assisted by man, the species doesn't colonize remote places. Natural dispersal rarely exceeds more than 1 km per year. The species can however become locally invasive because of a strong reproduction potential (Branquart, 2009).	
Potential to colonize natural habitats	3
Score = 3 : The non-native species often colonizes high conservation value habitats (i.e. most of the sites of a given habitat are likely to be readily colonized by the species when source populations are present in the vicinity) and makes therefore a potential threat for red-listed species. (Branquart, 2009).	
Potential impact on native species	3
Score = 3; The development of the non-native species often cause local severe (>80%) population declines and the reduction of local species richness. Those non-native species form long-standing populations and their impacts on native biodiversity are considered as hardly reversible. (Branquart, 2009).	
Potential impact on ecosystems	3
Score = 3; The impact on ecosystem processes and structures is strong and difficult to reverse. (Branquart, 2009).	
ISEIA index = 2 + 3 + 3 + 3 =	11

Invasion Risk = {Probability of Establishment} × {Consequence of Establishment} = {P × I × E × S} × {C × O × M} (2), where

P = Estimated probability of the organism being on, with or in the Pathway
I = Estimated probability of the organism surviving in transit and Introduction
E = Estimated probability of the organism colonizing and Establishing a population
S = Estimated probability of the organism Spreading beyond the colonized area
C = Estimated the Consequence of all possible ecological impacts if established
O = Estimated the Overall perceived impact from social and/or political influences
M = Estimated economic impact (i.e. Money) if established

Each of these element is given a risk rating of Low, Medium or High, with an indication of certainty: Very Certain (VC): firm conclusion; Reasonably Certain (RC): reasonably convinced; Moderately Certain (MC): more certain than not; Reasonably Uncertain (RU): reasonably indecisive; Very Uncertain (VU): a guess.

When out of the seven parameters, two are scored 'High" and four are 'Medium' (Table 4), the final ORP (Organism Risk Potential) is considered to be 'High'. An organism with a 'High' ORP is regarded as an organism of high concern, which forms an unacceptable risk (Leung & Dudgeon, 2008). The introduction of such species should be banned, control measures should be considered and according to Leung & Dudgeon (2008) prevention rather than mitigation is mandated.

7. Possibilities for management

7.1 Prevention

According to the literature that describes the invasion of *Didemnum vexillum* in New Zealand (Coutts & Forrest, 2007), along the American coast (Locke & Hanson, 2009), and in Great Britain (Kleeman, 2009), the main reason that efforts fail to prevent *D. vexillum* from being introduced and spread, is slow decision-making and lack of long-term commitment at a regional level. The rapid detection of the first colonies in an area and a lack of effective management tools, were not found problematic. Invasions of *D. vexillum* are often detected in an early stage (Coutts & Forrest, 2007; Griffith *et al*., 2009), as is also the case in the Dutch Wadden Sea (Gittenberger *et al*., 2009). There are several management tools and methods, that have proven to be suitable from a benefit-cost point of view, as long as they are applied in the early stages of the invasion. They focus mainly on preventing *D. vexillum* to be introduced, or obstructing

Table 4. Organism risk analysis of *Didemnum vexillum* being introduced into the Dutch Wadden Sea, according to Leung & Dudgeon (2008)

	Risk rating	Basis / source of risk rating	Certainty
P	High	*D. vexillum* is very likely to be on a fouled recreational vessel, a fishing vessel or in a mussel transport to the Dutch Wadden Sea from the Oosterschelde, where it has already settled. See paragraph 2.6	VU
I	Medium	The chance that a colony will survive being transported with mussels is lower than that it will survive the transport from the Oosterschelde to the Wadden Sea as fouling on a recreational or fishing vessel. See paragraph 6.2	RC
E	Medium	It is likely that *D. vexillum* will colonize and establish populations in harbours with a high salinity and many recreational vessels. Most of the habitats in the Wadden Sea are not ideally suitable for settlement however. See chapter 5 including figures 16-18.	RC
S	Medium	*D. vexillum* is expected to spread from Terschelling to the other harbours in the Wadden Sea and to European coastline to the north. It is uncertain to what degree it will spread to other areas in the Wadden Sea however. See paragraph 5.1	MC
C	High	If *D. vexillum* gets established in the Wadden Sea, its potential ecological damage is severe. See paragraph 6.1	MC
O	Low	*D. vexillum* is not known to cause social damage. See paragraph 3.1	MC
M	Medium	According to literature the economical impact can be severe if established. No economical damage has been reported from the Oosterschelde however, where it has been abundant and established from 1996 on. See paragraphs 2.3 and 6.2.	MC

it spreading further after an introduction. In the Dutch Wadden Sea fouled recreational vessels are considered to be the most likely vector responsible for importing *D. vexillum* , followed by shellfish transports (paragraph 2.5).

To minimize the chance that colonies are imported by recreational vessels, it is most important to raise more public awareness among both the owners of these vessels and the harbour masters (Kleeman, 2009; Locke, 2009). To achieve this goal, harbour masters should be taught how to monitor and deal with fouling on floating docks and recreational vessels entering the harbour. Already a simple course may reduce the chance of introductions. For example, recreational vessels, docks and pilings should not be cleaned in situ in summer time, because this will increase the chance that *Didemnum vexillum* (and other fouling organisms) will be able to settle and spread throughout the harbour. Heavily fouled vessels should not be allowed to enter a harbour at all, or should be obliged to clean their hull by surfacing the boat. Subsequently, while cleaning, one should make sure that the waste water with removed fouling organisms does not flow directly back into the harbour, but is treated in a way that ensures that *D. vexillum* colonies are killed (see the next paragraph about eradication methods).

Shellfish can be treated during transport to the Dutch Wadden Sea, to minimize the chance that *Didemnum vexillum* colonies are imported via this vector. Over the last five years various methods have been tested to eliminate *Didemnum* from seed-mussels (Denny, 2008; Piola *et al*., 2010). It turned out that fresh-water immersion for example proved ineffective or impractical against *D. vexillum* (Denny, 2008). In general, dipping or spraying the mussels appeared to be the most effective method, but that is very dependent on the timing, i.e. the duration of the spraying and the duration of the periods in between the spraying events (if repeated). The substance to be used for spraying is also critical of course. For example, fresh water, acetic acid and sodium hypochlorite (bleach) were tested for spraying in various concentrations (Coutts & Forrest, 2007; Denny, 2008; Piola *et al*., 2010). Denny (2008) concluded that dipping the mussels in a 0.5% solution of sodium hypochlorite for 2 minutes was a 100% effective treatment that left seed-mussels themselves relatively unaffected. Piola *et al*. (2010) found that single-spray treatments of 5 % acetic acid reduced the cover of *D. vexillum* by up to 100%, depending upon the exposure duration, while repeated spraying with short exposure periods (1 minute) achieved ~99% mortality. Although less effective than with chemicals, fresh water spraying can also radically reduce the chance of *D. vexillum* colonies surviving a shellfish transport to the Wadden Sea. In case of the Wadden Sea, a combination of spraying and dipping methods will most probably be the most cost effective, starting with for example spraying the mussels in the export area with a seawater/ low bleach concentration, and ending with spraying the mussels with fresh water from the IJsselmeer. Discarding the waste water of the spraying in the IIsselmeer also reduces the chance of *D. vexillum* being introduced into new area because this marine invader will not be able to survive the low salinities there.

In general one should take into consideration where the waste/fouling resulting from cleaning the shellfish during transport to the Wadden Sea, is discarded overboard. It can be done without any risk in the export area itself and in the IJsselmeer, but not anywhere in between, because this may induce the spreading of *Didemnum vexillum* along the Dutch coast into still uninfested areas like the Texelse stenen (see paragraph 5.1). In the heavily infested Georges Bank area along the US Atlantic coast, one of the important vectors that has furthered the spread there, is assumed to be the scallop fisheries. Fishermen returning from the field were cleaning their scallops on their way back, and discarded fouling, including *D. vexillum* colonies, overboard. It is assumed that these colonies survived and were able to settle in previously uninfested areas (Lengyel et al., 2009).

7.2 Eradication

Specifically aiming at eradicating *Didemnum vexillum*, a series of studies was performed in New Zealand (Coutts & Forrest, 2007; Pannel & Coutts, 2007). Many eradication methods were tried out, like smothering soft-sediment habitats with dredge spoil, smothering rip-rap habitats with geotextile fabric, wrapping wharf piles with plastic, air drying, chlorine dosing and water blasting. Depending on the area and structures that needed to be cleaned, some methods were more and others less effective (Coutts & Forrest, 2007). Much was learned and methods for effectively removing *D. vexillum* were optimized, but still eradication in New Zealand was not very successful because of the slow decision-making and a lack of long-term commitment at the regional level, as was described in the previous paragraph about prevention methods.

In general, all eradication efforts should be conducted in winter time, because *Didemnum vexillum* colonies tend to fragment and reproduce when handled in summer, which will induce their spreading.

The most effective method of eradicating didemnids on vessels and floating docks from a cost-benefit point of view was wrapping these structures in a geotextile fabric or another kind of plastic after which the openings in the plastic wrapping are closed with water tight tape. These wrappings can be left there for a week to a month, until all fouling organisms underneath suffocate and die. To speed up this process one can for example pump fresh water or a concentration of acetic acid into the wrappings (Coutts & Forrest, 2007; Kleeman, 2009; Pannel & Coutts, 2007) . When removing the wrappings, an obnoxious odor will be noticed, which is another reason why this approach should be planned in wintertime, when virtually no tourists are present. The estimated costs for cleaning a vessel or a floating dock segment as described above is about 1,000 Euro.

Another way of eradicating *Didemnum vexillum* from floating docks, is by lifting them out of the water and cleaning them, for example by spraying or drying. For maintenance purposes, including the removing of fouling, floating docks in several harbours along the Dutch coast are periodically cleaned anyway by lifting the docks from the water. In 2009, Joe Freisser, a student of Van Hall/Larenstein interviewed 53 Dutch harbour masters and/or owners and managers of hard artificial structures like floating docks along the Dutch coast, to make an inventory of the economical costs that are related to fouling organisms in general, and fouling non-native species in particular. This project was done for the companies GiMaRIS and MatureDevelopment. It turned out that the floating docks in the harbour of Terschelling are not regularly lifted out of the water for maintenance, while those in the harbour of Breskens and in the Wadden Sea harbour of Ameland are. In Ameland the pontoons are cleaned every year in November. For that purpose they are hauled from the water and cleaned by scraping off the fouling. The porous or leaking polyester pontoons are repaired and placed back in spring. In the harbour of Ameland this costs about 3.500 Euro yearly. Assuming that the cleaning costs are similar, the costs of cleaning the pontoons in the harbour of Terschelling is estimated to be about 10.500 Euro. Much depends however on the facilities that are available at a harbour and what kind of pontoon maintenance is followed. In the harbour of Breskens, the iron floating docks are taken out of the water about every 10 years by a crane, after which they are scraped, sand blasted and repainted before being placed in the water again. The costs related to a similar treatment of the pontoons in the harbour of Terschelling are estimated to be about 90,000 Euro.

The floating docks in the harbour of Terschelling differ from those in Ameland and Breskens in various ways by construction and material. It depends on the local conditions and possibilities in Terschelling, which of the methods to eradicate *Didemnum vexillum* will be most effective from a cost-benefit point of view.

Depending on the extent of the settlement and its expansion, it may be decided how *D. vexillum* can be eradicated from the tide pools at the jetty of Terschelling. Because of the restriction to these tide pools, the colonies can be eradicated in various ways, including hand-picking at low tide, and potentially spraying with for example fresh water.

7.3 Control

Controlling a population of *Didemnum vexillum* is not considered a realistic option. Some research has been done focusing on finding a predator that could act as a biological control species for *D. vexillum*, like the snail *Littorina littorea*, but this was eventually not considered to be an option (Carman *et al*., 2009a). In general the predators of *D. vexillum* eat too little of the colonies to have any effect on the population as a whole (Gittenberger, 2007).

7.4 Proposed management for the Dutch Wadden Sea

As was also concluded by Kleeman (2009) for the *Didemnum vexillum* infestation in 2008 in Holyhead marina, N Wales, Great Britain, which is a harbour like the one in Terschelling, there is a feasible option to eradicate *D. vexillum* and stop its spread within the Dutch Wadden Sea, as long as no time is wasted. Many examples worldwide show that waiting to act in the early stages of the infestation of *D. vexillum* will radically reduce the chances of a successful eradication . The Invasive Species Environmental Impact Assessment (paragraph 6.4) and the Leung & Dudgeon invasion risk analysis (paragraph 6.5) also indicate the high risk of *D. vexillum* if neglected as an invader to the Dutch Wadden Sea.

The management actions that are proposed below, are based on the results of this risk assessment. They are very similar to those proposed by Kleeman (2009) for Holyhead marina, Great Britain:

1. Monitor the distribution of *Didemnum vexillum* in the Dutch Wadden Sea

Timing: August/September 2010 (to be repeated in 2011 and 2012 to monitor spread and success of management efforts)

Action: Assessment of the presence of *Didemnum vexillum* in and in the proximity of Terschelling harbour, and in other high risk areas of the Dutch Wadden Sea, like other harbours with many recreational vessels and mussel beds south of Terschelling (Chapter 5; Figs. 16-17). The methods used for this assessment can be similar to those used during the Wadden Sea Survey in July/August 2009 (Gittenberger *et al*., 2009).

Duration: 1 week

Estimated costs: 6,000 Euro (excluding the potential costs of a boat with mussel dredge)

2. Course focused on reducing the risk of fouling, aimed at instructing harbour masters and boat-owners

Timing: August-October 2010 (to be repeated at regular intervals in the following years)

Action: Harbour masters and/or boat owners are made aware of the risks associated with fouling organisms, ways to minimize this risk, ways to minimize the amount of fouling on the pontoons and their boats, and ways to clean fouling from a boat or a pontoon from a cost-benefit point of view. Because the optimal methods may depend on local conditions, the one-day course should be given in the various harbours in the Wadden Sea separately.

Duration: 2 weeks (including travelling in between the harbours, and preparing the course).

Estimated costs: 15,000 Euro

3. Restrictions to be set in combination with a course focusing on reducing the risk of shellfish transports

Timing: August 2010

Action: Fishermen transporting shellfish to the Dutch Wadden Sea are advised on potential effective mitigation methods, which will minimize the risk that *Didemnum vexillum* (and other invasive species) are introduced to the Dutch Wadden Sea. The responsible ministry and/or local water manager sets restrictions, which will oblige that effective mitigation techniques are used that minimize the risk that invasive species are introduced by this vector. Fishermen should also be made aware of the high risk of discarding fouling organisms overboard during their trip to the Wadden Sea.

Duration: 2 weeks (including preparing the course; the 1-2 day course is repeated to a variety of audiences including fishermen and interested/involved politicians and officials).

Estimated costs: 15,000 Euro

4. Eradicating *Didemnum vexillum* from the Wadden Sea

Timing: November-March 2010

Action: Depending on the results of the assessment of *Didemnum vexillum* in the Dutch Wadden Sea in August/September, it is decided whether eradication at this stage is still feasible from a cost-benefit point of view. The duration and estimated costs indicated below are based on the assumption that *D. vexillum* is only present in the harbour of Terschelling. It is not unlikely that the population on the mussel beds south of Terschelling was already eradicated by the relatively cold winter of 2009-2010.

Duration: 2 months (including preparing the eradication , wrapping the docks in the water or lifting the floating docks out of the water)

Estimated costs: 10,000 - 50,000 Euro, strongly depending on local conditions and possibilities in Terschelling harbour and the extend of the *D. vexillum* population spread.

5. Evaluation of the eradication effort in November-March 2010

Timing: August/September 2011

Action: See Action 1: “Assess the distribution of *Didemnum vexillum* in the Dutch Wadden Sea”.

Duration: 1 week

Estimated costs: 6,000 Euro (excluding the potential cost of a boat with mussel dredge)

8. Acknowledgements

Joe Freijser, a Coastal Zone Management student of Van Hall Larenstein and Paul van der Heijden, MBA, of Maturedevelopment are thanked for their efforts concerning the assessment of the economical costs associated to fouling organisms on artificial structures along the Dutch coast. Prof. Dr. Edi Gittenberger of NCB Naturalis and Leiden University is thanked for checking and editing the text.

9. References

Branquart, E., 2009. Guidelines for environmental impact assessment and list classification of non-native organisms in Belgium. Version 2.6 (07/12/2009): 4 pp. ISEIA guidelines, Harmonia information system (version 2.5).

Bullard, S.G., Lambert, G., Carman, M.R., Byrnes, J., Whitlatch, R.B., Ruiz, G., Miller, R.J., Harris, L., Valentine, P.C., Collie, J.S., Pederson, J., McNaught, D.C., Cohen, A.N., Asch, R.G., Dijkstra, J. & K. Heinonen, 2007a. The colonial ascidian *Didemnum* sp. A: Current distribution, basic biology and potential threat to marine communities of the northeast and west coasts of North America. Journal of Experimental Marine Biology and Ecology 342: 99-108.

Bullard, S.G., Sedlack, B., Reinhardt, J.F., Litty, C., Gareau, K. & R.B. Whitlach, 2007b. Fragmentation of colonial ascidians: Differences in reattachment capability among species. Journal of Experimental Marine Biology and Ecology 342: 166-168.

Bullard, S.G. & R.B. Whitlatch, 2009. In situ growth of the colonial ascidian *Didemnum vexillum* under different environmental conditions. Aquatic Invasions 4(1): 275-278.

Carman, M.R., Bullard, S.G. & J.P. Donnelly, 2007. Water quality, nitrogen pollution, and ascidian diversity in coastal waters of southern Massachusetts, USA. Journal of Experimental Marine Biology and Ecology 342: 175-178.

Carman, M.R., Allen, H.M. & M.C. Tyrrell, 2009a. Limited value of the common periwinkle snail *Littorina littorea* as a biological control for the invasive tunicate *Didemnum vexillum*. Aquatic Invasions 4(1): 291-294.

Carman, M.R., Hoagland, K.E., Green-Beach, E., & D.W. Grunden, 2009b. Tunicate faunas of two North Atlantic-New England islands: Martha's Vineyard, Massachusetts and Block Island, Rhode Island. Aquatic invasions 4(1): 65-70.

Common Wadden Sea Secretariat, 2008. The Wadden Sea. Waddenzee – Wattenmeer. Nomination of the Dutch-German Wadden Sea as World Heritage Site. Volume One. CWSS, Wilhelmshaven: 1191 pp.

Coutts, A., 2002. A biosecurity investigation of a barge in the Marlborough Sounds. Cawthron Report No. 744.

Coutts, A.D.M. & B.M. Forrest, 2007. Development and application of tools for incursion response: Lessons learned from the management of the fouling pest *Didemnum vexillum.*

Daley, B.A. & D. Scavia, 2008. An integrated assessment of the continued spread and potential impacts of the colonial ascidian, *Didemnum* sp. A, in U.S. waters. NOAA Technical Memorandum NOS NCCOS 78: 61 pp.

Daniel, K.S. & T.W. Therriault, 2007. Biological Synopsis of the Invasive Tunicate *Didemnum* sp. Canadian Manuscript Report of Fisheries and Aquatic Sciences 2788: 53 pp.

Dehal, P., Satou, Y., Campbell, R.K., Chapman, J., Degnan, B., De Tomaso, A., Davidson, B., Di Gregorio, A., Gelpke, M., Goodstein, D.M., Harafuji, N., Hastings, K.E., Ho, I., Hotta, K., Huang, W., Kawashima, T., Lemaire, P., Martinez, D., Meinertzhagen, I.A., Necula, S., Nonaka, M., Putnam, N., Rash, S., Saiga, H., Satake, M., Terry, A., Yamada, L., Wang, H.G., Awazu, S., Azumi, K., Boore, J., Branno, M., Chin-Bow, S., DeSantis, R., Doyle, S., Francino, P., Keys, D.N., Haga, S., Hayashi, H., Hino, K., Imai, K.S., Inaba, K., Kano, S., Kobayashi, K., Kobayashi, M., Lee, B.I., Makabe, K.W., Manohar, C., Matassi, G., Medina, M., Mochizuki, Y., Mount, S., Morishita, T., Miura, S., Nakayama, A., Nishizaka, S., Nomoto, H., Ohta, F., Oishi, K., Rigoutsos, I., Sano, M., Sasaki, A., Sasakura,

Y., Shoguchi, E., Shin-i, T., Spagnuolo, A., Stainier, D., Suzuki, M.M., Tassy, O., Takatori, N., Tokuoka, M., Yagi, K., Yoshizaki, F., Wada, S., Zhang, C., Hyatt, P.D., Larimer, F., Detter, C., Doggett, N., Glavina, T., Hawkins ,T., Richardson, P., Lucas, S., Kohara, Y., Levine, M., Satoh, N. & D.S. Rokhsar, 2002. The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. Science 298 (5601): 2157-2167.

Denny, C.M., 2008. Development of a method to reduce the spread of the ascidian *Didemnum vexillum* with aquaculture transfers. ICES Journal of Marine Science 65, advance access published 8April 8, 2008: 6 pp.

Dijkstra, J., Sherman, H. & L.G. Harris, 2007b. The role of colonial ascidians in altering biodiversity in marine fouling communities. Journal of Experimental Marine Biology and Ecology 342: 169-171.

Fey, F., Brink, A.M. van den, Wijsman, J.W.M. & O.G. Bos, 2010. Risk assessment on the possible introduction of three predatory snails (*Ocinebrellus inornatus*, *Urosalpinx cinerea* , *Rapana venosa*) in the Dutch Wadden Sea. IMARES Report C032/10: 88 pp.

Gittenberger, A., 2007. Recent population expansions of non-native ascidians in The Netherlands. Journal of Experimental Marine Biology and Ecology 342: 122-126.

Gittenberger, A., 2009. Invasive tunicates on Zeeland and Prince Edward Island mussels, and management practices in The Netherlands. Aquatic Invasions 4(1): 279-281.

Gittenberger, A. & R.J. Leewis, 2008. Validatie van de methodiek voor de bepaling van kwetsbaarheid van watertypen voor exoten. TPS report nr. E002/08. GiMaRIS report nr. 2008.14: 45 pp. Ministry of Transportation and Water Management, Den Haag, The Netherlands.

Gittenberger, A., Rensing, M., Stegenga, H. & B.W. Hoeksema, 2009. Inventarisatie van de aan hard substraat gerelateerde macroflora en macrofauna in de Nederlandse Waddenzee. GiMaRIS report 2009.11: 63 pp.

Gittenberger, A., Rensing, M., Stegenga, H. & B.W. Hoeksema, in press. Native and non-native species of hard substrata in the Dutch Wadden Sea. Nederlandse Faunistische Mededelingen.

Goud, J. & E. Gittenberger, 2004. Live *Lamellaria* (Gastropoda,Lamellariidae) in The Netherlands. Vita Malacologica 2: 60.

Griffith K., Mowat S., Holt R.H.F., Ramsay K., Bishop J.D.D., Lambert G. & S.R. Jenkins. 2009. First records in Great Britain of the invasive colonial ascidian *Didemnum vexillum* Kott, 2002. Aquatic Invasions 4(4): 581-590.

Herborg, L.M., O'Hara, P. & T.W. Therriault, 2009. Forecasting the potential distribution of the invasive tunicate *Didemnum vexillum*. Journal of Applied Ecology 46: 64-72.

Hess, J.E., Swalla, B.J. & P. Moran, 2009. New molecular markers to genetically differentiate populations of *Didemnum vexillum* (Kott, 2002) – an invasive ascidian species. Aquatic Invasions 4(2): 299-310.

Holsteijn, H., 2004. A cowrie, *Trivia arctica* (Gastropoda, Triviidae), in the Dutch delta. Vita Malacologica 2: 61–62.

Kleeman, S.N., 2009. *Didemnum vexillum* – Feasibility of Eradication and/or Control. CCW Contract Science report No. 875: 51 pp.

De Kluijver, M., Leewis, R., Ingalsuo, S., Gijswijt, G., 1999. De Interactieve Duikgids, Nederland onder water gezien. Cd-rom. Expert Center for Taxonomic Identification. Amsterdam.

Kott, P., 2002. A complex didemnid ascidian from Whangamata, New Zealand. Journal Marine Biological Association UK 82: 625-628.

Lambert, G., 2007. Invasive sea squirts: A growing global problem. Journal of Experimental Marine Biology and Ecology 342: 3-4.

Lambert, G., 2009. Adventures of a sea squirt sleuth: unraveling the identity of *Didemnum vexillum*, a global ascidian invader. Aquatic Invasions 4(1): 5-28.

Leeuwen, S.J. van, Bogaardt, M.-J. & F.G. Wortelboer, 2008. Noordzee en Waddenzee: natuur en beleid. Achtergrondrapport bij de Natuurbalans 2008. PBL Rapport 500402013/2008: 150 pp. Planbureau voor de Leefomgeving (PBL), Bilthoven/Den Haag.

Leewis, R.J. & A. Gittenberger, 2008. Geoptimaliseerde methodiek: Kwetsbaarheid van watertypen voor exoten. TPS report nr. E003/08. GiMaRIS report nr. 2008.15: 10 pp. Ministry of Transportation and Water Management, Den Haag, The Netherlands.

Lengyel, N.L., Collie, J.S. & P.C. Valentine, 2009. The invasive colonial ascidian *Didemnum vexillum* on Georges Bank — Ecological effects and genetic identification. Aquatic Invasions 4(1): 143-152.

Leung, K.M.Y. & D. Dudgeon, 2008. Ecological risk assessment and management of exotic organisms associated with aquaculture activities. In M.G. Bondad-Reantaso, J.R. Arthur and R.P. Subasinghe (eds). Understanding and applying risk analysis in aquaculture. FAO Fisheries and Aquaculture Technical Paper. No. 519. Rome, FAO. pp. 67–100.

Locke, A., 2009. A screening procedure for potential tunicate invaders of Atlantic Canada. Aquatic Invasions 4(1): 71-79.

Locke, A. & M. Carman, 2009. An overview of the 2nd International Invasive Sea Squirt Conference: What we learned. Aquatic Invasions 4(1): 1-4.

Locke, A. & J.M. Hanson, 2009. Rapid response to non-indigenous species. 1. Goals and history of rapid response in the marine environment. Aquatic Invasions 4(1): 237-247.

McCarthy, A., Osman, R.W. & R.B. Whitlach, 2007. Effects of temperature on growth rates of colonial ascidians: A comparison of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus*. Journal of Experimental Marine Biology and Ecology 342: 172-174.

Mercer, J.M., Whitlatch, R.B. & R.W. Osman, 2009. Potential effects of the invasive colonial ascidian (*Didemnum vexillum* Kott, 2002) on pebble-cobble bottom habitats in Long Island Sound, USA. Aquatic Invasions 4(1): 133-142.

Minchin. D. & E. Sides, 2006. Appearance of a cryptogenic tunicate, a *Didemnum* sp. fouling marina pontoons and leisure craft in Ireland. Aquatic Invasions 1(3): 143-147.

Minchin, D., 2007. Rapid coastal survey for targeted alien species associated with floating pontoons in Ireland. Aquatic Invasions 2(1): 63-70.

Morris, J.A., Carman, M.R., Hoagland, K.E., Green-Beach, E.R.M. & R.C. Karney, 2009. Impact of the invasive colonial tunicate *Didemnum vexillum* on the recruitment of the bay scallop (*Argopecten irradians irradians*) and implications for recruitment of the sea scallop (*Placopecten magellanicus*) on Georges Bank. Aquatic Invasions 4(1): 207-211.

Osman, R.W. & R.B. Whitlatch, 2007. Variation in the ability of *Didemnum* sp. to invade established communities. Journal of Experimental Marine Biology and Ecology 342: 40-53.

Pannell, A. &A.D.M. Coutts, 2007. Treatment methods used to manage *Didemnum vexillum* in New Zealand. Prepared for Biosecurity New Zealand: 29 pp., 3 Appendices.

Pederson, J., Bullock, R., Carlton, J., Dijkstra, J., Dobroski, N., Dyrynda, P., Fisher, R., Harris, L., Niels, H., Lambert, G., Lazo-Wasem, E., Mathieson, A., Miglietta, M.P., Smith, J. & M. Tyrrell, 2005 Marine invaders in the Northeast: Rapid assessment survey of non-native and native marine species of floating dock communities, August 2003. MIT Sea Grant College Program.

Piola, R.F., Dunmore, R.A. & B.M. Forrest, 2010. Assessing the efficacy of spray-delivered 'eco-friendly' chemicals for the control and eradication of marine fouling pests. Marine Ecology Progress Series 398: 69-80.

Price, A., Collie, J. & D. Smith, 2005. 18S ribosomal RNA and cytochrome oxidase gene sequences of *Didemnum* sp., an invasive colonial tunicate. Papers from the summer undergraduate research fellowship program in oceanography at the university of Rhode Island. Graduate School of Oceanography and Department of Ocean Engineering: 46-53.

Sewell, J., Pearse, S., Bishop, J. & J.L. Evans, 2008. Investigations to determine the potential risk for certain non-native species to be introduced to North Wales with mussel seed dredged from wild seed beds. CCW Science Report 835: 88pp.

Small, K.S., Brudno, M., Hill, M.M. & A. Sidow, 2007. A haplome alignment and reference sequence of the highly polymorphic *Ciona savignyi* genome. Genome Biology 8(3): R41.

Stefaniak, L., Lambert, G., Gittenberger, A., Zhang, H., Lin, S. & R.B. Whitlach, 2009. Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. Aquatic invasions 4(1): 29-44.

Steward-Clark, S.E., Siah, A., Greenwood, S.J., Davidson, J. & F.C.J. Berthe, 2009. Development of 18S rDNA and COI gene primers for the identification of invasive tunicate species in water samples. Aquatic Invasions 4(4): 575-580.

Tyrrell, M.C. & J.E. Byers, 2007. Do artificial substrates favor nonindigenous fouling species over native species? Journal of Experimental Marine Biology and Ecology 342: 54-60.

Valentine, P.C., Carman, M.R., Blackwood, D.S. & E.J. Heffron, 2007a. Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat. Journal of Experimental Marine Biology and Ecology 342: 109-121.

Valentine, P.C., Collie, J.S., Reid, R.N., Asch, R.G., Guida, V.G. & D.S. Blackwood, 2007b. The occurrence of the colonial ascidian *Didemnum* sp. on Georges Bank gravel habitat – Ecological observations and potential effects on groundfish and scallop fisheries. Journal of Experimental Marine Biology and Ecology 342: 179-181.

Valentine, P.C., Carman, M.R., Dijkstra, J. & D.S. Blackwood, 2009. Larval recruitment of the invasive colonial ascidian *Didemnum vexillum*, seasonal water temperatures in New England coastal and offshore waters, and implications for spread of the species. Aquatic Invasions 4(1): 153-168.

Vazques, E. & C.M. Young, 2000. Effects of low salinity on metamorphosis in estuarine colonial ascidians. Invertebrate Biology 119: 433-444.