High risk exotic species with respect to shellfish transports from the Oosterschelde to the Wadden Sea

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Contents

Summary

This study presents the results of a literature review on 11 exotic marine species that are present in the Oosterschelde and could potentially be introduced into the Wadden Sea with shellfish transfers. Ten of the species result from a previous risk study, where they were identified as the species posing the highest risk. The Japanese oyster drill, that was recently observed in the Oosterschelde was also included in this study. The species discussed in the present study are: colonial tunicates *Didemnum* sp and *Botrylloides violaceus* the American oyster drill (*Urosalpinx cinerea*) and the Japanese oysterdrill (*Ocinebrellus inornatus*), the copepods *Myticola orientalis* and *M. ostreae*, the colonial bryozoan *Smittoidea prolifica*, *Marteilia refringens* and the macroalgae *Gracilaria vermiculophylla*, *Polysiphonia senticulosa* and *Undaria pinnatifida.* Four of these species (*Gracilaria vermiculophylla*, *Undaria pinnatifida*, *Didemnum vexillum* and *Botrylloides violaceus*) have recently been observed in the Wadden Sea.

Some of these species are known pest species and could have an impact on the Wadden Sea ecosystem and/or the shellfish culture. The information that is collected in this study can be used to get a more realistic estimation of the risks compared to the risk assessment study of 2008 which was based on a worst-case approach. Moreover, the results can be used in the development of mitigating measures to reduce the risks of introducing these exotic species with the shellfish transfer from the Oosterschelde to the Wadden Sea.

1 Introduction

The importing of shellfish from other countries into the Netherlands has resulted in the introduction of various exotic organisms into the Dutch coastal and marine waters and the Dutch aquaculture industry (Wolff 2005, Wijsman & Smaal 2006). Some of these exotic organisms have become established and some of these have had negative effects on the shellfish industry in various ways.

Investigations are being carried out concerning the feasibility and risks involved in the transportation of mussels from the Oosterschelde to the Wadden Sea (Wijsman & De Mesel 2009). As the Oosterschelde is known to contain more exotic species than the Wadden Sea (Wolff 2005, Gittenberger 2009b, Gittenberger et al. 2009, Wijsman & De Mesel 2009), there is a risk that harmful, exotic organisms present in the Oosterschelde may be transported with the mussels into the Wadden Sea, and thus affect the aquaculture industry there.

In 2008 the Ministry of LNV commissioned Wageningen IMARES to conduct a risk study on the introduction of exotic species with shellfish transports from the Oosterschelde and Voordelta to the Wadden Sea (Wijsman & De Mesel 2009). In this study an overview is given of the exotic species present in the Oosterschelde, but not yet in the Wadden Sea. Species experts then assigned 'risk scores' to each, according to the organisms ability to spread, become established and have negative impacts on the ecosystem (Wijsman & De Mesel 2008). A worst-case approach was used in this study. This means that in case of uncertainty or lack of information, the safe side (highest risk) was applied. Ten organisms had risk scores above 2.1, and were considered to have the highest potential of being introduced to the Wadden Sea with the transport of mussels (Wijsman & De Mesel 2008).

Recently, the lists of exotic species in the Wadden Sea has been updated with the results from a specific monitoring campaign (Gittenberger *et al.* 2009). Also the list of exotic species in the Oosterschelde has been updated with recent information (Gittenberger 2009b). The Japanese oyster drill (*Ocinebrellus inornatus*), one of the new exotic species that was observed in 2009 in the Oosterschelde (Faasse & Ligthart 2009), can pose a risk that is comparable to the risk of the American oyster drill (*Urosalpinx cinerea*).

Following the previous study, LNV commissioned Wageningen IMARES to produce a report providing more information on those species of highest risk. This report provides the details of the 10 high risk organisms from the risk study of Wijsman and de Mesel (2008), supplemented with the new exotic species *Ocinebrellus inornatus*. For each of the species a description is given on biology, distribution and spread, impact and control methods. The results of this study can be used to get a more realistic estimation of the risks of these 11 species compared to Wijsman and de Mesel (2008), which was based on a worst-case approach.

2   Materials and Methods

For the 10 high risk organisms, as identified by Wijsman and De Mesel (2009), supplemented with the Japanese oyster drill, a literature study was done to collect information for each species including taxonomy, biology, life history, distribution and spread, impact and control methods. The eleven species are:

1. *Didemnum vexillum*
2. *Botrylloides violaceus*
3. *Mytilicola orientalis*
4. *Myicola ostreae*
5. *Smittoidea prolifica*
6. *Marteilia refringens*
7. *Gracilaria vermiculophylla*
8. *Polysiphonia senticulosa*
9. *Undaria pinnatifida*
10. *Urosalpinx cinerea*
11. *Ocinebrellus inornatus*

A search for literature was done using on-line databases including ISI Web of Science (www.apps.isiknowledge.com), The Global Invasive Species Database (http://www.issg.org), the World Register of Marine Species (http://www.marinespecies.org/) and the Animal Diversity Web (http://animaldiversity.ummz.umich.edu/site/index.html). Other resources used included published journal articles, online articles and information websites.

Information from these resources was collated to produce an overview of each organism. In cases where details on a specific species name was limited, information was sought using a synonym if possible. Where information was still limited, or no synonym was known, details of other species from the same genus with probable similarities was used (this is mentioned in the text).

3   Results

An overview of the 11 organisms with a high risk potential for transportation from the Oosterschelde to the Wadden Sea follows below.

3.1   *Didemnum vexillum* Kott, 2002

Common names: ascidian, colonial tunicate, sea squirt
Risk score: 3.2 (Wijsman and De Mesel 2008)

Figure 1   *Didemnum vexillum* growing below the Zeelandbrug in the Oosterschelde (Image: A. Gittenberger, Gittenberger 2010) .

Phylum: Chordata
Subphylum: Tunicata
Class: Ascidiacea
Order: Aplousobranchia
Family: Didemnidae

Didemnum sp. is the general name given to a wide variety of species in this genus. Identification to species level is extremely difficult and impossible in the field because many morphological characters are difficult to study due to the small size of zooids, larvae, and spicules. Until recently, poor preservation techniques, inadequate sampling, both intra- and inter- colony variation, and even reproductive status of the colony at the time of sampling can obscure or remove important identifying characters. Precise identification is only possible through microscopic examination of internal body parts, molecular and genetic analysis (Kott 2002, Cohen 2005). With these new techniques, re-examination of previously identified samples have recently shown that various species described throughout the world, are in fact the same species (Kott 2002).

The species present in the Netherlands has been identified as *Didemnum vexillum* (Lambert 2009, Stefaniak et al. 2009).

3.1.1 Description and Biology

The genus *Didemnum* is distinguished from other ascidians by its distinctively lobed form. Within the genus there is a variety of morphologies including long, cylindrical rope- or beard- like colonies, often found hanging from hard man-made structures such as docks, ship hulls and lines, bulbous or branching structures on the seabed and more compact sheets encrusting rocks and other hard substrate, including itself. The colonies are often tan, cream, yellow, orange or pink in colour and can reach as much as a metre in length (Cohen 2005, Gittenberger 2010). Didemnids have also been shown to posses highly acidic surface tissue which acts as a chemical defence (Pisut & Pawlik 2002).

A colony of *Didemnum* consists of numerous individual zooids, each about 1 mm in length, embedded in a sheet-like matrix. Each zooid filters nutrients from the water and contributes waste products to be collectively expelled by the colony (Cohen 2005). As larvae, *Didemnum* sp. spend a few hours in the plankton before attaching head down to a firm surface and metamorphosing into the initial zooid of a new colony. *Didemnum* sp. are also able to reproduce whole colonies from broken reproductively active fragments that are transported to new locations. (Lambert 2002, Cohen 2005). Only a few weeks are required for *Didemnum* sp. to reach sexual maturity, and they have long breeding seasons. They easily tolerate and adapt to variations in temperature (surviving -2 to +24° C) and salinity as well as high levels of toxins (Cohen 2005, Global-Invasive-Species-Database 2005, Morris *et al.* 2009).

3.1.2 Distribution

Didemnum sp. grow in subtidal habitats such as bays, harbours and coastal waters, primarily on hard surfaces like rocks, gravel, all kinds of artificial structures and other organisms such as mussels, oysters, hydroids and tunicates (Cohen 2005, Global-Invasive-Species-Database 2005, Gittenberger 2010). While the native range of this genus is unknown, it has been reported as invasive globally from northern Europe (Ireland, Netherlands, France), the U.S. east coast (New Jersey, New York, Connecticut, Rhode Island, Massachusetts, New Hampshire, Maine), offshore of New England on Georges Bank, the U.S. and Canadian west coasts (California, Washington, British Columbia), Japan, and New Zealand (Cranfield et al. 1998, Cohen 2005, Gittenberger 2010). *Didemnum* sp. is gradually spreading to various temperate and tropical regions of the world. The reasons for this species sudden invasiveness are currently unknown. (Lambert 2002).

Since its first recorded presence in Georges Bank, New England, U.S.A in 1988, the population of *Didemnum vexillum* has increased substantially in the surrounding tidal lagoons and estuaries. The species is thought to have arrived in the area via international shipping, local boat traffic, and/or shellfish imports (Morris *et al.* 2009). During surveys of Georges Bank, *Didemnum* species were found covering large areas of the seabed. Colonies formed large mats on immobile pebble and cobble substrate 40 to 65 m deep (Kelly & Maguire 2008). Morris *et al.* (2009) found that larval bay scallops avoided settling on *D. vexillum* colonies, and suggested this may be due to the low pH of the ascidian's surface tissue. They further suggested that *D. vexillum* affects the recruitment of scallops by reducing the available and appropriate space for settlement.

In October 2001, an ascidian, later identified as *Didemnum vexillum* was recorded for the first time in Shakespeare Bay, New Zealand, after arriving on a heavily fouled ship's hull. As Shakespeare Bay is only 500 km from the heart of the New Zealand mussel industry, the arrival of *D. vexillum* was considered a major threat to the industry due to its ability to successfully invade areas with artificial structures and out-compete and smother mussels. The local councils agreed to investigate management options to restrict the spread of *D. vexillum* in the area. They discovered that *D. vexillum* had spread naturally from the ship's hull to local artificial structures in the bay such as barges, recreational vessels, moorings, salmon cages and wharf piles. *D. vexillum* was then accidentally translocated to aquaculture areas through the transport of salmon cages, and this initiated plans for eradication attempts. By early 2006, however, *D. vexillum* had spread from the salmon cages to an adjacent mussel farm and had devastating effects, increasing mussel mortality, preventing mussel spat from settling, and increasing the costs of equipment maintenance (Pannell & Coutts 2007).

Didemnum vexillum. was first officially reported in the Netherlands in 1991 as *Didemnum* sp. In 1998-1999, it rapidly increased and became very common in Lake Grevelingen and the Oosterschelde, spreading over much of the available hard substratum. Colonies expand quickly during the summer months, but generally die back over

winter (December and January), and are replaced by other organisms (ascidians, sponges, sea anemones) which settle before spring (Gittenberger 2010). *Didemnum vexillum* colonies grow larger and are more successful in Lake Grevelingen compared to the Oosterschelde. They are most common at depths between 3 and 12 meters in Lake Grevelingen and from just below low water to 14 m in the Oosterschelde (Gittenberger 2010).

Didemnum sp. has expanded significantly in the Netherlands during recent years, and now outcompetes many native organisms for space (Gittenberger 2007). The mussel industry in the Netherlands has also been affected by *Didemnum* sp., which is now commonly found fouling mussel lines (Gittenberger 2009c). Recently (2008) *Didemnum vexillum* was also observed in the Wadden Sea near Terschelling (pers. Comm. Rob Dekker, NIOZ). During a specific survey in the Wadden Sea the species was found on a jetty in the harbor of Terschelling (Gittenberger *et al.* 2009).

3.1.3   Vectors of translocation

The pelagic stage of the lifecycle of *Didemnum* sp. is generally less than a few hours and therefore, long-distance, natural dispersal is limited. However, as new colonies can grow from detached fragments, and these may be dispersed by tides and currents, colonies are easily dispersed via transportation of material associated with aquaculture, vessel traffic and fishing activities (Bower 2002, Lambert 2002, Kelly & Maguire 2008).

3.1.4   Impact

The rapid spread of *Didemnum vexillum*. colonies alters marine habitats as they overgrow organisms such as tunicates, sponges, macroalgae, hydroids, anemones, bryozoans, scallops, mussels, oysters, seaweeds, limpets, barnacles, and other species of sea squirts. The colonies are likely to smother the siphons of infaunal and bottom-dwelling shellfish and may cover grounds needed by fish to lay eggs. (Cohen 2005, Gittenberger 2010).

Colonies of *Didemnum* have been reported as nuisance species throughout the world due to their rapid growth and spread and their ability to foul marine habitats, ship hulls and structures associated with shellfish aquacultures and fishing grounds (Cohen 2005, Global-Invasive-Species-Database 2005). Kelly & Maguire (2008) reported a significant link between artificial structures and local spread. They suggest that these structures provide reservoirs for its incursion to the seabed habitats.

3.1.5   Control

There are no recorded cases of completely successful control or eradication of invasive *Didemnum* species (Kelly & Maguire 2008). In Shakespeare Bay, New Zealand novel methods were developed for treating various substrata to control the spread or completely eliminate *D. vexillum* before the spawning period. These methods included smothering soft-sediment habitats with uncontaminated dredge spoil, wrapping wharf piles with plastic, smothering rip-rap habitats using a geotextile fabric, and various other approaches based on water blasting, air drying or chlorine dosing. Many of these methods were successful in eradicating *D. vexillum* from the substrata, however the program failed to fully eradicate the organism from the entire region (Pannell & Coutts 2007).

3.2 *Botrylloides violaceus* Oka, 1927

Common names: gewone slingerzakpijp, violet tunicate, chain sea squirt
Risk score: 2.2 (Wijsman and De Mesel 2008)

Figure 2 *Botrylloides violaceus* over growing a mussel line (Image: Gittenberger 2009c)

Phylum: Chordata
Subphylum: Tunicata
Class: Ascidiacea
Order: Stolidobranchia
Family: Styelidae

3.2.1 Description and Biology

Botrylloides violaceus is a sessile colonial ascidian with a rubbery exterior which forms flat sheets that are usually oval in shape but also occur in meandering, branching, twisted rows or chain-like arrangements. Each colony is usually either dark brown, red, orange, purple or yellow (Snowden 2008). The colonies are generally 2-3 mm thick and can reach up to 300 mm in diameter.

Each colony encompasses numerous individual oval or tear-drop shaped zooids, 1-2 mm long, and arranged in elongated clusters within a firm matrix. Each zooid has 16 small tentacles surrounding an open aperture through which it filter feeds. Waste products are then combined with that of neighbouring zooids and expelled from the colony (Cohen 2005). Networks of transparent blood vessels can often be seen running through the matrix with numerous dead ends (Cohen 2005).

The tunicate can reproduce both sexually and asexually as all species of the *Botrylloides* genus are hermaphrodites, and sexual reproduction can be either viviparous or ovoviviparous. Each zooid contains a brood pouch which contains a single egg when the colony is reproductively active. Larvae are tadpole-shaped and relatively large (1 mm in body diameter) when hatched from these brood pouches. They spend less than a day in the water column before settling and attaching onto a firm surface where they metamorphose into the first zooid of a new colony. Once settled, the larvae may reproduce asexually and bud new zooids to form the colony (Prince-William-Sound-Regional-Citizens'-Advisory-Council 2004, Cohen 2005).

Botrylloides violaceus can be found along marine coastal protected areas in the shallow sub-tidal zones (Prince-William-Sound-Regional-Citizens'-Advisory-Council 2004). It grows on various hard surfaces such as docks, ship hulls, buoys, ropes, pilings, rocks, blades of eel grass (*Zostera marina*), macroalgae and is often found encrusting mussels, barnacles, bryozoans and solitary ascidians (Cohen 2005, Snowden 2008). *B. violaceus* is known to tolerate salinities ranging from 26-34 ppt, and temperatures of 8-25° C (Cohen 2005).

3.2.2   Distribution

The native range of *Botrylloides violaceus* is believed to be Japan. It has since spread to the East and West coast of the United States the Mediterranean Sea (first collected in Venice Lagoon, Italy in 1993); Queensland, Australia (reported in 1985) (Cohen 2005, Gittenberger 2009a). *B. violaceus* was first reported in the Netherlands in 2000 in the Westerschelde near Breskens and has since spread through the Oosterschelde estuary at Yerseke and Wemeldinge (Faasse & De Blauwe 2002, Wolff 2005, Gittenberger 2009a). During a specific survey in the Wadden Sea the species was found at several locations (Gittenberger *et al.* 2009).

3.2.3   Vectors of translocation

Due to its ability to reproduce both sexually and asexually from small fragments, *Botrylloides violaceus* can be introduced to new locations very easily through the release of oyster shells and spat from the oyster industry and biofouling on artificial structures such as ship hulls and fisheries equipment. Colonies can also be transported through rafting on broken leaves and other debris to which they are attached (Prince-William-Sound-Regional-Citizens'-Advisory-Council 2004). It is thought to have been introduced to the Netherlands due to biofouling on a pleasure craft (Wolff 2005).

3.2.4   Impact

As few species are capable of settling and living on colonies of *B. violaceus*,, its presence reduces the available space for other fouling organisms. Their short distance dispersal allows them to quickly invade established communities and become dominant. There have been reports that the ascidian can reduce post-settlement survivorship and growth of oysters, but reports have also shown that the fouling of *B. violaceus* on mussel ropes does not substantially increase the mussel weight and the effect on harvesting is negligible (Prince-William-Sound-Regional-Citizens'-Advisory-Council 2004, Gittenberger 2009c)

3.2.5   Control

In late August 2002, mussel growers in Savage Harbour, Canada reported the arrival of three heavily fouled barges. A diver survey of the ship hulls identified the presence of *Botrylloides violaceus*. The authorities considered the risk of spread of *B. violaceus* high due to their previous experience with invasive tunicates and local aquaculturalists strongly requested its elimination. The authorities considered various options for controlling it, such as bringing the barges onshore, scraping the hulls, and disposing of the removed material in a landfill; or towing the barges offshore over deep water for pressure-washing of the hull. Bringing the barges onshore for cleaning was considered impractical due to the risk of accidental hull scraping while lifting the barges from the water and the high financial cost (estimated CAN$120 000). Instead they opted to tow the barges offshore for cleaning, which was estimated to cost close to CAN$30 000. The barges were thus towed ~2.5 km off shore 1-1.5 weeks after their arrival and power-washed (Locke *et al.* 2009b).

Unfortunately, by the next year, the species had already spread to bivalve aquaculture facilities in the harbour. By late 2004 mussel growers in Savage Harbour were reporting extensive fouling on mussel stocks. *B. violaceus* was reported to have spread to areas such as the Northumberland Strait where mussel spat had been transported from Savage Harbour in 2004. This spread was most likely due to processing, as it originally occurred within the immediate vicinity of the processing plant discharge. By 2005 it had spread further to Seacow Head near Bedeque Bay and was reported to be fouling floating vegetation far offshore and the equipment of lobster fishermen. By 2006 it had spread across Northumberland Strait to Cape Tormentine, New

Brunswick. The authorities decided to attempt to contain the species by restricting the transport of mussels between infested and uninfested areas. They began experimenting with possible control options. These included chemical compounds that had been effective against the invasive clubbed ascidian *Styela clava*, but these proved less successful with *B. violaceus*. They then adopted containment as a temporary control mechanism while more research was conducted, but no known effective methods of control have since been adopted (Locke *et al.* 2009b).

Various control methods have been investigated for colonial tunicates like *Botrylloides violaceus*. These involve the application of chemicals via sprays or immersion and pressure washing ship hulls and equipment. In Prince Edward Island (PEI), Canada, 5 % acetic acid has been found to be effective as a spray treatment for colonial ascidians, but also to increase mussel mortality. This mortality can be reduced between 5-20 % by shaking the mussels to cause them to close their valves, but mortality is still high, and the addition of this acid can affect the local environment (Locke *et al.* 2009a).

Lime has also been used as a biological control for colonial ascidians in aquaculture fisheries. Two forms have been used, quicklime (calcium oxide, produced by heating limestone) and hydrated lime (calcium carbonate, produced by adding water to quicklime). Hydrated lime has been used to control predatory sea stars on mussel spat for years and the method has recently been adapted for the control of colonial tunicates. This method consists of briefly immersing the mussels in a trough filled with a saturated solution of hydrated lime in seawater, or alternatively, as a spray (Locke *et al.* 2009a). The use of these treatments must be considered along with the associated financial and environmental costs and with the known consequences of unmanaged invasive tunicates (Locke *et al.* 2009a).

Pressure washing tends to be the preferred method by many mussel growers in Prince Edward Island due to its low cost and reduced potential impact on the local environment associated with chemical treatments. However, pressure washing is not always feasible in estuaries, and has been shown to be ineffective against the eventual spread of *B. violaceus* in Savage Harbour and surrounding areas due to the species ability to regenerate from small fragments which may resettle after the treatment (Locke *et al.* 2009a, Locke *et al.* 2009b).

3.3 *Mytilicola orientalis* Mori, 1935

Common name: oyster redworm, parasitic copepod
Risk score: 2.8 (Wijsman and De Mesel 2008)

Figure 3.   *Mytilicola* sp. (Image: http://wwz.ifremer.fr/envlit/region/basse_normandie
/activites/conchyliculture/biologie_et_physiologie)

Phylum: Arthropoda
Class: Maxillopoda
Subclass: Copepoda
Order: Poecilostomatoida
Family: Mytilicolidae

3.3.1   Description and Biology

Mytilicola orientalis is a parasitic copepod that infects the stomach and intestines of bivalves such as oysters and mussels. The species is reddish coloured, elongate and shows sexual dimorphism with males growing to approximately 4 mm long and 0.55 mm wide. The male's abdomen is not segmented and the mouth parts have an extra pair of maxillipeds compared to the female. Females are much larger than males and can grow up to 10-12 mm long and 1.33 mm wide. The female's head is separate from its thorax and the first pair of antennae has four segments while the second pair has two. The species has five segments in the thorax and triangular lateral appendices. The genital segment is wider than the abdomen and is fused with the thoracic segments.

Females carry egg sacs that are 7 mm in length and contain about 200 eggs (Grizel 1985, Bower 2002). Hatching is likely to occur within seven days and the nauplius and metanauplius instars to appear on the eighth day (Hockley 1951). The stages of development consist of two naupilii moults and five copepodid stages. During the nauplius and metanauplius stages the larvae are active and swimming in the water column with two pairs of biramous thoracic limbs. The planktonic phase most likely lasts three to four days and the total free-living stage is 10-14 days (Dethlefsen 1985). The first copepodid stage is probably the parasitic stage and at this point the organisms settle into the digestive system of a bivalve where it reaches maturity (Hockley 1951).

The abundance of *M. orientalis* is positively correlated with mussel body size and density. Larger mussels and dense mussel populations from mudflats are likely to harbour larger populations of *M. orientalis* than mussel populations with smaller individuals and in exposed sites (Goater 1996).

Mytilicola orientalis is present in the digestive system of many bivalve species, with the original Asian hosts including the Pacific oyster *Crassostrea gigas*. It can also occur in other oyster species such as *Ostrea edulis*, *Ostrea conchaphila* as well as a wide range of other marine bivalves including mussels, clams and cockles (Grizel 1985, Bower 2002).

3.3.2   Distribution

M. orientalis is native to north-Eastern Asia, particularly Japan and Korea in seed oysters and has spread widely along the west coast of North America (including Canada's west coast) as well as France, Ireland and the Netherlands and possibly other European locations where *C. gigas* spat has been introduced (His 1977, Grizel 1985, Bower 2002). *M. orientalis* was introduced to the Pacific coast of the USA with shipments of *C. gigas* as early as 1938 and to France in the early 1970s (Carlton 1979, Grizel & Heral 1991).

In the 1960s a new policy in Britain and Ireland restricted shellfish and fish imports from unapproved areas, including the oyster growing areas in France. These oysters had originated from wild stocks which had been imported from Japan in the 1970s and *M. orientalis* had been introduced as well by association, although the parasite did not occur in every estuary where *C. gigas* was present, at least until 1977. However, the 1993 EU directive 67/91/EEC, which was designed to improve trade within Europe, did not adequately consider ecological matters when it enabled a wider movement of half-grown *C. gigas* that had been cultivated in France. Following this policy change, *M. orientalis* was introduced into the Netherlands and the south coast of Ireland with imports of half-grown oysters and became established, infecting a range of shellfish including the farmed mussel *Mytilus edulis*. Despite reports of little harm resulting from this introduction, unexplained summer mortalities of oysters were reported during warm summers in Ireland for the first time (Steele & Mulcahy 2001, Leppakoski *et al.* 2002).

3.3.3   Vectors of translocation

With a relatively short pelagic phase and its dependence on bivalve hosts, *Mytilicola orientalis* is restricted in its ability to spread and disperse naturally. However, its spread has been greatly facilitated by the global transport of shellfish in the aquaculture industry, particularly in association with the Pacific oyster *Crassostrea gigas* (Carlton 1979). This is also the most likely vector for its arrival in the Netherlands, although the timing of its arrival into the Oosterschelde is unknown except that it was first reported in 1993 (Stock 1993, Wolff 2005).

As the spread of *Mytilicola orientalis* is primarily dependent on the movement of its hosts rather than natural dispersal, the transport of infected bivalves for aquaculture has caused its spread to new environments. When the population of the native flat oyster *O. edulis* declined and the Portuguese oyster, *Crassostrea angulata*, was devastated by a viral disease in France during the mid 20th century, large scale imports of *C. gigas* were brought in with broodstock coming from British Columbia (Canada) and spat from Japan to replace oyster stocks between 1971 and 1975.

3.3.4   Impact

While some reports suggest that the pathology of *Mytilicola orientalis* is negligible or minimal in most cases (Steele & Mulcahy 2001), the copepod has been shown to affect its bivalve host by altering the morphology of the epithelial lining of the gut and, when present in numbers, it can produce pea-size swellings of the rectum. It has also been reported to cause a loss of condition in its host (Steele & Mulcahy 2001, Bower 2002). Mann (1956) showed that in mussels, there was a 10 to 20 % loss of gonad. Williams (1969) also found that infected mussels had reduced gonad size and possible retarded spawning. However, Steele and Mulcahy (2001) reported that over a two year study there were no effects of *Mytilicola orientalis* on the condition, growth, sex, stage or glycogen content in a population of *Crassostrea gigas* despite 14 % of oysters being infected with up to 20 copepods in a single host (Steele & Mulcahy 2001).

3.3.5 Control

The Pacific oysters that were imported to France during the mid 20th century were subjected to brine dips to kill the organisms attaching to the outside of the shell. This approach, however, was not effective against *Mytilicola orientalis* and other organisms which dwell within the shell of the oyster (Grizel & Heral 1991, Leppakoski *et al.* 2002).

There are currently no known methods of prevention or control for the spread of *Mytilicola orientalis*. Bower (2002) merely suggests that bivalves from areas known to be affected (currently or historically) should not be moved to areas with no record of *M. orientalis*.

3.4 *Myicola ostreae* Hoshina & Sugiura, 1953

Synonym: *Mytilicola ostreae* Wilson C.B., 1938 (unaccepted synonym (Chew *et al.* 1967, Ho & Chad 2009))
Common name: parasitic copepod
Risk score: 2.8 (Wijsman and De Mesel 2008)

Figure 4.   Individual from the family Myicolidae. (Image: (Bower 2002))

Phylum: Arthropoda
Class: Maxillopoda
Subclass: Copepoda
Order: Poecilostomatoida
Family: Myicolidae

The taxonomy of *Myicola ostreae* is somewhat unclear and some records indicate that the species is of the genus Mytilicola while others even suggest it is identical to *Mytilicola orientalis* (Chew *et al.* 1967, Ho & Chad 2009). As the latest records of this species refer to it as *Myicola ostreae* (Ho & Chad 2009), the same will be done in this report.

3.4.1   Description and Biology

Myicola ostreae is a parasitic copepod which infects the gills of various bivalve species. The copepod is elongate and females grow to approximately 1.7 mm in length and carry a pair of multiseriate egg sacs containing about 105 eggs, each of which measures around 135µm in diameter (Ho & Kim 1992, Poulin 1995). Myicolids can attach themselves strongly to the gills of their host to the point where removal of them is virtually impossible without causing damage to the host. (Bower 2002).

Little is known about the biology of *Myicola ostreae*, but it is presumed to share many of its features with other members of the Myicolidae family, which go through a relatively short pelagic phase with six free-swimming

nauplial stages and five parasitic copepodid stages prior to maturing to the adult stage (Batista *et al.* 2009). The first copepodid stage is the infective stage where individuals search for a suitable host and attach themselves to the branchial chamber inside the mantle. The specific infestation mechanism of *M. ostreae* is currently unknown, but it is likely to be similar to other parasitic copepods of bivalves in which the free-swimming copepodid enters the host through by inhalant current.

Myicola ostreae lives attached to the gill inside the branchial chamber of various bivalve species, particularly the oysters *Crassostrea virginica*, *C. gigas*, *C. angulata*, the European flat oyster *Ostrea edulis*, *Ostrea tulipa*, the razor clam *Sinonovacula constricta* and other species of bivalves (Bower 2002, Batista *et al.* 2009). Batista et. al. (2009) reported a difference in preference of *M. ostreae* between two hosts, *C. gigas* and *C. angulata* and their hybrids. They found that *M. ostreae* occurred more prevalently and in higher intensity in *C. angulata* than *C. gigas*. They suggested that this preference was due to the defense mechanism of *C. gigas* whereby parasites were encapsulated at the gill surface (Batista *et al.* 2009).

3.4.2   Distribution

M. ostreae is native to Japan and Korea, where the Pacific oyster *Crassostrea gigas* is endemic (Stock 1993, Batista *et al.* 2009). The spread of *Myicola ostreae* is closely linked to that of *Mytilicola orientalis* because they share the same host species and therefore the two parasitic copepods have been transported together. *M. ostreae* was most likely introduced to the Atlantic in 1972 with the mass importation of *C. gigas* from Japan to France and was first recorded from Bassin d'Arcachon and the Gironde estuary and has since spread to several European countries (Wolff 2005, Batista *et al.* 2009). By the 1980s, it had spread to the Mediterranean Sea.

M. ostreae was first recorded in the North Sea in 1992 where it was found in the Netherlands at Schelphoek in the Oosterschelde estuary by Stock (1993). However, *M. ostreae* may have been present in the area much earlier as Stock apparently conducted the first official investigation of parasitic copepods on *C. gigas* in the Netherlands (Streftaris *et al.* 2005, Wolff 2005, Chad 2009).

3.4.3   Vectors of translocation

Due to its dependence on bivalve hosts, and its probable short pelagic phase, *M. ostreae* is unlikely to disperse far naturally. However, the accidental translocation of the species with the movement of shellfish has greatly facilitated its dispersal and is the most likely vector of translocation for this species (Stock 1993, Wolff 2005, Chad 2009).

When the EU directive 67/91/EEC was implemented in 1993, the policy restricting the movement of shellfish and fish around Europe was relaxed. The resulting mass transport of *C. gigas* brought with it the accidental mass introduction of the oyster's parasites, including *M. ostreae*, to other areas in Europe such as Ireland and the North Sea. Although it was most probably already present in the Netherlands prior to the change in policy, the mass import of *C. gigas* greatly facilitated its population growth (Stock 1993, Leppakoski *et al.* 2002).

3.4.4   Impact

Most myicolid species are believed to be relatively harmless as they live commensally within their host (Bower 2002). Despite the fact that most species are strongly attached to the gills of their bivalve host, Bower (2002) reports that there is a low prevalence and intensity of infection. However, *Myicola ostreae* has been found to cause gill lesions in *Crassostrea gigas* and *C. angulata* in France (Bower 2002). Batista *et al.* (2009) reported that gill lesions and yellow/green aureoles were observed in the regions of the host's gills where *M. ostreae* were attached. Although these gill lesions were apparently not severe and had little impact on the host, they suggested that *M. ostreae* can reduce host fitness and/or allow the introduction of pathogens particularly in hosts with high intensity of parasite infestation (Batista *et al.* 2009).

3.4.5   Control

There are currently no known methods of prevention or control of *Myicola ostreae*. Due to the little effect *M. ostreae* is likely to have on its host, prevention or control is rarely of high priority (Bower 2002).

3.5   *Smittoidea prolifica* Osburn, 1952

Common name: Colonial bryozoan
Risk score: 2.1 (Wijsman & De Mesel 2008)
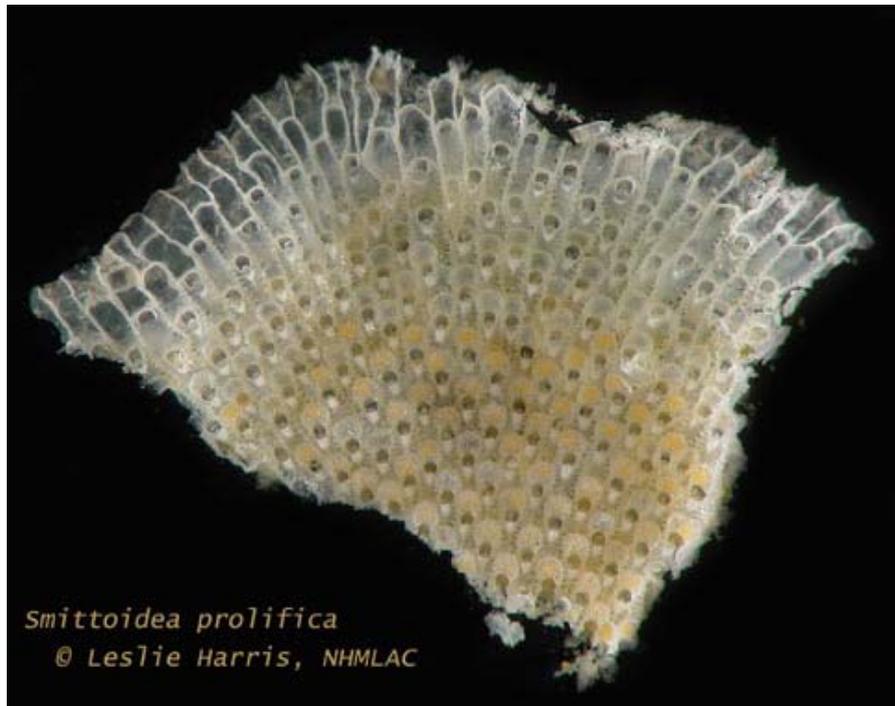
Figure 5.   *Smittoidea prolifica* (Image: Leslie Harris http://www.californiabiota.com/cabiota/smittoidea_prolifica.htm)

Phylum: Ectoprocta
Class: Gymnolaemata
Order: Cheilostomata
Family: Schizoporellidae

3.5.1   Description and Biology

Smittoidea prolifica is an encrusting colonial bryozoan. The surface begins smooth, but develops a pinkish calcareous crust over time. Each colony is made up of individual zooids which grow to 0.5-0.7 mm in length. The zooids are centrally perforated with large marginal pores and are separated from each other with robust ridges. Each zooid contains a median tooth, or lyrula, which may vary in size between ¼ and ½ of the width of the mouth opening. Inside the coelomic cavity of each zooid is a polypide, which encompasses the internal organs of the zooid including 12 tentacles, a tentacle sheath, a U-shaped digestive tract, musculature and nerve cells. The mandible is shaped as a half circle and is perpendicular to the surface of the zooid. Young zooids contain 2-4 oral spikes (Nieuwkoop & Sutasurya 1981, De Blauwe 2009).

Almost every zooid is reproductively active and hermaphroditic, except the youngest two or three rows. Each zooid contains a brood chamber which remains in contact with the neighbouring zooid. Each brood chamber has a pore, usually round, although they are often irregularly shaped as if two or three pores had merged into one (De Blauwe 2009).

Smittoidea prolifica can reproduce both asexually and sexually. Colonies usually form during summer by asexual reproduction through budding. This involves the formation of a single bud attached to the mother zooid. The outer

surface of the zooid forms a mass of orange coloured embryonic cells which protrude into the coelomic cavity. A mass of yolk-rich cells are encompassed in the walls of the developing zooid while the outer wall secretes a calcareous capsule around the embryo to protect it from desiccation during its development (Nieuwkoop & Sutasurya 1981, De Blauwe 2009).

Sexual reproduction usually only occurs when new colonies develop. In spring, the embryonic cells of an asexually produced bud develop in a calcareous capsule, in the wall of which develops a polypide bud. The capsule then breaks open and a new primary zooid is formed. This is the sexual form which produces eggs and sperm. Fertilised eggs develop into pelagic larvae which then settle onto appropriate substrate and develop into a new colony (Nieuwkoop & Sutasurya 1981, De Blauwe & Faasse 2004).

Smittoidea prolifica can be found in a widely variable range of habitats, including sublittoral boulder and shellgrounds, shallow intertidal pools and brackish water bodies without an open connection to the sea. Likewise, *S. prolifica* can be found on a variety of substrates down to 45 fathoms including wood, the underside of boulders, wharf piles, buoys and other floats, living bivalves, empty shells and algae.(De Blauwe & Faasse 2004).

3.5.2   Distribution

Smittoidea prolifica is native to the Pacific, particularly the Pacific coast of North America as its type locality is the gulf of California (De Blauwe & Faasse 2004). Its only known location in the Atlantic is the coast of the Netherlands where is was first officially recorded in 1998. However, due to taxonomic difficulties, it may have been misidentified as *Smittoidea reticulata* in earlier records, in which case it has been known in the Netherlands since 1995 or even earlier (De Blauwe 2009). It is likely that *S. prolifica* has spread to other locations in Europe due to the species adaptability to a range of habitats and substrates, and its ability to grow and reproduce rapidly (De Blauwe & Faasse 2004).

In the Netherlands, *S. prolifica* has primarily been recorded in the Oosterschelde area and does not appear to have spread very far since its first introduction, yet is very common where it does occur. *S. prolifica* has not been found in waterways connecting two major ports in the world, Rotterdam and Antwerp, which lie just north and south of its known range in the Netherlands respectively. This is notable because the Westerschelde provides very similar environments and habitats to that of the Oosterschelde.

3.5.3   Vectors of translocation

The tendency of *S. prolifica* to settle and grow very well on shellfish can facilitate the species' introduction to new regions through the transportation of shellfish for aquaculture purposes. Additionally, as larvae settle easily on artificial floats and wharf piles, it is possible that they also settle on ships and therefore introductions via biofouling may occur as well. However, the lack of spread in the vicinity of the ports of Antwerp and Rotterdam indicate that spread via biofouling may be less likely (De Blauwe & Faasse 2004).
Smittoidea prolifica is most likely to have been one of the 32 species to have been introduced to the North Sea with the Pacific oyster *Crassostrea gigas*. It appears that *Smittoidea prolifica* has a limited ability to disperse naturally and that there has been little facilitated transport within its current range in the Netherlands (De Blauwe & Faasse 2004).

3.5.4   Impact

De Blauwe and Faasse (2004) conducted a study of the abundance and distribution of *Smittoidea prolifica* within and around the Oosterschelde. They reported that in none of the locations they surveyed was *Smittoidea prolifica* the dominant species and never appeared to over-grow other invertebrates. *S. prolifica* never occupied more than a low percentage of the available substrate and it was always out-numbered by other bryozoan species. They concluded that the apparent ecological consequences of the introduction of *Smittoidea prolifica* were negligible at the moment (De Blauwe & Faasse 2004).

3.5.5   Control

No known methods have been developed for the control and prevention of spread of *Smittoidea prolifica*.

3.6   *Marteilia refringens* (Grizel 1974)

Common names of disease: Aber disease, Digestive gland disease of the European oyster, Marteiliosis
Risk score: 2.8 (Wijsman & De Mesel 2008)
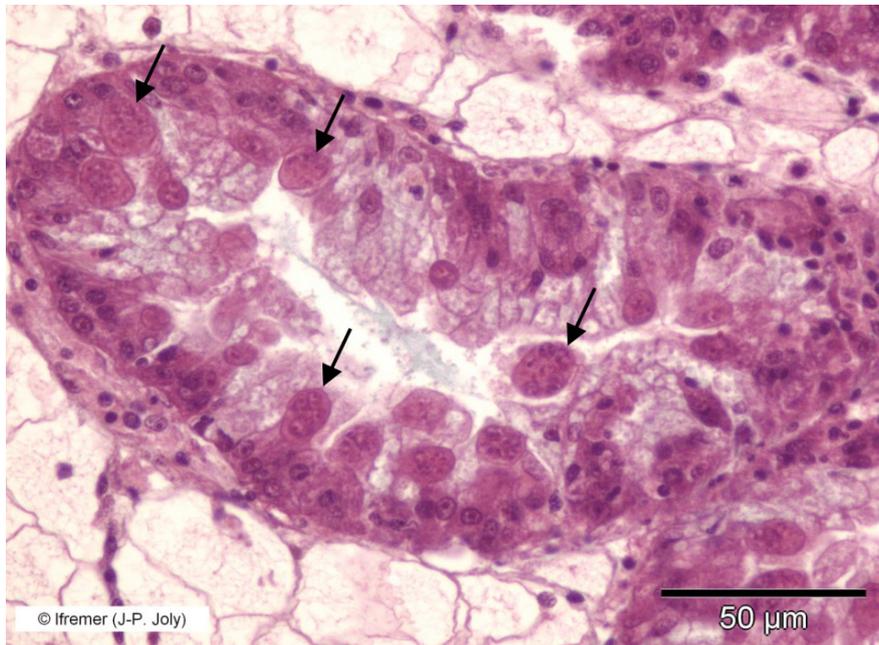
Figure 6.   *Marteilia reringens* in the digestive diverticulum of an infected European flat oyster *Ostrea edulis* [H&E staining] (Image: http://www.ifremer.fr/crlmollusc/images/m_refringens_h&e.jpg)

Kingdom: Protista
Infrakingdom: Rhizaria
Phylum: Paramyxea
Class: Marteiliidea

There has been some debate over the identification of *Marteilia refringens* and whether it is in fact identical to the similar *M. maurini*. Initially the discrimination between *M. refringens* and *M. maurini* was based on histological criteria and the target host (*M. refringens* infects oysters and *M. maurini* infects mussels), but since *M. refringens* was detected in the mussel *Mytilus galloprovincialis* and the evidence that the same parasite can have a different morphology in different hosts, the identification of the species is questionable (Virvilis & Angelidis 2006). However molecular diagnosis by Lopez-Flores *et al.* (2003) indicate that these are two different strains of the same species. Therefore, in this report the parasite will be referred to as *M. refringens* but the two will ultimately be considered to be the same species.

3.6.1   Description and Biology

Marteilia refringens is a haplosporidium protozoan parasite that affects the digestive system of many bivalve species, particularly the European flat oyster *Ostrea edulis* and the mussel *Mytilus galloprovincialis* (Lopez-Flores *et al.* 2003, AGDAFF–NACA 2007). The parasite itself can only be viewed microscopically after smears from digestive gland tissue of the host are fixed in acetone or methanol and then stained with an appropriate colour stain (see Figure 8).

Marteilia sp. can be identified during sporulation (spore production) by its internal cleavage which produces cells inside the parent cells. This is a unique feature of *Marteilia* sp. among all the Protista (Ifremer 2009). The parasite *Marteilia refringens* is 5–8 µm in size in the early stages, but can grow up to 40 µm during sporulation. The

cytoplasm of the cells when stained is basophilic and the nucleus is eosinophilic, while the secondary cells, or sporoblastsare is surrounded by a bright halo (Ifremer 2009).

Different stages of the *Marteilia refringens* can be observed in infected bivalves. Young plasmodia are found mainly in the epithelia of labial palps and the stomach. Sporulation takes place in the digestive gland tubules and ducts. After sporulation, propagules are released into the lumen of the digestive tract and later excreted into the environment in faeces. Spores can survive from several days up to 2-3 weeks depending on the environmental conditions (Ifremer 2006).

Little is known about the life cycle of *Marteilia refringens* but it has been shown to require a planktonic copepod as an intermediate host prior to infecting a bivalve (López-Flores *et al.* 1998). A study of the parasite at Marennes-Oléron Bay, France showed that *M. refringens* overwintered in the oyster *Ostrea edulis* while eggs of the copepod *Paracartia grani* rested in the benthos. When temperatures increased in spring to above 12° C, the release of *M. refringens* sporangia in the oyster feces was synchronized with the hatching eggs of *P. grani* and the copepods then became infected. By June, July and August the oysters became infected, coinciding with the highest temperatures (above 17° C) and highest abundance of *P. grani* (Audemard *et al.* 2004, Carrasco *et al.* 2007).

3.6.2   Distribution

The area of origin in unknown (Wolff 2005). The present distribution of parasite is known to stretch along the Atlantic coast of Europe from southern United Kingdom to Portugal, Mediterranean Sea including the northern Adriatic Sea, Persian Gulf and the Gulf of Thermaikos in northern Greece; and maybe the east coast of Florida in *Argopecten gibbus* (Menzel 1991, Lopez-Flores *et al.* 2003, Virvilis & Angelidis 2006, Bower 2007, Berthe 2008).

Infections by *Marteilia refringens* was first reported in the flat oyster *Ostrea edulis* in 1968 in Aber Wrach, French Brittany (Menzel 1991, Carrasco *et al.* 2007). The disease spread progressively to different oyster farming areas along the French Atlantic coast from 1973-1975. In the following years, infected oysters were transported around Europe for aquaculture purposes and the disease spread further afield to include Spain and the Netherlands (Menzel 1991).

3.6.3   Vectors of translocation

The fact that the life cycle of the *Marteilia refringens* involves pelagic copepods indicates that this parasite is capable of spreading naturally as sporangia with its intermediate host. This is evidenced by the apparently unfacilitated spread along the French Atlantic coast after its first reported presence (Menzel 1991).

However, it seems that long distance dispersal of the parasite is only possible when facilitated by the transport of its primary bivalve host. This is shown by the reported introductions of the parasite to new locations in Europe coinciding with the mass transport of shellfish (Menzel 1991). Furthermore, biofouling by infected hosts and ballast water may also provide vectors for the parasite's spread. The introduction of *M. refringens* in the Gulf of Thessaloniki is suspected to have occurred through either biofouling of oysters on ship hulls or in sporangia stages in the ballast water (Virvilis & Angelidis 2006).

3.6.4   Impact

Marteilia refringens is considered to be a potentially lethal pathogen. Since the early 1970s, it has been responsible for large scale oyster mortalities and economic losses in the European oyster industry (Audemard *et al.* 2004).

A bivalve infected with *Marteilia refringens* can appear to be emaciated, and have a discoloured digestive gland and areas of dead tissue cells (Virvilis & Angelidis 2006, AGDAFF–NACA 2007). Heavy infections can also cause a reduction in the efficiency of absorption of the host, leading to retarded gonad and storage tissue development resulting in a loss of condition in the infected host and potentially severely increased mortality rates (Bower 2007). In Galicia, Spain, the average prevalence of infection in *Mytilus galloprovincialis* by *M. refringens* was reported to range up to 35% between 1985 and 1989 and in some areas the increased mortality rates severely

affected the mussel farming industry (Bower 2007). In the Gulf of Thessaloniki, Greece, populations of the flat oyster, *Ostrea edulis* dramatically decreased from 1994 to 1998, and by 1999 the oyster was so rare in the area that fishing completely stopped. Virvilis and Angelidis (2006) suggest that the high prevalence of *Marteilia* sp. (46.47 %) in the oysters, along with the intensity of the infection is almost certainly the cause of their near or total local extinction.

However, *Marteilia refringens* can occur in some oysters and mussels without causing disease. A pathogenic host response may be dependent on environmental stress or difference in disease resistance of the stock (AGDAFF– NACA 2007). Some high mussel mortalities (up to 100%) associated with heavy infection by the parasite have been reported in France. These occurred only in mussels (*Mytilus edulis*) bought in Northern European countries for transport to France. In Galicia, Spain, it was reported that mussels (*M. edulis* and *M. galloprovincialis*) from areas where *M. refringens* was already present were infected with the parasite, but showed no serious effects, while mussels from areas where the parasite was not present were severely affected by its introduction (Bower 2007, Ifremer 2009).

3.6.5   Control

Although *Marteilia refringens* is an OIE/EU listed agent (EU Directive 91/67/EC), there are few available methods for its control (Berthe 2008). Attempts to control the parasite can be made by growing oysters in high salinity (35-37 ppt) to limit the development of the parasite (Ifremer 2009).

Generally, however, only prevention strategies are currently possible. Prevention involves restricted transport of shellfish from infested areas to locations with no record of the parasite, or restricted transport of shellfish during the warmer months when parasite transition occurs (Berthe 2008, Ifremer 2009). Robledo *et al.* (1994) suggested that the collecting mussel seed from areas free of *Marteilia* sp. may reduce the prevalence of the parasite in cultured stocks. Mussels from the inner part of two rías in Galicia, Spain, and those held at shallower depths (2 m rather than 5 m) in one ría had a higher mean prevalence of infection. Thus, culture rafts located in the outer zones of the rías contributed to minimizing the impact of this parasite on the mussel culture industry in Galicia (Robledo *et al.* 1994).

3.7   *Graciliaria vermiculophylla* (Ohmi) Papenfuss, 1967

Common names: Graceful Red Weed, Swedish: Grov agaralg, Perukalg
Synonym: *Gracilaria asiatica*, *Gracilaria verrucosa*
Risk score: 2.4 (Wijsman & De Mesel 2008)

Figure 7.   *Gracilaria vermiculophylla* (Ohmi) Papenfuss (Image: (AlgalBase 2009))

Phylum: Rhodophyta
Class: Florideophyceae
Order: Gracilariales
Family: Gracilariaceae

3.7.1   Description and Biology

Gracilaria vermiculophylla is a common macroalage which can grow 15-100 cm in length. Its colour can vary from reddish-brown to a deep wine-red depending on the availability of sunlight. The algae consists of a central stem and has irregularly spaced branches. The base of the stem of large specimens may be hollow and wider than the branches which measure about 2-5 mm in diameter. Branching can be thick or sparse depending on the habitat of the algae (AlgalBase 2009, Franzén 2009).

Gracilaria vermiculophylla can form tangled mats but usually occurs as loose-lying individuals on mud or fine sand. It can also attach to rocks and shells or as epiphytes to other individuals. The alga is commonly found in shallow inlets or eelgrass (*Zostera marina*) beds, but can occur as deep as five meters. It is a very tolerant species and can survive extreme salinities (2-40 psu), temperatures (5-30° C), and without sunlight and water coverage for more than six months (Thomsen *et al.* 2007, AlgalBase 2009, Franzén 2009).

Reproduction in *G. vermiculophylla* occurs in a diurnal rhythm according to environmental conditions. The whole individual may be either male or female, or both male and female organs may occur on the same plant. Male gametes are produced in pits (conceptacles) that are usually more than 75 µm deep (Kain & Destombe 1995, Franzén 2009). The female gamete is fertilised *in situ* and the zygote develops into a diploid carposporophyte, a spore-producing structure entirely dependent on the female gametophyte. These carposporophytes produce numerous genetically identical carpospores, each of which can develop into a diploid tetrasporophyte.

Tetrasporophytes reproduce through meiosis, which produces genetically variable tetraspores. This life history has been completed in culture and takes 5-12 months (Kain & Destombe 1995). Alternatively, new plants can regenerate from small fragments that break off, disperse and settle on appropriate substrate (Franzén 2009).

In Asia *G. vermiculophylla* is cultivated as a raw material for the production of agar, the jelly-like thickening agent used as a biological medium for growing bacteria (Franzén 2009).

3.7.2   Distribution

Gracilaria vermiculophylla is native to East and South-East Asia. In the last decade the alga has been introduced via biofouling or ballast water to the coast of Sweden; the west and east coasts of Jutland in Denmark; in the south-west Baltic; Kiel, and along the North Sea coast of Germany, as well as the coasts of northern Spain, Morocco, Portugal, France and the Netherlands. It also occurs along the east and west coasts of the United States (North Carolina, Virginia and California) and Mexico (Franzén 2009).

The ability of *G. vermiculophylla* to disperse naturally through spore release or fragment regeneration, its high tolerance of a range of environmental conditions and its ability to recruit onto patchy hard substratum make it a highly successful invader. It spreads easily and becomes established quickly (Thomsen *et al.* 2007).

In 2003 *G. vermiculophylla* was reported for the first time on the Swedish west coast and also in Horsens Fjord in Denmark. Over the next few years, many permanent and abundant populations became established in numerous soft-bottom, low energy bays and lagoons, and in less than five years it had become the most abundant algae at several invaded locations in Denmark, Sweden and Germany (Thomsen *et al.* 2007, Nyberg *et al.* 2009).

In the Dutch Wadden Sea, *G. vermiculophylla* has been reported to be spreading since the 1980s, but at the time it was identified as the synonym *G. verrucosa*. The same species is now suspected to be spreading on the tidal flats of Niedersachsen (Nehls 2006). During a specific survey in the Wadden Sea the species was found at several locations (Gittenberger *et al.* 2009).

3.7.3   Vectors of translocation

It is not known exactly how *Gracilaria vermiculophylla* was introduced into Europe and the United States in the mid 1990s. However, it is likely that the alga arrived either with oyster transplantation, as a biofouling organism on ship hulls or in ballast water and sediment (Rueness 2005, Franzén 2009, Nyberg *et al.* 2009).

Franzén (2009) suggests that natural dispersal on currents might also be possible, although large specimens do not float. She also suggests that dispersal may occur as a result of fragments becoming initially entangled with nets, lines and anchors and later being freed. She also suggest that the alga probably reached Sweden and Denmark through secondary introductions from southern or central Europe.

Local scale dispersal may occur through the transport of fishing gear and boats. In North Carolina, local spread of *G. vermiculophylla* was facilitated by the culling of trawl catches outside the trawl pull area or movement of small vessels and fouled gear such as nets, lobster pots, lines and anchors within the inland waters or between boat launching sites (Freshwater *et al.* 2006). Tomsen *et al.* (2007) suggest that local dispersal may also be facilitated by grazers causing fragmentation of the alga, and these fragments travelling on water currents, settling and regenerating in new locations.

3.7.4   Impact

Gracilaria vermiculophylla is known as a 'habitat-former' or 'ecosystem engineer', it alters the local environment and creates new habitats for various other epiphyte and invertebrate organisms and has potentially large impacts on the ecosystem metabolism (Thomsen *et al.* 2007, Nyberg *et al.* 2009). On the west coast of Sweden, Nyberg *et al.* (2009) found a variety of native species, from more than ten phyla, utilizing the invasive, but established *G. vermiculophylla* as habitat, probably for shelter, substratum for attachment, or as feeding grounds. They observed no negative impacts of the presence of *G. vermiculophylla*, but suggested that if it continues to spread and accumulate as large mats in eelgrass beds, where it often occurs, there may be a change in community

structure in the area because of the increased shading, smothering and potential for anoxia (Franzén 2009, Nyberg *et al.* 2009).

Gracilaria vermiculophylla is a fouling species, and can therefore become a nuisance species. Since it first arrived in North Carolina in 2000, there have been reports of the alga causing problems for commercial fisheries by fouling fishing gear, increasing the time and cost involved in maintenance. Although *G. vermiculophylla* generally remains on the seabed, large specimens may also become entangled in the propellers of small boats The alga was also reported as a major problem in the in the lower Cape Fear River where it clogged the cooling water intake screens of the Brunswick Nuclear Plant. (Freshwater *et al.* 2006). The algae has been found to passively attach to mussels by the byssus threads in the Wadden Sea, but does not appear to have a major negative effect (Nehls 2006).

3.7.5   Control

There are few methods of protection or control of *Gracilaria vermiculophylla* where it is invasive. Some have suggested that its use in the production of agar should be exploited as a means of controlling further population expansion while producing an economically viable product (Villanueva *et al.* 2009).

Experiments on a similar species, *Gracilaria salicornia* investigating the control of algal growth found that the algae was resistant to all treatments involving salinity and temperature. Only concentrated algacides resulted in the desired high mortality rates (Smith *et al.* 2004). Manual removal is the only feasible control option for *G. salicornia*, and most probably *G. vermiculophylla* as well. However it is a very labor intensive technique and evidence suggests that not only will the alga regenerate quickly, but the removal activity could produce fragments which may disperse and grow into new plants elsewhere (Smith *et al.* 2004).

3.8   *Polysiphonia senticulosa* Harvey, 1862

Common names: Red Macroalgae, Mutsu-itogusa (Japanese)
Synonyms: *Polysiphonia morrowii*, *Orcasia senticulosa* (Harvey) Kylin 1941, *Polysiphonia pungens* Hollenberg 1942. Also known with the genus *Orcasia*
Risk score: 2.4 (Wijsman & De Mesel 2008)

Figure 8.   *Polysiphonia senticulosa* Harvey, 1862 with close up of frond ends (inset). (Image: http://uni2008.web.fc2.com/awajiikimono/syoujyoukenori.jpg)

Phylum: Rhodophyta
Class: Rhodophyceae
Order: Ceramiales
Family: Rhodomelaceae

Polysiphonia senticulosa Harvey was originally described in 1862 from Washington State, and then later from Alaska, as *P. pungens* Hollenberg. However, the correct name for this species is probably *P. morrowii* Harvey, described from Hokkaido, Japan. Although the suggested conspecificity of *P. morrowii* and *P. senticulosa* has been questioned, (Kudo & Masuda 1988, Maggs & Stegenga 1999), the distinctions between the two are relatively vague and may be the result only of different geographical locations and environments (Kim *et al.* 1994), therefore in this report they will be considered conspecific and referred to as *P. senticulosa*.

3.8.1   Description and Biology

Polysiphonia senticulosa is a densely tufted red alga with slender and elongate branches, growing up to 25 cm high. The base is attached to the substrata by unicellular, disc-shaped rhizoids which grow irregularly as outgrowths of pericentral cells. The algae grows as a thallus, i.e. without a true stem, roots, leaves or vascular system. The thallus consists of four pericentral cells which lack an outer cortex. The primary branches grow alternately and come to a sharply pointed apex. The auxiliary branchlets grow from the axils of the branches (Kim *et al.* 1994).

Polysiphonia senticulosa grows abundantly on hard surfaces such as rocks, barnacles, concrete, wharfs and jetties and even on brown algae (Kim *et al.* 1994, Parmentier 1999). The alga is relatively resilient and can withstand the wave and tidal forces of the intertidal zone (Kelly *et al.* 2009).

Growth of the alga usually occurs in autumn and spring followed by a die-back in winter (Kim *et al.* 1994). Fragments of the alga that survive the winter temperatures, or spend months buried under sand can regenerate into healthy plants when environmental conditions become more favourable (Kelly *et al.* 2009).

Reproduction in *Polysiphonia* is a complicated process involving three phases of the plant; male, female and tetrasporophyte. Diploid tetrasporophytes form tetrasporangia which appear as round balls, one each in a segment of the branches. Each tetrasporangia divides into four gametophytes from which either male or female plants are formed. Tetrasporophytes and gametophytes appear identical other than the reproductive organs. Male reproductive organs (spermatangia) are formed at the apex of the gametophyte branches. Each spermatangia grows to 150 µm long and is full of spermatia. Spermatia rely on water currents to travel to the carpogonium (the female reproductive organ). A spermatium merges with the carogonium and the nucleus of the male cell moves to the egg cell where a zygote is formed and develops into a vesicle called a cystocarp. Diploid carpospores are formed inside the cystocarp and eventually escape through a hole and grow into a tetrasporophyte. (Kim *et al.* 1994, Parmentier 1999). Both tetrasporangial and sexual reproduction occur in November to June, in summer and autumn (Maggs & Stegenga 1999).

3.8.2 Distribution

Polysiphonia senticulosa was first described from Orcas Island in Washington, USA and is native to both the north-west and north-east Pacific ocean, including Japan, Korea, China and Russia (Nelson & Maggs 1996, Curiel *et al.* 2002).

The first report of *P. senticulosa* in Europe was at Gorishoek, in the Netherlands in 1993. Since then it has become a common species in the Oosterschelde and is particularly abundant in the oyster ponds of Yerseke Oesterbank (Maggs & Stegenga 1999). It was reported for the first time in the Mediterranean Sea in 1999 off the island of Choggia, and within a year the alga was found near Venice (Curiel *et al.* 2002). The alga has also spread to New Zealand, Australia, and along the coast of northern Europe (Nelson & Maggs 1996, Curiel et al. 2002, Guiry 2009).

Maggs and Stegenga (1999) suggest that it is likely that the species will spread further in Europe.

3.8.3 Vectors of translocation

The durability of *Polysiphonia senticulosa* and its ability to regenerate after unfavourable environmental conditions, along with its tendency to attach to hard substratum suggest that it can also travel long distances, and is likely to be translocated via biofouling on ship hull or in ballast water (Nelson & Maggs 1996).

As the alga is known to attach to artificial structures as well as brown algae and shellfish, it is also likely to be translocated by association with fishing equipment and shellfish (Kim *et al.* 1994, Parmentier 1999). In Choggia the alga was first reported where imported fish and shellfish were handled (Curiel *et al.* 2002), suggesting that the fisheries industry facilitated the introduction of the algae.

3.8.4 Impact

There is little information about the impact of *P. senticulosa*, but it is worth noting that various *Polysiphonia* species have caused serious problems where they have been introduced.

In North Carolina, a bloom of *P. breviarticulata* became a nuisance species when it was observed in the spring and summer of 1988. Large quantities of the alga washed ashore and into the sounds between Cape Lookout and Myrtle Beach (Kapraun & Searles 1990). In the Mediterranean, the introduced alga *P. (= womersleyella) setacea*, which forms dense carpets on all sublittoral surfaces, altering the habitat has become one of the most rapidly spreading algal species in the area (Maggs & Stegenga 1999).

Polysiphonia brodiei has frequently been reported as a nuisance species in California and New Zealand as it fouls slow moving vessels, such as barges. The alga also frequently fouls ropes, buoys and harbour structures including pylons and boat ramps (NIMPIS 2002).

3.8.5   Control

There is little information on prevention or control methods for *Polysiphonia senticulosa*, but it is worth considering those for other *Polysiphonia* species. For example, in the near-shores of the Tuggerah Lakes on the central coast of New South Wales, macrophytes, including *Polysiphonia mollis*, had become a nuisance in the shallow recreational areas. Shallow dredging was investigated as a means of controlling the offending macrophytes. Four months after the dredging all macrophytes had re-established in the shallowest areas, but had failed to recolonise the deeper areas, even after 12 months (Collett *et al.* 1981). Collett *et al.* (1981) suggested that this was not a particularly efficient method of control.

3.9 *Undaria pinnatifida* (Harvey) Suringar

Common names: Apron-ribbon vegetable, Japanese kelp, Asian kelp, haijiecai (Chinese), miyeuk (Korean), qundaicai (Chinese), wakame (Japanese)
Risk score: 2.3 (Wijsman & De Mesel 2008)

Figure 9. *Undaria pinnatifida* (Harvey), Suringar. (Image: www.algaebase.org/webpictures/Undaria.jpg)

Phylum: Chromophycota
Class: Phaeophyceae
Order: Laminariales
Family: Alariaceae

3.9.1 Description and Biology

Undaria pinnatifida is a brown laminarian kelp which can grow to lengths of 1-3 m. The algae is golden brown to green and has a 1-3 cm wide strap-like midrib which runs the full length of the thallus. The edges of the midrib expand into a thin membranous blade about 50-80 cm long. The blade is dotted with white cryptostomata and dark gland cells. The base of the midrib is bare and forms a stipe. As the alga matures, a thickened, fluted sporophyl develops along each edge of the stipe and they bend laterally around it in a spiral with interleaved folds (Global-Invasive-Species-Database 2007b).

Undaria pinnatifida is an opportunistic algae and is able to rapidly colonize new or disturbed substrata. It grows on hard surfaces such as rocks cobble stones, mudstone, shells (both of living and dead organisms), even seagrass and other seaweed. Artifical substrates like rope, pylons, buoys, ship hulls, bottles, pontoons and plastic are also colonised by *U. pinnatifida*. The alga generally occurs in dense strands and forms thick canopies (Global-Invasive-Species-Database 2007b). Up to 200–250 individual plants have been recorded per square metre, with a biomass of over 10 kg, wet weight (Franzén 2006).

The alga can withstand a wide variety of wave exposure, but grows best in sheltered areas. It is most common at 1-3 m depth, but can be found from the low tide level down to 15-18 m depth in clear water. It grows best in low temperatures, particularly below 12° C, and has been found to degrade in temperatures above 20° C and die in

temperatures above 23° C. It can survive a wide range of sunlight exposure, but can not tolerate areas of low salinity with a major input of freshwater (Franzén 2006, Global-Invasive-Species-Database 2007b).

Reproduction in *U. pinnatifida* can be either sexual or asexual. It has an annual lifecycle with two separate life stages; the diploid macroscopic sporophyte stage (the large visible plant), and the microscopic haploid gametophyte stage (CSIRO 2007, Global-Invasive-Species-Database 2007b). Sporophytes grow during winter and release microscopic spores when water temperatures rise. These spores eventually settle and germinate into motile gametophytes which then produce sperm and eggs. Once the eggs are fertilized they grow into the sporophyte (Global-Invasive-Species-Database 2007b).

Undaria pinnatifida is a commercially cultivated food plant in Japan and Korea. In these countries around 200 000 tonnes of fresh or dried plant are consumed annually (CSIRO 2007, Global-Invasive-Species-Database 2007b).

3.9.2 Distribution

Undaria pinnatifida is native to the north-western Pacific coast including Japan, Korea, south-eastern Russia and eastern parts of China (Franzén 2006). It has been introduced to Australia, New Zealand, Argentina, the Mediterranean Sea and the North Atlantic including Brittany France, Britain, Spain, Belgium and the Netherlands (Global-Invasive-Species-Database 2007b).

Undaria pinnatifida was first recorded in Australia in 1988 near Triabunna on the east coast of Tasmania. Over the next decade it spread along 100 km of the Tasmanian east coast and also to Victoria, most likely due to international shipping (CSIRO 2007).

After the accidental introduction of *U. pinnatifida* to the Mediterranean in 1971, (most likely due to oyster imports from Japan) attempts were made to cultivate the algae. Cultivation was originally in French Mediterranean waters, but eventually the algae was deliberately introduced for farming to the Atlantic coast as north as the coast of Brittany. By 1987, naturally occurring individuals were found outside cultivation areas in Brittany and by 1990 the algae had spread (probably associated to oyster transport) to Ria de Arosa on the Atlantic coast of Spain. Since 1998 the algae has been reported around the shores of Italy, including the canals of Venice and in the Mar Piccolo in southern Italy. Its appearance in Italy is probably due to shellfish transport or biofouling on ship hulls coming from France. In 1994 the algae was reported in Britain, again presumably due to shipping from France (Wallentinus 2004, Franzén 2006).

Undaria pinnatifida was first recorded in the Netherlands in 1999. It was found in the Oosterschelde as a 60 cm long sporophyte on oyster shells in an oyster farm near Yerseke. Later in the same year it was found near Strijenham. Rapid colonization was observed, up to 5-6 ha in some places. Plants were also washed ashore on the northern shore and were also found in small densities in the saltwater Lake Grevelingen, where it probably arrived via oyster pots (Wallentinus 2004). During a specific survey in the Wadden Sea *Undaria pinnatifida* was found on a floating jetty in Terschelling harbor (Gittenberger *et al.* 2009).

3.9.3 Vectors of translocation

There are many means by which *U. pinnatifida* may be transported to new locations. The primary vector held responsible for the global spread of the species is the accidental translocation through aquaculture and fisheries activities. This is how the algae is assumed to have arrived in Europe, Australia and the USA (Global-Invasive-Species-Database 2007b). Other vectors likely to facilitate its spread include the accidental release of the alga when imported for human consumption; the release of the both spores or whole plants in ballast water discharged from vessels and biofouling on ship hulls (Franzén 2006, Global-Invasive-Species-Database 2007b).

Local scale dispersal occurs very easily for *U. pinnatifida*. The tendency for the alga to easily colonize areas cleared by storms, urchin grazing, pollution or abrasion by gravel and sand make it a very successful invader (Stuart 2004). In addition, while the microscopic gametophytes are non-motile and have limited dispersal capabilities, the motile spores can disperse meters along the shore. The dispersal of fragments of whole plants which have come free from their substrate can range over several kilometres (Stuart 2004). Furthermore, as a gametophyte, *U. pinnatifida* can survive months of darkness, desiccation and varying temperatures. It can therefore be transported unknowingly over long distances attached to fishing equipment (Franzén 2006).

3.9.4 Impact

Undaria pinnatifida is noted as among the 100 worst invaders in the world (Global-Invasive-Species-Database 2007b). When the alga colonizes new areas, it can become the dominant canopy-forming species which can limit light availability and water movements and smother or exclude local native species, thereby altering the ecosystem structure (Stuart 2004, Franzén 2006, Williams & Smith 2007). Stuart (2004) reported that colonisation by *U. pinnatifida* can increase biodiversity in areas otherwise sparsely populated with native algae, but may reduce biodiversity where it displaces other algal species and reduces spatial heterogeneity.

Where *U. pinnatifida* is abundant, it can become a nuisance species. It is known to block water intakes and can be unsightly. When plants wash up on the shore, they can from unpleasant, foul smelling banks of rotting plant material (Franzén 2006).

Furthermore, *U. pinnatifida* also causes problems to commercial fisheries. The large, dense canopies, and the ability of *U. pinnatifida* to attach to shells can obscure the shellfish on the seabed during harvest. The alga fouls ropes and lines, buoys, pontoons, cages, jetties, wharfs, ship hulls and various other fishing equipment, which increases labour and harvesting costs and may limit water circulation through cages and slow the growth of mussels (Franzén 2006, Global-Invasive-Species-Database 2007b). In the Oosterschelde, *U. pinnatifida* has been found growing on shellfish and due to its slippery fronds, it has made retrieval of oysters from pots and cleaning equipment before harvest more difficult (Wallentinus 2004).

3.9.5 Control

Various methods for the control of *U. pinnatifida* have been investigated. Control methods at Big Glory Bay, Stewart Island, New Zealand include an attempt to sterilise floating structures with sodium hypochlorite. Despite the intensive technique, it failed to kill all the *U. pinnatifida* present. Later, brominated microbiocide was investigated as a potential algaecide, but also proved to be ineffective. Other techniques involved heat treatments, but ultimately manual removal of the sporophyte stage proved to significantly reduce the population, but eradication was not achieved (Stuart 2004). Forrest and Blakemore (2005) found that *Undaria* gametophytes were completely removed from mussel shells when water blasted for two seconds at pressures greater than 2000 psi. They also found that plantlets died within 10 minutes of immersion in freshwater, and suggested this would be an effective method of treating mussel stock prior to transport.

The use of acetic acid for the treatment of fouling organisms such as *U. pinnatifida* have been used with relative success without harming mussels (Forrest *et al.* 2007). In New Zealand, Forrest *et al.* (2007) reported that *U. pinnatifida* gametophytes and plantlets were killed with one minute exposure to 0.1-2 % acetic acid. However, they found sporophylls to be more resilient to the effects of acetic acid as they were still viable after treatment.

In Monterey Harbor, California, USA, a formal *Undaria* management programme implemented in 2002 involved volunteer divers manually removing the sporophytes from the pilings. The technique was not overly successful as the alga continued to spread around the harbor. Eradication was then identified as an unfeasible option due to the likelihood of new introductions. A proposed management option to develop a market for the alga was then suggested, but the amount of algae in the harbor was found to be too low to be commercially viable. Techniques using UV light or high-pressure, heated water to kill harmful spores and bacteria quickly and efficiently are being investigated, but currently only monitoring programmes are being carried out (Global-Invasive-Species-Database 2007b).

Natural control of *U. pinnatifida* has been observed in Europe where grazing on both sporophytes and gametophytes by sea urchins (*Paracentrotus lividus, Evichinus esculentus* and *Psammechinus miliaris*) and abalone (*Haliotis tuberculata*) has been found to have a negative effect on the abundance of *U. pinnatifida* (Stuart 2004). In Belgium, although *U. pinnatifida* was still present in the marina of Zeebrugge in 2003, it has not spread since 2000. This was suggested to be due to the grazing of coots (*Fulicra atra*) (Wallentinus 2004).

3.10   *Urosalpinx cinerea* (Say, 1822)

Common names: Eastern oyster drill, American oyster drill, American tingle, American whelk tingle, Atlantic oyster drill
Risk score: 2.1 (Wijsman and De Mesel 2008)

Figure 10. Above: *Urosalpinx cinerea* (Image: http://shell.kwansei.ac.jp/~shell/pic_book/data54/r005315.html)
Below: *Urosalpinx cinerea* egg capsules with juvenile snails in Gorishoek, The Netherlands (Image: A.H.M. Ligthart (Faasse & Ligthart 2009))

Phylum: Chordata
Class: Gastropoda
Order: Neogastropoda
Family: Muricidae

3.10.1 Description and Biology

The American oyster drill, *Urosalpinx cinerea*, is a small muricid gastropod, growing up to 35 mm long with larger specimens generally found to be female. It has a knobbly, rugged shell of 5-6 whorls with rounded shoulders and 9-12 rounded axial ribs per whorls. The shell is often streaked and may be yellow, gray, white, brown and occasionally orange. It has an oval aperture with a short, open canal at the base containing 2-6 teeth. The flesh is grey, yellow, reddish-brown, or purple in colour (Williams 2002, Cohen 2005, Global-Invasive-Species-Database 2008).

The American oyster drill is known to feed upon different prey species, including oysters, mussels, slipper limpets, barnacles and bryozoa (Anonymous 2009). Prey is located from a distance by chemoreceptors. To feed, *U. cinerea* crawls over its prey (e.g. an oyster) and grips with its foot from which it secretes a softening agent onto the shell and bores a hole through the shell of its prey using its file-like radula. It then inserts its proboscis into the tissue of the oyster and secretes a muscle relaxant which induces the oyster the open, leaving the animal exposed. Barnacles are penetrated through the soft parts between the plates. *U. cinerea* can then easily feed on the soft tissue of its victim (Nichols & Cooke 1971, Buchsbaum *et al.* 1987, Williams 2002, Cohen 2005).

Breeding in *Urosalpinx cinerea* occurs in the spring and summer when the water temperature rises. After fertilisation, the female deposits 20-40 translucent capsules, containing 5-12 eggs each, on a suitable substrate. The surface of the substrate to which the egg cases are to be attached is carefully cleaned by the use of the radula. Living oysters are preferred to empty shells as a substrate for the eggs. The eggs have a vase-like structure with more or less parallel sides and a short peduncle, attached to the substratum by a basal disk (Anonymous 2009). In the British waters, egg laying is done primarily during May and June, but it is not uncommon for freshly laid capsules to be found in August (Cole 1942). Cole (1942) reported that spawning increased as temperatures rose above 12° C while Cohen (2005) reports that spawning only begins when the water temperature exceeds 20° C for at least a week. After approximately 6-8 weeks the well developed but tiny young emerge from the eggs and begin feeding on various shellfish species and occasionally diversify to include encrusting ectoprocta. This diverse diet of juveniles reduces intra-specific competition for food (Franz 1971). Sexual maturity is reached after two years and individuals can live for up to eight years (Williams 2002, Cohen 2005).

Urosalpinx cinerea occurs in intertidal and shallow subtidal waters in bays, marshes and estuaries, to a maximum depth of about 15 m. It flourishes particularly well in rocky areas and oyster beds and in high salinities, but can tolerate salinities as low as 13 ppt (Williams 2002, Cohen 2005).

3.10.2 Distribution

The native range of *Urosalpinx cinerea* is reportedly the Northwestern Atlantic from the Gulf of St. Lawrence to southeastern Florida (Williams 2002, Gittenberger 2009a). It has been introduced to the Pacific Coast of North America, southern Great Britain and recently the Netherlands (Global-Invasive-Species-Database 2008).

The date and means of the introduction of *Urosalpinx cinerea* to the Netherlands is unknown, but there is no record of its presence in the Netherlands prior to 2007 (Faasse & Ligthart 2007). Faasse and Ligthart (2009) reported that the population of *U. cinerea* at Gorishoek has been steadily growing despite regular collections by hand. In the Gorishoek area, egg capsules have not been counted since mid 2008, but at that time more egg capsules than adult individuals were recorded. They also suggest that cold weather poses no problem for the species as, after a particularly cold spell in the winter 2009, where temperatures dropped to 0-1° C, eight specimens were collected within two hours.

Urosalpinx cinerea, was introduced to Willapa Bay, Washington, USA prior to 1948, most likely with imported eastern oysters (*Crassostrea virginica*) in the early 1900s. The species is now established and widespread in Willapa Bay and has become an economically significant pest of oyster aquaculture that is particularly damaging to juveniles. Oyster growers have attempted to control *U. cinerea* by manually removing adults and egg capsules, but even local eradication has proven difficult and in some cases growers have abandoned oyster beds due to the intense predation (Cohen 2005). Despite this, unauthorised movement of shellfish has also occurred and facilitated the potential spread of unwanted organisms. In 1997 a seized importation of *C. virginica* at Shannon

Airport, Ireland, coming from Long Island Sound, USA and flown from New York, contained eggs of *U. cinerea* (Leppakoski *et al.* 2002).

U. cinerea is thought to have been introduced into the Thames Estuary, England from eastern North America with the translocation of the eastern oyster *Crassostrea Virginica* around 1880. At the time, identification of the species was problematic and it was not officially identified as non-indigenous in England until 1928 (Locke & Hanson 2009). By the 1950s *U. cinerea* was abundant along the Essex coast and was reported for the first time as a problem to oyster growers in Essex and parts of Kent (Edwards 2006). Over the next decade *U. cinerea* had a devastating effect on the mortalities of juvenile European flat oysters (*Ostrea edulis*) so that by 1954 large-scale control methods were being investigated. (Edwards 2006, Locke & Hanson 2009). The introduction of tributyl tin (TBT) antifouling biocides drastically reduced the population of *U. cinerea* and the species is now rare on the Essex coast. The decline of native oyster fisheries, resulting in poor spatfalls and inappropriately muddy substratum also prevented population recovery to the point where during a population survey in 2006 Edwards (2006) did not find a single specimen; adult, juvenile or egg capsule, and in a questionnaire survey shellfish aqua culturists also reported an absence of *U. cinerea* (Edwards 2006).

Urosalpinx cinerea is most likely now established in the Netherlands and the population is growing, but the distribution remains relatively localised and there is little evidence to suggest it has spread to locations further than a few hundred meters away. Faasse and Ligthart (2009) found the population in the Oosterschelde dispersed less than 200 m in a year and a half. Using lab-experiments, Cole (1942) reported a maximum individual migration speed of 50 meter in 48 hours.

3.10.3 Vectors of translocation

Due to the lack of a pelagic phase in its lifecycle, and therefore reduced risk of translocation via natural means, the primary vector of translocation of *Urosalpinx cinerea* is through commercial shellfish transfers (Faasse & Ligthart 2007, Global-Invasive-Species-Database 2008, Buhle & Ruesink 2009, Locke & Hanson 2009).

Carriker (1957) reports that newly hatched oyster drills in the laboratory are able to cling tenaciously to and float on minute bits of debris or mucus. They may also be transported with small pieces of seaweed, seagrass or saltmarsh plants (Carriker 1957, Anonymous 2009). Oyster drills might also hitch-hike with other species like crabs and hermit crabs.

3.10.4 Impact

Urosalpinx cinerea is a common and important pest to the commercial oyster industry. The species is a major threat to native oysters wherever they occur as they have been found to inflict over 60 % mortality of the annual seed crop (Williams 2002, Global-Invasive-Species-Database 2008). In England, for example, *U. cinerea* has been reported to feed on the native oyster (*Ostrea edulis*), with each snail estimated to consume about 40 spat per year (Eno *et al.* 1997, Cohen 2005). During its lifetime (at least six years (Cole 1966), a single individual is capable of consuming 240 young oysters. Furthermore, its ability to reproduce in large numbers (approximately 25 capsules laid per year, each containing around ten eggs), and that when the young emerge they are well developed and ready to eat, foregoing a vulnerable pelagic, larval stage, the species is able to populate new areas quickly and efficiently (Cole 1966). However, spread by natural means is slow due to the lack of a pelagic stage and limited adult mobility although movement and dispersal has been facilitated by the translocation of mussels and oysters (Eno *et al.* 1997).

Where the species is introduced and has no natural predators or parasites, it can also have a competitive advantage over the native species and affect the structure of local communities. For example, in the Netherlands, populations of the native dog whelk (*Nucella lapillus*) have been adversely affected by the intense competition with the recent invasion of *U. cinerea* (Faasse & Ligthart 2007, Global-Invasive-Species-Database 2008).

3.10.5 Control

Various control methods have been implemented in areas invaded and affected by *Urosalpinx cinerea*. For example, in England these methods included mechanical or suction dredging, but these proved ineffective (Locke & Hanson 2009). Traps have been used during the summer to control the species and on the Essex oyster beds,

bounties were paid for bucket loads of *U. cinerea* (Eno *et al.* 1997). However, individual removal by hand was considered too labor-intensive and experimental traps were impractical for large-scale removal.

In the 1970s, the Ministry of Agriculture & Fisheries in England developed legislation to control the import and export of molluscs into and around the UK. The Molluscan Shellfish (Control of Deposit) Order was released in 1974 introducing a strict licensing system to help control and reduce the risks of pests and diseases in the country. *Urosalpinx cinerea* was one of the three species of prime importance and highest risk in this legislation (Cole 1966, Edwards 2006).

Between the 1940s and 1970s, over 1000 chemical compounds were investigated as eradication methods (Locke & Hanson 2009). These involved immersing *U. cinerea* and their host oysters in solutions of freshwater, formalin, potassium permanganate, chlorol (10% chlorine), phenol (0.15% in seawater) and copper sulphate or as chemically impregnated barriers (McEnnulty *et al.* 2000). However, all resulted in either killing the oysters as well or having severe environmental costs (Locke & Hanson 2009).

Immersion in fresh water has been successful in killing the drills (McEnnulty *et al.* 2001). The salinity at which the animals die depends on the environment they are acclimatised to (Federighi 1931). Federighi (1931) reported on 10 day salinity tolerance experiments with *U. cinerea* from populations originating in habitats with differing salinities. Drills from areas in Hampton Roads, Virginia, where the salinity in summer was 15-20 ppm had a salinity death point of 11.7-12.5 ppm. However, drills collected from Beaufort, North Carolina, where salinity was over 30 ppm had salinity death points of 15.6-17.6 ppm. He concluded that with lower environmental salinity, the smaller the difference between environmental salinity and the salinity death point becomes (Federighi 1931).

After the attempt of eradication was abandoned, the oyster industry had to alter its husbandry methods. Juvenile oysters were reared in trays or bags, behind barriers or under plastic netting for protection from predators, increasing the costs of maintenance and equipment required for the industry. Furthermore, in 1974 the Molluscan Shellfish (Control of Deposit) Order prohibited further transfer all molluscs between specific areas except under highly restrictive license conditions. This order was effective in controlling further spread of *U. cinerea* (Locke & Hanson 2009).

Between 1987 and 1990 the population of *U. cinerea* in England was almost wiped out due to the use of vessel antifouling paints containing TBT (Edwards 2006, Faasse & Ligthart 2007). TBT was found to cause imposex, a masculinisation in females to the point where oviducts were deformed, copulation and egg capsule formation was inhibited so that females were effectively sterile. No viable spawn was observed during the four summers of 1987-1990 (Gibbs *et al.* 1991). This appears to have been the only method somewhat successful in the control of the species. In the Netherlands, however, there has been a ban on the use of TBT since 1993 for ships travelling shorter than 25 m to reduce the effect of the chemicals on the environment.

3.11   *Ocinebrellus inornatus* (Recluz, 1851)

Common names: Japanese oyster drill, Asian drill, Asian oyster drill
Synonyms: *Ocenebra japonica* (Dunker, 1860), *Ceratostoma inornatum* (Recluz, 1851)
Risk score: no score at present
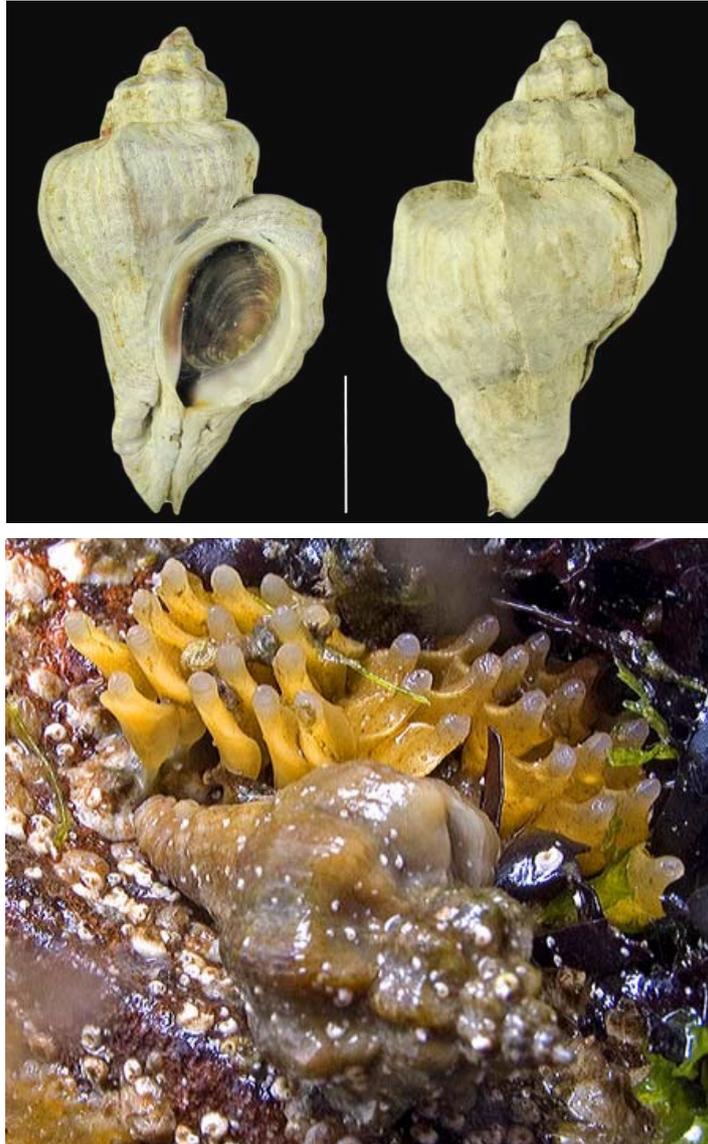
Figure 11.   Above: *Ocinebrellus inornatus* (Recluz, 1851) (Image: http://www.bily.com/pnwsc/web-content/Gastropod%20Identification.html). Below: *Ocinebrellus inornatus* (Recluz, 1851) with egg capsules (Image: http://www.cryptosula.nl/photos/Ocinebrellusinornatusweb.jpg)

Phylum: Mollusca
Class: Gastropoda
Order: Neogastropoda
Family: Muricidae

Although this species is more commonly known as its synonym *Ceratostoma inornatum*, in this report it will be referred to as *Ocinebrellus inornatus*.

3.11.1 Description and Biology

The Japanese oyster drill, *Ocinebrellus inornatus*, is a muricid gastropod with a range of phenotypic variation in the species, but generally it has a solid looking, knobbly shell which can grow up to 5 cm long. Females tend to be larger with a higher growth rate compared to males (Martel *et al.* 2004). The shell has 5-6 whorls, each with about eight low axial ribs that come to points at the apical edge of the body whorl, but less so on the whorls of the spire. Faint spiral ridges can often been seen on the shell as well. The aperture is oval with a thick outer lip and the canal is relatively short and open in the early stages of development, but closes as the individual matures (Global-Invasive-Species-Database 2007a, Goud *et al.* 2008, Eissinger 2009, Faasse & Ligthart 2009). The colour of the shell ranges from white to yellow to brown and there is no exterior periostracum (Eissinger 2009).

The diet of *O. inornatus* consists of benthic bivalves, including young oysters, mussels, clams and cockles, as well as barnacles. Feeding is most likely similar to that of *U. cinerea* which grips its prey with its foot and secretes a softening agent onto the shell before boring a hole with its radula. It then inserts its proboscis into the tissue of the prey and secretes a muscle relaxant to induce the bivalve the open, leaving the animal exposed for feeding. (Nichols & Cooke 1971, Buchsbaum *et al.* 1987, Williams 2002, Cohen 2005). Predation of a bivalve by *O. inornatus* is easily identified by the distinctive 2 mm hole left in the shell (Global-Invasive-Species-Database 2007a, Buhle & Ruesink 2009, Eissinger 2009). To drill through the shell of a Pacific oyster (*Crassostrea gigas*), an adult *O. inornatus* can take between one day on oysters 2.5 cm in diameter, to around two weeks for an oyster 5 cm long (Committee-on-Nonnative-Oysters-in-the-Chesapeake-Bay 2004, McCoy 2009).

Ocinebrellus inornatus has a simple two stage life history. Mature adults aggregate in both Spring and Autumn and lay clumps of bright yellow egg capsules (each containing about 10 young about 2 mm long) on any emergent hard substratum they can find. After about three weeks juveniles emerge looking like miniature adults and begin to feed. Juveniles grow rapidly (more than 2 mm per month, although growth rates decrease with increasing size), and reach reproductive maturity the following year when they are about 27 mm long (Buhle *et al.* 2004, Martel *et al.* 2004, Eissinger 2009, McCoy 2009). McCoy (2009) reported that adult survival rates of *O. inornatus* were low (less than 10 % annually) and suggested that this short life span was compensated for by high fecundity.

The oyster drills are usually found in estuarine and benthic marine habitats at a range of temperatures. The species is capable of surviving cold winters in the Netherlands with temperatures of 0-1° C (Faasse & Ligthart 2009). It is typically found on substratum including gravel, mud, sand and shells, particularly where *C. gigas* is present (Buhle *et al.* 2004). Although adult *C. gigas* are not preyed upon by *O. inornatus* because they have shells too thick to drill through, they are highly conducive to the survival of *O. inornatus*. The oysters provide complex, three-dimensional habitats and offer a refuge from predation and abundant food source in terms of juvenile oysters and attached barnacles (Buhle & Ruesink 2009)

3.11.2 Distribution

Ocinebrellus inornatus is native to the Sakhalin and Kurile Islands up to Japan and from North of China to Korea (Garcia-Meunier *et al.* 2003, Global-Invasive-Species-Database 2007a). Its presence has been reported in Australia, but it is unknown whether it is native there or introduced (Global-Invasive-Species-Database 2007a). In the 20th century, it was accidentally introduced to the Pacific coasts of North America, including Pugnet Sound (1924), British Columbia (1931), Oregon (1930-1934) and California (1941) (Garcia-Meunier *et al.* 2003). *O. inornatus* was then introduced into the bay of Marennes-Oléron on the French Atlantic coast in 1995. and later spread northward to the Golfe de Morbihan, south Brittany (Garcia-Meunier *et al.* 2003, Martel *et al.* 2004, Faasse & Ligthart 2009). Genetic studies found the source population of the French introduction probably came from the United States (Martel *et al.* 2004).

It was first reported in the Netherlands in the Oosterschelde in 2007 but had been misidentified as the European oyster borer *Ocenebra erinacea* (Faasse & Ligthart 2007), so it may have been present in the area prior to this time. *O. inornatus* can now be easily found in the oyster ponds in Yerseke (pers. obs.) and the population appears to be growing in the Oosterschelde. Faasse and Ligthart (2009) reported that more *O. inornatus* were found

during two hour searches in Yerseke in 2008 and 2009 than in 2007, despite temperatures as low as 0-1° C in 2009. Furthermore, in 2008 the species was found in a new location near Gorishoek. So far *O. inornatus* has not spread as far north as the Wadden Sea (Dame & Olenin 2005).

3.11.3 Vectors of translocation

Due to the lack of a pelagic phase in its lifecycle, and therefore reduced risk of translocation via natural means, the primary vector of translocation of *Ocinebrellus inornatus* is accidental transfer with the movement of commercial shellfish (Martel *et al.* 2004, Faasse & Ligthart 2009).

Natural dispersal of *Ocinebrellus inornatus* is limited by its lack of free-swimming larval stages. Without the ability to travel in the water column, juveniles are restricted to the immediate local area (Buhle & Ruesink 2009). However, this limitation is easily counter-balanced by aquaculture activities, which play an important role in expanding the range of the species (Martel *et al.* 2004). The intensive oyster translocation activity that followed the introduction of *O. inornatus* into France via the transportation of live oysters from the USA and the success of the Pacific oyster in the new environment reportedly facilitated the spread of *O. inornatus* within and beyond Marennes-Ole´ron Bay (Martel *et al.* 2004, Faasse & Ligthart 2009). Similarly, Buhle (2009) reported that the spatial spread of *O. inornatus* within Willapa Bay, Washington, USA, was largely the result of transferring oysters and shells by growers.

3.11.4 Impact

Where it is introduced *Ocinebrellus inornatus* can devastate native bivalve populations. In Netarts Bay, Oregon a major component of the diet of *O. inornatus* is the native clam *Macoma balthica* and the native cockle *Clinocardium nuttalli* (Carlton 1979). If the prey of *O. inornatus* is fundamental to the local ecosystem, providing habitats and food for other native species, the predation of *O. inornatus* may alter the local environment. Additionally, if *O. inornatus* outcompetes and replaces native species, it may indirectly cause a trophic cascade that ultimately alters the community structure and biomass of the coastal ecosystem (Williams & McDonald 2008). Williams and McDonald (2008) report anecdotal evidence that *O. inornatus* has replaced the native dogwhelk *Nucella lamellosa* in Willapa Bay.

Furthermore, *O. inornatus* can prevent the restoration of native environments recovering from a disturbance (Buhle & Ruesink 2009). On the coast of British Columbia and Washington the drill was reported to hamper efforts to restore beds of native Olympia oysters (*Ostreola conchaphila*) (Committee-on-Nonnative-Oysters-in-the-Chesapeake-Bay 2004).

The major impact of *Ocinebrellus inornatus* is economical, as it can decimate stocked shellfish populations. In British Columbia and Washington, where *O. inornatus* was introduced in cases of oyster seed from Japan, it began attacking the farmed oysters as well as the Manila clam (*Venerupis philippinarum*). The drill caused about 25 % mortality in oyster stocks. Productions costs increased by about 20 % and profits decreased by about 55 % (Committee-on-Nonnative-Oysters-in-the-Chesapeake-Bay 2004, Global-Invasive-Species-Database 2007a, Buhle & Ruesink 2009). In Netarts Bay, Oregon the Japanese clam (*Venerupis philippinarum*) was introduced for aquaculture purposes, but due to predation by *O. inornatus*, it only became established after an intensive planting programme (Carlton 1992).

3.11.5 Control

In the aquaculture industry, prevention of the introduction of *Ocinebrellus inornatus* is obviously the preferred method of control. The West Coast states of the USA adopted regulations in 1945 to prohibit the transfer of oyster drills among oyster plantings within the state. An inspection programme was implemented in Washington and California in 1947 where authorities in Japan checked oyster seed shipments for pest species prior to packing. Inspections were again made when the shipment arrived in the USA. Along with these regulations and inspections, the transition to hatchery-produced seed has helped to prevent the spread of *O. inornatus* (Committee-on-Nonnative-Oysters-in-the-Chesapeake-Bay 2004, McCoy 2009).

There have been several attempts to control the impact of *O. inornatus* where it is introduced. The most common control method for the drill is manual removal (Buhle *et al.* 2004, McCoy 2009). McCoy (2009) reported that

oyster growers in Puget Sound and Willapa Bay, Washington would go out on the tide-flat with buckets and pick up as many drills as they could. Unfortunately these efforts were not successful in eradicating or reducing the distribution of the drills (McCoy 2009). White (2007) suggested that *O. inornatus* eggs could be easily removed from oyster shells with a screwdriver (in (Global-Invasive-Species-Database 2007a))

Due to the two stage life history of *O. inornatus*, manual removal of eggs and adults can differ in efficiency. The destruction of eggs decreases fecundity, while the removal of adults reduces adult survival. Removal of juveniles is not feasible because of the difficulty in finding them due to their small size. Modeling by McCoy (2009) showed that removal of egg capsules is more effective in reducing the population than removal of adults (although removal of both is obviously optimal). However, Buhle *et al.* (2004) reported that although eggs are more numerous (they found about 150 eggs to each adult) and have a higher population elasticity, so that removing them would have a greater impact on the population, it is much more effective to control *O. inornatus* invasions by reducing adult survival rather than by reducing fecundity. This is because adults are much easier to find than the eggs (particularly in low reproduction seasons) and therefore decrease the costs of the technique.

Several other methods to remove *O. inornatus* on a larger scale have also been investigated. Buhl *et al.* (2004) reported that destroying the eggs of *O. inornatus* by burning effectively controlled their numbers. However, the obvious risks involved with this technique are likely to become an issue.

Immersion in freshwater is a successful method for killing *O. inornatus*. This simple and cost-effective technique targets the eggs of *O. inornatus* without harming the oysters (Mueller & Hoffmann 1999). However, immersion in freshwater can cause juvenile mussels to drop off mussel ropes as well as killing other epibiota (McEnnulty *et al.* 2001). Mueller and Hoffmann (1999) reported that the drills would detach from the substrate when immersed in water with salinity between 7.2-18 ppt and experimented with immersing drills in freshwater (0 ppt). The length of time required for immersion before the drills detached increased with increase size of the drill. They suggested that drills with shells equal to or larger than 40 mm in length took between 1.4 to 20.2 minutes to detach when immersed in freshwater, although one in 10 was expected to remain attached after this time.

4   Quality Assurance

IMARES utilises an ISO 9001:2000 certified quality management system (certificate number: 08602-2004-AQ-ROT-RvA). This certificate is valid until 15 June 2010. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

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Justification

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The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: dr. I.G. de Mesel
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