Freshwater immersion as a method to remove *Urosalpinx cinerea* and *Ocinebrellus inornatus* from mussel seed

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Contents

Summary

A simple experiment to test whether immersion in freshwater for ≤24 hours would kill two oyster drills, *Urosalpinx cinerea* and *Ocinebrellus inornatus* was conducted to test the proposal of using freshwater to rid mussel seed of the drills before translocation from the Oosterschelde to the Wadden Sea. Freshwater failed to kill any individuals of either species, but did cause them to detach from the substratum for the entire time of immersion. Immersion in freshwater is therefore not recommended as a method to control the drills. Rinsing in freshwater may be an option to remove the drills without killing them, but has no guarantee of 100 % success. Further investigation may result in the development of other treatment options.

Uitgebreide samenvatting

Op basis van de beleidslijn schelpdiertransport is het verplaatsen van schelpdieren vanuit de zuidwestelijke delta naar de Waddenzee, het zogenaamde Zuid-Noord transport, niet toegestaan. Met het verplaatsen van schelpdieren bestaat namelijk de kans dat er onbedoeld exoten, die al wel in de Oosterschelde voorkomen, maar nog niet in de Waddenzee, worden geïntroduceerd in de Waddenzee.

In de studie duurzame schelpdiertransporten, dat in 2008 is uitgevoerd in het kader van het VPT programma van het ministerie van LNV, is er uitvoerig onderzoek gedaan naar de risico's van introductie van exoten naar de Waddenzee met het Zuid-Noord transport. Een van de (65) risicosoorten die bij deze studie naar voren kwam is de Amerikaanse oesterboorder (*Urosalpinx cinerea*). In 2009 is er ook een andere exotische oesterboorder, de Japanse oesterboorder (*Ocinebrellus inornatus*) in de Oosterschelde aangetroffen. Om te voorkomen dat deze soorten in de Waddenzee terecht komen wordt er onderzocht of de risico's zijn te mitigeren.

Een van de voorgestelde mitigerende maatregelen zijn het spoelen, of het geheel onder zetten van de lading mosselen met zoetwater teneinde ongewenste organismen te doden. In deze studie is er in kleinschalige laboratoriumexperimenten onderzocht of behandeling met zoetwater effectief is voor het bestrijden van oesterboorders.

Japanse- en Amerikaanse oesterboorders zijn verzameld van de dijkglooiing bij Gorishoek. Tevens zijn er Japanse oesterboorders verzameld uit het in- en uitwater kanaal van de oesterputten in Yerseke. De oesterboorders zijn geconditioneerd in aquaria die met lucht zijn doorborreld. Tijdens de experimenten werden 20 exemplaren in met lucht doorborrelde aquaria geplaatst. De aquaria waren gevuld met zoetwater (100%) of een mengsel van zoetwater (90%) en zoutwater (10%). Tevens was er aquarium met zoutwater als controle. De behandelingen zijn uitgevoerd gedurende 2 uur en 24 uur. Na behandeling zijn de oesterboorders weer teruggeplaatst in 100% zeewater om de sterfte te meten.

Tijdens de experimenten is geen sterfte van oesterboorders als gevolg van de behandeling met zoetwater vastgesteld. Blijkbaar zijn de oesterboorders goed in staat zich af te sluiten van het zoetwater met behulp van hun operculum. Ze kunnen dit minimaal 24 uur volhouden. Uit literatuuronderzoek is gebleken dat de Amerikaanse oesterboorders het meer dan 5 dagen kunnen volhouden in zoetwater. In de praktijk is het niet mogelijk om de mossellading zo lang onder zoetwater te zetten omdat er dan sterfte optreedt onder de mosselen.

De conclusie van dit onderzoek is dat behandeling met zoetwater geen effectieve methode is om de Japanse en Amerikaanse oesterboorders die mogelijk met de schelpdiertransporten worden meegenomen af te doden. Omdat de oesterboorders tijdens de behandeling met zoetwater los moeten laten van het substraat om het operculum te kunnen sluiten zal het wel eenvoudiger worden om de oesterboorders mechanisch te verwijderen.

1 Introduction

Recent discussions about the feasibility of transporting mussel stock from the Oosterschelde to the Wadden Sea for farming have raised concerns about the risks of introducing new harmful exotic species to the Wadden Sea. Two muricid gastropods, the American oyster drill, *Urosalpinx cinerea* (Figure 1) and the Japanese oyster drill, *Ocinebrellus inornatus* (Figure 2) are recent introductions in the Netherlands (Faasse & Ligthart 2007, 2009) and pose a risk of being introduced into the Wadden Sea with shellfish transports.

Figure 1: The American oyster drill, *Urosalpinx cinerea*.(Image: httpwww.anemoon.organemoonanemoon-forumalgemeen607746581354933131urosalpinxml2.jpg)

Figure 2: The Japanese Oyster drill, *Ocinebrellus inornatus*. (Image: http://www.ifremer.fr/lerpc/PGSauriau/Alien%20species/Images/ocinino.jpg)

The drills both have a simple life history, with young emerging directly from egg capsules laid on the substratum (Cole 1942, Buhle et al. 2004, Martel et al. 2004, Eissinger 2009, McCoy 2009). The oyster drills have no pelagic phase, and therefore have a reduced risk of translocation via natural vectors. The risk of introduction of *U. cinerea* and *O. inornatus* is mainly associated with commercial shellfish transfers (Faasse & Ligthart 2007, Global-Invasive-Species-Database 2008, Anonymous 2009, Buhle & Ruesink 2009, Locke & Hanson 2009).

U. cinerea is native to Northwestern Atlantic from the Gulf of St. Lawrence to southeastern Florida (Williams 2002, Gittenberger 2009a), and has primarily been introduced to the Pacific Coast of North America and southern Great Britain (Global-Invasive-Species-Database 2008). *O. inornatus* is native to the Sakhalin and Kurile Islands up to Japan and from northern China to Korea (Garcia-Meunier et al. 2003, Global-Invasive-Species-Database 2007) and has primarily been introduced to the Pacific coasts of North America and the French Atlantic coast (Garcia-Meunier et al. 2002, Martel et al. 2004, Faasse & Ligthart 2009).

These drills are thought to have arrived in Europe in association with the Pacific oyster, *Crassostrea gigas,* which was imported from Japan to supplement failing stocks of the European native oysters, *Ostrea edulis* in the late 20th century (His 1977, Grizel 1985, Bower 2002, Smaal et al. 2009). The drills were first recorded in the Netherlands in 2007 where they were found in the oyster ponds in Yerseke and along the dike slopes of Gorishoek, in the Oosterschelde. Since then populations of both species have gradually been growing (Faasse & Ligthart 2007).

Both species are known pests to the aquaculture industry and have the potential to decimate oyster and mussel stocks. In England, *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). In British Columbia and Washington, where *O. inornatus* was introduced in cases of oyster seed from Japan, the drill caused about 25 % mortality in farmed oyster stocks. Production costs increased by about 20 % and profits decreased by about 55 % (Committee-on-Nonnative-Oysters-in-the-Chesapeake-Bay 2004, Global-Invasive-Species-Database 2007, Buhle & Ruesink 2009).

Both *U. cinerea* and *O. inoratus* are present in the Oosterschelde and unknown in the Wadden Sea (Gittenberger et al. 2009, Wijsman & De Mesel 2009). Therefore both species pose a potential risk of being introduced in the Oosterschelde with the shellfish transports. The recent deployment of mussel seed capture devices (MZI's), both in the Wadden Sea and the Delta area have increased the urgency for the Dutch mussel sector to transfer mussels from the southwestern Delta to the Wadden Sea. The reason for this is that growth rate of the mussels in the Oosterschelde is lower compared to the Wadden Sea because the shellfish production in the Oosterschelde is close to the carrying capacity of the system. For the transfer of mussels from the Oosterschelde to the Wadden Sea a permit is required based on the nature conservation act of 1998.

In order to evaluate the tenability of the current legislation, a risk study was conducted in 2008 (Wijsman & De Mesel 2009) to assess the risks of introducing exotic species into the Wadden Sea with the shellfish transfers. *U. cinerea* was indicated as one of the risk species. After this study, the other oyster drill, *O. inornatus*, was identified in the Oosterschelde (Faasse & Ligthart 2009, Gittenberger 2009b). The risk of this Japanese oyster drill was also suggested to be high (Gittenberger 2009b).

To mitigate concerns about transporting *U. cinerea* and *O. inornatus* with mussels between the Oosterschelde and the Wadden Sea, it was suggested that transported stock be rinsed or immersed in freshwater during harvest or *en route* before entering the Wadden Sea. Mussels would preferably be translocated between sites within 24 hours so treatment would occur on board the transport vessel. The effectiveness of this approach on the survival of both oyster drills is investigated by Wageningen IMARES. The results of this study are presented in this report

2 Materials and Methods

Specimens were collected from two locations within the Oosterschelde (Figure 3). *O. inornatus* was collected primarily from the out-flow of the oyster pits in Yerseke Figure 4, while *U. cinerea* and some *O. inornatus* was collected from the dikes in Gorishoek. Searching was done haphazardly under rocks and amongst the oysters *Crassostrea gigas*.

Figure 3: Location of sampling locations of oyster drills in the Oosterschelde.

Figure 4: Oysterponds in Yerseke

For the experiment 20 specimens of varying sizes were placed in each of three plastic containers with holes to allow water flow and lids to prevent the animals escaping. The containers were placed in a tank of seawater and left to allow the animals to acclimatize and attach to the walls (Figure 5). Once the animals were attached, the

containers were removed from the water and gently placed in separate tanks filled with either freshwater, 90% freshwater and 10% seawater, or the control with only seawater (approx. 30 ppt). All tanks had an equal and constant air supply and kept at room temperature.

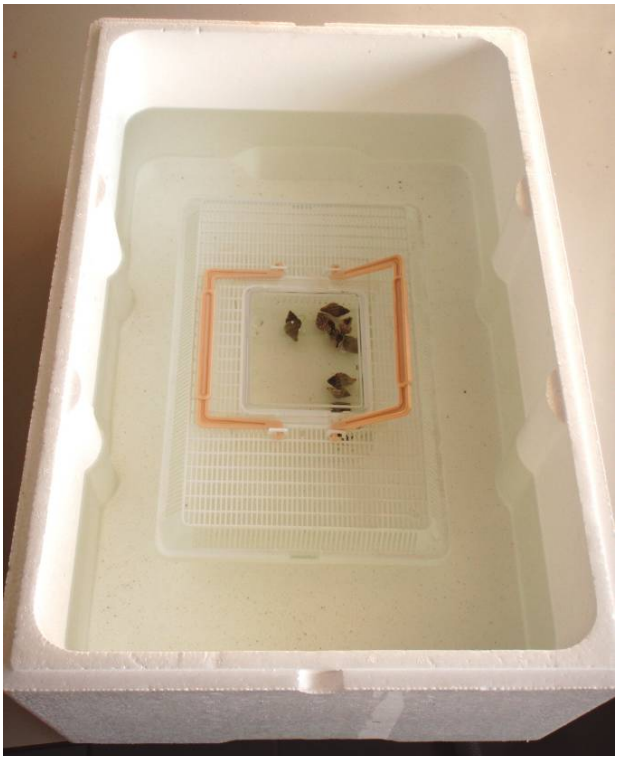

Figure 5. Example of experimental setup. Specimens were placed in a permeable container and then immersed in water.

The initial reaction of the animals was observed and recorded and then the animals were left for two hours. After this time, the animals in the freshwater treatments were removed and placed in seawater. Those that had reattached to the sides of the container or at least started moving within two hours were considered alive, while those that had not moved were considered dead. The experiment was then repeated with fresh specimens immersed in the water treatments for 24 hours.

3 Results

When immersed in the two freshwater treatments, all specimens of both species detached from the walls and closed their operculum. Small individuals detached much sooner than larger individuals, but all had detached within ten minutes and remained closed for the entire duration of the experiment.

When replaced into salt water after two hours, all individuals of both species remained closed for at least 30 minutes. After two hours, all individuals had reattached to the walls of the container and began moving around.

The same initial reaction was observed when the experiment was repeated for 24 hours. Similarly, after at least 30 minutes of re-immersion into saltwater following the 24 hours, all individuals of both species had reattached to the container walls and were moving around (Table 1).

Table 1. Mortality after immersion in freshwater for 2 and 24 hours.

Species	Treatment	Number dead		Observations
		2 hrs.	24 hrs.	
Ocinebrellus inornatus	Control	0	0	All remained attached to container walls
	90%	0	0	most detached immediately. All were detached within 5 minutes
	100%	0	0	most detached immediately. All were detached within 5 minutes
Urosalpinx cinerea	Control	0	0	All remained attached to container walls
	90%	0	0	no immediate detachment, larger specimens began detaching after 7-8 minutes
	100%	0	0	detachment after 3-4 minutes

4 Discussion

From the results of this study, it can be concluded that immersion in freshwater treatments for 24 hours or less fails to kill either *Urosalpinx cinerea* or *Ocinebrellus inornatus*. The oyster drills are very tolerant to varying salinities, as well as to exposure to air. The hard shell and the operculum serve as refuges in adverse conditions. The reported invasive success of these two species (Garcia-Meunier et al. 2003, Martel et al. 2004, Global-Invasive-Species-Database 2008) is itself indicative of their resistance to a wide range of conditions.

Other studies investigating salinity tolerance in oyster drills have shown similar results. While there are no known in-depth studies on the salinity tolerance of adult *O. inornatus*, Zachary and Haven (1973) looked at the survival of *U. cinerea* in different salinity treatments. They reported mortality decreased sharply at constant salinities above 10.0 ppt and suggested that the salinity range from 9 ppt to 10 ppt is the transition zone between quickly lethal conditions to all individuals and conditions causing only partial mortality over longer periods. In constant salinities of 8.0 ppt salinity, 92 % mortality was observed in 15 days and 100 % after 20 days, while at 10 ppt salinity, 50 % of the drills died in 10 days and 40 % were still alive after 40 days. However, as the shortest unit of time used where salinity could be lethal to any specimens was 0-5 days, a period of 24 hours is obviously too short to expect any significant effect on drill mortality regardless of salinity levels. Freshwater immersion of mussel seed for 24 hours is therefore unlikely to effectively eliminate the two oyster drill species in the transport of mussels from the Oosterschelde to the Wadden Sea.

While freshwater failed to kill the oyster drills within 24 hours, it may still prove to be a useful treatment in removing them. Within a few minutes of immersion of the two freshwater treatments, all individuals from both species detached from the substratum and closed their operculum. With no physical attachment to the substratum, they could be washed away if the substratum was rinsed. Mueller and Hoffman (1999) investigated the detachment behaviour in freshwater of *O. inornatus* (referred to as *Ceratostoma inornatum*). They also found that detachment occurred within the first few minutes following freshwater immersion, and that larger individuals took longer to detach than smaller conspecifics.

However, the effectiveness of rinsing mussels in freshwater to remove oyster drills is limited by the risk of drills remaining on the mussels if they become caught in crevices Furthermore the egg capsules of the oyster drills which are attached to hard substrates do not detach from their substrate in freshwater. Even if only a small number of drills are introduced to the new area along with the mussel seed, they can be expected to establish a growing population. Furthermore, Immersion in freshwater for a longer period may prove to be an inefficient treatment for mussel seed regardless of its effect on oyster drills as it can cause juvenile mussels to detach from mussel ropes, and reduce the quantity of mussel seed successfully arriving at their destination (McEnnulty *et al.* 2001).

Further investigations into the removal of oyster drills with minimal effect on mussel seed would provide more information and potentially more treatment options. Simple options for treatments such as the effects of increased salinity above that of natural sea water, or temperature are likely to be ineffective as the levels causing mortality in the drills will likely also kill the mussels (Hanks 1957, Beal 1993). Other more involved, and potentially more expensive options such as changes in levels of pH, ammonia or nitrate may be more successful as these are known to be effective against saltwater snails in aquariums (AllExperts 2009). Further investigation is needed to determine whether such treatments may be effective against *Urosalpinx cinerea* and *Ocinebrellus inornatus* and their effect on mussel seed.

In conclusion, Immersion in freshwater for 24 hours fails to cause any mortality in either species and while rinsing in freshwater may remove live drills, it does not guarantee 100 % removal. Therefore, freshwater treatments alone are unlikely to be an effective method of preventing the unintentional transportation of *Urosalpinx cinerea* and *Ocinebrellus inornatus* from the Oosterschelde to the Wadden Sea with mussel seed. Further investigation is recommended to determine whether a more effective treatment is feasible.

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 December 2009. 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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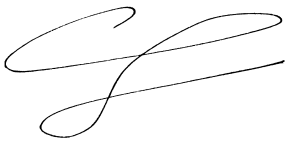
Approved: A.C. Smaal
Senior Scientist

Signature: 

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