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The invasive quagga mussel *Dreissena rostriformis bugensis* (Andrusov, 1879) (Bivalvia: Dreissenidae) in the Dutch Haringvliet, an enclosed freshwater Rhine-Meuse estuary, the westernmost record for Europe

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Here the westernmost record in Europe of the invasive quagga mussel *Dreissena rostriformis bugensis* is discussed, i.e. in the Haringvliet, an enclosed freshwater Rhine-Meuse estuary in The Netherlands. In 2006 and 2007 small numbers of quagga mussels were found in between large numbers of zebra mussels, *Dreissena polymorpha*. They were recorded during the periodical analyses of settlement plates of the international biofouling monitoring project SETL. Identifications were done on the basis of morphology and confirmed by DNA-barcoding techniques. The quagga mussels show a patchy distribution in the Dutch Haringvliet and Hollands diep. No settlement of quagga and zebra mussels was recorded between the second half of March to the first half of June both in 2006 and 2007. The main settlement period of quagga and zebra mussels in the research area is concluded to be from the second half of June until the end of December. The calculated maximum shell growth rates of both invasive mussel species are similar to somewhat higher than those recorded in literature, i.e. 0.077 mm/day to 0.141 mm/day.

Key words: Bivalvia, *Dreissena*, quagga mussel, zebra mussel, invasive species, shell growth rate, SETL, DNA-barcoding, The Netherlands.

INTRODUCTION

The quagga mussel, *Dreissena rostrifomis bugensis* (Andrusov, 1879) and the zebra mussel, *Dreissena polymorpha* (Pallas, 1771) are Ponto-Caspian species (Son, 2007) that have invaded lakes and rivers throughout North America (Berkman et al., 1998; Mills et al., 1993; Molloy et al., 2007; Stokstad, 2007; Wilson et al., 2006) and Europe (Popa & Popa, 2006; van der Velde & Platvoet, 2007; Zhulidov et al., 2005). They have caused substantial economical and ecological damage by for example increasing the maintenance costs of overgrown man-made structures like water corridors and pipes in cooling water circuits next to causing dramatic changes in native species communities e.g. by filtering plankton and other suspended matter out of the water column (Dermott & Munawar, 1993; Ricciardi et al., 1996; Karatayev et al., 1997; Vanderploeg et al., 2002; Ricciardi, 2003). In the 1980's Northern America (May & Marsden, 1992; Mills et al.,1993) and Eastern Europe

(Orlova et al., 2004; Zhulidov et al., 2005) were invaded, first by the zebra mussel and a few years later by the quagga mussel. Even though the quagga mussel has spread into the Don and Volga river basins of Russia in the early 1980's (Orlova et al., 2004; Zhulidov et al., 2005), the species remained absent from Western European waters until the twenty-first century (Molloy et al., 2007; van der Velde & Platvoet, 2007).

The fouling community study SETL was started in March 2006 by the ANEMOON foundation in cooperation with the Smithsonian Marine Invasions Laboratory of the Smithsonian Environmental Research Center (SERC). The materials and methods were based on those developed and used by the SERC (e.g. Hines & Ruiz, 2001). Volunteers and professionals of research organisations throughout Western Europe, North America and New Zealand are periodically checking fouling communities on standardized 14 x 14 cm PVC plates. Even though most SETL-localities are marine (e.g. Gittenberger & Schipper, 2008), two are positioned in fresh water. Here we discuss the finds of the quagga and zebra mussel in the Netherlands at these two localities.

MATERIAL AND METHODS

Ten 14 x 14 cm PVC plates per locality are hung at a depth of 1 metre below the water surface. They are periodically replaced, checked for fouling species, and photographed in overview and detail. Two SETL-localities in The Netherlands are situated in fresh-water, i.e. one on a fixed jetty at the Tiengemeten ferrydock of Nieuwendijk in the Haringvliet (fig.1, loc. A, 51°45′ 15″ N, 4°19′ 03″ E), and one on a floating dock off Numansdorp in the Hollands Diep (fig.1, loc. B, 51°43′ 02″ N, 4°26′ 13″ E). The deployment periods of SETL-plates at these localities are shown on the timeline in figure 2. The locality of the first record of the quagga mussel in The Netherlands by Molloy et al. (2007), i.e. in 2006, is also shown in fig. 1. The Haringvliet and Hollands Diep (fig. 1AB) are both part of the Rhine-Meuse estuary. Since 1970 they are separated from the North Sea by sluices. These sluices are only opened at high discharge of river water. As a consequence the area receives only freshwater from the Rhine and Meuse and sea water influences are minimal (Smit et al., 1997). The specimens from the PVC plates were identified on the basis of their conchology, independently by Edmund Gittenberger and by the two authors from the Institute of Biology Leiden, Leiden University and the National Museum of Natural History Naturalis, The Netherlands. The shell length of the largest specimen per deployment period

Fig. **1**. SETL localities A and B in the Rhine-Meuse estuary. **A**, Nieuwendijk, Haringvliet; **B**, Numansdorp, Hollands Diep; **C**, first record of *Dreissena rostriformis bugensis* in The Netherlands (Molloy et al., 2007). Satellite imagery by TerraMetrics, Inc. Copyright 2008, http://www.truearth.com.

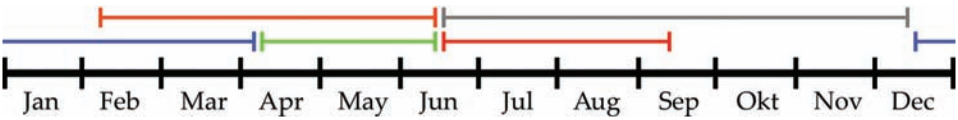

Fig. **2**. PVC plate deployment periods for SETL-localities A and B (Fig. 1). Brown bar, March 16 2006 – June 16 2006; Grey bar, June 16 2006 - December 16 2006; Blue bar, December 16 2006 - April 6 2007; Green bar, April 6 2007 - June 15 2007; Red bar, June 15 2007 - September 20 2007.

(fig. 2) was measured for both the quagga mussel and the zebra mussel. These shell lengths in combination with the number of days that the plates were deployed were used to calculate the shell growth rates of the species. These shell growth rates are underestimates of the maximum growth rate because it was assumed that the mussels settle on the plates on the first day of deployment into the water. Voucher specimens of the quagga mussels were fixed in 96% ethanol. To confirm their identity by their DNA barcode, i.e. the marker cytochrome oxidase subunit I (COI), the DNA of two specimens, i.e. one collected in 2006 and one in 2007, was isolated using the Dneasy Tissue Kit (Qiagen). PCR was done as in Gittenberger & Gittenberger (2006). The primers used for the amplification of the COI marker were LCO1490 (forward), i.e.5'- GGTCAACAAATCATAAAGATATTGG-3' (Folmer et al., 1994), and HCO2198 (reverse), i.e. 5'- TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al., 1994). An ABI Prism 377 DNA sequencer was used to analyse the DNA by direct sequencing. To check the identity of the specimens as *Dreissena rostrifomis bugensis* (Andrusov, 1879) a BLAST search was done with the retrieved sequences on the NCBI GenBank.

RESULTS

Quagga mussels were encountered in 2006 and 2007 during the seasonal checks of the SETL-project (figs 2-3, table 1). The quagga mussels were encountered on the settlement plates that were deployed from the fixed jetty at the ferrydock of Nieuwendijk in the

Figs **3A-B**. *Dreissena rostriformis bugensis* specimen found on December 16th 2006 (RMNH 106720, Genbank EU651840). 3A: The specimen is highlighted on the bottom centre on a SETL plate in between numerous *Dreissena polymorpha* specimens. 3B: Detailed photograph of the *D. rostriformis bugensis* specimen.

Deployment period Retrieval date Deployment period	Jun. 16, 2006 - Dec. 16, 2006 183 days	Dec. 16, 2006 - Apr. 6, 2007 111 days	Jun 15, 2007 - Sep. 20, 2007 97 days
Quagga mussel, *Dreissena rostriformis bugensis* (Andrusov, 1897)			
max. shell length	14 mm	12 mm	10.5 mm
max. growth rate	0.077 mm/day	0.108 mm/day	0.108 mm/day
Zebra mussel, *Dreissena polymorpha* (Pallas, 1771)			
max. shell length	16 mm	16 mm	13.7 mm
max. growth rate	0.087 mm/day	0.087 mm/day	0.141 mm/day

Table **1**. Maximum shell growth rates of quagga and zebra mussels on a depth of one metre in the Haringvliet river, The Netherlands. Maximum shell growth rates are underestimates of the real maximum growth rates, because it is assumed that the mussels settled on the day of SETL-plate deployment. The growth rates are based on measurements of the largest specimen on the plates (n=1).

Haringvliet (fig. 1A). No specimens were found on the ten plates that were deployed from the floating dock off Numansdorp in the Hollands Diep (fig.1B). No quagga and zebra mussels were recorded on plates that were deployed between the second half of March to the first half of June in both 2006 and 2007 (fig. 2). The first two specimens of *Dreissena rostrifomis bugensis* were discovered on two out of ten Nieuwendijk plates that had hung underwater from June 16 to December 16, 2006 (fig. 2, table 1). The quaggas were found amidst hundreds of zebra mussels (fig. 3). On plates that were deployed from December 16, 2006 to April 6, 2007, again two quagga mussels were encountered amidst some, i.e. about eight, zebra mussels. Four specimens, on three different plates, were found after the deployment period from June 15 to September 20, 2007. They settled in between numerous zebra mussels.

For each deployment period in which quagga mussels and zebra mussels were found the shell growth rate was calculated (table 1). The recorded shell growth rates varied from 0.077 mm/day to 0.141 mm/day. In general zebra mussels were larger in size and therefore had a higher shell growth rate compared to the quagga mussels. This result may at least partly be biased by the much higher numbers of zebra mussels that were encountered. Both species showed the lowest shell growth rate during the deployment period of June 16 to December 16, 2006. During the deployment period of June 15 to September 20, 2007, the zebra mussel showed the highest shell growth rate, i.e. 0.141 mm / day.

Two identical, 336 bp sequences were retrieved from the two specimens of *Dreissena rostrifromis bugensis* of which the DNA was analysed (GenBank accession numbers EU604834 and EU651840). A BLAST search on the NCBI GenBank, revealed five 100% identical COI sequences of *D. rostriformis bugensis*, i.e. EF080861-EF080862 (Molloy et al., 2007), DQ840132- DQ840133 (Gregory et al., 2006), and U47651 (Baldwin et al., 1996).

DISCUSSION & CONCLUSSIONS

Origin. -- The origin and import vector of the quagga mussel specimens in The Netherlands remains unknown. The low variance in the mitochondrial COI gene of *D. rostriformis bugensis* causes little room to speculate about its dispersal pathway on the basis of molecular results only (Molloy et al., 2007). However, the discoveries of quagga mussels upstream in the Danube River by Popa & Popa (2006) and in the Main River near the Main-Danube canal by van der Velde & Platvoet (2007) suggest that this species could have reached Western Europe through these connected water bodies.

According to our observations quagga mussel and zebra mussels settle in the Haringvliet at least seven months of the year, i.e. starting in the second half of June and proceeding well into December (fig. 2, table 1). This settlement period exceeds those known from literature. Zebra mussels have for example been known to settle from the end of June to late October (a period of roughly 4 months) in the Great Lakes of North America (Mackie , 1991; Mackie & Schloesser, 1996).

Growth rates. -- The shell growth rates estimates for both the quagga mussel and the zebra mussel in the Haringvliet (table 1) resemble those calculated for the zebra mussel in the Haringvliet by Smit et al. (1992). Smit et al. (1992) measured a maximum shell growth rate of 0.107 mm/day (end of May – start of June), which is very similar to the shell growth rate of the quagga mussel, i.e. 0.108 mm/day, in the periods from December 16, 2006 to April 6, 2007 and June 15, 2007 to September 20, 2007 (table 1). The maximum shell growth rate found for the zebra mussel, i.e. 0.141 mm/day in the period June to September 2007, is considerably higher of those measured by Smit et al. (1992) in the Haringvliet. The shell growth rates calculated in the present study were averaged over 3 and 6 month periods in comparison to the two week periods used in the measurements of Smit et al. (1992). This means that our shell growth rates are more averaged and, at times, would probably have been higher than those calculated by Smit et al. (1992). This difference is supported by prior investigations that already indicated that zebra mussel shells grow faster in shallower waters (Kornobis, 1977; Bij de Vaate, 1991). The settlement plates that are used in the present study are suspended about one metre below the water level while the cages deployed by Smit et al. (1992) were at a depth of 5 m.

Patchy distribution. -- Quagga mussels were found east of Numansdorp (fig. 1, loc. B) by Molloy et al. (2007) in the Hollands Diep (fig. 1, loc. C) and in three separate occasions (table 1) they were found west of Numansdorp in the Haringvliet at Nieuwendijk (fig. 1, loc. A). On the SETL-plates deployed at Numansdorp no quagga mussels were found. According to Mackie (1991), veligers of dreissenids are present in the water column over a 3-4 week period. They should be able to reach Numansdorp from either Nieuwendijk or Hollands Diep localities (fig.1). It is therefore unlikely that quagga mussels were missed on the plates at the Numansdorp locality just by chance. Possibly the recorded patchy distribution can be explained by a habitat preference. A more detailed study of the abiotic and biotic parameters associated with the studied habitats is necessary to test this hypothesis. Two differences in habitat between the Nieuwendijk (fig. 1A) and Numansdorp (fig. 1B) localities that may have had an influence are [1] the non floating vs. floating structures on which the plates were hung at respectively Nieuwendijk and Numansdorp. Differences in epifauna compositions have been linked to floating and non-floating structures in the past by e.g. Holloway & Connel (2002); [2] the eutrophication due to the active agricultural water discharge through a water outlet about ten metres away from the SETL-locality at Nieuwendijk.

Invasion risk. -- Our finds of individual quagga mussels on SETL-plates proves that the quagga mussel has established itself for now in the Haringvliet, the Netherlands. Whether and how the species will spread through The Netherlands waters remains uncertain and is hard to predict (Côté & Reynolds, 2002). It will be discussed in more detail by van der Velde et al. (in press.). In the Haringvliet the spread of the species may be influenced in the near future by the recently made political decision to open the Haringvliet sluices to the North Sea on a more permanent basis. This may cause an increase in the salinity in the area (Smit et al., 1997).

It is not unlikely that the quagga mussel will start to replace and dominate the zebra mussel in The Netherlands as it has done already in Eastern Europe, e.g. localities in the Lower Volga Delta and Middle Volga (Orlova et al., 2004), as well as in Northern America, i.e. in the U.S.A., e.g. in the Great Lakes of North America (Mills et al., 1996, Mills et al., 1999; Ricciardi & Whoriskey, 2004; Wilson et al., 2006), Lake Erie (Mills et al., 1993), and the southern Lake Ontario (Mills et al., 1999), and in Canada, e.g. the Canadian nearshore of Lake Ontario (Wilson et al., 2006) and the Soulanges Canal, upper St. Lawrence River (Ricciardi & Whoriskey, 2004). According to Ricciardi & Whoriskey (2004) in Canada 'The mechanism of this shift remains unknown, but may be related to a greater bioenergetic efficiency for quaggas, which attained larger shell sizes than zebra mussels at all depths'. Whether the same shift will occur in the Netherlands, and more specifically at the localities of Nieuwendijk and Numansdorp, is not clear, but will be monitored by the SETL-project that is aimed to proceed indefinitely with the help of the volunteers of the ANEMOON foundation.

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