

Rapid Communication**Distribution of the dwarf surf clam *Mulinia lateralis* (Say, 1822) in the Wadden Sea after first introduction**

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OPEN ACCESS**Abstract**

During the SIBES project, a large-scale monitoring of the marine benthos of the Dutch and western German Wadden Sea, three specimens of the bivalve *Mulinia lateralis* (Say, 1822) (Bivalvia: Mollusca: Mactridae), a common opportunistic species native in the east coast of North America, were found in August 2017 in the Ems-Dollard estuary. Present records backdate of two months the arrival of this species in Europe and first report it for the German Wadden Sea. Further specimens were then found in 2018, confirming its establishment in the area. An analysis of favourable habitat characteristics for *M. lateralis* showed that large parts of the Dutch Wadden Sea are a potential suitable habitat for this species.

Key words: Dutch Wadden Sea, invasive, bivalve, potential distribution, SIBES, Mactridae

Introduction

The Wadden Sea is a large intertidal ecosystem that stretches from the Netherlands to Denmark. It is the largest wetland area and is recognised as a heritage site of international importance in the Netherlands and Germany (Ramsar status, UNESCO World Heritage Site). It is known for its habitat variability and unique (benthic) biodiversity. The invasion of non-native species can alter the synergetic community currently present in this ecosystem. Thus, the detection of invasive species in the Wadden Sea is of key importance, and large-scale monitoring programs are a requisite for this.

SIBES (Synoptic Intertidal Benthic Surveys) is a spatially comprehensive large-scale monitoring campaign of the macrozoobenthos and sediment composition of the Dutch and western German intertidal Wadden Sea, carried out by the department of Coastal Systems of the Royal Netherlands Institute for Sea Research (Bijleveld et al. 2012; Compton et al. 2013). It covers the entire intertidal of the Dutch Wadden Sea and consists of gridded samples taken at 500 m intervals and additional random samples, making a total of ~ 4,500 samples per year. Complementary, in the western German Wadden Sea, samples are taken at 1000 m intervals with the addition of

random samples (~ 1,000 samples per year). Samples are collected annually, since 2008, from the end of June to the beginning of September.

In August 2017, three specimens of a bivalve similar to *Spisula subtruncata* (da Costa, 1778) were found in the Ems-Dollard estuary during the SIBES programme. However, suspicion was raised about this identification, as this common North Sea species is rarely found in the Wadden Sea. When more specimens were found in 2018, it was identified as *Mulinia lateralis* (Say, 1822), a western Atlantic species that was never found before in European coastal waters. This morphological identification was later confirmed by DNA-barcoding. In the meanwhile, Craeymeersch et al. (2019) first published records of *M. lateralis* for the Netherlands; however, their findings in October 2017 in the southwestern Dutch coastal zone (the Voordelta) were two months later than the SIBES records in the Wadden Sea.

This present paper deals with additional records of *M. lateralis* in the Wadden Sea, backdating of two months the detection date of this species in Europe and first reporting this taxon for Germany. Furthermore, on the basis of habitat characteristics and community composition of the locations of findings, an attempt is made to outline its potential future distribution range in the Dutch Wadden Sea.

Materials and methods

Sample collection and processing

Sampling locations were accessed either by foot or from a small boat, depending on the tide. Sediment cores (~ 25 cm depth, core surface of 0.018 m²) were sieved over a 1 mm round mesh sieve in the field. Large shellfish (> 8 mm) were stored in the freezer for later identification and biomass determination. All other organisms remaining on the sieve were stored in a 4% buffered formaldehyde solution for later identification to species level or the finest taxonomic level possible, and for counting and biomass determination (Compton et al. 2013; Christianen et al. 2017). Sediment cores of the top 4 cm of the sediment were stored in the freezer and freeze-dried for up to 96 hours till dry. Subsamples ranging between 0.5 and 5 grams of homogenized sediment, depending on the estimated grain size were sieved over a 2 mm sieve and collected in 13 ml PP Autosampler tubes prior to grain-size analysis. RO water was added and the sample was shaken vigorously on a vortex mixer for 30 seconds. Median grainsize and the percentage silt (fraction < 63 µm) of sediments were determined using a Coulter LS 13 320 particle size analyser and Autosampler. This apparatus measured particle sizes in the range of 0.04–2,000 µm in 126 size classes, using laser diffraction (780 nm) and PIDS (450 nm, 600 nm and 900 nm) technology. The optical module “Gray” was used for the calculations. The sediments were analysed according to the “biological

approach”, i.e. the organic matter and calcium carbonate was not removed from the samples. (Compton et al. 2013; Folmer et al. 2017).

Salinity data were not obtained in the field but acquired through model simulations as described in Duran-Matute et al. (2014). In short, a three dimensional numerical simulation was carried out with the General Estuarine Transport Model (GETM), at a horizontal resolution of 200 m and with terrain-following vertical coordinates with 30 layers. For the years 2009–2010, meteorological forcing, freshwater discharge, and boundary conditions for tidal forcing and storm surges were imposed. Indeed, we are aware the salinity data are not from recent years and conclusions based on these data will be made with caution. However, changes in salinity in the Dutch Wadden Sea are considerable both on a short time scale (tidal) and on longer time scales (seasonal-centennial) (van Aken 2008). Therefore, salinity measurements will always be rough indicators of the long-term salinity trends at the specific locations.

Morphological identification

Among the bivalves sorted out from the SIBES samples, a few shells found looked very similar to the common North Sea species *Spisula subtruncata*. Because the shells looked more globose and because of the rarity of *S. subtruncata* in the inshore parts of the Wadden Sea, suspicion was raised about the identification. Thus, we compared our specimens with the identification key provided by Craeymeersch et al. (2019) for all seven Mactridae of the NE Atlantic Ocean, namely *Mactra glauca* Born, 1778, *Mactra stultorum* (Linnaeus, 1758), *M. lateralis*, *Rangia cuneata* (G.B. Sowerby I, 1832), *Spisula elliptica* (T. Brown, 1827), *Spisula solida* (Linnaeus, 1758), and *S. subtruncata*.

Molecular identification

Tissues samples from all specimens found (Supplementary material Table S1) were collected from the foot of the bivalves and stored in 96% ethanol. Genomic DNA was extracted using the GenElute™ Mammalian Genomic DNA miniprep kit (Sigma-Aldrich Inc.). Each individual extract was amplified for two barcode regions; the 16S rRNA gene and the Cytochrome c oxidase subunit I (COI) gene, respectively, using the 16Sar and 16Sbr primer pair (Palumbi 1996) and the Mul2L and Mul1R primer pair (Hare et al. 2000). All polymerase chain reactions (PCR) were performed in a 50 µl reaction volume, containing 0.5 µM of each primer, 0.25 µM dNTPs, 2U BioTherm™ + Taq DNA Polymerase (Biotherm™ Inc.), 1x PCR buffer (Biotherm™ Inc.) and 2 µl of DNA extract. PCR reactions were subjected to five minutes at 94 °C, followed by 35 cycles each comprised of 60 seconds at 95 °C, 60 seconds at 45 °C, and 2 minutes at 72 °C, respectively, and one final extension step for seven minutes at 72 °C. The PCR products were

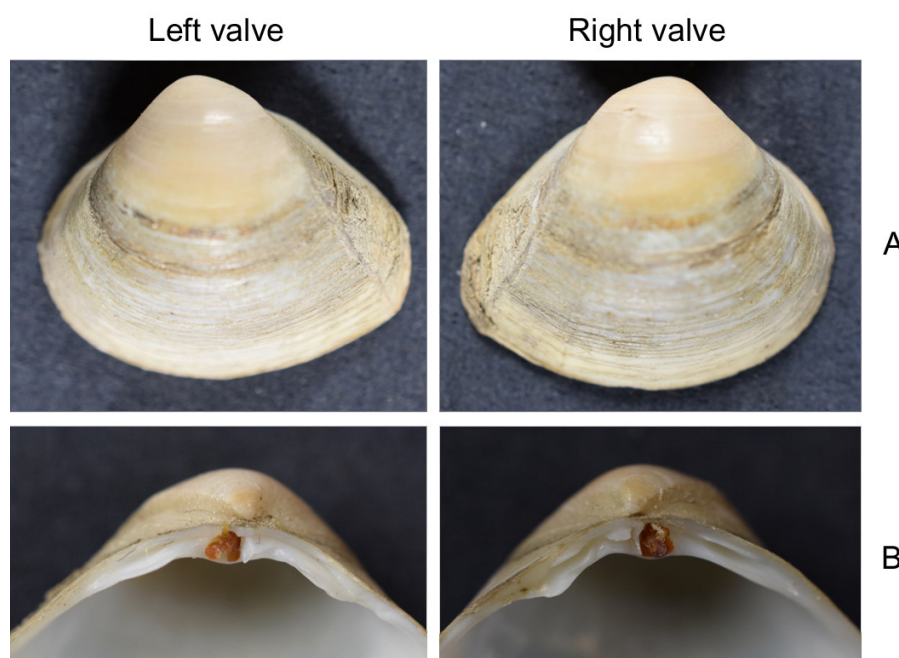


Figure 1. Pictures of key morphological identification characteristics of *Mulinia lateralis*: (a) the outside of the valves with the radial ridge and (b) the anterior cardinal tooth which is parallel to the edge of the shell and the non-serrated lateral teeth. The specimen at the picture had a length of 16 mm.

Sanger sequenced in both directions by BaseClear (Leiden, Netherlands). Forward and reverse sequences obtained by the Sanger procedure were aligned using Geneious™ (version. R9, Kearse et al. 2012). The consensus sequence was obtained with a highest quality threshold. Consensus sequences were aligned with reference sequences from the entire NCBI nucleotide (NT) database using Blast (Morgulis et al. 2008).

Results

Morphological and molecular identification

Careful examination under the microscope, and especially of the hinge, made clear that our specimens belonged to *Mulinia lateralis*, first described by Say (1822) as *Mactra lateralis*. Three clear characteristics in which it differs from *S. subtruncata* are: 1) a radial ridge at the outside along posterior ends of valves; 2) the cardinal teeth of the right valve almost parallel with the edge of the shell above it; and 3) the lateral teeth of the hinge smooth and not serrated (Figure 1). Comparison with the clear identification key provided by Craeymeersch et al. (2019) confirmed the identification of this specimens as *Mulinia lateralis*. Blast queries for both genes generated sequences with a 100% identical score to *Mulinia lateralis*. All specimens listed in Table S1 were stored as voucher-specimens and the sequences of the processed specimens were submitted to Genbank.

Distribution in the Wadden Sea

The dwarf surf clam *Mulinia lateralis* was found in the Wadden Sea at several stations in 2017 and 2018 (Table S1, Figure 2). Most specimens were

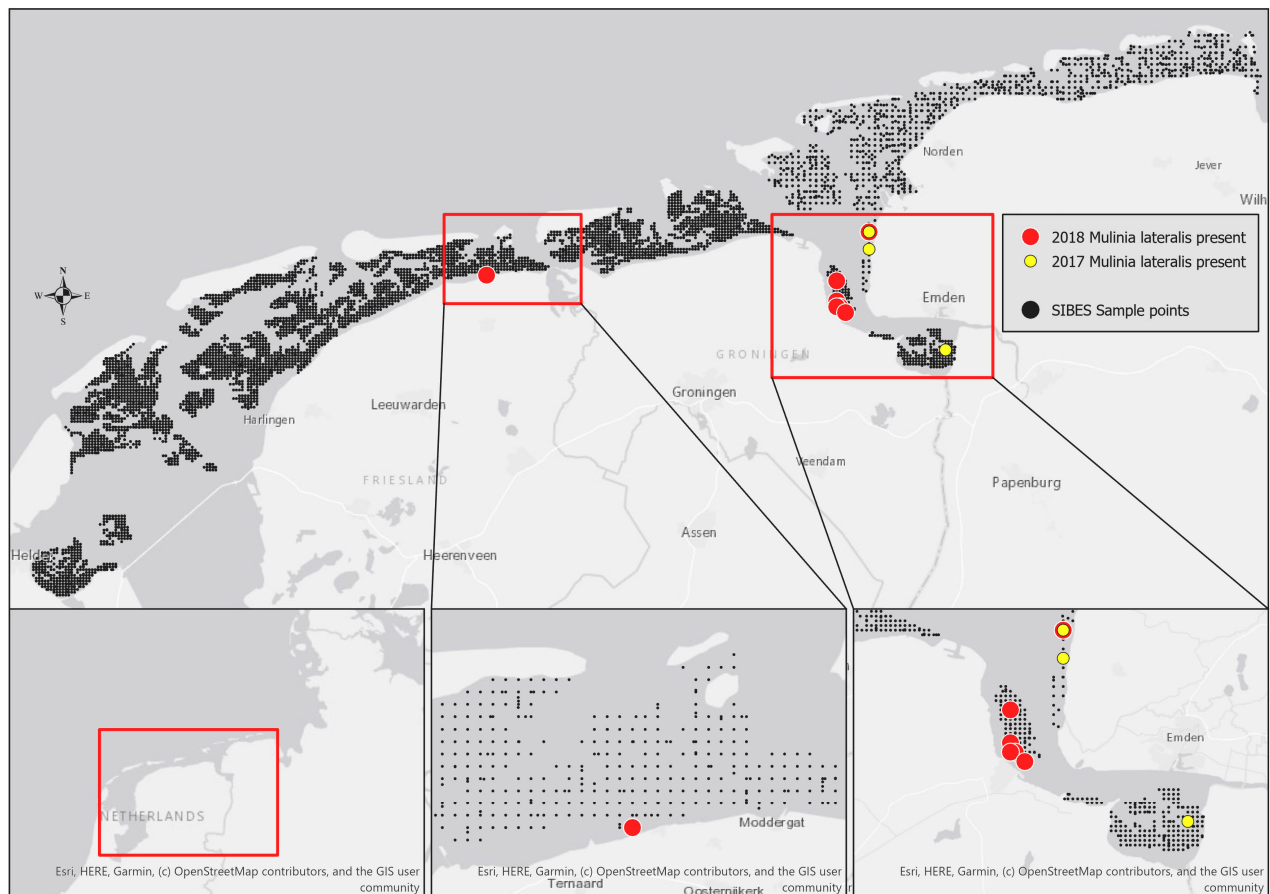


Figure 2. Overview of the 5600 sampled stations in the Wadden Sea (black dots), with the locations where *Mulinia lateralis* was found indicated with yellow and red dots. Bottom-middle shows locations at the Frisian coast; bottom-right shows locations in the Ems-Dollard estuary.

found in the Ems-Dollard estuary, both in the Dutch and German part. One specimen was found at the Frisian coast. All specimens ranged in sizes between 13–17 mm and were detected in the field during collection. However, not all samples collected in 2017 and 2018 have been analysed in the lab yet, and more specimens, especially smaller ones, could show up later. Craeymeersch et al. (2019) also mention findings in the Wadden Sea, namely 5 locations in the Ems Dollard estuary, 3 between Ameland and Friesland, and 2 in the subtidal parts of the western Wadden Sea.

The environment in the Dutch Wadden Sea where *M. lateralis* was found (Table S1) consisted of soft mud with median grain sizes ranging between 72–106 μm , silt contents (% < 63 μm) ranging between 27–44% and a salinity range of 12.4–26.8‰ (based on Duran-Matute et al. 2014). Values of salinity were obtained by spatially joining the sample points and raster data of the salinity in ArcGIS Pro (version 2.2.4 ESRI, Redlands, California, USA). The specimens were found only in sediments with high percentages of silt and a low median grain size relative to the other intertidal SIBES sampling points, while, for salinity, they were found at a much broader range. Hence, the distribution of *M. lateralis* seems to be mainly driven by sediment characteristics rather than salinity, at least within the Dutch Wadden Sea.

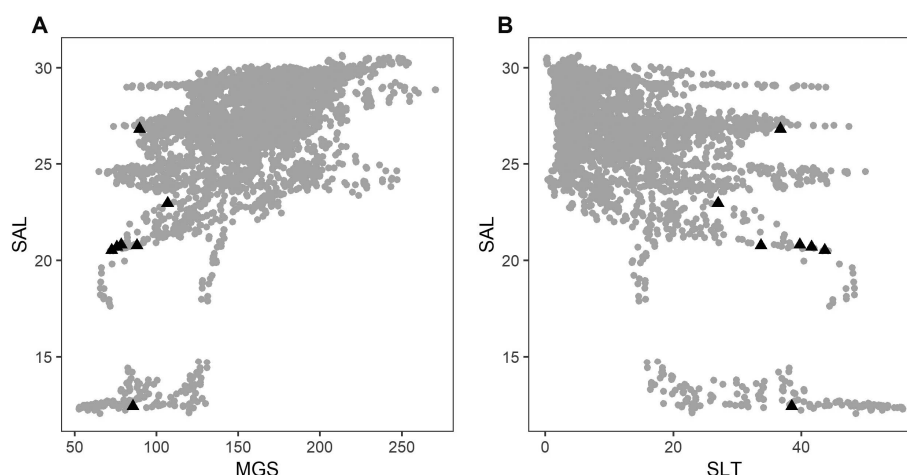


Figure 3. Scatterplot of all SIBES sampling points and their salinity (SAL) and (A) median grainsize (MGS) or (B) silt content (SLT) measurements. The triangle shaped points depict sampling points at which *Mulinia lateralis* was found.

Community composition

Mulinia lateralis was found living together with the polychaetes *Alitta succinea* (Leuckart, 1847), *Heteromastus filiformis* (Claparède, 1864), and *Tharyx* sp., the gastropod mollusc *Peringia ulvae* (Pennant, 1777) and the bivalve mollusc *Mya arenaria* Linnaeus, 1758, and the amphipod *Corophium volutator* (Pallas, 1766). *Alitta succinea* and *C. volutator* are especially known to tolerate low salinities and thrive at relatively fine sediments (Compton et al. 2013; Folmer et al. 2017). Also *H. filiformis*, *Tharyx* sp., *M. arenaria*, and *P. ulvae* are mostly associated with fine sediments (Folmer et al. 2017). Other species found in the samples, but less frequently, were the polychaetes *Pygospio elegans* Claparède, 1863, *Polydora cornuta* Bosc, 1802, and *Marenzelleria viridis* (Verrill, 1873), the bivalves *Limecola balthica* (Linnaeus, 1758) and *Cerastoderma edule* (Linnaeus, 1758), and the gastropod *Retusa obtusa* (Montagu, 1803).

Potential distribution

According to literature, adult *Mulinia lateralis* are tolerant to low salinities, and often occur in estuaries where the salinity varies from 18 to 30 PSU (Lippson and Lippson 1984). Also, larval development was shown normal between 15 to 35 PSU (Calabrese 1969). Salinity in the Wadden Sea naturally ranges from low salinity close to the coast versus higher salinity near the inlets with the North Sea, ranging from 12 to 31 PSU (based on Duran-Matute et al. 2014). Therefore, salinity does not seem to inhibit the spread of *M. lateralis* within the Dutch Wadden Sea. Median grain size and silt content however seem to have a high influence on the potential distribution of *M. lateralis* within the Dutch Wadden Sea. Literature showed *M. lateralis* is natively found in areas with relatively fine sediments (Walker and Tenore 1984); this is in accordance with the data shown in Figure 3. Median grainsize

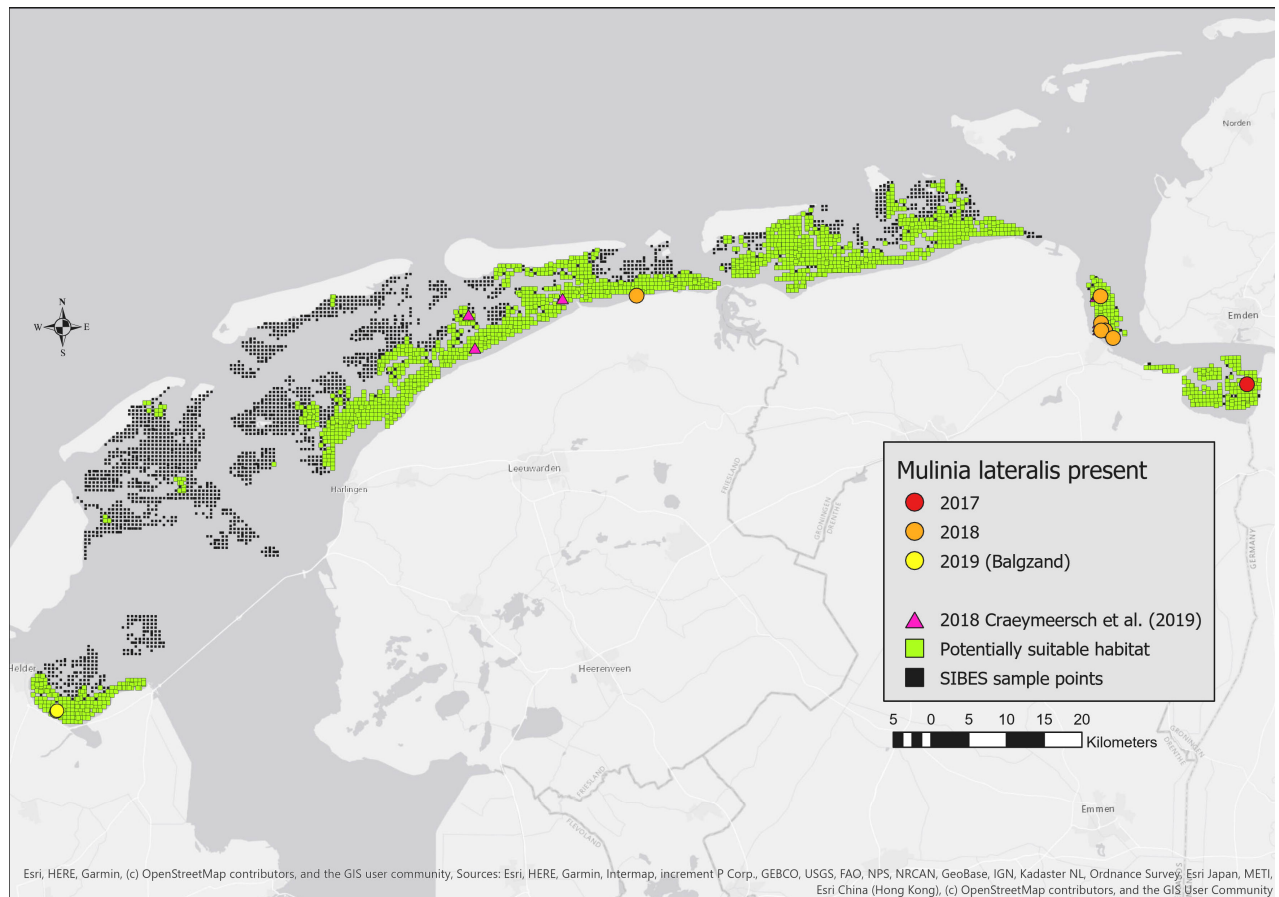


Figure 4. Potential suitable habitat of *Mulinia lateralis* in the Dutch Wadden Sea based on the environmental data as collected at the 2017 and 2018 sample locations, with addition of the locations from Craeymeersch et al. (2019).

distribution in the Wadden Sea roughly increased from south to north, as well as from east to west (Folmer et al. 2017).

Based on the environmental data collected in the SIBES program (median grainsize and silt content) a habitat suitability map for *M. lateralis* was constructed (Figure 4). The environmental variables median grainsize, silt content, and salinity were measured or calculated for each SIBES sample location. The range of these variables were used to obtain a potentially suitable habitat. Figure 4 shows which SIBES sampling locations would potentially be suitable habitats for *M. lateralis* based on the range of the environmental variables as measured at the sampling locations of 2017 and 2018. Also the intertidal locations of the Wadden Sea published in Craeymeersch et al. (2019) were taken into account. The addition of the environmental data of these nine extra locations widened the range of median grainsize (up to 164 μm) and silt content (as low as 6%), and indicated that *M. lateralis* also thrived in areas with coarser sediments.

Within the SIBES programme, only larger specimens of *M. lateralis* were identified so far. This is merely due to the sampling strategy rather than the presence of juvenile specimens. Larger bivalves were separated from the rest of the sieved sediment fraction in the field. These larger bivalves were

then analysed directly whereas the bulk of the sieved residue samples of 2017 and 2018 still needs to be processed. Therefore, further detections of small specimens (i.e. specimens < 8 mm shell length) cannot be excluded. Moreover, during the 2019 winter sampling campaign of the macrozoobenthos programme on long-term variability, population dynamics, and production with bi-annual sampling at Balgzand, western Dutch Wadden Sea, since the early 1970-ies (for methodological description, see Beukema and Cadée (1997) and Dekker and Beukema (2007)) two more specimens of *M. lateralis* were found, roughly 400 meters away from each other (Figure 4). Both specimens, though quite different in size (± 16 and 4 mm shell length) showed no growth line as depicted in Figure 1, and therefore were considered to originate from a 2018 settlement. Both specimens were detected close to the mainland coast and within the area described as potentially suitable to *M. lateralis* (Figure 4).

Discussion

According to literature, *Mulinia lateralis* has a short generation time reaching sexual maturity in 1–2 months and can produce up to 2 million eggs each time (Guo and Allen 1994; Lu et al. 1996). As it grows and reproduces quickly, it can become dominant in areas where conditions are optimal (Chalermwat et al. 1991), or where competition is diminished by either a catastrophe (Santos and Simon 1980) or poor water quality and low oxygen levels (McKeon et al. 2015). Of the benthic community of the St. Lucie Estuary, eastern Florida, it had the most variable occurrence of all species, indicating that this species is very tolerant to different environmental conditions (McKeon et al. 2015). But in places with better conditions and a higher bivalve diversity its presence was reduced, suggesting that it is not a strong competitor. Predation is probably the major factor controlling adult population size, at least during warmer months (Virnstein 1979), and numbers increase drastically in the absence of predators.

The first detections of *M. lateralis* in North-west Europe did occur at two spatially separated areas (i.e., Voordelta by Craeymeersch et al. (2019) and Ems-Dollard (this paper)) around the same time period. The detections of both locations involved adult specimens. This dual detection is of particular interest considering their short life cycle, up to two years (Lu et al. 1996). Therefore, both records are assumed to have settled in the same year, 2016. It can be discussed if *M. lateralis* settled at both locations at the same time or possibly earlier settlements were missed either in the Voordelta or Ems-Dollard. The spatial range in which *M. lateralis* has been detected since then increased rapidly including detections in the Western Wadden Sea within two years after its first detection, assuming the first detection in the Ems-Dollard estuary was directly after its first settlement here. In comparison, the polychaetous worm *Marenzelleria viridis* (Verrill,

1873) was detected in the Western Wadden Sea six years after its first detection in the Ems-Dollard (Essink and Dekker 2002).

In conclusion, *M. lateralis* seems to become a successful invader in the Wadden Sea. In 2017, only one specimen was detected in the Dutch Wadden Sea, whereas in 2018 it was found by SIBES at six locations (at eleven when including the locations of Craeymeersch et al. 2019) and in 2019 it was also found at Balgzand during the bi-annual sampling programme there by NIOZ (Beukema and Cadée 1997; Dekker and Beukema 2007). A considerable part of the Dutch Wadden Sea can be described as potentially suitable habitat and a fast spread towards these potentially suitable locations can be expected.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Records of *Mulinia lateralis* in the Dutch (NL) and German (DE) Wadden Sea including (abiotic) metadata according to the detection locations and GenBank accession numbers of the sequenced specimens.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2019/Supplements/BIR_2019_Klunder_etal_Table_S1.xlsx