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ORIGINAL RESEARCH ARTICLE

Limited success of the non-indigenous bivalve clam *Rangia cuneata* in the Lithuanian coastal waters of the Baltic Sea and the Curonian Lagoon

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Winter conditions;
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Natural spread.

Summary The gulf wedge clam, common rangia *Rangia cuneata*, with a native origin in the Gulf of Mexico has spread to north European brackish and freshwaters. This semitropical species is able to survive in conditions of low winter temperatures in boreal environment of the Baltic Sea. Its expansion within lagoons and sheltered bays in the southern and eastern parts of the Baltic Sea appears to be with natural spread and its discontinuous distribution is likely to have been with shipping, either within ballast water or as settled stages transported with dredged material. In this account, we report on the occurrence of *R. cuneata* in Lithuanian waters. We compare habitats of the common rangia in the Curonian Lagoon and in the exposed coastal waters of the Baltic Sea. We notice high mortality of the species in the Lithuanian waters in comparison to the neighboring Vistula Lagoon. Based on finding of small specimens of *R. cuneata* attached to the spiked watermilfoil *Myriophyllum spicatum*, we indicate a risk of local spread with movements of fishing equipment and snagged plants on anchors or boat trailers removed from the water. We discuss the possibility of further spread of the common rangia to similar environments in the Baltic Sea and elsewhere in Europe.

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1. Introduction

The common rangia *Rangia cuneata* (G.B. Sowerby I, 1832) (Bivalvia, Mactridae), has a native origin within the Gulf of Mexico and extended its range northwards to the Chesapeake Bay by the 1960s and arrived to the lower reaches of the Hudson River (Carlton, 1992). It is spreading within north European brackish waters (AquaNIS, 2018; Verween et al., 2006; and references therein). It was first found, and well established, in Belgium during 2005 (Verween et al., 2006) and has since spread to estuaries in southern regions of the North Sea (Bock et al., 2015; Gittenberger et al., 2015; Kerckhof et al., 2018; Neckheim, 2013; Wiese et al., 2016) and has been found in freshwater in Britain (Willing, 2015).

It entered the south-eastern Baltic Sea about 2010, in the waterway leading to the port of Kaliningrad, Vistula Lagoon region in Russia (Ezhova, 2012; Rudinskaya and Gusev, 2012). In 2011 it was recorded in the Polish part of the Vistula Lagoon (Janas et al., 2014; Warzocha and Drgas, 2013; Warzocha et al., 2016). It was in 2013 when it was recorded at an early stage along Lithuanian coastal waters (Solovjova, 2014), and further to the north in Pärnu Bay, Gulf of Riga, Estonia (Möller and Kotta, 2017). In 2015 it appeared on the German Baltic coast (Wiese et al., 2016) and in a Swedish Baltic fjord in 2016 (Florin, 2017). In this account, we present data on the first record and spread of *R . cuneata* in Lithuanian waters. We compare habitats of the common rangia in the Curonian Lagoon and in the exposed coastal waters of the Baltic Sea, and discuss the possibility of its further spread to similar environments in the Baltic Sea and elsewhere in Europe.

2. Material and methods

2.1. Study area

The Baltic Sea area along the exposed coast of the Curonian Spit is mesohaline, with a stable salinity (Table 1). From June to September where the epilimnion extends down to 20—30 m (Olenin and Daunys, 2004). In winter, ice usually occurs along this shoreline, but does not extend offshore where wedge clam rangia is found. Coastal currents in the south-eastern Baltic are usually from south to north and on account of its exposure the seabed is well oxygenated (Olenin and Daunys, 2004) and is densely packed with fine sand at all stations with the exception of an admixture of mud near the Curonian Lagoon outlet.

The Curonian Lagoon is exposed to irregular inflows of marine water, causing abrupt changes in salinity within the Klaipėda Strait and extending up to 40 km within the lagoon, where otherwise the average annual salinity is 2.5 (Dailidienė and Davulienė, 2008). Due to the shallow depths, the lagoon rapidly heats up in spring and remains warmer during the summer than the surface water of the open sea (Gasiūnaitė et al., 2008). The lagoon has no seasonal thermocline and in winter there is ice-cover (Table 1) which in recent years occurs for fewer days on account of warmer winter conditions. Whereas the Klaipėda Strait is always ice-free. Localized anoxia events may take place during ice coverage and overnight during summer (Gasiūnaitė et al., 2008). The main sediments within the Lagoon are sand and silt where there are shell deposits. In the port area of the Klaipėda Strait bottom sediments are influenced by constant dredging and water flow from being at the lagoon entrance. When compared with the exposed coast, the lagoon is more eutrophic by having seasonal phytoplankton blooms and high accumulations of organic carbon in bottom sediments (Remeikaitė-Nikienė et al., 2016).

2.2. Morphological identification

R. cuneata can be confused with the Baltic clam *Limecola balthica* (Linnaeus, 1758) especially for specimens <10 mm shell length (Fig. 1). These two species can be distinguished based on the identification descriptions of the common rangia (Abbott and Morris, 2001; Janas et al., 2014; Leal, 2000; Verween et al., 2006): (a) a thicker and more convex shell; (b) the prominent and anteriorly curved umbo; (c) internal ligament of the left shell with chondrophore, typically with two fused cardinal teeth forming an 'inverted V'.

2.3. Molecular identification

For molecular identification DNA was extracted from a specimen, which was clearly identified by morphological features as *R. cuneata* (shell length 27 mm) using InnuPREP DNA Mini Kit (Analytik Jena AG, Germany) according to manufacturer's instructions. The identification was performed using a species-specific molecular marker developed by Ardura et al. (2015) for *R. cuneata*. For amplification of a 205 bp long fragment of the 16S rRNA gene the *R. cuneata*-specific forward primer RC-16Sar: 5′-AATTTCTTCTAATGATGTGAGG-3 (Ardura et al., 2015) and universal revers primer 16Sbr: 5′-CCGGTCTGAACTCAGATCACGT-3′ (Palumbi, 1996) were used.

Table 1 Environmental parameters at stations with living and vacant *Rangia cuneata* in 2013—2018.

Parameter	Exposed coast	Curonian Lagoon
Depth range [m]	13—17	0.3—11.2
Salinity [PSU]	6.40—7.48	0.18—7.27
Temperature [°C]	1.05—20.35	0.52—21.76
Duration of ice cover, days per year	None	30—64
Dissolved oxygen [mg L^{-1}]	4.44—13.28	4.70—14.64
Oxygen saturation [%]	40—119	48—126

Salinity, temperature, dissolved oxygen, and oxygen saturation are indicated for the near bottom layer. (Lithuanian Environmental Protection Agency (LEPA) monitoring data.)

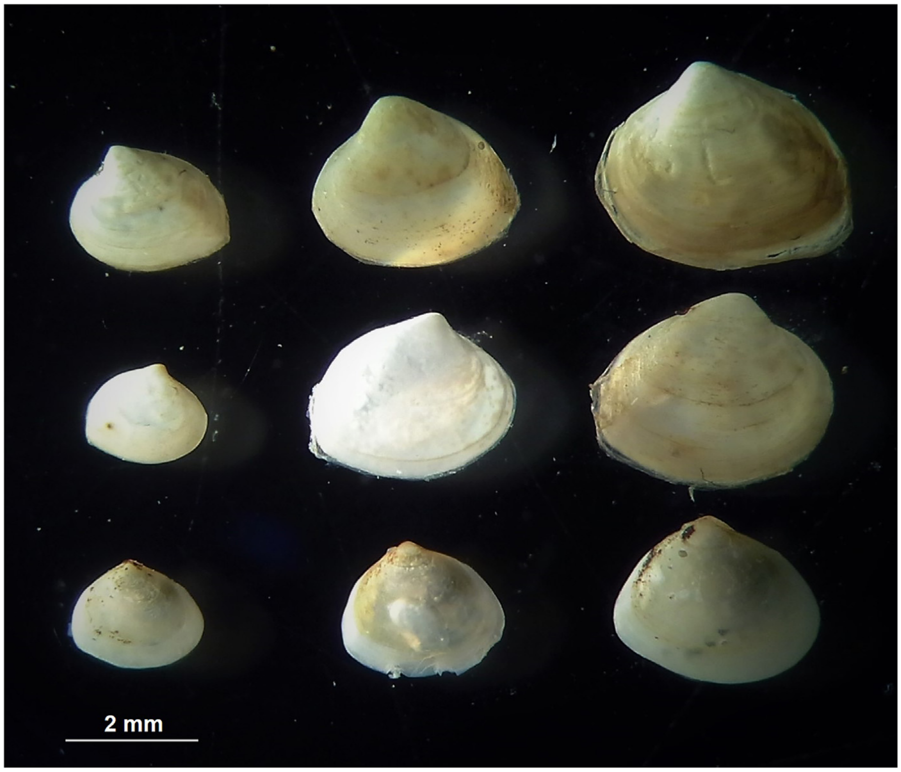

Figure 1 A view of small (<10 mm) shells of *Rangia cuneata* (two above rows) and *Limecola balthica* (row below) under dissecting microscope (Photo: S. Solovjova).

Amplification was carried out in six replicates of *R. cuneata* using InnuTaq DNA polymerase (Analytik Jenna AG, Germany) in 20 μl reactions containing 2 μl of DNA, 2.5 mM $MgCl_2$, 250 μM of each dNTP, 1.25 μg μl^{-1} BSA and 0.7 pmol of each primer. The PCR protocol consisted of a denaturation at 95°C for 3 min followed by 35 cycles at 95°C for 30 s, 54°C for 30 s, 72°C for 45 s followed by a final extension at 72°C for 5 min and a 4°C hold. PCR products were visualized on a 1.5% agarose gel stained by SybrSafe. To exclude the possibility of false positives, species-specific PCR was performed with three other bivalve mollusks species occurring within the sampling area: *L. balthica*, *Dreissena polymorpha* (Pallas, 1771) and *Mytilus* spp. Linnaeus, 1758. To exclude the possibility of PCR inhibition causing negative PCR results, a PCR with universal primers 16Sar and 16Sbr described by Palumbi (1996) was performed.

2.4. Sampling at monitoring stations

The annual benthic monitoring program, which began in 1980, involves 22 stations at depths of 13—117 m within the Lithuanian sector of the southeastern Baltic and 10 stations in the Curonian Lagoon (Fig. 2). Further samples, taken monthly from May to November were at selected stations in the Curonian Lagoon. Three of these are located in the dredged area of the Klaipėda Strait at depths 7—12 m and elsewhere in the Lithuanian part of the Lagoon at 2—5 m. The most recent survey was undertaken in September 2018.

A 71 kg Van Veen grab sampler with a sample area of 0.1 m^{-2} was used capable of penetrating sediments to 10—15 cm at the coastal stations and to *ca*. 10—20 cm within the Lagoon. Three to five samples were taken at each station, each separately sieved using a 0.5 mm mesh size. Retained material was fixed in a 4% formaldehyde solution and later examined using a stereomicroscope at 7.8× to 120×, according to the procedure of HELCOM (2014). In total, approximately 230 Van Veen grab samples were taken in the Lithuanian part of the Baltic Sea and 140 in the Curonian Lagoon were the common rangia was expected to occur. Samples were examined for live common rangia and also their vacant shells. All rangia were counted and measured for live wet weight (with shells, without water in the mantle cavity) using an analytical balance (0.0001 g). A digital caliper (0.01 mm) was used to measure the length of the living and vacant shells.

Salinity, temperature and oxygen concentration were measured at each station using a Multi Water Sampler.

2.5. Additional sampling

In the Curonian Lagoon additional samples were taken during 2015 close to the shoreline, at depths <0.8 m, using a standard Boettger hand net (sampler steel frame 25 × 25 cm; net sieve mesh size 0.5 mm) near Juodkrantė, Preila, Nida and in the Klaipėda strait (Fig. 2). A basket dredge (260 mm open diameter, 300 mm height, 180 mm bottom diameter, diagonal mesh size 5 mm; Minchin, 2014) was used in the Lagoon each month from May to September 2018 in areas where vacant shells and living specimens were obtained using grab sampler.

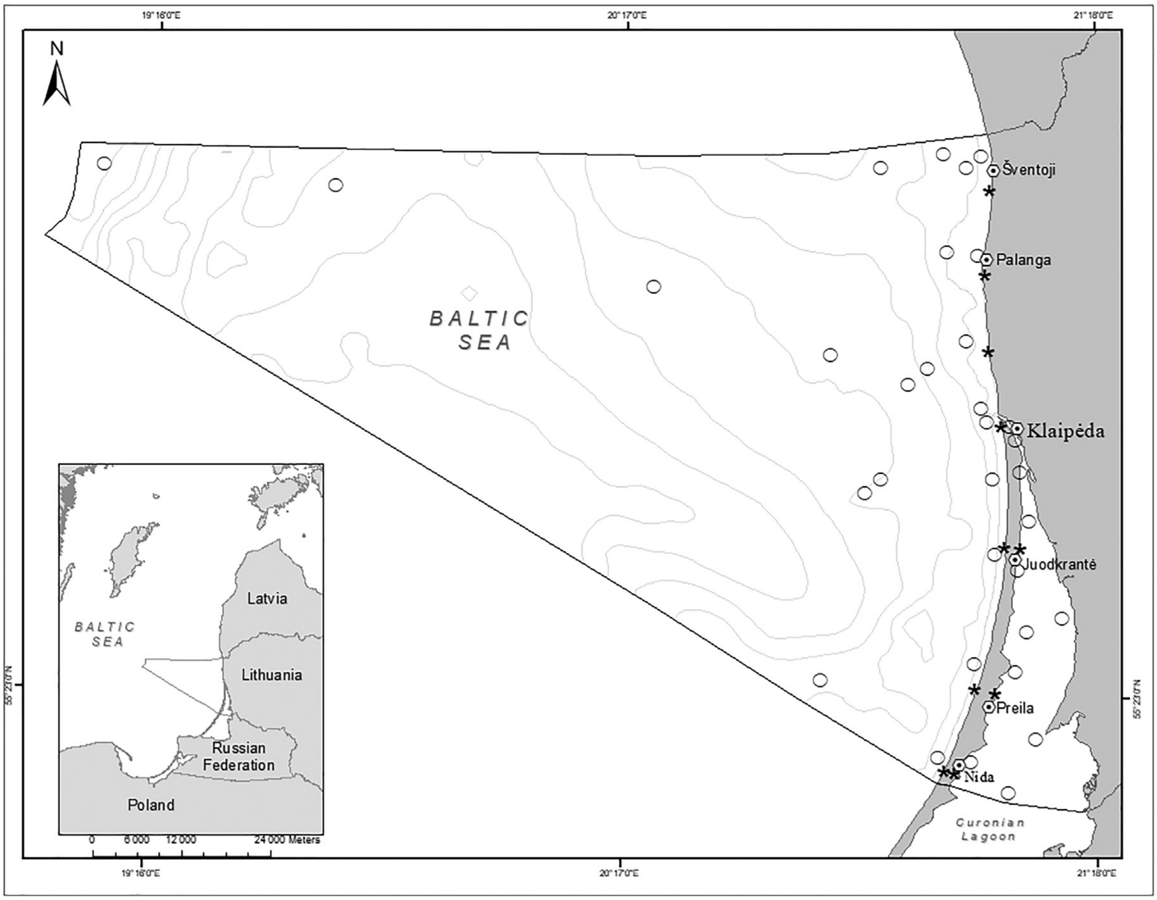

Figure 2 Map of the benthic monitoring stations (opened circles) in the Lithuanian part of the Baltic Sea and in the Curonian Lagoon. Places of littoral excursions and additional nearshore sampling shown by asterisks. A line designates the Lithuanian Exclusive Economic Zone; isobaths indicate 10 m depth intervals.

Shore surveys were undertaken in 2016—2018 for beached shells of *R. cuneata* on either side of the Curonian Spit and to the north of Klaipėda (Fig. 2). Observations were performed for more than 50 sampling occasions along 50 m belt transects between the water's edge to the highest accumulations of shore drift.

3. Results

3.1. Distribution and size structure

R. cuneata was first recognized in Lithuanian marine waters in May 2013, at two coastal stations west of the Curonian Spit. Here two small living individuals and vacant shells were found (Fig. 3, left). At the same time at the entrance to the Curonian Lagoon, only vacant shells were detected.

Living individuals were found only once at the coastal marine stations after 2013, whereas in the Klaipėda Strait they were detected in all years, except 2017 (Fig. 3, right). Generally, the number of living rangia detected remained low with a maximum of four individuals taken in a Van Veen sample in 2016. It is noteworthy that two living 4—5 mm specimens were found 20 km from the Lagoon entrance during September 2015. These were byssally attached to the spiked watermilfoil *Myriophyllum spicatum* L. at the depth 0.3—0.6 m. The detailed information on *R. cuneata* findings in the Lithuanian waters together with environmental data is presented in Table S1 in Appendix. Therewith, environmental conditions data one year before *R. cuneata* found are presented in Table S2 in Appendix.

The vacant shells did not increase over the following years at marine coastal stations, whereas in the Lagoon their numbers were gradually growing (Fig. 4). Also, the maximum size of the living mollusks and vacant shells in the Lagoon was four times greater than at exposed marine sites. Living individuals attained 27 mm shell length and weighing 5.4 g and vacant shell up to 37 mm (Fig. 5). Size distribution of the vacant shells in the Curonian Lagoon is shown in Fig. 6.

3.2. Shore surveys

During the initial period of observation (2016—2017) only shells of common mollusks were found: *L. balthica*, *Mya arenaria* Linnaeus, 1758 and *Cerastoderma glaucum* (Bruguière, 1789) at the seaside exposed sandy coast. Beached shells within the Lagoon consisted of the freshwater bivalves — *Anodonta cygnea* (Linnaeus, 1758), *Unio tumidus* Philipsson, 1788, *D. polymorpha* and gastropods — *Viviparus viviparus* (Linnaeus,

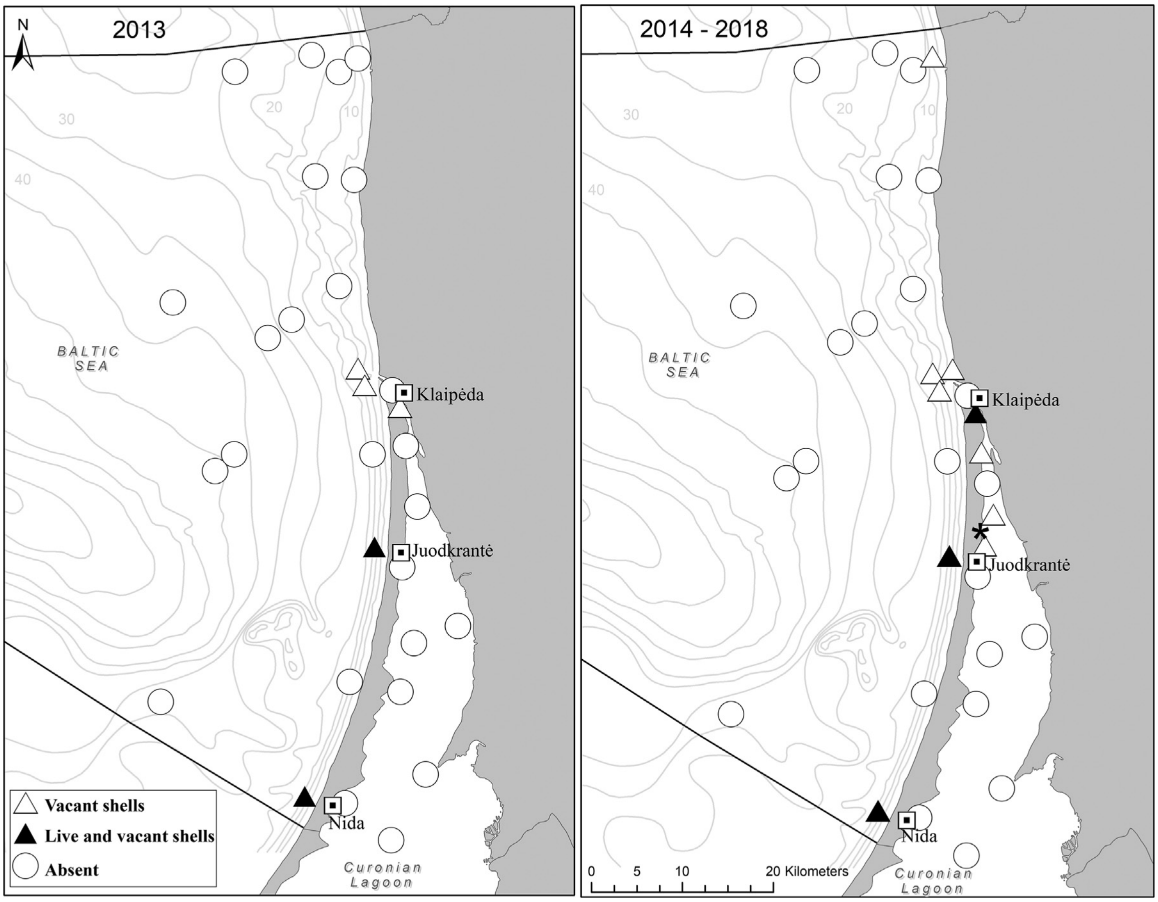

Figure 3 First records of *Rangia cuneata* in the Lithuanian waters in 2013 (left); findings of *R. cuneata* in 2014—2018 (right): ▲ findings of both live mollusks and vacant shells; △ only vacant shells, * live mollusks attached to the spiked watermilfoil *Myriophyllum spicatum*, ○ stations with no presence of *R. cuneata*.

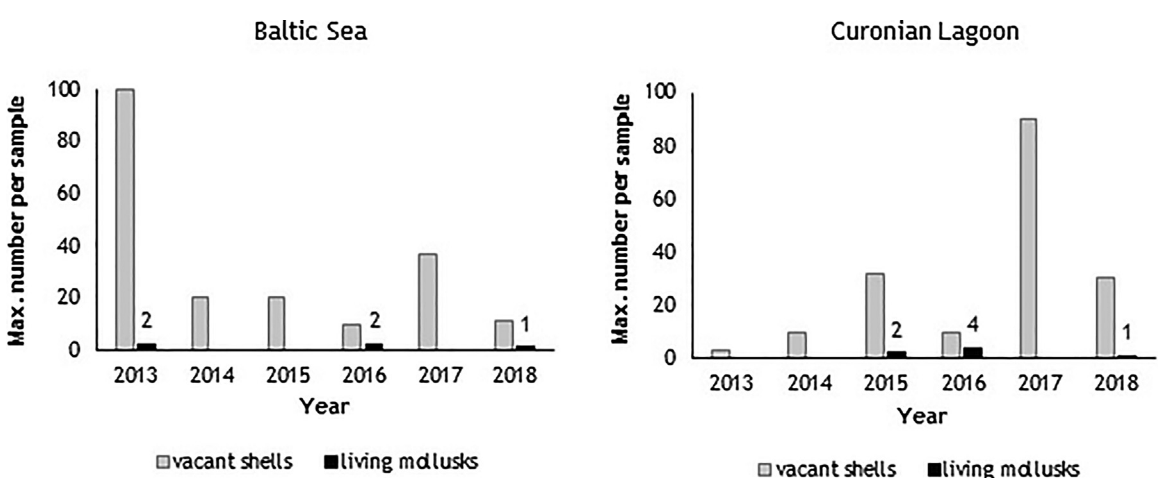

Figure 4 Dynamic of the maximum number of the vacant shells and living mollusks in grab samples at the monitoring stations at the southeastern Baltic and in the Curonian Lagoon. The number of living mollusks found is indicated.

1758), *Radix auricularia* (Linnaeus, 1758) and *Planorbarius corneus* (Linnaeus, 1758). For the first time, one beached shell of *R. cuneata* was found in 2018 April (shell length 27 mm) on the seashore to the north of Klaipėda (Fig. 3). In October, after storm events, 15 vacant shells (shells length 26—35 mm) were found. Remarkably, they were found in a debris, most probably transported by currents from the Curonian Lagoon as they contained remains of freshwater organisms (*A. cygnea*, *U. tumidus*, *V. viviparus*, canes, reeds, *etc.*) not occurring in the marine area.

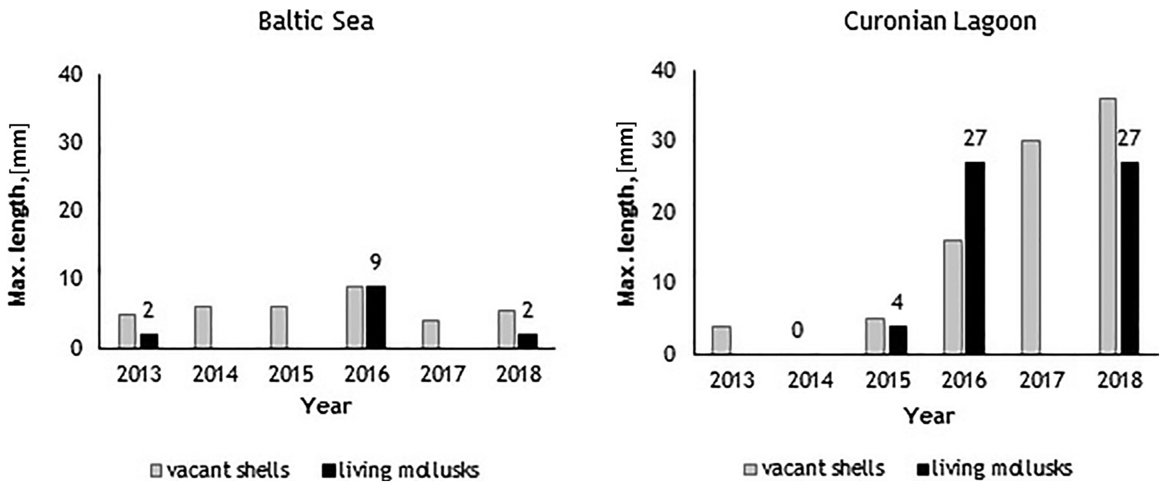

Figure 5 Dynamic of the maximum shell length, mm of *Rangia cuneata* in the southeastern Baltic and in the Curonian Lagoon. Numbers indicate the maximum size of living mollusks found in the particular year.

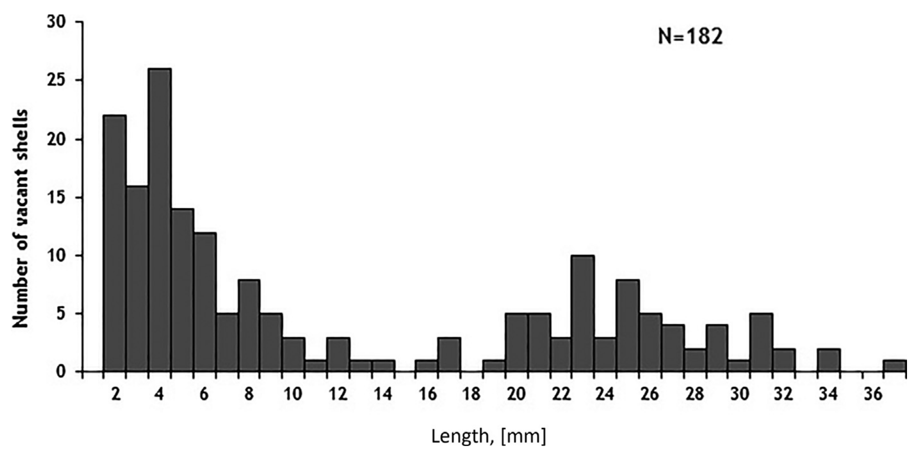

Figure 6 Length-frequency distribution of *Rangia cuneata* collected in the Klaipėda Strait, Curonian Lagoon in May—September 2018.

3.3. Molecular species identification

Universal 16S rRNA gene-targeted primers yielded positive PCR amplifications in all samples — the amplicons were approximately 500 bp long that is the expected size (Fig. 7b). Amplification using *R. cuneata*-specific primers was detected only when DNA from *R. cuneata* specimen was used as a template. Only one PCR product of the expected size (approximately 200 bp) was obtained with no additional bands (Fig. 7a). Amplification using DNA extracted from other bivalve mollusks did not get any result.

4. Discussion

4.1. Living conditions and status of the common rangia population in the Lithuanian waters

The high number of small vacant shells in the initial period and slow growth of the common rangia in the marine coastal area (Figs. 4 and 5) indicates that conditions here generally are less suitable than in the Curonian Lagoon. This may be due to higher hydrodynamic action at the exposed marine coast, hard-packed sediments, less organics and reduced food resources than in the Curonian Lagoon, where the bottom sediments are softer and richer with organic material (Remeikaitė-Nikienė et al., 2016). Temporal anoxia, which may occur during the ice coverage and in summer at night in the Lagoon (Gasiūnaitė et al., 2008) is an unlikely limiting factor for common rangia as adult specimens have the ability to live anaerobically for up to 2 weeks (Risk Assessment, 2017). However, even in the Curonian Lagoon, the abundance of the common rangia remains low (only seven live specimens found during five years since the first detection) than compared to the neighbouring Vistula Lagoon (max. abundance up to 4040 ind. m^{-2}; Rudinskaya and Gusev, 2012) and coastal waters of the Gulf of Gdańsk (540 ind. m^{-2}; Janas et al., 2014). At the same time the number of vacant shells on sites is attaining a hundred per sample.

According to Rudinskaya and Gusev (2012) the rangia yearlings in the Vistula Lagoon can reach 14 mm in size, therefore finding of numerous vacant shells (<10 mm) in

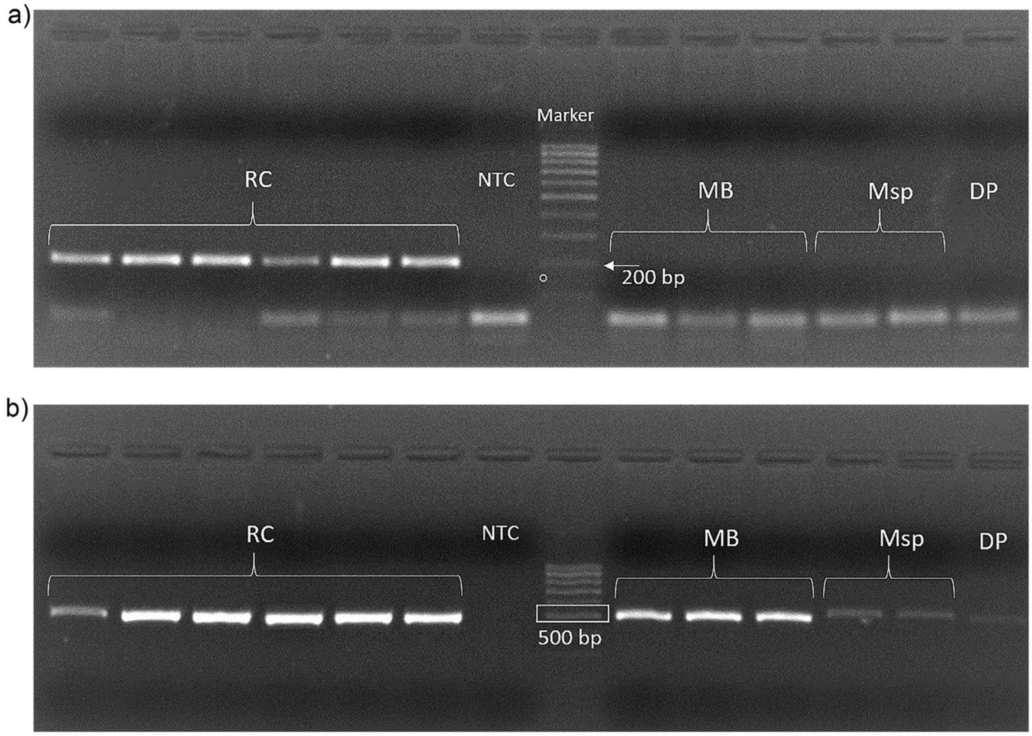

Figure 7 (a) Agarose gel of PCR products obtained with *Rangia cuneata*-specific primers. RC: *Rangia cuneata*, MB: *Limecola balthica*, Msp: *Mytilus* spp., DP: *Dreissena polymorpha*, NTC: non-template control, Marker: DNA size marker (100 bp DNA ladder). (b) Agarose gel of PCR products obtained with universal primers (Palumbi, 1996). RC: *Rangia cuneata*, MB: *Limecola balthica*, Msp: *Mytilus* spp., DP: *Dreissena polymorpha*, NTC: non-template control, Marker: DNA size marker (100 bp DNA ladder).

our samples implies high mortality of the juvenile *R. cuneata*. Interestingly, in the same samples where numerous vacant shells of *R. cuneata* were found, the number of vacant shells of common local species, such as *L. balthica*, *C. glaucum*, *D. polymorpha* and unionids never exceeded a few per sample. Kornijów et al. (2018) experimentally established that non-predatory mass mortality of the common rangia in the Vistula Lagoon occurs in spring, when water temperature begins to exceed approximately 10°C and not during or immediately after the end of winter at lower temperature. It is not clear what is causing high mortality of the common rangia in the Curonian Lagoon, where seasonal temperature variation and the salinity range are similar to the Vistula Lagoon (Chubarenko and Margoński, 2008).

In US populations, gametogenesis of *R. cuneata* is triggered by a spring rise in temperature to at least 10°C and can take place over at least a 7-month period if conditions remain suitable (Risk Assessment, 2017). Another source indicates that gametogenesis initiates at the water temperature above 15°C (Cain, 1975, cit. by Verween et al., 2006) and with salinities above 0 or below 15 (Hopkins, 1970, cit. by Verween et al., 2006). It is important, that the key trigger to *R. cuneata* spawning is an abrupt salinity change from either a higher or lower salinity to a range between 3 and 10 (Risk Assessment, 2017), which is the case in the Curonian Lagoon (Dailidienė and Davulienė, 2008; Gasiūnaitė et al., 2008).

The size structure of the vacant shells of *R. cuneata* in the Curonian Lagoon indicates presence of at least two cohorts: the yearlings (<14 mm) and the adult specimens, which age may be 2—4 years, according to Rudinskaya and Gusev (2012). Thus, it is likely that the population in the Curonian Lagoon has established itself and will continue to increase, while in the exposed marine coastal area, where no shells greater than 9 mm were found, the population status of *R. cuneata* (established/not established) remains unclear.

4.2. Possible vectors of introduction and further dispersal

The distribution pattern of the Atlantic rangia in northern Europe supposes involvement of shipping vectors in its spread, such as ballast water or sediments transported by dredgers. In European waters *R. cuneata* was found in limnic-to-mesohaline conditions with salinity 0.0 to 10.3, and in a wide range of temperatures, from 0 to 25°C (Bock et al., 2015; Janas et al., 2014; Möller and Kotta, 2017; Rudinskaya and Gusev, 2012; Verween et al., 2006; Wiese et al., 2016; and the present study).

Based on the distribution pattern of *R. cuneata* along the Curonian Spit and small size of shells found in 2013 we suspect that planktonic larvae were brought by currents from the region of the Vistula Lagoon and/or Gulf of Gdańsk during 2012. In these southern regions the populations are well established and occur at high densities (Ezhova, 2012; Janas et al., 2014; Rudinskaya and Gusev, 2012; Warzocha and Drgas, 2013; Warzocha et al., 2016).

The arrival of *R. cuneata* in Lithuania due to a possible natural spread differs to its arrival to the Estonian and Swedish coasts (Florin, 2017; Möller and Kotta, 2017). These two localities form the most northern known populations within the Baltic Sea. Both are found in areas adjacent to ports, and possible means of introduction are beyond what might be expected with the natural drift of rangia larvae but most likely by ballast water or with craft involved in the dredging port channels. We are aware of dredging activities having taken place to the ports of Kaliningrad, Pärnu but also at Klaipėda. Further human-mediated spread with fishing equipment and snagged weed on retrieved anchors is possible, because, as found in this study, small individual can attach to macrophytes. In a separate study, small clams (<1 mm) were observed attached to a hydroid colony (Hoese, 1973, cit. by LaSalle and de la Cruz, 1985).

Möller and Kotta (2017) assumed, that some predicted climate change scenarios might allow this species to flourish in the northern Baltic Sea. From here, it may spread elsewhere by both natural and anthropogenic means. We suggest coastal lagoons and estuarine areas will be more suitable for such colonization due to the greater levels of eutrophication and higher water temperatures than in the open sea. We predict that there will be further expansions within estuarine areas of western and southern Europe and possibly the Black and Caspian Sea regions. Populations may already exist in some areas where to date they have not been revealed.

In this study, beached rangia were not found for some five years after its first known occurrence, which was established from the monitoring samples and based on identification of small specimens requiring a dissecting microscope. The small shells that will have occurred on exposed marine coasts would be unlikely to be detected in shore surveys. In most of other cases (Bock et al., 2015; Janas et al., 2014; Rudinskaya and Gusev, 2012; Verween et al., 2006; Willing, 2015), the first detection of the new mollusk occurred during a routine benthic monitoring or specialized monitoring program aimed at detection of non-indigenous species.

5. Conclusion

The semi-tropical non-indigenous clam *R. cuneata* continues its spread within the boreal environment of the Baltic Sea. Both human-mediated vectors (ballast water or dredged material) and natural spread by coastal currents appear to be involved in the process. Coastal bays and lagoons seem most suitable for colonization, probably due to higher levels of eutrophication and availability of food resources, and higher summer water temperatures than in the open sea. Further spread of *R. cuneata* to similar environments in Europe and perhaps in the Black and Caspian Seas is possible, either from the Baltic or from the North Sea or perhaps the Americas.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.oceano.2019.01.005.

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