Manila clams *Ruditapes philippinarum* spreading north and establishing in the European Wadden Sea

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ABSTRACT

In an era of accelerating biological globalization and climatic warming, it is vital to understand how introduced species integrate. Pacific Manila clams *Ruditapes philippinarum* were introduced for aquaculture to Europe in the 1970s, spread and became harvested from the Mediterranean Sea to British coasts. From the Dutch Rhine Delta, Manila clams immigrated stepwise into the southern Wadden Sea, and by long distance jump dispersal they arrived in the northern Wadden Sea in the 2010s. Encounters remained few until 2021, when live clams were often found in intertidal seagrass beds and around mixed beds of Pacific oysters with native mussels. Shell lengths reached up to 74 mm with a longevity of 7–8 years. Adult abundances remained low (<10 clams m^{-2}), although larval numbers substantially increased in 2022 and 2023. Strong predation on spat may constitute ecological resistance to the immigrant. Haplotype diversity at mtDNA *COI* gene fragments is high and its composition is mixed from dominant haplotypes of other European sites, suggesting multiple introductions or an unknown source with an already mixed population. Currently, this is the most northern (55° N) population of *R. philippinarum* in continental Europe, but with the rapid expansion of this genetically variable population, further northward spread can be expected.

1. Introduction

Coastal waters accumulate more and more species from overseas, mainly vectored by shipping and aquaculture (Anton et al., 2019; Bailey et al., 2020; Cuthbert et al., 2022; Olenin and Minchin, 2023). Also, in the European Wadden Sea with an extensive belt of sedimentary tidal flats, there are no signs of reaching species saturation (Buettger et al., 2022). Recent climate warming seems to facilitate establishment, and the trophic guild of suspension feeders has particularly diversified by introduced mollusks (Reise et al., 2023). The species spectrum of this geologically young, marginal sea of the NE Atlantic seems to be still expandable. Therefore, little ecological resistance is expected to the newly arrived Manila clam *Ruditapes* (syn. *Venerupis, Tapes*) *philippinarum* (Adams and Reeve, 1850; syn. *Tapes japonica* Deshayes, 1853), originating from subtropical to boreal East-Asian coasts.

Regarded as a valuable seafood, *R. philippinarum* has been widely introduced, and arrived in France via British Columbia in 1972 and 1975 (Flassch and Leborgne, 1992; de Montaudouin et al., 2016) and Britain in 1980 (Humphreys et al., 2015). Feral populations established and spread, and spat was traded into the Mediterranean Sea and to Iberian coasts (i.e., Pranovi et al., 2006; Campos and Cachola, 2006; Chiesa et al., 2017). An introduction to Norway in 1987 did not result in a persistent population (Mortensen and Strand, 2000; and personal communication).

In the Dutch Oosterschelde, Manila clams were encountered since 2008 (Faasse and Ligthart, 2008; Titselaar, 2008), with subsequent spread within the Rhine delta (Troost et al., 2023) and Belgian coast (Kerckhof, 2016). Unlike further south, these feral populations remained unexploited. A common feature of dispersal seems to be that Manila clams established preferentially in estuaries, lagoons or semi-enclosed bays and harbors. This can be explained by favorable conditions for larval retention after a founder population had arrived (Herbert et al., 2012).

Genetic analyses support the historical evidence of European Manila

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clams originating from central Japan, and then transferred via hatcheries in British Columbia to France and Britain, followed by multiple translocations within Europe (Chiesa et al., 2017; Cordero et al., 2017). Compared to East Asian coasts from southern China to northern Japan, as well as clams from the American Pacific coast, the European populations display a lower genetic diversity. This suggests strong founder effects (Mao et al., 2011; Mura et al., 2012; Cordero et al., 2017; Tan et al., 2020), even though haplotype diversity was still high due to rare haplotypes (Chiesa et al., 2017). This seems to apply particularly for the northern edge of the European range, where only low genetic variability was observed at two British sites (see Chiesa et al., 2017).

Life history traits vary with geographic region (i.e., Ponurovsky and Yakovlev, 1992; Nakamura et al., 2005; Gillispie et al., 2012). Spawning occurs at 14–26 °C (optimum 20–22) and once or twice per year. Optimal salinities range from 24 to 35. Larval development takes 2–3 weeks. Settlement on sediment surface occurs at about 200 μm size. Spat attaches with byssus to coarse sand, pebbles or mollusk shells, and adult clams remain below sediment surface with a siphon about half of shell length. Longevity can be up to 13 years at northern sites.

In the Wadden Sea, the only close relative is *Venerupis* (*pullastra*) *corrugata*. This is more rectangular in shape with prominent concentric stripes, while *R. philippinarum* is more elliptic with prominent radial rips. Dark pigmentation patterns on shells of both species may look similar but tend to be more extensive and variable in Manila clams. Thus, the handsome Manila clams can be readily identified and recorded by citizen scientists when visiting tidal flats in the Wadden Sea, because the two valves cling together as shell doublets for a long time (Fig. 1, Goulletquer, 1989). This study summarizes their observations reported to www.beachexplorer.de, www.waarneming.nl and Strandwerkgemeenschap (rien-tien@ziggo.nl). We supplement this with own observations, measurements and genetic analyses.

In Venice lagoon, population development and harvesting of Manila clams altered the entire ecosystem (Pranovi et al., 2006), while in other regions ecological effects remained modest (i.e., Byers, 2005; Humphreys et al., 2007; Bidegain and Juanes, 2013) and foraging birds even benefitted (Caldow et al., 2007). In the northern Wadden Sea, we ask whether Manila clams face ecological resistance, may benefit from climate warming, whether genetic diversity is reduced at the northern edge of its range, and from where it might have come.

2. Methods

2.1. Study area and climate change

The Dutch-German-Danish Wadden Sea with 4700 km^2 of mud and sand flats, emerging twice daily at low tide, came into existence about 7000 years ago when rapid post-glacial sea level rise declined and the remaining slow rise tended to be balanced by sedimentation (Reise et al., 2010). Climate is maritime with modest summer temperatures and mild winters. In the northern Wadden Sea, air temperature has increased since the 1880s by 1.8 °C, with a current annual mean at 9.3 °C (DWD, 2023). Water temperature has risen by more than 1 °C during the last 40 years, with a highest increase in autumn (Rick et al., 2023), and a marked decline in ice winters since 1990 (de Amorim et al., 2023). Sea surface temperatures vary mostly between 0 and 20 °C with an average at about 10 °C. Salinity fluctuates between 26 and 32. In the northern Wadden Sea, seagrass beds cover about 20% of the tidal flat area (Dolch et al., 2013). These occur mainly around mean tide level, while at low tide level mussel beds abound, since the 2000s intermixed with introduced Pacific oysters (so-called oyssel beds; Reise et al., 2017a,b). At these two intertidal habitats most *R. philippinarum* were encountered.

2.2. Citizen science observations

Records of citizen scientists on *R. philippinarum* reported to the websites www.beachexplorer.de and www.waarneming.nl covered the entire North Sea coast from the Dutch Rhine Delta to the Danish part of the Wadden Sea. Such records do not represent a systematic survey but rely on volunteer contributions, tending to report new and unusual findings. In the case of a conspicuous, hitherto unseen species in this region, the range extension of *R. philippinarum* may be documented reliably. In particular, a regional mortality event in early 2022 with

Fig. 1. Shell doublets of *Ruditapes philippinarum* collected in an intertidal seagrass bed near Hallig Hooge (northern Wadden Sea) in April 2022.

moribund clams and fresh shell doublets appearing at the sediment surface, helped to visualize the distribution of clams otherwise hidden in the sediment. Our observations focused on a coastal stretch of about 100 km from the island of Rømø (southern Denmark) to Eiderstedt peninsula (northern Germany; see Fig. 2).

2.3. Field sampling with statistical analyses

The mortality event in the northern Wadden Sea early 2022 with many fresh shell doublets and live but moribund clams at the sediment surface, supplemented by live specimens raked out of the sediment at the same sites (see below), allowed us compiling size-frequency distributions without disturbing much of the sediment. Shell length was measured to the nearest millimeter and annual rings were counted when visible. Differences in shell length between fresh shell doublets (recently deceased) and living *R. philippinarum* specimens gathered from late 2021 to early 2022 at four sites within the northern Wadden Sea (for site descriptions see Table 1) were examined via pairwise Mann-Whitney U-tests for each site individually (Table S1). For living and dead specimens together, shell length distributions were analyzed with Kruskal-Wallis test. Combining surface collections of shell doublets with live specimens raked out of the sediment, has skewed size spectra to larger shell sizes. However, differences in mean shell size were small and consistent between sites (see results and Table S1).

For quantitative sampling of adult clams, a hand rake with four prongs, each 1 cm wide and spaced by 3 cm, was employed. Such an instrument is used in Korea by professional clam diggers on tidal flats, and was also employed by Ruesink et al. (2014). Raking depth was approximately 10 cm at square plots of 0.04 or 0.25 m^2 (Table 1). At the site where adults were raked at an oyssel bed in Königshafen (see Kh in Fig. 2), an attempt was also made to sample spat or juveniles by sieving 50 random cores of 10 cm^2 (5 cm deep) with a 1-mm mesh in August 2022. Because this was in vain, a further set of 30 random cores of 40 cm^2 (10 cm deep) was taken in the same area. At this site, the volume of shell hash in the upper 10 cm of 6 sediment cores, each of 135 cm^2, retained with a 1-mm mesh, was estimated to 36.5 ± 11.6 % [range 24–55], measured as water displacement of mollusk shell hash in a

Table 1

Sites and habitats in the northern Wadden Sea where sediment was raked for *Ruditapes philippinarum* to a depth of approximately 10 cm and where fresh shell doublets were collected from sediment surface concurrently. Estimated submergence time is given for semi-diurnal tide cycle.

Site description	Habitat	Submergence time	Raked plots	Date
Föhr-Southwest	Seagrass belt	~40 %	15 × 0.04 m^2	September 2021
	Bare sand flat	~40 %	15 × 0.04 m^2	September 2021
Hooge-West	Seagrass bed	~50 %	10 × 0.04 m^2	April 2022
	Seagrass bed	~75 %	10 × 0.04 m^2	April 2022
Königshafen Sylt	Oyssel bed cluster	~90 %	60 × 0.25 m^2	Jan./Febr. 2022
	Oyssel bed cluster	~90 %	100 × 0.25 m^2	November 2022
	Oyssel bed cluster	~90 %	40 × 0.25 m^2	May 2023
	Oyssel bed cluster	~90 %	40 × 0.25 m^2	January 2024

calibrated cylinder.

At the same site in Königshafen, a transect perpendicular to the shore was surveyed across the belt of oyssel beds in May 2022. We collected fresh shell doublets found at sediment surface on 12 random plots of 16 m^2 at 5 intervals as a proxy for abundance: bare flat >30 m landward of the oyssel bed belt, <30 m landward, within oyssel beds, <30 m seaward and >30 m seaward of the oyssel bed belt. Shell sizes were measured as described above. Kruskal-Wallis test was employed to investigate differences in shell size from transect intervals, and Mann-Whitney U-tests were used to find significant differences between paired positions.

2.4. Larval metabarcoding

Cytochrome oxidase I (COI) metabarcoding of zooplankton caught on the seaside border of Königshafen was used to monitor larval occurrence of *R. phillipinarum*. Samples were collected bi-weekly from
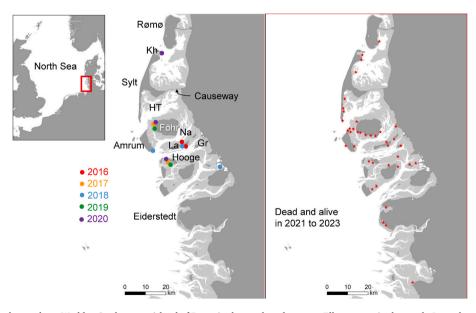

Fig. 2. *R. philippinarum* in the northern Wadden Sea between island of Rømø in the north and to outer Elbe estuary in the south. Inset shows the position in the North Sea (red rectangle). Left: Initial encounters of shell doublets and one living clam between November 2016 and December 2020. Right: sites with living clams and paired valves found in 2021–23 (without repeat records from same sites). Based on www.beachexplorer.org/arten/ruditapes-philippinarum (74 reports) and own observations until end of 2023. Tidal flats are shown in light grey. Location names mentioned in the text are indicated. Königshafen (Kh) and Hooge were sampled for genetic analyses. Hörnum Tief (HT), Norderaue (Na), Langeness (La), Gröde (Gr). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2019 to 2024 by filtering 10 l of surface seawater with an 80 μm mesh. Larvae were collected by centrifugation of the sample and removing the sea water supernatant. Bivalve larvae were counted using a stereo microscope in the retained material. Samples with >50 bivalve larvae, were used for DNA extraction. DNA was extracted using the QIAamp DNA micro kit following the manufacturers' instructions (Qiagen, Hilden, Germany). Illumina MiSeq library preparation followed the manufacturer's instruction. In short, partial COI sequences were amplified using 0.9 μl of the primers mICOIinf_F 5′-TCGTCGGCAGCGTCA-GATGTGTATAAGAGACAG-GGWACWGGWTGAACWGTWTAYCCYCC-3′ and HCO2189-R 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGA-CAG-TAAACTTAGGGTGACCAAAAAATC-3′ containing the Illumina index adapter overhangs (underlined), 7.1 μl of water, 10 μl of KAPA HiFi HotStart polymerase (Roche, Mannheim, Germany) and 2 μl of sample with the using the cycling condition described by Closek et al. (https://www.protocols.io/view/environmental-dna-edna-coi-met abarcoding-illumina-rm7vzey2vx1w/v1.). After bead purification with AMPure XP beads (Beckman Coulter, München, Germany), sample specific barcodes were added in a second index PCR using the Illumina Nextera XT index primer set 1 (Illumina, Berlin, Germany). After a second round of bead purification, successful adapter addition was checked and PCR product concentration was determined on a Qiaxcel gel electrophoresis system (Qiagen, Hilden, Germany). PCR products were then equimolarly pooled and paired-end sequenced on the Illumina MiSeq sequencer of the AWI Genomics platform. After demultiplexing of the reads primer sequences were removed and the dada2 pipeline (Callahan et al., 2016) was used to convert reads into amplicon sequence variants (ASV) with default parameters except for the filter and trim step where following parameters were used: trimLeft = 26,26, truncLen = 290,250, maxN = 0, maxEE = 2,10, truncQ = 10.

Taxonomy was assigned to the ASVs using the taxa_assign function of dada2 using the MZG_dbAll database (https://metazoogene.org/). Species were assigned when bootstrap support exceeded 80%. The estimated number of *R. phillipinarum* larvae was then calculated by the proportion of all reads assigned to this species from all reads assigned to Bivalvia, and then this proportion multiplied by the number of bivalve larvae used in the extraction, assuming that the number of reads is to some degree proportional to the number of individuals. To compare temporal dynamics of the non-indigenous *R. philippinarum* to a native species with a similar ecology, the estimated number of cockle larvae *Cerastoderma edule* was calculated analogously.

2.5. Genetic analysis

To investigate genetic variability and delineate potential introduction sources mtDNA *COI* haplotypes of 79 *R. philippinarum* clams were sequenced. Clams were collected from a seagrass bed west of Hooge (see Figs. 2 and 13 clams in April 2022 and 18 in September 2023) and adjacent to an oyssel bed in Königshafen (see Figs. 2 and 23 clams in 2022 and 25 in 2023). Tissue from mantle and foot was frozen at −20 °C before DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). Partial COI sequences were amplified using 1 μl of the primers COI-ALT_LIV_F: 5′-AACMAATCATAAAGATATTGG-3′ and COI-ALT-LIV_R: 5′-AACTTCRGGRTGACCAAAAA-3′ at 5 μM (Chiesa et al., 2017), 8 μl of water and 10 μl of HotStarTaq DNA polymerase mastermix (Qiagen, Hilden, Germany). Cycling used an initial 3 min denaturation at 95°, followed by 35 cycles of 30 s at 95 °C, 55 s at 48 °C, and 45 s at 72 °C and concluded with a final extension at 72 °C for 10 min (Chiesa et al., 2017). PCR products were purified using the MinElute PCR purification kit (Qiagen, Hilden, Germany) and bi-directional Sanger-sequencing was performed by eurofins genomics (Ebersberg, Germany). After sequencing forward and reverse sequences were assembled and primer sequences were trimmed. Sequences were then aligned with reference sequences provided by Chiesa et al. (2017) and the alignment was trimmed to a length of 491 bp to include only complete sequences. The R-library *pegas* was used to identify haplotypes and

estimates haplotype frequencies for all four samples. The haplotype nomenclature of Chiesa et al. (2017) was adopted and haplotype frequencies of other European populations were estimated from the information provided (Chiesa et al., 2017). Nucleotide and haplotype diversities were calculated and compared to the other populations using genetic_diversity_diffs v1.0.6 (https://github.com/laninsky/genet ic_diversity_diffs). The relationship of the northern Wadden Sea populations to potential European source populations was investigated visually by ordinating the matrix of pairwise Bray-Curtis distances in a principal coordinate analysis (PCoA).

3. Results

3.1. Arrival and spread in the Wadden Sea

Based on BeachExplorer.org data only 12 records of *Ruditapes philippinarum* were reported in the period 2016 to 2020 (Fig. 2 left). All shell doublets found in 2016–2019 were from adults 30–50 mm shell length (2–7 years old), and a living clam in 2020 was of 40 mm (Björn Nadarzinski, personal communication). The first two shell doublets were collected in seagrass beds north and south of Langeness in November 2016, and had reached an age of 3–4 years. Records south of the island of Sylt are spread over a distance of 30 km. The site where a living clam was found in 2020 (Fig. 2: Königshafen at the northern end of Sylt) is > 50 km seaway away from the previous observations. An insurmountable causeway between Sylt and the mainland blocks inshore dispersal northward, forcing larvae to drift along the western shore of Sylt before entering the Wadden Sea again between Sylt and Rømø.

This pattern of rare and widely spread encounters in the 5 years between 2016 and 2020 changed abruptly in 2021, when plenty of paired valves and living clams were recorded at several more sites, now spanning almost 100 km of coastline, with more than 1300 km^2 of intertidal flats (Fig. 2 right). Twenty records were reported in 2021, 30 in 2022 and 28 in 2023. These encounters are mainly from an area stretching from southern Sylt to Eiderstedt peninsula. A smaller cluster occurred at northern Sylt, separated by the causeway mentioned above. An outlier was found 30 km south of Eiderstedt peninsula near the outer Elbe estuary in July 2022 (Fig. 2 right).

Independent from Manila clams colonizing the northern Wadden Sea, a stepwise spread occurred from the Dutch Rhine Delta along the coast towards the southern Wadden Sea (Fig. 3; Strandwerkgemeenschap and www.waarneming.nl). There, a first fresh shell was found in Mok Bay at Texel in April 2017. On Balgzand near Den Helder, four living clams were found in June and August 2019, with shell lengths from 4 to 41 mm (Rob Dekker, personal communication). More clams were found in 2020–2023 between Texel and Ameland, and since February 2023 also at the German islands of Borkum, Juist and Norderney (www.beachexplorer.de). Finally, in January 2024, shell doublets were collected at Schillig near Wilhelmshaven (Benedikt Wiggering, personal communication; most eastward red arrowhead in Fig. 3), continuing the stepwise spread eastward.

3.2. Distribution, size and abundance

In the northern Wadden Sea, most *R. philippinarum* – shell doublets and living – were primarily encountered in intertidal seagrass beds or intertidal sediments rich in mollusk shell hash down to spring low tide level. Subtidal habitats to the west and east of northern Sylt were intensively surveyed with an oyster dredge in 2020–2023 without catching any shell doublets or live Manila clams, while the related *V. corrugata* was occasionally caught. In Königshafen, the shallow subtidal zone down to 1 m below spring low tide was surveyed for shell doublets on occasions of exceptional low tides and none were found (2022–2023), while plenty of shell doublets and living clams occurred at a cluster of oyssel beds in the adjacent lower intertidal zone. No shells were found at exposed beaches.
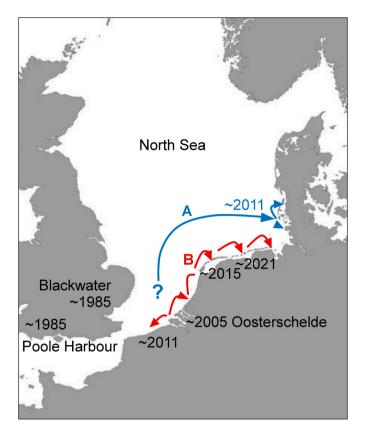
Fig. 3. Two introduction and/or dispersal routes into the Wadden Sea, (A) with a long distance jump dispersal from an unknown donor region towards the northern Wadden Sea and (B) a stepwise dispersal from the Rhine Delta towards the southern Wadden Sea. Years suggest approximate dates of arrival (dates outside the Wadden Sea from Faasse and Ligthart, 2008; Humphreys et al., 2015; Kerckhof, 2016).

Fig. 4. Length-frequency distributions of shells of *R. philippinarum,* grouped into 5-mm size intervals, collected September 2021 (Föhr), in January and February 2022 (Königshafen) and April 2022 (Hooge). Plots increase in tidal submergence time from top to bottom (see Table 1). Upper left in each panel: Mean shell length (± standard deviation) and number of shells measured (N). Dashed vertical lines mark size range 25–50 mm.

Mean shell length of size spectra (shell doublets and live specimen together) collected in September 2021 and early 2022 differed significantly between sites (Kruskal-Wallis test, $\chi 2 = 124.6$, df = 42, p < 0.001), suggesting an increase in mean lengths with longer inundation times (Fig. 4). Shell length of living clams were usually slightly smaller than those of fresh shell doublets (Table S1). In Fig. 4 the frequencies per 5-mm size interval refer to living and recently deceased clams together, and all populations are composed of several age classes.

Shells smaller than 15 mm in length were not detected, and shells 15–20 mm length were rare except September 2021 in a 100-m belt of seagrass close to shore surveyed at SW Föhr (see Figs. 2 and 4 above). Raking the sediment on 15 random plots of 400 cm² each, revealed a mean clam density of 1.0 ± 1.5 [range 0–4], corresponding to 25 clams per m². Annual growth rings suggested an age of 2–3 years. Seaward, the seagrass belt thinned out and gave way to a bare lugworm flat with muddy sand where no clams could be found.

An extensive seagrass bed west of Hooge was sampled for clams at mean and at low tide level in April 2022 (see Figs. 2 and 4). At this site, raking the sediment on 20 plots of 400 cm² revealed a mean clam density of 0.1 ± 0.3 [0–1], corresponding to 2.5 clams per m² which is comparatively low. Size-frequency and annual growth rings suggest a strong recruitment event in 2020, and moderate to low recruitment in 2019 and in the years before. Growth rings on largest clams indicate first recruitment in 2016 at this site.

In Königshafen at the northern tip of Sylt (see Kh in Fig. 2), clams and shell doublets were found at low tide level in association with clusters of oyssel beds in January/February 2022 (Fig. 4). Annual growth rings indicated recruitment in 2016–2020. The largest living clam was encountered in May 2022 (shell length 64 mm) and presumably
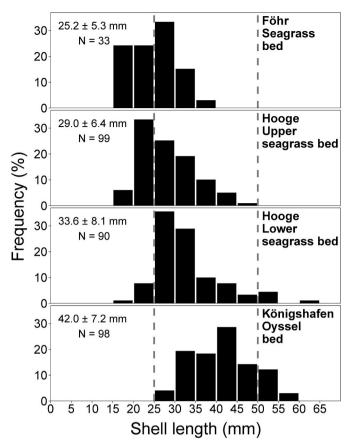
recruited in 2015, and the largest shell doublet found reached a length of 74 mm.

A transect perpendicular to the shore was surveyed across the oyssel bed belt in Königshafen in May 2022 by collecting fresh shell doublets found at sediment surface. No clams were encountered on bare flats >30 m landward and >30 m seaward of the oyssel bed belt. At the transect positions abundance (± standard deviation) was similar: at the landward edge (<30 m from beds), mean abundance was 7.1 ± 2.3 [range 5–11] per 16 m², within the belt 10.8 ± 7.7 [2–25], and seaward (<30 m) 7.8 ± 4.2 [3–18]. This corresponds to 0.5 shell doublets per m² on average. Shell lengths of *R. philippinarum* collected did not show significant differences between transect intervals (Kruskal-Wallis test, $\chi 2 = 45.8$, df = 32, p = 0.054). However, in pair-wise comparisons, shells at the seaward edge were significantly larger than those within the bed and at the landward edge (pairwise Mann-Whitney *U* test, both p < 0.001). The smallest average shell size was found within the bed and were smaller than those at the landward edge (pairwise Mann-Whitney *U* test, p = 0.038).

Abundance of living clams obtained by raking plots of 0.25 m² at the cluster of oyssel beds in Königshafen was very patchy (range 0–11 per plot), low overall (<10 per m²), and increased from 2022 to 2024 (Table 2). Mean and range of shell sizes remained similar over the years, even though density increased four-fold over the last 8 months.

3.3. COI metabarcoding

From all samples taken during the regular monitoring of bivalve

Table 2
Abundance and size of living *Ruditapes philippinarum* at a cluster of oyssel beds in Königshafen. 240 plots of 0.25 m^2 were raked 10 cm deep in the years 2022–2024. SD = standard deviation.

Date	Plots	$\sum$m^2	Abundance on 0.25 m^2 mean $\pm$ SD	Shell length in mm mean $\pm$ SD
Jan/Febr. 2022	60	15	0.33 $\pm$ 0.65	38.3 $\pm$ 5.4
Nov. 2022	100	25	0.50 $\pm$ 0.73	40.2 $\pm$ 5.2
May 2023	40	10	0.68 $\pm$ 0.86	40.1 $\pm$ 9.4
Jan. 2024	40	10	2.45 $\pm$ 2.52	39.5 $\pm$ 6.1

larvae in the tidal basin of northern Sylt, 77 samples covering a period from April 2019 to July 2023 had more than 50 larvae (max: 3196) and were chosen for DNA extraction and sequencing. The 35′264 bivalve larvae used for DNA extractions yielded a total of 96′178 reads, which were assigned to 16 species. After conversion of read proportion to estimated larvae numbers, 9518 larvae were assigned to *C. edule* and 4754 larvae were assigned to *R. philippinarum*. In total we identified 29 ASVs that were assigned to *R. philippinarum*. Out of these only asv1244 showed an average sequence divergence to the other *R. philippinarum* ASVs of more than 30% and probably represents a M-type mitochondrium of this DUI (Doubly Uniparental Inheritance) species (Plazzi and Passamonti, 2010). With 100 reads this ASV was comparatively rare (~2% of reads assigned to *R. philippinarum*) and removal of this ASV did not influence our conclusions. Since it still identifies *R. philippinarum* correctly we left it in the data set. The temporal dynamics differed between the two species within years as well as over the whole period (Fig. 6). *C. edule* was observed in 41% of all samples with similar occurrence between years (2019: 16.7% of samples not including the spring main spawning event, 2020: 37.5 %, 2021: 40%, 2022: 51.9%, 2023: 50%). The majority of larvae were found in the earlier months of the years (April–June) with only few observations in later months (August, September). *R. philippinarum,* was found in 32% of the samples but frequency and total numbers increased over the whole period (2019: 8.3%, 2020: 6.3%. 2021: 30%, 2022: 37.0%, 2023: 83.3%). In earlier years (2019–2020) larvae were mainly observed in later months (September, October) but extended to early summer in later years, especially in 2022 when larvae were detected throughout the whole year except winter (Fig. 6).

3.4. Recruitment and mortality

Size-frequency distributions suggest little or no recruitment in 2021 (Figs. 4 and 5), and no spat of clams was detected by sieving sediment cores in summer 2022. By chance, a small clam (14.5 mm shell length), crawling on submersed mud during low tide was encountered end of August 2022. It might have recruited in that summer but remained the only one found. Although the raking method was not suitable for clams <20 mm in shell length, larger numbers, if present, would have been noticed. This was not the case, although plenty of larvae were sampled in 2022 and 2023 in the plankton (Fig. 6).

Most shell doublets found on sediment surface were undamaged. At the oyssel bed in Königshafen, out of 167 only 7 (4 %) had scars at the posterior end, reminiscent of damages inflicted by oystercatchers hammering on cockles and mussels. In a saltmarsh just behind a stone revetment on Gröde (see Fig. 2), 24 shell doublets of *R. philippinarum* were collected. These lay scattered at a breeding ground of oystercatchers, probably brought there for feeding chicks, however, shells lacked damages.

Almost all shell doublets collected between January and May 2022 were fresh, either still with flesh inside or with a polished inner surface, indicating substantial mortality in Königshafen as well as near Hooge. This ceased in summer. In August, out of 83 paired valves collected at sediment surface in Königshafen, only 11 (13 %) appeared to be fresh, while all others already had a film of green cyanobacteria and/or
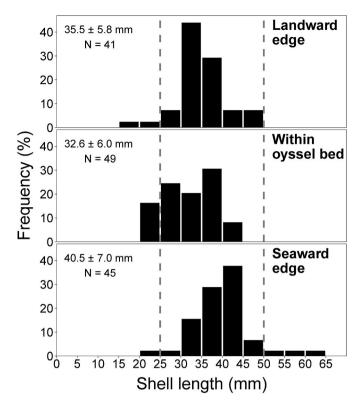

Fig. 5. Length-frequency distributions of fresh shell doublets of *R. philippinarum,* grouped into 5 mm size intervals, at three positions of an oyssel bed cluster in Königshafen sampled in May 2022. Upper left in each panel: Mean shell length ($\pm$ standard deviation) and number of shells measured (N). Dashed vertical lines mark size range 25–50 mm.

attached juvenile barnacles (*Austrominius modestus*) on the inner side. In 2023, only very few fresh shell doublets were encountered, suggesting that the mortality event was confined to the first half of 2022.

V. corrugata co-existing in low numbers with *R. philippinarum* at the oyssel bed cluster in Königshafen died concurrently. A disease agent could not be detected macroscopically (i.e., no signs of brown ring disease; Paillard, 2004). When storing 10 surfaced Manila clams and 8 cockles found in May 2022 in a cooler for 2 days, all clams died while cockles stayed alive.

3.5. COI haplotype composition

A total of eight unique haplotypes was found in the 79 sequenced individuals from the Northern Wadden Sea. The four most frequent ones (RpCOI1 – RpCOI4, Genbank accession numbers KU252866 - KU252869 were previously described by Chiesa et al. (2017) while four novel haplotypes were found that were only observed once (RpCOI14 – RpCOI17, Genbank accession numbers PQ062665 - PQ062668, Fig. 7A). Haplotype and nucleotide diversities were high and did not differ significantly between the sites and years within the northern Wadden Sea populations (all p values > 0.06, Table 3). In comparison to the other European populations, haplotype diversity was higher in the northern Wadden Sea than in the Adriatic and UK populations, but was similar to the Atlantic populations (Table 3). Nucleotide diversity was also significantly higher in the Wadden Sea than in the UK populations, probably reflecting the low number reported for individuals sampled in the UK (16 individuals, Chiesa et al., 2017). Haplotype RpCOI4 was the most common haplotype in all Wadden Sea populations (Fig. 7A). This haplotype was also dominant in the UK populations (Chiesa et al., 2017) supporting an introduction route via UK hatcheries. However, haplotypes RpCOI1 (dominant in the Adriatic) and RpCOI2 and RpCOI3 characterizing the Atlantic populations were also frequent (Fig. 7A).
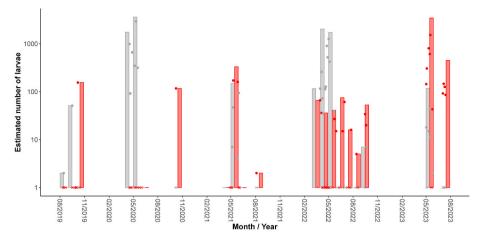
Fig. 6. Estimated numbers of larval *R. philippinarum* and *C. edule* in 10 l of surface seawater. Samples were collected bi-weekly in the tidal basin near Königshafen in 2019–2023, but only samples with >50 larvae were sequenced. Dots show the numbers of larvae for each day while bars show the monthly sums, and x indicates that >50 larvae were found on that day but were not assigned to either of the two species.

Consequently, the Wadden Sea population took a central position in the PCoA ordination (Fig. 7B), which could either indicate multiple introductions from different sources or a previously uncharacterized source population with mixed haplotype composition.

4. Discussion

4.1. Two routes into the Wadden Sea

Multiple translocations of Pacific Manila clams within Europe have taken place (Chiesa et al., 2017). Faasse and Lighthart (2008) suggest that *R. philippinarum* might have hitchhiked mussel (*Mytilus edulis*) transports from Poole Harbour (southern England) into the Oosterschelde (Dutch Rhine Delta). Similarly, seed mussels were sent from the Thames Estuary and the British Channel to the northern Wadden Sea several times between 2007 and 2011. These could have introduced Manila clams, already established at these sites at that time (see Humphreys et al., 2015). British seed mussels were meant to compensate recruitment failures in the northern Wadden Sea but were stopped by German court decision because of risking the introduction of non-natives (Hamburger Abendblatt, Dec 19, 2011). Perhaps too late, and *R. philippinarum* had already sneaked in unnoticed with those shipments.

High frequency of RpCOI4, dominating British populations (Chiesa et al., 2017), might support this route. However, high haplotype diversity in the northern Wadden Sea contrasts with a low diversity reported from Poole Harbour and Southampton (Chiesa et al., 2017). The low diversity in the British sites in the previous study might represent an artefact of analyzing only 16 clams, which limits the chance to find rare haplotypes observed in the 79 specimen used here. An alternative origin may be the Rhine Delta. Mussel culturing in the northern Wadden Sea is run by a Dutch company linked to the shellfish hub of Yerseke in the Oosterschelde. With commuting ships and equipment, *R. philippinarum* might have been transferred unintentionally. Screening more clams from sites in southern England and from the Rhine Delta should be a next step in solving the riddle of the origin, and perhaps the foundation of the population in the northern Wadden Sea.

From shell sizes and widely scattered records over the first 5 years in the northern Wadden Sea, we suggest that introduction most likely happened between 2010 and 2012. Presumably, this occurred somewhere south of the islands Sylt or Föhr, where in the tidal inlets Hörnum Tief and Norderaue most mussel culture plots are maintained. Subsequent dispersal by larval drift may explain the widely distributed records from the northern Wadden Sea including northern Sylt during the long initial phase of clam scarcity up to 2020. According to Herbert et al. (2012) larval drift over >50 km seaway without suitable habitats as stepping stones would be a challenge. However, a rather similar haplotype composition found south and north of Sylt at the mtDNA *COI* fragments do not suggest distinct source regions for the introduced clams.

The gradual spread from the Rhine Delta along the Dutch coast into the western Wadden Sea probably occurred by stepwise larval drift between 2013 and 2015, including a further spread throughout the southern Wadden Sea, covering a distance of 250 km in roughly a decade. This narrows the gap left between routes A and B (Fig. 3) to <80 km across the Weser and Elbe estuaries. Since survival of *R. philippinarum* has shown to be sensitive to low salinity events (Woodin et al., 2020; Román et al., 2024), estuaries in the central Wadden Sea may serve as a barrier. However, a further spread northward through the Danish Wadden Sea and beyond is to be expected.

4.2. Population development in the northern Wadden Sea

Compared to other locations with feral populations (i.e., Jensen et al., 2004; Pranovi et al., 2006; Dang et al., 2010; Humphreys et al., 2015; and reviewed in Chiesa et al., 2017), establishment in the northern Wadden Sea was a rather slow process spanning an entire decade. The difference may be explained by a small versus large founder populations. In addition, in the northern Wadden Sea adverse conditions may prevail with narrow windows of opportunity, suggesting that risk of failure was high over many years despite of individual longevity. Lack of benthic juveniles while planktonic larvae were abundant, points to high mortality at or shortly after settlement.

Predation on the spat of Manila clams may constitute strong ecological resistance in introduced regions (Byers, 2005; Ruesink et al., 2014; Bidegain and Juanes, 2013; Harris, 2016) as well as at coasts of origin (Ishii et al., 2001; Toba et al., 2007). Protection against predators with netting is common practice in clam cultures on tidal flats (Humphreys, 2010). The shore crab *Carcinus maenas* can crush young Manila clams up to a size of 24 mm shell length (Harris, 2016; Domínguez et al., 2021) and oystercatchers may take over from 10 mm shell length upwards (Caldow et al., 2007). So far, we found no direct evidence for the former but observed some clam shells damaged by oystercatcher attacks. On the other hand, previous experimental exclusions of crabs and shrimp in the northern Wadden Sea had demonstrated strong pressure on young cockles (Reise, 1985; Strasser, 2002).
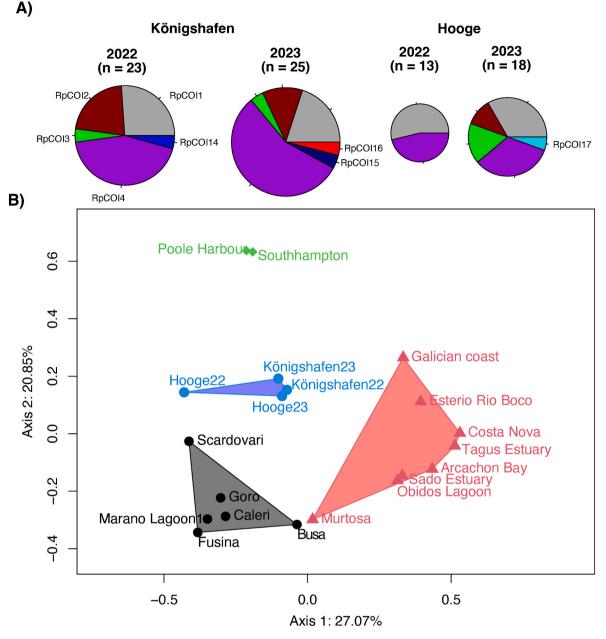
Fig. 7. A) COI haplotype composition of *R. philippinarum* sampled at two sites in two consecutive years in the Northern Wadden Sea. Pie chart size is proportional to the number of haplotypes sampled. B) Principal coordinate analysis (PCoA) ordination of haplotype frequencies in the Wadden Sea (blue) and European regions (red: Atlantic, grey: Adriatic, green: UK) based on pairwise Bray-Curtis distances. Data for European populations was estimated from Chiesa et al. (2017). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Occurrence in the northern Wadden Sea is confined to the intertidal zone and there often to seagrass beds and the vicinity of mussel beds intermixed with Pacific oysters. Few fresh shell doublets were encountered in pure mud or strongly rippled sand, and none in the subtidal zone irrespective of habitat structure. In view of the recent arrival, this may be a transitory pattern and habitat use might increase if the population expands. However, the current habitat use might also represent conditions advantageous for *R. philippinarum*. Reciprocal benefits between *R. phlippinarum* and the intertidal seagrass were detected (Tsai et al., 2010; Román et al., 2024). Wiesebron et al. (2022) simulated storm induced sediment erosion in an experimental flume, and found that *R. philippinarum* is more susceptible than cockles *C. edule* because the latter are more agile diggers. Seagrass rhizomes and abundant shell hash may also offer clam recruits protection against predation.

Large mean size of clam individuals in the northern Wadden Sea may

be due to the absence of commercial harvesting. Slightly smaller mean size at the landward side of a belt of oyssel beds compared to the seaward side where more food arrives in unfiltered flood currents, indicate at what scale competition for suspended food might be expected in the tidal zone. The apparent absence of Manila clams in the subtidal zone of the northern Wadden Sea might suggest that the subtidal zone is still too cold. In summer, intertidal sediments become warmer than subtidal bottoms (Reise, 1985). In the northern Wadden Sea, this may be an advantage when assuming that high temperature facilitates larval metamorphosis, settlement and juvenile growth. Humphreys et al. (2015) wrote that it remains unlikely that *R. philippinarum* can naturalize in British waters north of the annual 10 °C contour line unless warming sea temperatures allow an expansion. In the northern Wadden Sea at 55°N, mean annual water temperature has increased from 9 to 10 °C in the past decades (Rick et al., 2023). This may have eased the

Table 3
Haplotype and nucleotide diversities in Wadden Sea populations of *R. philippinarum*. The upper four lines give the estimates for single sites and years within the Wadden Sea, while the lower four lines compare all Wadden Sea populations together with European populations (data assembled from Chiesa et al., 2017). Significant differences between the Wadden Sea and the European populations based on 1000 bootstrap replicates are given in bold (*: 0.05 < p < 0.01, **: 0.01 < p < 0.001, ***: p < 0.001).

	Population	N	Haplotype diversity	Nucleotide diversity
Wadden Sea	Königshafen 2022	23	0.72332	0.00422
	Königshafen 2023	25	0.65333	0.00385
	Hooge 2022	13	0.53846	0.00219
	Hooge 2023	18	0.77778	0.00333
Europe	Wadden Sea	79	0.68808	0.00358
	UK	16	**0.125*****	**0.00128****
	Adriatic	111	**0.40825***	0.00306
	Atlantic	96	0.63862	0.00381

establishment by reaching optimal spawning temperatures for *R. philippinarum* more frequently. Introduced species often took advantage from climate change when establishing and spreading in a new range (i.e., Stachowicz et al., 2002; Walther et al., 2009; Sorte et al., 2013; Beck et al., 2024), including introductions in the Wadden Sea, such as the Pacific oyster *Magallana gigas* as a prominent example (Reise et al., 2023).

4.3. Ecological integration

Introduced species may be benefit and burden at the same time, depending on interest and perspective (Kourantidou et al., 2022). Manila clams have established in the northern Wadden Sea but overall abundance is still too low for recognizing effects on other species or the ecosystem. However, what would be the long-term perspective? Functionally, Manila clams overlap with the related but rare *V. corrugata*. However, in the northern Wadden Sea, this species occurs mainly below mean low tide level, while *R. philippinarum* remained above spring low tide level. Thus, only marginal interaction can be expected. In NW-Spain the two species occur together in intertidal seagrass beds (Román et al., 2024). There, Manila clams are less at risk to predatory shore crabs than *V. corrugata* and cockles, presumably due to their stronger shell (Domínguez et al., 2021).

Cockles are one of the most common benthic suspension feeders in the Wadden Sea but population density is highly variable in space and time (Reise, 1985; Beukema and Dekker, 2019). Thus, competition may be marginal besides overlapping food (Dias et al., 2019). The same may apply to competition with *Mya arenaria*. This species is most abundant in the estuarine regions of the Wadden Sea where *R. philippinarum* would be limited by low and fluctuating salinity (see Nakamura et al., 2005). Furthermore, in the Wadden Sea, bivalves tend to be more often restrained by recruitment failure rather than limiting resources (Beukema and Dekker, 2022). Consequently, ecological resistance to introduced Manila clams may be primarily caused by predation rather than competition. For the Dutch delta region, it has been suggested that Manila clams could be an alternative prey to mollusk feeding birds, if the abundant cockles continue to suffer from recent heat waves in summer and begin to decline (Kamermans and Leopold, 2021; Glorius et al., 2023). The same may hold for the Wadden Sea (Sneekes et al., 2015).

In France, Italy, Spain and Portugal, *R. philippinarum* was introduced with little opposition and unrestrained farming proceeded (but see Galil et al., 2013), while in Britain the imported clam stock was initially reared in enclosed hatcheries before release into the coastal environment was eventually permitted (Humphreys, 2010). Climate warming then facilitated spawning and the population went feral.

The spread of Manila clams contributes to biological globalization.

Divergence from pristine species compositions increases and climate warming may shift this further. The perception of an ecosystem with co-evolved interactions between its components is gradually replaced by an 'ecoglomerate' with spontaneously formed interactions. It remains to be shown how much this affects ecosystem functionality and resilience.

5. Conclusions

The Manila clam arrived in the European Wadden Sea in the early 2010s. First by long distance jump dispersal into the northern Wadden Sea, and then by stepwise immigration from the Rhine delta into the southern Wadden Sea.

Haplotype composition at mtDNA *cytochrome oxidase I* in the northern Wadden Sea population is diverse and represents a mix of common haplotypes found throughout Europe. The source population therefore remains unresolved and the observed pattern either suggest multiple introductions from different sources or one from an already mixed population.

Spread in the north commenced slowly initially, but spatial expansion in recent years was rapid. Although larval abundances increased notably, adult abundances remained low, probably relegated by strong predation on early recruits.

Currently, the population of *R. philippinarum* at 55°N in the northern Wadden Sea constitutes the northern frontier of its European range, expected to expand further with climate warming.

CRediT authorship contribution statement

Karsten Reise: Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization. **K Mathias Wegner:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Conceptualization. **Rainer Borcherding:** Validation, Project administration, Data curation. **Sarah Brand:** Validation, Investigation, Formal analysis. **Christian Buschbaum:** Supervision, Resources, Conceptualization. **Andreas M Waser:** Visualization, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2024.108940.

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