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Research Article

Establishment and population features of the non-native Atlantic rangia, *Rangia cuneata* (Mollusca: Bivalvia), in northwestern France

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Abstract

The presence of shells of the Atlantic rangia, *Rangia cuneata*, a brackish-water species native from the Gulf of Mexico also known as gulf wedge clam, was reported in 2017 on the French coasts of the English Channel, in the waterway that connects Caen to the sea. However, no information was available on whether a population of this alien species had successfully established in the region. Here, only empty shells—except for one live individual—were sampled in that waterway, and the sampling was shifted to the nearby marina of Ouistreham, where water is mesohaline ($6.89 \pm \text{SD } 0.06$ PSU). In spring 2017, the mean density in the marina reached 110.45 ± 86.08 ind m^{-2} , largely dominating the benthos community. The population was mostly composed of fairly large individuals, with no young-of-the-year found inside the marina. The modal values of the size frequency distribution ranged between 35 and 40 mm shell length. The growth patterns determined from the annual rings suggest a maximum lifespan of eight completed years. Following the assumption that colonization occurred at the larval stage, as in other European countries, this population may have established in 2009, *i.e.* only four years after its first detection in Europe, in the Antwerp harbour. The specimens collected are the largest recorded in European waters, suggesting a highly suitable environment for the species in the region. Given the invasive potential of Atlantic rangia within the last decade, a close monitoring of this population and of the spread of the species in French and European waters appears necessary to determine its impacts on these ecosystems.

Key words: the Atlantic rangia, alien species, Normandy (F), brackish waters, density, growth, reproduction

Introduction

The Atlantic rangia, *Rangia cuneata* (G.B. Sowerby I., 1832; AphiaID: 156991), also known as wedge clam or gulf clam, is a bivalve species belonging to the Mactridae, often considered as semi-tropical. This burrowing and non-selective filter-feeder species has a bipartite, benthopelagic life cycle. *Rangia cuneata* tolerates a wide range of salinity but is typically found in estuaries with salinities < 18 PSU (Swingle and

Bland 1974; LaSalle and de la Cruz 1985). In its area of origin (Gulf of Mexico), the species is more abundant in oligohaline (0.5–5 PSU) waters where it experiences less predation and competition due to a relatively low species diversity (LaSalle and de la Cruz 1985). Adults can live anaerobically for up to 2 weeks (Hopkins et al. 1973), but a recent study showed that the blue crab *Callinectes sapidus* exerted a higher predation on clams stressed by hypoxic conditions, thus suggesting an indirect and negative effect of hypoxia (Howard et al. 2017). In Gulf estuaries, the Atlantic rangia is a good indicator of the oligohaline zone where periodic episodes of near freshwater salinity reduce competition and predation and allow this clam with broad generalised adaptations and tolerances to dominate the benthos (Poirrier and Caputo 2015). The species displays a clear preference for fine-medium sand but can also tolerate mud and mixtures of sand and clay bottoms (Tenore et al. 1968; Peddicord 1977; Sundberg and Kennedy 1993). *Rangia cuneata* grows faster in sandy areas, with a maximum expected length of 75 mm (Wolfe and Petteway 1968; Wong et al. 2010), although one 94 mm individual has been found in Louisiana (LaSalle and de la Cruz 1985). The average lifespan is around 4 to 5 years, exceptionally reaching 10 (Wolfe and Petteway 1968) to 15 years (Hopkins et al. 1973). Despite its muddy taste, the Atlantic rangia is harvested and marketed for human consumption in eastern Mexico, where it has a relatively high economic value (Wakida-Kusunoki and MacKenzie 2004).

Densities in harvest locations of the species' native area range between 15 and 29 ind m⁻² (Wakida-Kusunoki and MacKenzie 2004) and represents 15.9%, 40.0% and 99.9% of the total biomass in three coastal lakes (Wong et al. 2010). The highest densities were actually reported in the USA but outside of the native range of the species, with 300–600 ind m⁻² in Virginia and the North Carolina, and up to 1,200 ind.m⁻² in the Upper Chesapeake Bay with an overall maximum of 10,000 ind m⁻² (Hopkins et al. 1973). Such high densities suggest that *R. cuneata* can play a critical role on the ecological processes of shallow, brackish water systems (e.g. Cerco and Noel 2010) and is thus locally considered as a key species (Hopkins et al. 1973; Wong et al. 2010). The Atlantic rangia has been reported as a biofouling species, causing clogging issues in industrial cooling water systems (Verween et al. 2006).

The species was first discovered in Europe in the Antwerp harbour in 2005 (Verween et al. 2006), and then spread to the brackish and freshwater habitats of northern Europe (Solovjova et al. 2019). In 2010, the species had already entered the southeastern Baltic Sea through the waterway of the port of Kaliningrad, Russia (Ezhova 2012), and reached the Russian part of the Vistula Lagoon (Rudinskaya and Gusev 2012). In 2011, it was detected on the Polish side of the Vistula lagoon (Warzocha and Drgas 2013), followed by the Curonian Lagoon in Lithuania (Solovjova 2014) and the Nord-Ostsee Kanal, Brunsbüttel, in Deutschland, in 2013 (Wiese et al. 2016). In 2014, *R. cuneata* had established in the Rotterdam harbour in the

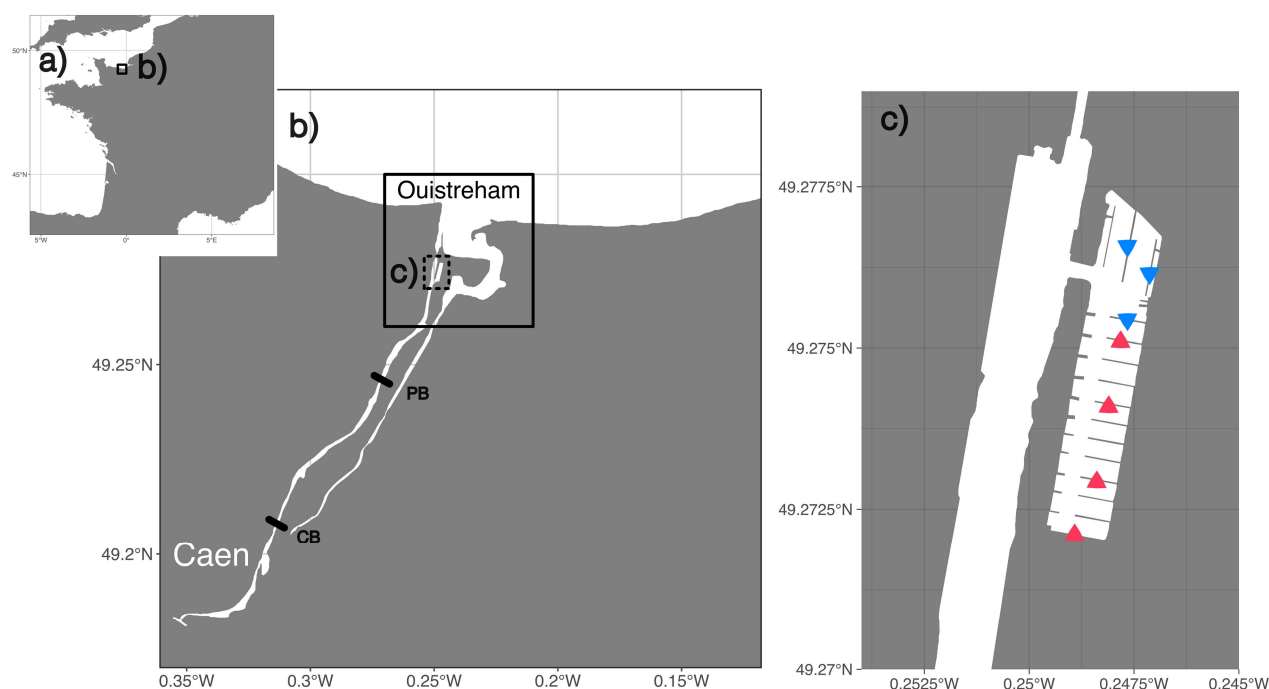


Figure 1. a) Localisation of the study site in Normandy (France). b) Focus on the waterways between Caen and the English Channel, with the localisation of the Colombelles bridge (CB) and Pegasus bridge (PB). The dashed rectangle indicates the Ouistreham marina. c) Sampling sites in the Ouistreham marina, blue triangles indicate samples collected on 20th April and red triangles samples from 3rd May 2017.

Netherlands (Gittenberger et al. 2015), and, one year later, it had reached Great Britain (Willing 2015; ICES WGITMO 2016). The most recent new detection came from the Pämu Bay, in Estonia, in 2016–17 (Möller and Kotta 2017; ICES WGITMO 2018).

In France, Dewarumez et al. (2011) suggested that the Atlantic rangia might have reached the northernmost French estuaries due to the proximity with the Belgian coasts. The species was mentioned again recently “on the banks of the Canal de Caen à la mer à Benouville – Ranville on 9th August 2017” (Kerckhof et al. 2017) but only limited biological information were collected (i.e. “empty shell length between 3.5 and 5 cm”). Thus, there is still no information on its potential repartition and population characteristics (Gouletquer 2016). In this context, the present study confirms the presence of an established population of *R. cuneata* on the French coasts, and describes the population’s characteristics (i.e. density, size structure and growth) and provides new insights on the reproduction of this diecous species through histological analysis.

Materials and methods

Study site

The study site was located on the French coast of the English Channel, in the waterway—built in the 19th century—leading to the Ouistreham marina (49°20′5N; 00°14′7W) and the Caen harbour (Figure 1a). The waterway is characterised by a marked gradient of salinity from seawater to mesohaline water in Ouistreham and to freshwater in the Caen harbour (Figure 1b)

(Charles et al. 2018). The Ouistreham marina is closed by a lock, and has a capacity of 650 berths distributed over 15 fixed pontoons (Figure 1c). Salinity varies between 5 and 10 PSU, averaging $6.89 \pm \text{SD } 0.06$ between March and May 2017, and water temperature at 1 m depth 11.41 ± 0.21 °C. On April 18–20, 2017 and May 3, 2017, it averaged 13.97 ± 0.12 °C and 14.58 ± 0.37 °C, respectively. The substratum was analysed in triplicate with metal sieves with square meshes of 50 µm, 250 µm, 500 µm, 1 mm and 2 mm. The substratum was composed of silt and mud (particle size < 50 µm: $34.99\% \pm 10.64$) and fragments of various bivalve shells, barnacle plates and reefs of the annelid *Ficopomatus enigmaticus* (Fauvel, 1923) with a size > 2 mm on average ($37.23\% \pm 6.42$). The depth, ca. 3.5 m, is homogeneous throughout the marina.

Sampling design

All samples presented in this study were collected with a 6.7-kg Van Veen grab (Eijkelpomp®, Giesbeek, The Netherlands) that collects 2 L of substrate volume on a surface of 260 cm². All samples were passed through a 1 mm mesh sieve and all live individuals and empty shells (either one or two valves) of *R. cuneata* were picked up and brought back to the laboratory.

During the first exploratory phase, between April 17 and 18 2017, samples were collected in the waterway from the banks (visual survey only) and from two bridges (20 samples from each) located about 12 km and 6 km from the entrance of the channel, respectively (Figure 1b). Only empty shells were collected at these sites, except for one live individual. The sampling effort was thus shifted to the Ouistreham marina. Nine sediment samples were collected from three different pontoons randomly selected on April 20, 2017 and eight samples were collected from four other pontoons on May 3, 2017 (Figure 1c).

Several specimens of *R. cuneata* sampled in this study have been deposited and registered in the collection of the Muséum National d'Histoire Naturelle (Paris, F), under the identification number: MNHN-2012-25155.

Samples processing and histological analysis

The length (the distance between the anterior and the posterior edges) of all shells was measured to the nearest 0.01 mm. The total weight of live individuals was obtained to the nearest 0.01 g following the standard procedures for bivalves. The clams were then opened and the flesh removed for histology purpose. The emptied shells of live clams were later weighted to the nearest 0.01 g (shell weight). The number of annual rings reflecting the slowdown or the stop of growth during winter periods was determined from the shells of live individuals. For each clam, the number of rings was linked to its size and total weight to determine the size- and weight-at-age distributions. When the number of individuals was high enough ($N \geq 10$), the distance between the hinge and every ring (partial height) and the distance

between the opposite edges and each ring (partial length) were measured to assess the shell's growth over successive years. No outliers resulting from sensitive identifications were detected with the Grubbs' test (or Extreme Studentized Deviate method, ESD; GraphPad Prism® 2019; Grubbs' test; $P > 0.05$). A theoretical growth curve was computed by fitting the standard Von Bertalanffy equation (Von Bertalanffy 1938) to the length-at-age distribution of the population. The size of the empty shells collected was included to compute the population's lifespan.

Histological analyses were conducted on the central cross-sections of the clams fixed in Davidson's solution. Tissue samples were processed for histology, with 3 μm thick paraffin-embedded sections stained according to the trichrome of Prenant Gabe (eosin, hematoxylin and light green). The gender and gametogenesis stage were determined for each histological slide, considering six stages: stage 0, sexual resting; stage 1, beginning of gametogenesis, mainly gonidia in mitosis; stage 2, active gametogenesis, with all types of germinal cells and highly expanding follicles; stage 3, sexual maturity and possible beginning of spawning; stage 4, active spawning; and stage 5, spent/post-spawning. The course of the clams' gametogenesis was synthesised with a sexual maturity index (SMI), as:

$$SMI = (N_{st\ 1}/N) \times 0,5 + (N_{st\ 2}/N) \times 1 + (N_{st\ 3}/N) \times 2 + (N_{st\ 4}/N) \times 1,5 + N_{st\ 5}/N \times 0,25$$

where N is the total number of clams, and $N_{st\ x}$ the number of clams showing the gametogenesis stage x .

Statistical analyses

The clam densities were computed as the number of individuals per surface unit of sampled substrate ($1\ \text{m}^{-2}$). The data did not meet the homoscedasticity and normality assumptions even with transformations. Differences in clam density between pontoons were thus tested with the non-parametric Kruskal-Wallis and pairwise Wilcoxon rank sum *post hoc* tests, with Holm correction for multiple testing. Analyses of variance (ANOVAs) and Tukey *post hoc* tests were used to test for differences in size increments between rings among age classes and years. A χ^2 test was used to compare the proportion of gametogenesis stages between the two sampling periods, and to test for differences in the balance of sex-ratio by applying the Yates correction. A standard significant threshold of $P < 0.05$ was selected for all analyses. Statistical analyses were conducted in R 3.6.1 (R Core Team 2019), with the package *tidyverse* for data manipulation and graphics (Wickham 2017).

Results

Densities of *Rangia cuneata*

In the waterway between Caen and Ouistreham, a fairly high sampling effort provided no live clams or empty shells from the Benouville bridge,

Table 1. Average $\pm$ standard deviation of shell length measured in *Rangia cuneata* sampled in Ouistreham marina in April and May 2017. Other biometric parameters (shell height, total weight and shell weight) are also provided for completeness.

Sampling dates (2017)	Length (mm)	Height (mm)	Total weight (g)	Shell weight (g)	% of shell weight / total weight
20 April (N = 49)	39.17 $\pm$ 8.08	31.05 $\pm$ 6.91	23.28 $\pm$ 13.28	16.46 $\pm$ 9.66	70.09 $\pm$ 2.78
3 May 2017 (N = 72)	35.97 $\pm$ 9.12	29.18 $\pm$ 7.64	20.16 $\pm$ 14.72	14.51 $\pm$ 10.83	70.04 $\pm$ 5.22
Both dates (N = 121)	37.26 $\pm$ 8.82	29.94 $\pm$ 7.38	21.42 $\pm$ 14.18	15.30 $\pm$ 10.37	70.06 $\pm$ 4.38

and only 29 empty shells (non-disarticulated valves or only left or right valves) were collected from the more inland site at the Colombelles bridge (mean salinity = 5.31 ± 0.03 PSU; Figure 1). These shells were on average 36.71 mm (shell length), with a single live clam of 32.31 mm and 10.92 g (total weight); no shell was found in the surrounding banks.

In contrast, the species was abundant in the Ouistreham marina, with 95 empty shells and 112 live clams collected (49 live individuals from 27 samples on April 20, 2017, and 63 individuals from 32 samples on May 3, 2017). Among the 46 samples containing live clams and/or empty shells, 22 contained both live and dead individuals, 17 live individuals only, and 7 only empty shells. When present, the overall density of live clams was 110.45 ± 86.08 ind m^{-2} . In the samples collected from the pontoons, the density of live clams ranged from 8.55 ± 16.96 ind m^{-2} to 132.48 ± 94.02 ind m^{-2} . Densities varied between pontoons (Kruskal-Wallis test; $H_6 = 19.99$, $P = 0.003$) but *post hoc* tests did not reveal significant differences (lowest $P = 0.07$), suggesting no particular spatial distribution at the scale of the marina.

Size and growth

Biometric parameters were measured on 121 live clams, 112 from our samples and on 9 additional ones from an abandoned fishing net in the marina. No “apparent growth” was observed between sampling dates (Table 1). The 121 individuals were therefore pooled to improve the quality of the size-frequency distribution. The smallest and largest individuals were 9.80 mm and 56.76 mm in length and 0.16 g and 65.74 g in total weight, respectively. The proportion of shell weight over total weight ranged from 53.13% to 76.76%, with a mean value of $70.06 \pm 4.38\%$, highlighting the disproportionate contribution of the shell to the total weight of the individuals. The modal values of shell length and total weight were 35–40 mm and 5–10 g, respectively (Figure 2). Among 119 exploitable specimens, between 1 to 8 rings marked the slowdown or stop of growth during the winter period (Figure 3). In the Ouistreham marina, the current maximum lifespan of *R. cuneata* is therefore 8 completed years (see Table 2 for the biometric parameters for each determined age class and Figures 4 and 5 for growth in size and weight over time). The results on extreme ages should be taken with caution since the age classes 1 and 8 completed years are each represented by only two individuals. The youngest age class (1 annual ring) had a total weight of 1.72 ± 0.64 g, while the oldest age class (8 annual rings) weighted 56.86 ± 12.57 g. The contribution of shell weight to the total

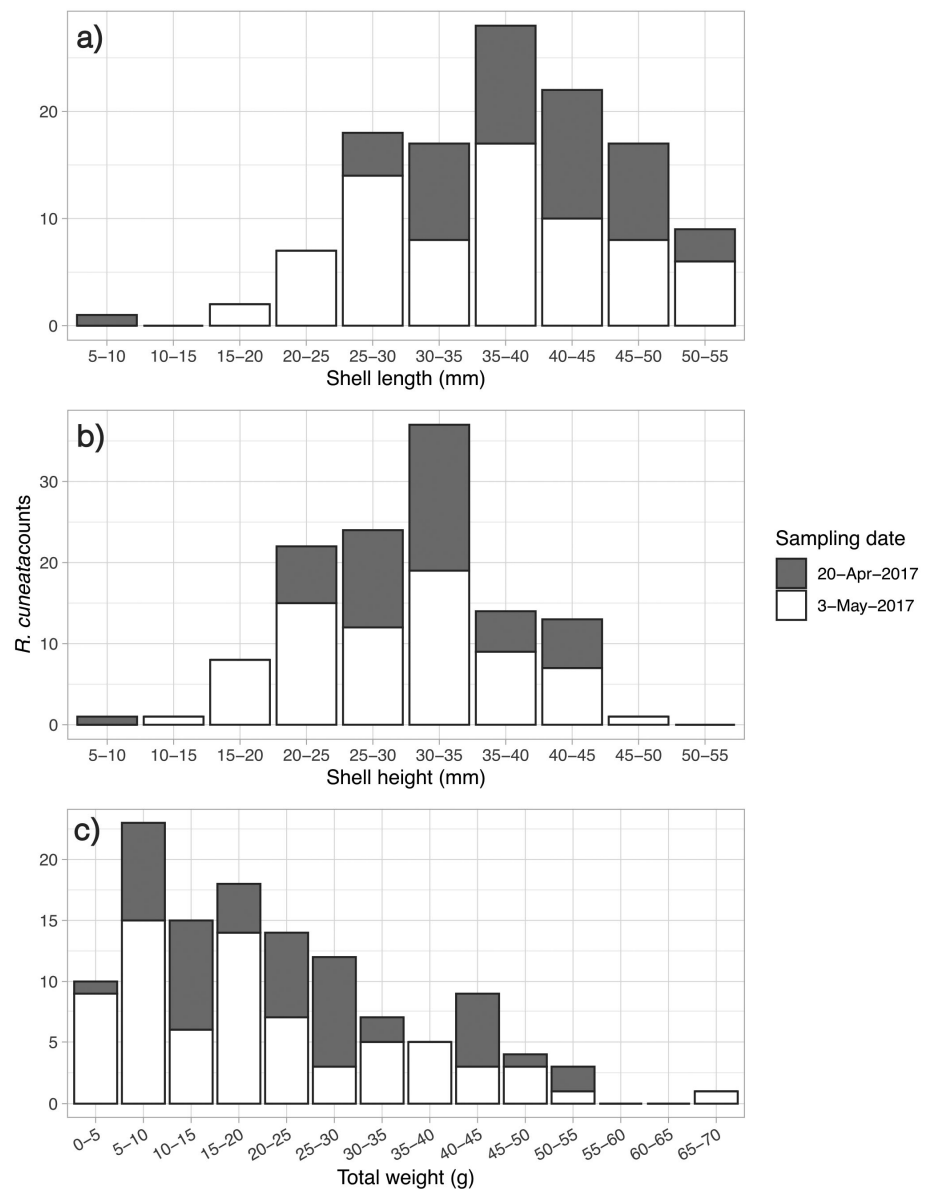


Figure 2. Size and weight frequency distributions of *Rangia cuneata* in the Ouistreham marina (N = 121), split per sampling date: a) shell length, b) shell height, and c) total weight.



Figure 3. Pictures of *Rangia cuneata* sampled in the Ouistreham marina, Normandy, France. The main taxonomic features are the long posterior lateral tooth, the small but distinct pallial sinus, the tenuous pallial line and beak in front of the midline clearly turned inwards and forward. Photos by K. Costil.

Table 2. Average $\pm$ standard deviation of shell length measured in *Rangia cuneata* sampled in Ouistreham marina and showing from 1 to 8 shell rings (corresponding to 1 and 8 completed years old). Other biometric parameters (shell height, total weight and shell weight) are also provided for completeness.

Ring number (assessed age)	Length (mm)	Height (mm)	Total weight (g)	Shell weight (g)	% of shell weight / total weight
Ring 1 (1 y) (N = 2)	18.30 $\pm$ 1.68	15.21 $\pm$ 1.33	1.72 $\pm$ 0.64	0.96 $\pm$ 0.37	55.28 $\pm$ 1.34
Ring 2 (2 y) (N = 9)	23.91 $\pm$ 2.23	19.23 $\pm$ 1.97	4.42 $\pm$ 1.35	2.82 $\pm$ 1.03	62.90 $\pm$ 7.48
Ring 3 (3 y) (N = 12)	27.72 $\pm$ 1.40	22.09 $\pm$ 1.63	7.22 $\pm$ 1.88	5.00 $\pm$ 1.45	68.75 $\pm$ 3.35
Ring 4 (4 y) (N = 36)	34.06 $\pm$ 3.24	27.13 $\pm$ 2.97	13.85 $\pm$ 4.30	9.81 $\pm$ 3.19	70.50 $\pm$ 2.80
Ring 5 (5 y) (N = 31)	40.98 $\pm$ 2.53	32.58 $\pm$ 1.92	24.08 $\pm$ 4.52	17.14 $\pm$ 3.63	70.89 $\pm$ 2.96
Ring 6 (6 y) (N = 17)	46.26 $\pm$ 3.46	37.46 $\pm$ 2.75	36.82 $\pm$ 6.83	26.56 $\pm$ 4.80	72.27 $\pm$ 1.80
Ring 7 (7 y) (N = 10)	49.70 $\pm$ 2.33	41.10 $\pm$ 2.12	46.83 $\pm$ 5.03	33.92 $\pm$ 3.68	72.45 $\pm$ 1.80
Ring 8 (8 y) (N = 2)	54.28 $\pm$ 3.51	44.37 $\pm$ 3.86	56.86 $\pm$ 12.57	41.27 $\pm$ 9.02	72.60 $\pm$ 0.19

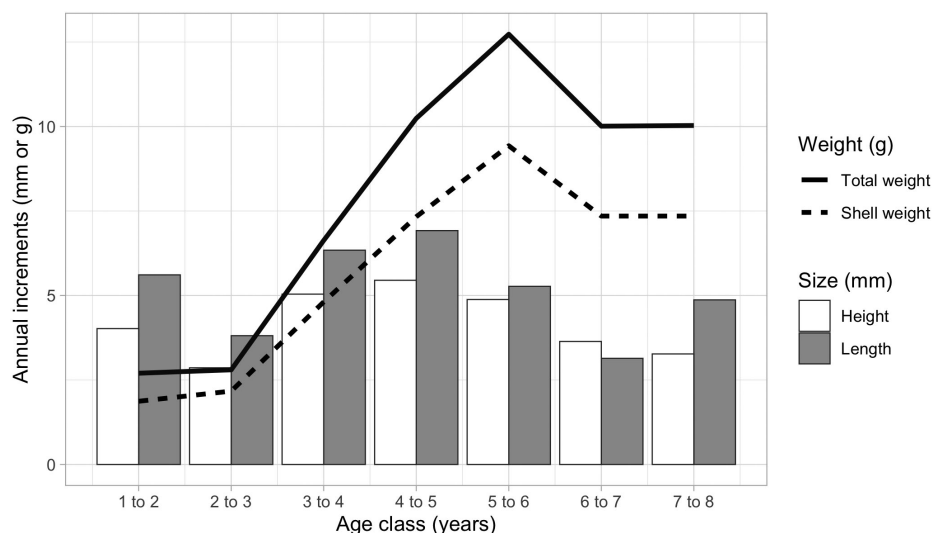


Figure 4. Yearly growth increments in size and in weight of *R. cuneata* sampled in Ouistreham marina between successive ages (e.g. “1 to 2” indicates clams from 1 completed year to 2 completed years). The values correspond to the differences in the mean biometric values of each age class.

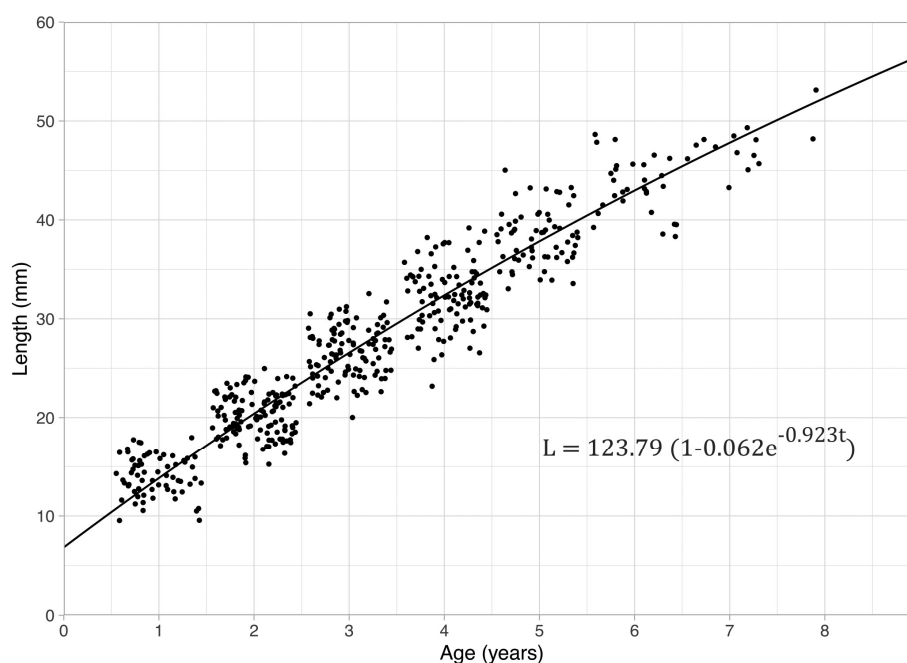


Figure 5. Theoretical growth of the *R. cuneata* population in Normandy, expressed by the Von Bertalanffy growth curve. Points represent the lengths of size groups 1–8 (with a 0.45 offset around each age class to display all points), used to fit the Von Bertalanffy growth curve. The parameters of the growth curve (L_{inf} , k and t_0) are provided in the equation.

Table 3. Average $\pm$ standard deviation of shell length increments (L) and height (H) in mm between 2 successive annual rings for the age classes of *Rangia cuneata* sampled in spring 2017 with ≥ 10 individuals. For each age class, the average growth increment was computed from all individual increments ($N_{\text{increments}} = 24$ to 124). For each year, the “overall mean” was obtained by assigning the same weight to each age class ($N_{\text{class}} = 2$ to 5).

Age classes		2016	2015	2014	2013	2012	2011	Mean values
7 rings (N=10)	L	3.63 $\pm$ 1.40	4.95 $\pm$ 1.40	4.72 $\pm$ 1.19	6.08 $\pm$ 1.77	6.32 $\pm$ 2.06	6.44 $\pm$ 2.15	5.36 $\pm$ 1.92
	H	3.74 $\pm$ 1.16	4.53 $\pm$ 1.23	3.62 $\pm$ 0.68	5.11 $\pm$ 1.40	4.34 $\pm$ 1.23	4.59 $\pm$ 1.15	4.32 $\pm$ 1.23 N=60
6 rings (N=17)	L	4.06 $\pm$ 1.61	5.55 $\pm$ 1.96	6.87 $\pm$ 1.86	6.91 $\pm$ 1.37	5.44 $\pm$ 1.73		5.77 $\pm$ 1.99
	H	4.17 $\pm$ 1.06	4.69 $\pm$ 1.13	4.93 $\pm$ 1.14	5.27 $\pm$ 1.21	4.39 $\pm$ 1.10		4.69 $\pm$ 1.17 N=85
5 rings (N=31)	L	4.72 $\pm$ 1.42	5.04 $\pm$ 1.60	6.80 $\pm$ 1.83	6.52 $\pm$ 1.72			5.77 $\pm$ 1.86
	H	4.14 $\pm$ 1.17	3.70 $\pm$ 1.18	4.92 $\pm$ 1.72	5.39 $\pm$ 1.73			4.52 $\pm$ 1.59 N=124
4 rings (N=36)	L	5.03 $\pm$ 1.73	5.81 $\pm$ 1.34	6.28 $\pm$ 1.47				5.71 $\pm$ 1.59
	H	4.15 $\pm$ 1.32	4.26 $\pm$ 1.13	4.68 $\pm$ 1.06				4.37 $\pm$ 1.19 N=108
3 rings (N=12)	L	5.66 $\pm$ 1.50	5.55 $\pm$ 1.53					5.61 $\pm$ 1.48
	H	4.40 $\pm$ 1.24	4.47 $\pm$ 0.66					4.44 $\pm$ 0.98 N=24
Overall mean (N=5)	L	4.62 $\pm$ 0.80	5.38 $\pm$ 0.37	6.17 $\pm$ 1.00	6.50 $\pm$ 0.42	5.88 $\pm$ 0.63		N=20
	H	4.12 $\pm$ 0.24 N=5	4.33 $\pm$ 0.39 N=5	4.54 $\pm$ 0.62 N=4	5.26 $\pm$ 0.14 N=3	4.36 $\pm$ 0.03 N=2	N=1	

weight increased throughout life, and particularly over the first 3 years. Shells with 3 to 7 rings were the most abundant ($N \geq 10$ for each class) and enabled the analysis of growth patterns at finer scales (Table 3). Growth was the slowest between years 2 and 3, while it became remarkably rapid during the completed years 3 to 5 (Figure 4). Due to the diverse number of individuals collected between age classes and their small variations in shell growth, the overall mean growth for each year from 2011 to 2016 was calculated using the mean annual values instead of individual measurements. Growth has been more rapid in 2013 compared to 2016 (ANOVA: $F_5 = 3.95$, $P = 0.02$, and Tukey *post hoc* test; $P < 0.05$). The mean length of the 95 dead individuals from the marina was 36.08 ± 11.60 mm [min=11.26 mm, max=58.00 mm]. The life expectancy of the adult population established in Ouistreham is therefore in the range of 4–5 years. Fitting the Von Bertalanffy growth curve to the length-at-age distribution shows no clear plateau, with a large maximum expected length ($L_{\text{inf}}=123.4$ mm) and a k of 0.062 yr^{-1} (Figure 5).

Reproduction

The gender and gametogenesis stage from the 110 histological slides suggest a slightly imbalanced sex-ratio in favour of males (60% of males vs 40% of females) but was not significantly different from a balanced sex-ratio (χ^2 test with Yates correction; $P > 0.05$). Four gametogenesis stages were observed on each sampling date (from stage 2, active gametogenesis to stage 5, post-spawning), globally reflecting a period of sexual maturity and spawning throughout spring (Figure 6). While no significant difference in the proportions of gametogenesis stages was detected between the two sampling periods (χ^2 test; $P = 0.086$), the temporal increase in the proportion

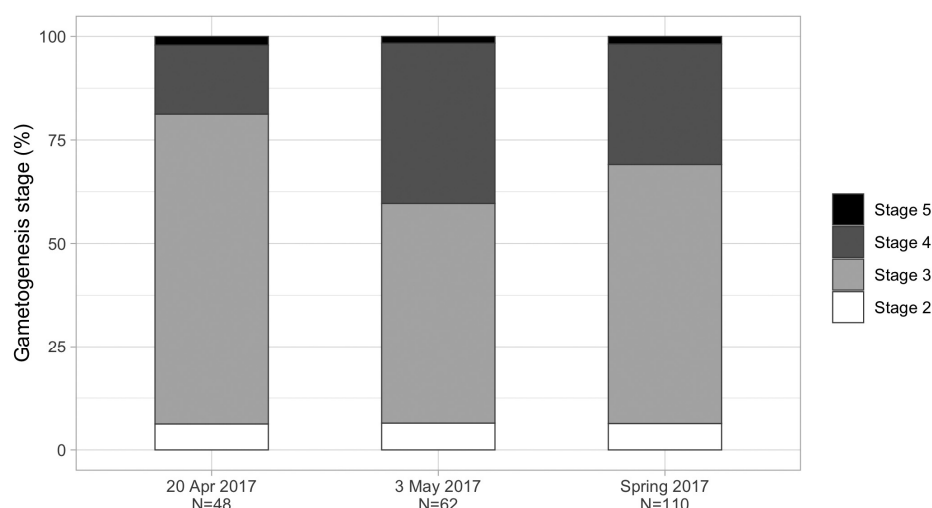


Figure 6. Proportion of gametogenesis stages in the *Rangia cuneata* population established in the Ouistreham marina. Stage 2: active gametogenesis, stage 3: sexual maturity and possible spawning, stage 4: active spawning, and stage 5: post-spawning.

of stage 4 (active spawning; from 16.67% to 38.71%) and the slightly lower SMI (1.71 vs 1.81) illustrates the progress in the reproductive cycle even over just these 13 days.

Discussion

Population densities and ecological relevance

We provide evidence that a population of *R. cuneata* is now consistently established in Normandy, France, following the species' colonisation of the brackish waters of various European countries, from Belgium to further north in the Baltic region, between 2005 and 2016. Here, the presence of large clams with developed taxonomic features left no doubt on the early species' identification, as opposed to the first specimens found in the Baltic Sea (Rudinskaya and Gusev 2012).

In northern Europe, the density of *R. cuneata* displays large spatial and seasonal fluctuations, ranging from 80 to 4,040 ind m⁻² throughout the year and between 119 and 94 ind m⁻² in April and May, respectively (Rudinskaya and Gusev 2012). These densities are comparable with our estimates in Normandy during these months (110.45 ± 86.08 ind.m⁻²) and confirm the healthy state of this newly settled population. With fluctuating mesohaline waters of high turbidity, subtidal but shallow zones (< 6 m depth) and soft bottom rich in organic matter (e.g. in Lasalle and de la Cruz 1985; Wakida-Kusunoki and MacKenzie 2004), the Ouistreham marina may provide optimal conditions for the species. The colonisation by *R. cuneata* may have also been facilitated by the steadier local climate compared to the Gulf of Mexico, where violent climatic events and variability, such as hurricanes and ENSO shifts, can induce drastic decline in the clam populations in its native range (Poirrier and Caputo 2015).

No information is available on the benthic fauna in the marina prior to the introduction of the Atlantic rangia, preventing any pre/post impact

assessment. Currently, a single species, *Mya arenaria* L., was found associated with Atlantic rangia and in low densities (6 individuals in total), indicating that *R. cuneata* already dominates the benthos' biomass in the Ouistreham marina, as it does in its native region (Wong et al. 2010). The establishment of Atlantic rangia will thus likely or may already have a substantial impact on the ecosystem functioning by altering the availability of trophic resources and accumulating contaminants like heavy metals (Lewis and Chancy 2008; Cerco and Noel 2010).

Insights on the species' life cycle

Little information is available on the reproductive cycle *R. cuneata* in the European countries where it has been recently introduced. Here, the sex-ratio of the population appeared balanced, although estimated on a limited number of individuals and only in spring. A balanced sex-ratio was also observed in Louisiana, USA (Fairbanks 1963) but not on the eastern coast of the US where females predominated (Cain 1975). The smallest clam we examined was a 17.11-mm female and was already in active gametogenesis (stage 2), in line with observations in its native habitat (Cain 1975). Our study took place during the reproduction period since the great majority of the individuals were fully ripe or spawning. The water temperature remained below 10 °C in March (Charles et al. 2018) and reached 14 °C by the end of April (13.97 °C; this study), which thus appears sufficient to trigger spawning. Seasonality in the reproductive cycle is hardly comparable between Normandy and the Gulf of Mexico, where the populations handle temperatures over 30 °C. Still, the species spawns mostly from late spring to fall (Fairbanks 1963; Wakida-Kusunoki and MacKenzie 2004; Drescher 2017), and the gametogenesis is initiated at temperatures as low as 15 °C (Cain 1975; Jovanovitch and Marion 1989). In the Vistula Lagoon of the Baltic Sea, the “reproduction starts by the end of May, when water temperature reaches 15 °C and continues until October” (Rudinskaya and Gusev 2012). Here, it is induced at temperatures below 14 °C. Given the wide thermal tolerance of *R. cuneata*, the reproduction in environments cooler than in its native range may thus simply traduce the species' natural capabilities, and not be the result of acclimation.

While temperature may initiate the gametogenesis, changes in salinity trigger the release of gametes (Fairbanks 1963; Cain 1975; LaSalle and de la Cruz 1985; Jovanovitch and Marion 1989) with an exception of a study conducted in a Mississippi (USA) estuary, where no clear impact of the salinity was detected (Drescher 2017). Here, the salinity varied between 5 and 10 PSU and we confirmed that gametogenesis and spawning are effective in Normandy under normal temperature and salinity conditions. Larger variations in salinity are likely to occur punctually with the marina's management procedures, which could trigger the release of gametes.

Whether the observed spawning was partial or complete and the population has another gamete-releasing period remain unknown, however, and can only be addressed by extending the species' monitoring throughout the year.

Pediveliger larvae become competent *ca.* 0.375 mm shell length and the growth is particularly fast after settlement: by the end of the first year, the shells are >10 mm (Fairbanks 1963; Rudinskaya and Gusev 2012). While the youngest age classes are generally the most abundant (e.g. in Lithuania; Solovjova et al. 2019), here, small individuals were seldom found: only three were smaller than 20 mm, while the modal size was within 35–40 mm. The soft substrate, mainly composed of large fragments (size > 2 mm) and clay-silt (size < 50 μ m) sediments at the sampling sites are favourable for the adults (LaSalle and de la Cruz 1985) but may be suboptimal for the settlement stage (Sundberg and Kennedy 1993). It is unknown whether the sediment type has become finer in recent years, but the current granulometry could be limiting the settlement. Other potential nursery may be revealed by mapping the nature of the sediment in this sector. It would also enable to determine whether the recent lack of yearlings is due to a reproductive failure or a lack of settlement.

In the species' native areas, the shell length can reach 70 mm (Wolfe and Petteway 1968), with an overall largest individual of 94 mm (LaSalle and de la Cruz 1985). While we caught the largest individuals reported in European water (56.76 mm for a live clam and 58.00 mm for a dead one), the growth seems limited compared to the Gulf of Mexico (Figures 2 and 4). Here, the Von Bertalanffy growth curve suggests a maximum length of 124 mm (Figure 5) but this unrealistic estimate, along with the absence of a clear plateau, may result from the truncated length-at-age distribution in the samples with too few very young and old specimens. The first introduced clams reported in the harbour of Antwerp measured from 4 to 40 mm (Verween et al. 2006) and were probably small due the recent introduction. Still, several studies in the Baltic Sea reported a maximum length of only 34 mm (Möller and Kotta 2017), 35.24 mm (Rudinskaya and Gusev 2012), 36.1 mm (Janas et al. 2014) and 37 mm (Solovjova et al. 2019); individuals in the Ouistreham marina are thus already > 30% larger than elsewhere in Europe.

The increments in the shell size of this new alien population is a proxy of age and growth rate in its native range (Drescher 2017). In Normandy, eight age classes were identified, with accurate information on growth for 3-year and older clams. Growth was the slowest between the second and third year but became stable after the 3rd year (mean yearly shell increments: 5.5. mm in length; Table 3). In Louisiana, annual growth rates range from 0 to 9.7 mm (Gooch 1971 and Bedinger 1974, *In* LaSalle and de la Cruz 1985), with yearly shell length increments of 5–9 mm for 2-year-old individuals and 4–5 mm for 3-year-old ones (Fairbanks 1963; Wolfe and Petteway

1968). While the parameters of the theoretical Von Bertalanffy growth curve indicate a slower growth than in the American populations for the first years (Figure 5), the shell length of the specimens we collected (1-y: 18.30 mm, 2-y: 23.91 mm, 3-y: 27.72 mm) still falls within the size ranges observed in the aforementioned studies and the yearly growth rate at later ages is even slightly larger. The growth of Atlantic rangia in these French waters does thus not appear to constrain the development of the population, and even larger individuals will likely emerge in the future. This newly established population therefore appears well developed and largely adapted to the local environmental conditions.

Possible introduction pathway and history

The most likely introduction pathway and spread of *R. cuneata* in Europe is through early life stages in ballast water (Verween et al. 2006), as for many other aquatic species (Katsanevakis et al. 2013). The proximity of the Caen harbour in the nearby waterway and the overall spread of the species in northern Europe also supports shipping, through ballast water and sediment transport by dredgers, as the main introduction vectors (Rudinskaya and Gusev 2012; Janas et al. 2014; Solovjova et al. 2019). The Caen harbour is the 10th largest in France with 3.2 million tons of goods transported every year, mostly by cargo ships to and from the North Sea and the Baltic Sea (Caen harbour authorities *pers. comm.*). Assuming an introduction as larvae in ballast water, a life expectancy of 4–5 years (Table 3) and a maximum lifespan of 8 completed years (Table 2), the colonisation of the area should have occurred at least 8 years ago, in 2009.

At finer scale, the dominance of empty and small shells in the waterway from Caen to the sea, where it was first noticed (Kerckhof et al. 2017), indicates that we have likely missed the core of a second population. Given the low mobility of the species, the distance between this area and other colonised brackish ecosystems, and that no dredging sediment from the marinas are deposited close to Ouistreham (Caen harbour authorities *pers. comm.*), a population may definitely be living in the canal and has yet to be found. It is noteworthy that two other notable alien species, the Australian tubeworm *Ficopomatus enigmaticus* (Fauvel, 1923) and the dark false mussel *Mytilopsis leucophaeata* (Conrad, 1831) were also first recorded in France in this same canal. These two species have now populated all the brackish waters in the area (Charles et al. 2018), and their shells and tubeworm fragments significantly contribute to the sediment of both the waterway and the marina (this study). We confirmed that at least one population of *R. cuneata* is now well established in Normandy; its spread in France should thus be assessed, in particular around harbours with regular maritime connections with Caen, and the Atlantic rangia placed under environmental monitoring as an introduced species. With climate change

facilitating invasions through changes in coastal hydrology, salinity regimes, water quality and species richness, such early surveys are key for understanding and anticipating current and future changes in the ecosystems.

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