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Assessment of size at first maturity for *Ruditapes philippinarum* from Arcachon Bay (French Atlantic coast): New insights for fishery management

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Abstract :

Regarding fisheries, management strategies aim at regulating access to the resource and at enforcing resource conservation in order to achieve sustainable exploitation. In this context, Minimum Conservation Reference Size is a technical measure based on scientific knowledge of the species characteristics (sexual maturity, individual growth and natural mortality). Reproductive capacity is generally apprehended by using the size at first maturity SL50, the size at which 50% of the individuals are mature. The SL50 was unknown for the Arcachon Bay Manila clam population (Ruditapes philippinarum). This study provides a first estimate based on the histological analysis of a large sample of individuals (1420 clams ranging from 10 to 41 mm) from four distinct bay inner sites and collected when the majority of the individuals are mature (from June to August). Our methodological choice was made to allow comparison with other European R. philippinarum deposits but also to be recognized by the EU; an interest of practices harmonization was besides revealed for bivalves. Following a logistic regression analysis, the SL50 was estimated to be 26.7 mm for the totality of the samples considered, with moderate spatial variability. If only sexed individuals were taken into account in the estimation, the SL50 was 24.5 mm for females and 21.6 mm for males. All sites and hypsometric levels combined, it is estimated that SL50 is reached at an average age of 1.6 years, 32 mm at 2.3 years and 35 mm at 2.9 years with high variability at the scale of site-hypsometric level pairs. Considering the SL50 and the spawning activity usually observed intra-basin, at least half of the clam population would have spawned at least once before their potential catch if the MCRS was reduced from 35 mm (current regulations) to 32 mm (regulations under study). This information constitutes essential knowledge to be included in Joint Recommendations when considering a MCRS revision in a European context that recognizes a more important place dedicated to regionalization.

Highlights

► SL_{50} was determined for *R. philippinarum* based on gonad histology and logistic regression analysis. ► SL_{50} is an essential parameter to assess the sustainability of a MCRS. ► Current MCRS (35 mm) is 29–34% higher that the estimated size at first maturity. ► Practices for histological analysis of bivalves reproduction would need to be harmonized.

Keywords : sustainable exploitation, regional regulation, MCRS, gonad histology, SL50, bivalves

1. Introduction

Fishery management aims at creating the conditions required for a sustainable exploitation of marine resources by ensuring the renewal of a stock whilst optimizing (or even maximizing) its production. In order to adjust the fishing pressure to the growth and reproduction potential of a resource, management strategies are developed to regulate access to the resource by, for example, implementing fishing permits and to enforce resource conservation by reducing fishing efforts, limiting catches and introducing technical measures. Amongst them, the Minimum Conservation Reference Size (MCRS) is established to ensure that individuals can mature and reproduce at least once before their potential capture (Small, 2021). In Europe, the MCRS is determined by the European Commission (provided by EU Regulation No. 1380/2013). For the Manila clam, *Ruditapes philippinarum* (A. Adams & Reeve, 1850), the MCRS refers to the anteroposterior length of the shell and was first set at 40 mm in 1998, for the waters of the North Atlantic, the Channel and the North Sea, except Skagerrak/Kattergat (EC Regulation No. 850/1998). In 2008, this reference size was modified to 35 mm (EC Regulation No. 40/2008 confirmed by EC Regulation No. 227/2013; the last one in force is EU No. 2019/1241). However, it should be noted that a specific MCRS was established for the particular case of the Basse Normandie in France (Orders of 15/07/2010 JORF n°0171 and 14/02/2013 JORF n°0038) for which the MCRS was modified to 40 mm. For the Mediterranean waters, it is set to 25 mm (EC Regulation No. 1967/2006 and EU No.

2019/1241) with for example a MCRS fixed at 30 mm for the littoral of the Etang de Berre (Prefectural order n°R93-2020-04-06-001).

Initially introduced for aquaculture purposes, *R. philippinarum* is now considered as a neonatural resource within the Arcachon Bay (French Atlantic coast). This wild species is economically important with a first sale value around one million euros. It represents 99% of the clam population and it is exploited by 60-70 licensed fishermen with annual catches around 300–450 tons per year (Caill-Milly et al., 2021; Sanchez et al., 2021). Since 2003, the management process applied to Manila clam is based on a collaborative approach involving professional structures, French administration and scientists with co-organized regular surveys to assess clam stock and research programs to better understand the population dynamics (Caill-Milly et al., 2021). Since 2014, the Manila clam stock is relatively stable, around 7000 - 8000 tons. In contrast, the exploitable part (individuals measuring more than 35 mm) is variable depending on years: 807 t in 2014, 490 t in 2018 and 507 t in 2021 (Sanchez et al., 2021).

For *R. philippinarum* population from the Arcachon Bay, the question of the adequacy of the MCRS in force (applied to all the clam deposits in Europe) has been raised for several years. For instance, this population differs from most other European populations for its particular growth pattern and its specific morphology, being less elongated and more globular than most European clam populations (Dang, 2009; Dang et al., 2010; Caill-Milly, 2012, Caill-Milly et al., 2014). On the request of the Maritime Fisheries and Aquaculture Branch (DGAMPA) of the French Ministry, a review on the biological characteristics of the species (including sexual maturity, individual growth, natural mortality) with a focus on the local observations was undertaken in 2020 (Caill-Milly et al., 2020). The goal was to assess the possible impacts of a MCRS reduction on the Arcachon Bay population. The review fuelled the preparation by the South West Waters Advisory Council (SWWAC) of a Joint Recommendation (JR) to the European Commission (EC) suggesting the possibility to reduce the MCRS of *R. philippinarum* from 35 mm to 32 mm for Arcachon Bay. In spring 2021, the EC advisory committee, the Scientific, Technical and Economic Committee for Fisheries (STECF), issued an unfavourable opinion on this revision (STECF, 2021). It stated that the size at the first maturity was unknown for *R. philippinarum* in the Arcachon Bay. Moreover, the committee argued that fishery management and monitoring measures were not detailed in the JR.

To respond the STECF shortcomings and to resubmit an application in 2022, this short note assesses the size at which 50% of the individuals are mature (SL_{50}) of *R. philippinarum* and its intra-basin spatial variability. In parallel, the CRPMEM (Regional committee for fishery and aquaculture) Nouvelle-Aquitaine - the regional professional organization - is coordinating a synthesis on the management of the intra-basin clam fishery since 2000.

2. Material and methods

For the Arcachon Bay, the reproductive cycle of *Ruditapes philippinarum* is similar to that reported from other sites in France (Laruelle et al., 1994). A detailed description of the reproductive patterns of *R. philippinarum* in the Arcachon Bay was produced by Dang (2009), based on observations made in the years 2006 and 2007: the gametogenesis starts in March; gonad maturation begins in April-May and spawning events occur between May and October. In 2006 and 2007, the majority of individuals were mature during three months, from May to July and from June to August according to the sites within the Bay (Dang, 2009; Dang et al., 2010).

To match the maturation period described above, clams were collected from four sites inside the Bay from June to August 2021. Since the objective was to estimate the SL_{50}, the sampling covered three month in order to take into account possible lag regarding maturation among sites. The sites were identical to those chosen by Dang (2009) and the sampling was carried out with 400 individuals at Andernos, 370 at Gujan, 450 at Lanton and 200 at Île aux Oiseaux. For further details, the map of study sites can be found in Dang et al. (2010). The clams were mostly collected by fishermen by hands at low tide by scouring the sediment on the first 10 cm; a part also came from the biannual survey performed to assess clam stock [sampling at high tide with a Hamon grab that collects a sediment core of 0.25 m^2 (0.5 m x 0.5 m) on a 0.2 m depth].

In the laboratory, the anteroposterior length (*L*) was measured with an electronic caliper (Digital IP67 Mitutoyo, accuracy 0.01 mm). Each clam was shelled and the visceral mass was separated from the gills and the siphons to keep only the central part of the flesh, containing the gonads. Biological samples were fixed in a 4% buffered formaldehyde for 24 hours at 4°C (Martoja and Martoja, 1967). Tissues were dehydrated in a graded series of ethanol, cleared and embedded in paraffin. The embedded samples were cut with a microtome (Leica RM 2125RTS) to obtain 5-µm sections which were stained with hematoxylin-eosin (Martoja and Martoja-Pierson, 1970).

Histological samples were analyzed under a light microscope (Nikon Eclipse E200) to determine individuals' sex and gonad developmental stages, according to Drummond et al. (2006) and Moura et al. (2018): stage 0 (Resting or Inactive), stage 1 (Early developing or Early active), stage 2 (Late developing or Late active), stage 3 (Ripe), stage 4 (Partially spent), stage 5 (Spent/Resorbing). The different stages are illustrated in Fig.1. The main criteria for identification included the presence/absence of follicles and the identification and proportion of gametes at different degrees of maturity in each of the observed follicles (e.g., spermatogonia, spermatocytes, spermatids, spermatozoa, in the case of males and primary oocytes, growing oocytes, mature oocytes and residual oocytes, in the case of females). When different degrees of maturity were observed between follicles of the same individual, the maturity stage assigned for that individual corresponded to the most observed stage between follicles (Moura et al. 2018).

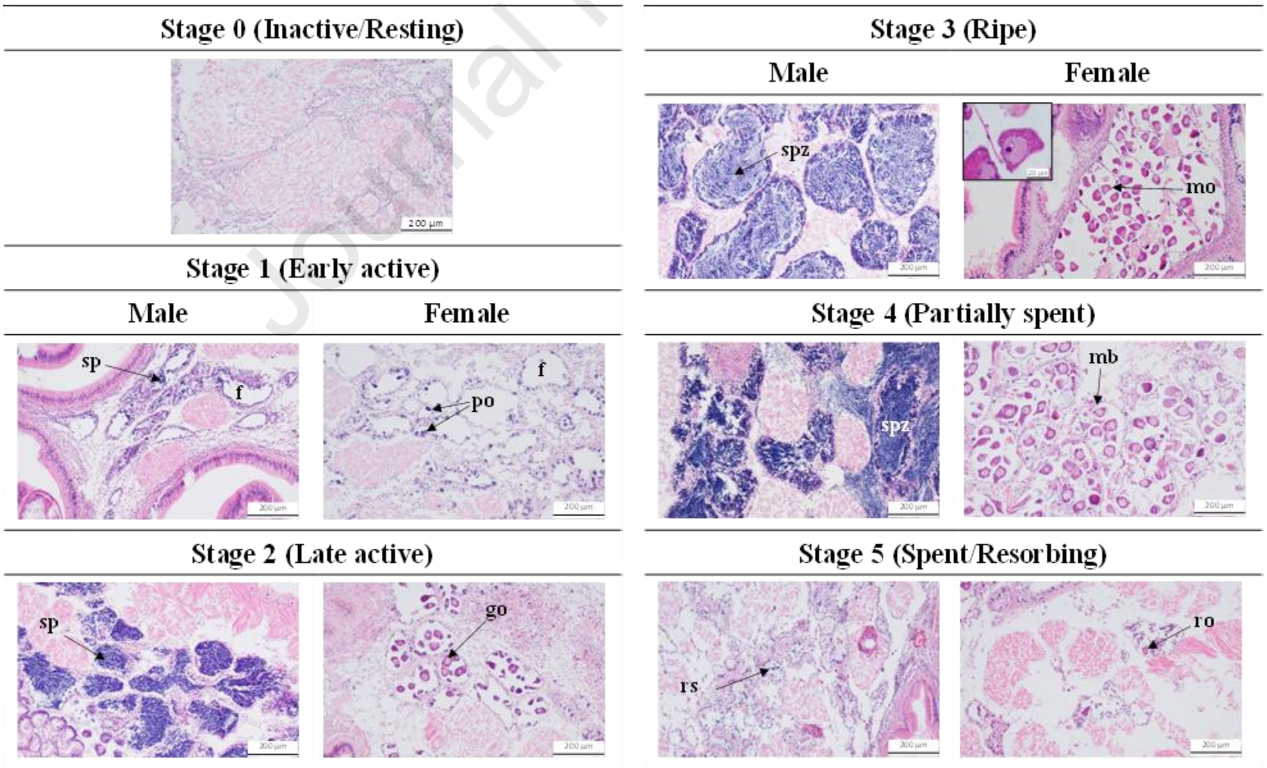

Figure 1: Micrographs of the six gonad developmental stages for *R. philippinarum*. f: follicles; sp: spermatocytes; po: primary oocytes; go: growing oocytes; spz: spermatozoa; mo: mature oocyte; mb: basal membrane breakdown; rs: residual spermatozoa; ro: residual oocytes.

Based on the same scale used (6 stages), the considered mature stages may differ according to the authors. For Maia et al. (2014; 2021), the mature stages are stages 3 to 5, whereas Moura et al. (2018) only consider stages 3 and 4. In other species, such as *Scrobicularia plana* (da Costa, 1778), the stages classified as mature are stages 3, 4 and 5 (Maia et al., 2016). In our case, in addition to stages 3 and 4, we considered the stage 5 as mature as Maia et al. (2014) since residual gametes are observed and it corresponds to the end of a cycle.

For the estimation of the SL_{50}, individuals were classified into two categories as immature (stages 0, 1 and 2) and mature (stages 3, 4 or 5). This metric was estimated from a logistic function relating the proportions of mature individuals and length, according to the equation (King, 1995):

$$P = \frac{1}{(1 + e^{-(a+b*L)})}$$

where *P* is the probability of an individual of being mature at a determinate *L* length with *L* the anteroposterior length of the individual (mm); *a* (intercept) and *b* (slope) are parameters estimated.

The SL_{50} was estimated by:

$$SL_{50} = \frac{-a}{b}$$

Calculations regarding the SL_{50} were done in R, using the sizeMat package (Torrejon-Magallanes, 2020) on the totality of the sample, by site and for each sex. The logistic regressions were performed as generalized linear models followed by a non-parametric bootstrapping procedure with 999 iterations.

The potential impact of a possible decrease of the MCRS on the spawning peaks was assessed using the Von Bertalanffy Growth Function (VBGF) equations related to the four sites within the Bay and determined by Dang et al. (2010) using enclosure field experiments. For each site, Dang et al. (2010) used four enclosures located at different hypsometric level (*i.e.* elevations above chart datum), from enclosure 1 (named Q1 for the lowest level) to enclosure 4 (named Q4 for the highest level).

3. Results

Histological sections were performed on 1420 gonad samples with clams ranging from 10 to 41 mm with 1238 for which the gonad developmental stages could be determined (mean value 27.6 mm; standard deviation 5.5 mm). Sections could not be interpreted for 182 individuals, mainly due to the absence or low quantity of mantle tissue in the sample section, in particular in the case of small individuals. Effectives by size class are well distributed for the whole area and by site, except for Île aux Oiseaux, where only few individuals smaller than 26 mm could be collected. All sites combined, the overall sex ratio was not significantly different from the theoretical 1:1 sex ratio (observed $\chi^2 = 2.68$, p-value = 0.1013). By site, there was also a balance between males and females with the exception of Andernos site where males had predominance over the females (χ^2 observed = 9.39; p = 0.0021; male:female = 1.5:1). For the sampling period (June to August 2021) and for the whole area, all gonad developmental stages were observed (Fig. 2a and b). Most individuals below the size class [22-24 mm[were inactive or resting. Cumulated proportions of stages 0, 1 and 2 seem to increase for individuals above 38 mm (Fig. 2a). Overall, the percentage of immature (stages 0, 1 and 2) and mature (stages 3, 4 and 5) individuals were 46% and 54% respectively. For males, stage 3 (Ripe) was the most observed stage (54%) while 49% of females were in stage 4 (Partially spent).

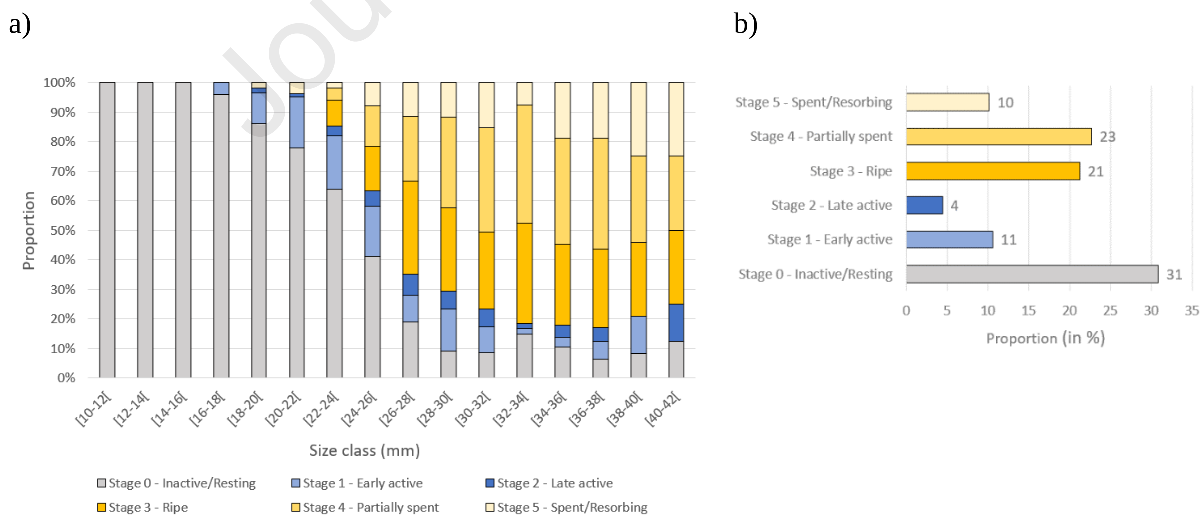

Figure 2: Proportion (%) of the different gonad developmental stages of *R. philippinarum* individuals observed in the Arcachon Bay from June to August 2021 a) according to size classes; b) all sizes considered.

Using the 1238 samples from the 4 inner-sites of the Arcachon Bay, the SL_{50} was estimated at 26.7 mm (confidence intervals 26.2 - 27.2 mm) (Fig. 3).

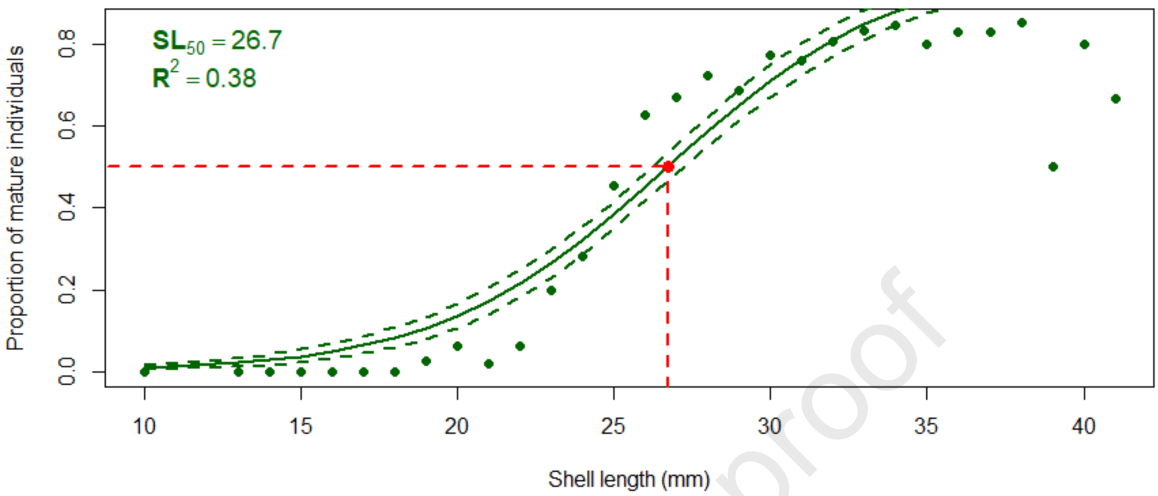

Figure 3: Proportion of mature individuals in relation to shell length for the estimation of the SL_{50} for *R. philippinarum* from Arcachon Bay [dashed red line: SL_{50}; solid line: fitted model; dashed lines: confidence intervals CI (95%); SL_{50} was assessed using the R package sizeMat (Torrejon-Magallanes, 2020).

Spatially (sites considered separately), the estimated SL_{50} varied moderately, with a minimum value of 25.0 mm for Île aux Oiseaux (CI: 21.1 - 26.7 mm) and a maximum value of 28 mm for Andernos (CI: 27.0 - 28.9 mm); the size ranges of individuals for the different sites are specified in Table 1. The quality of the estimate for Île aux Oiseaux is low with an R^2 of 0.13. Lanton and Gujan have similar values with 26.1 mm (CI: 25.3 - 26.8 mm) and 26.3 mm (CI: 25.2 - 27.4 mm). For females, SL_{50} is estimated at 24.5 mm (CI: 22.3 - 25.9 mm) while for males it is estimated at 21.6 mm (CI: 19.2 - 23.0 mm) (Table 1).

Table 1. Estimated size at first sexual maturity of *R. philippinarum* by site and by sex in the Arcachon Bay and estimated age at 26.7 mm (SL_{50}), 32 mm and 35 mm lengths by sites and by hypsometric levels (Q1 the lowest level and Q4 the highest level) according to Von Bertalanffy equations established by Dang et al. (2010); * Minimum ; ** Maximum.

	Length (min-max; mm) (median)	**SL_{50} (mm)**	**Confidence intervals (mm)**	**R^2**	**Sample number**	**Hypsometric levels**	**Estimated age for 26.7 mm (SL_{50}) (years)**	**Estimated age for 32 mm – suggested new MRCS (years)**	**Estimated age for 35 mm - current MCRS (years)**
Lanton	10 – 41 (27)	26.1	25.3 – 26.8	0.57	357	Q1	2.1	3.0	3.6
						Q2	1.6	2.2	2.7
						Q3	2.0	2.8	3.5
						Q4	1.9	2.7	3.3
Andernos	14 – 40 (27)	28.0	27.0 – 28.9	0.41	362	Q1	2.2	3.0	3.7
						Q2	0.9*	1.2*	1.6*
						Q3	1.2	1.7	2.3
						Q4	1.2	1.7	2.3
Gujan	15 – 41 (27)	26.3	25.2 – 27.4	0.26	338	Q1	1.2	1.7	2.2
						Q2	1.9	2.7	3.4
						Q3	2.4**	3.4**	4.3**
						Q4	1.9	2.8	3.6
Île aux Oiseaux	19 – 40 (30)	25.0	21.1 – 26.7	0.13	181	Q1	1.2	1.8	2.4
						Q2	1.3	1.9	2.5
						Q3	1.3	1.9	2.5
						Q4	1.8	2.7	3.7
4 Sites	10 – 41 (27)	26.7	26.2 - 27.2	0.38	1238	Average for all sites and levels combined	**1.6**	**2.3**	**2.9**
Males	17 – 40 (29)	21.6	19.2 – 23.0	0.21	453				
Females	19 – 41 (30)	24.5	22.3 – 25.9	0.19	405				

For the four sampling sites selected in the present study, growth modelling was already available (Dang et al., 2010). Therefore, individuals' age was estimated based on their anteroposterior length, using the Von Bertalanffy equations (Dang et al., 2010). On average, individuals of 26.7 mm (SL_{50}) were estimated to be 1.6 years whilst individuals of 35 mm (current MCRS) and 32 mm (suggested new MCRS) were 2.9 and 2.3 years old, respectively (Table 1). Note the high variability at the scale of site-hypsometric level pairs. If the MCRS is reduced to 32 mm, more than 50% of the clam population would still be able to spawn at least once before their potential catch.

4. Discussion

The SL_{50} was unknown for the *R. philippinarum* population of the Arcachon Bay. This study provides a first estimate based on the histological analysis of a large sample of individuals from four distinct bay inner sites and collected when the majority of the individuals are mature. Following a logistic regression analysis, the SL_{50} was thus estimated to be 26.7 mm for the totality of the samples considered, with moderate spatial variability. If only sexed individuals were taken into account in the estimation, the SL_{50} was 24.5 mm for females and 21.6 mm for males. Our results also show that almost all the clams under 20 mm in length were not mature. Holland & Chew (1974) reported similar result considering that the species was sexually mature from a shell length of about 20 mm. For large individuals (above 38 mm), the higher proportion of immature stages may suggest the start of a second cycle as already described for the Arcachon Bay by Dang et al. (2010). It is also mentioned for Italy [in Venice lagoon by Meneghetti et al. (2004) in Dang et al. (2010); in lagoons of the river Po delta by Sbrenna and Campioni (1994)] and for the North-East Pacific [Holland and Chew (1974) and Bourne (1982) in Dang et al. (2010)].

In the available literature on *R. philippinarum*, the size at first sexual maturity is estimated either from histograms representing the percentage of mature individuals as a function of size, or by using a logistic function relating the proportion of mature individuals and length. In the latter case, the SL_{50} is then available. Thus, for different deposits in Europe (England, Ireland, Portugal) or in the world (South

Korea, Sea of Japan), the size at first sexual maturity for *R. philippinarum* is generally observed between 15 and 25 mm (Holland & Chew, 1974; Ponurovsky & Yakovlev, 1992; Chung et al., 2001; Tumnoi, 2012; Maia et al., 2014; Maia et al., 2021). Based on information available for Europe, the estimated SL_{50} was only available for Portugal; with a SL_{50} of 20 mm in Ria de Aveiro and 29 mm in Tagus estuary (Moura et al., 2018; Maia et al., 2021). In the first case, they were estimated separately for each of the two sexes; in the second for both sexes combined. Methodological differences are also noted between Europe and works conducted in South Korea and Japan, the species' area of origin (Appendix). Despite these methodological differences, the results obtained for Arcachon are consistent with the SL_{50} estimates described in the literature.

Different values of SL_{50} among locations within the Bay were observed. These differences may be linked to environmental factors known to influence gametogenesis and spawning events: temperature, photoperiod, trophic resource availability and salinity (Devauchelle, 1990; Toba & Miyama, 1995; Le Pennec & Benninger, 2000; Delgado & Pérez Camacho, 2007) combined with those influencing growth: temperature, trophic resource, duration of immersion (Yamamoto et al., 1956; Maître-Allain, 1982; Goulletquer et al., 1987). They may act on an intra-site scale but also on a inter-site scale.

Our methodological choice was made to allow comparison with other European deposits of *R. philippinarum* but also to be recognized by the EU because the stages presently considered as mature are identical to those of *Chamelea gallina* (Linnaeus, 1758) in Adriatic Sea (Bargione et al., 2021). For this species, a consequent work (including SL_{50} assessment for each sex) has been carried out before a revision of its MCRS in 2020. For such local stocks with strong local and regional components regarding management, there is not the same dynamic of harmonization of practices as that instigated for several years by ICES thanks to the Workshop for MATurity staging CHairs (WKMATCH) and the Workshop for Advancing Sexual Maturity Staging in Fish (WKASMSF) for demersal and pelagic fishes, some cephalopods and crustaceans. This is lacking for bivalves.

Finally, the current MCRS at 35 mm is higher by 29-34% than the overall and all sexes combined SL_{50} (26.7 mm); a MCRS at 32 mm would be higher by 18-22% than this SL_{50}. Moreover, a MCRS at 32 mm for Arcachon Bay would remain above the upper bound of the confidence intervals calculated for the SL_{50} for all four sites and for each site in all cases. All sites and levels combined, it is estimated that SL_{50} is reached at an average age of 1.6 years, 32 mm at 2.3 years and 35 mm at 2.9 years with high variability at the scale of site-hypsometric level pairs. Considering the SL_{50} and the spawning activity usually observed intra-basin, at least half of the clam population would have spawned at least once before their potential catch if the MCRS was reduced to 32 mm. With such a modification, the exploitable stock (calculated for 2021) would change from 507 t to 2 148 t (representing respectively 6% and 25% of the total biomass).

At European level, fishery management is set by the Commission based on scientific knowledge and recommendations provided by the STECF. In some cases, species characteristics may differ geographically and demand for more specific management measures at national or regional scales. MCRS modifications have already been suggested and sometimes applied in previous cases where the species growth and reproductive capacity differed in specific areas (Gosling, 2003; Bargione et al., 2021; Small, 2021). Regionalization of fishery management is a marked evolution of the last Common Fisheries Policy (EU Regulation No. 1380/2013). Differences (regarding MCRS but not only) already existed between Mediterranean and Atlantic North East waters (EC Regulation No. 40/2008; EC Regulation No. 227/2013; EC Regulation No. 1967/2006): an additional regional division appeared in the 2019 regulations (EU No. 2019/1241) regarding the North Atlantic, the Channel and the North Sea with north-western and southern waters for examples. This evolution illustrates a recognition of regional specificities regarding fisheries (with a regionalization process introduced in 2013) and provides an additional regulatory framework for its effective implementation.

5. Conclusion

The data collected and analyzed in this study provide valuable information on the biology of the species. In the case of the Arcachon Bay, the *R. philippinarum* population differs from most other European populations by its particular growth pattern and morphology (Dang, 2009; Dang et al., 2010; Caill-Milly, 2012; Caill-Milly et al., 2014). By complementing knowledge already acquired previously, this work allows a better understanding of the functioning of the clam population in the Arcachon Bay and a comparison with other sites. The implementation of management measures within the Bay is based on biological traits and fishery knowledge (Caill-Milly et al., 2021). Regarding management implications, the estimated SL_{50} for *R. philippinarum* from Arcachon Bay was unknown so far and constitutes an essential parameter to be included in Joint Recommendations when considering a MCRS revision. As illustrated by the European regulation from 2019 (EU No. 2019/1241), the regionalization of fishery management can be a solution to reach effective regulations in specific populations. Indeed, the current MCRS set at European scale for *R. philippinarum* greatly differs from the low SL_{50} value newly calculated for the Arcachon Bay population and suggest the need for some more specific management measures for this population.

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Figure captions

Figure 1: Micrographs of the six gonad developmental stages for R. philippinarum. f: follicles; sp: spermatocytes; po: primary oocytes; go: growing oocytes; spz: spermatozoa; mo: mature oocyte; mb: basal membrane breakdown; rs: residual spermatozoa; ro: residual oocytes.

Figure 2: Proportion (%) of the different gonad developmental stages of *R. philippinarum* individuals observed in the Arcachon Bay from June to August 2021 a) according to size classes; b) all sizes considered.

Figure 3: Proportion of mature individuals in relation to shell length for the estimation of the SL_{50} for *R. philippinarum* from Arcachon Bay [dashed red line: SL_{50}; solid line: fitted model; dashed lines: confidence intervals CI (95%); SL_{50} was assessed using the R package sizeMat].

Tables

Table 1. Estimated size at first sexual maturity of *R. philippinarum* by site and by sex in the Arcachon Bay and estimated age of *Ruditapes philippinarum* at 26.7 mm (SL_{50}), 32 mm and 35 mm lengths for four sites (Andernos, Gujan, Lanton and Ile aux Oiseaux) in Arcachon Bay and four hypsometric levels (Q1 the lowest level and Q4 the highest level) according to Von Bertalanffy equations established by Dang et al. (2010); * Minimum ; ** Maximum.

- *SL_{50}* was determined for *R. philippinarum* based on gonad histology and logistic regression analysis
- *SL_{50}* is an essential parameter to assess the sustainability of a MCRS
- Current MCRS (35 mm) is 29-34% higher that the estimated size at first maturity
- Practices for histological analysis of bivalves reproduction would need to be harmonized

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: