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Effect of stocking density on growth and survival of juvenile Manila clams (*Ruditapes philippinarum*) farmed in suspended lanterns in a North Italian lagoon

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ABSTRACT

To evaluate the effect of stocking density on pre-fattening growth and survival, Manila clam seeds from three different batches (B1, B2, and B3) were farmed in plastic net lanterns at two sites (western and northern) in Sacca degli Scardovari (Italy). Different stocking densities were compared, i.e. EXTRA (50,000 clams m^{-2}), HIGH (30,000), and MEDIUM (20,000) for B1 and B2 clams; HIGH, MEDIUM, and LOW (10,000 clams m^{-2}) for B3 clams. At the end of pre-fattening at the western site, the shell length decreased with increasing stocking density of both B1 (14.2 mm vs. 13.6 mm vs. 12.9 mm at HIGH vs. MEDIUM vs. EXTRA density; $P < 0.001$) and B2 (14.9 mm vs. 13.6 mm vs. 12.5 mm at MEDIUM vs. HIGH vs. EXTRA density; $P < 0.001$) clams. At the northern site, the shell length decreased in the following manner: MEDIUM to HIGH to EXTRA density for B1 clams ($P < 0.001$) and MEDIUM to EXTRA to HIGH density for B2 clams ($P < 0.001$). The same trend was recorded for B3 clams at both western (16.1 mm vs. 14.3 mm vs. 12.7 mm at LOW vs. MEDIUM vs. HIGH density; $P < 0.001$) and northern (15.6 mm vs. 13.9 mm vs. 13.2 mm; $P < 0.001$) sites. The stocking density did not affect the survival rate at the northern site and of the B3 clams, whereas the survival rate significantly decreased from MEDIUM and HIGH to EXTRA density for B1 (84.8 % and 85.4 % vs. 52.8 %; $P < 0.05$) and B2 (92.5 % and 87.6 % vs. 67.0 %; $P < 0.01$) clams at the western site where pre-fattening ended one week later than at the northern site. In the present conditions, clams in suspended lanterns reached the minimum sowing size (shell length 11 mm, weight 0.3 g) in 10 weeks. However, an increase in stocking density decreased clam growth and drastically increased mortality when water conditions became less favourable.

1. Introduction

Shellfish farming is the second main aquaculture practice in the world, and it accounted for 20 % of the global aquaculture production in 2017 (FAO, 2020). Manila clam (*Ruditapes philippinarum* [Adams & Reeve, 1850]) cultivation accounts for 20 % of the global shell market value, with an annual harvest of 17 million tonnes. Italy is the second largest producer of Manila clams worldwide, and the leading producer in Europe, accounting for 95 % of the total European yield, with an annual clam production of 33,500 tonnes. Most Italian clam farming sites are located in the highly productive coastal lagoons of the Northern Adriatic Sea, such as Sacca degli Scardovari (Rovigo, Italy). These environments provide optimal conditions for clam growth and reproduction, which led to their spontaneous, rapid, and extensive spread in the 1990s (Pellizzato, 1990; Pellizzato and Da Ros, 2005). In 2019, Manila clam production in the Po Delta lagoons (including Sacca degli Scardovari) of the Veneto Region reached 10,643 tonnes (Veneto Agricoltura, 2020).

However, poor management of clam resources in the past few decades has resulted in uncontrolled harvesting and overfishing, which has led to a drastic decline in clam and seed availability in particular (Pellizzato and Da Ros, 2005; Ponti et al., 2017). Although clam seeds in

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North Italian lagoons have always been sourced primarily (95 %) from the wild (Turolla, 2008), the declining natural seed stocks have led to increasing use of hatchery-produced seeds (Parisi et al., 2012), as already witnessed for *R. philippinarum* in Spain (Cordero et al., 2017; Ramón et al., 2005; Royo et al., 2005a, 2005b) and Portugal (Cordero et al., 2017) and other bivalve species, including *Ruditapes decussatus* (Jara-Jara et al., 1997), *Venerupis corrugata* (Cigarría and Fernández, 2000), and *Mercenaria mercenaria* (Castagna, 2001; Whetstone et al., 2005).

Hatchery-produced Manila clam seeds (average size 2.0–3.5 mm) require a pre-fattening phase to attain the minimum sowing shell length (SL) of 10–18 mm (Claus et al., 1983; Dethier et al., 2019a), to minimize subsequent losses during the fattening phase owing to environmental factors (i.e. sea currents) and predation by other organisms, such as crabs (*Carcinus maenas* and *Carcinus aestuarii*) and fish (*Sparus aurata*) (Dethier et al., 2019a; Munroe and McKinley, 2007; Palazzi, 2015).

In Italy, the pre-fattening of Manila clams is generally managed by local farmers, without any technical knowledge or standardized methods (Boscolo Brusà et al., 2011; Palazzi, 2015). Limited scientific information is available; few preliminary studies comparing different clam culture techniques (i.e. floating systems, suspended *poches*, suspended net lanterns, seabed covered by plastic net) have identified suspended lanterns as an efficient strategy (Boscolo Brusà et al., 2003; Palazzi, 2015). However, these results were obtained on few experimental units (as lanterns and/or individual clams) in the Venice Lagoon for pre-fattening (Palazzi, 2015) and fattening (Boscolo Brusà et al., 2003). On the other hand, information about clam growth ad survival during pre-fattening in the area of the Po River Delta, highly specialized in clam production, is missing. These data would be necessary to standardize the farming technique, with special reference to optimal stocking time, stocking density, and pre-fattening duration, in view of the maximization of productive and economic results in terms of quantity of sowable seeds and ratio with respect to stocked hatchery-produced seed.

Thus, we evaluated the effects of different stocking densities on the growth and survival of Manila clams cultured in suspended net lanterns at two farming sites in Sacca degli Scardovari during the pre-fattening phase. Largely different stocking densities (from 10,000–50,000 clams m^{-2}) in three batches of Manila clams stocked in the lagoon at three different times (during March and April) were evaluated under field conditions and using a high number of experimental units (115 suspended lanterns). Our general aim was to standardize the clam culture techniques under the conditions prevalent at one of the most productive Italian lagoons.

2. Materials and methods

2.1. Study area

The study was conducted in Sacca degli Scardovari (44°51′N, 12°24′E; Porto Tolle, Rovigo, Italy), which is a large lagoon situated between two terminal branches (Gnocca and Tolle) of the Po River, in the southern part of its delta (Fig. 1). The lagoon occupies an area of 32 km^2, with an average depth of 1.5 m (Mistri et al., 2018). A wide mouth protected by partially submerged sand banks connects the bay to the sea (Munari et al., 2013). Tidal ranges are relatively small (yearly maximum range of ~1 m at spring tide), and the bottom of the bay is permanently covered with water (Munari et al., 2013).

Nutrient-rich agricultural run-offs flow into the northern part of the lagoon, whereas the southern part is influenced by exchanges with seawater and hosts several shellfish farms (mainly clams and mussels). Eutrophication causes seasonal blooms of opportunistic macroalgae in the most sheltered areas in late spring and summer (Natali and Bianchini, 2018).

2.2. Clam seeds and rearing system

A total of 241 kg of Manila clam seeds (SL 3.0 mm, weight 0.04 g) were purchased from a commercial French hatchery (Novostrea Bretagne, Sarzeaualle, France) and transported to Sacca degli Scardovari, in March-April 2018. Three different batches of seeds (B1, B2, and B3) were delivered at different times (Table 1). Once received, seeds from all batches were distributed inside the experimental lanterns (115 in total). Each lantern consisted of 10 storeys (surface area 0.2 m^2/storey, corresponding to 2.0 m^2 per lantern; total lantern length 150 cm), raschel net mesh of 2.0 mm × 3.0 mm, steel ring of 50 cm diameter, with a galvanized cross-bar and anticorrosion coating.

2.3. Experimental arrangement

Two different farming sites were identified (Fig. 1), i.e. the western site in the south-western part of the lagoon, characterized by the greatest hydrodynamism, and the northern site in the northern-most part of the basin, situated in a region with a weak hydraulic regime. A "pergola" for mussel farming was used at both sites, consisting of rows of beams spaced 2 m apart on which the lanterns were hung at a distance of 1 m. The depth was approximately 2.5 m at both sites.

Seeds were distributed on the lantern storeys at four different stocking densities, i.e. 50,000 clams m^{-2} (EXTRA), 30,000 clams m^{-2} (HIGH), 20,000 clams m^{-2} (MEDIUM), and 10,000 clams m^{-2} (LOW). The stocking density was EXTRA, HIGH, and MEDIUM in lanterns with batches B1 and B2, and HIGH, MEDIUM, and LOW in lanterns with batch B3. The numbers of lanterns at each site and their stocking density are summarized in Table 2. Clams at the northern site were collected a week earlier than those at the western site (18 June vs. 22 June), as required by the farmer. Thus, the pre-fattening duration varied depending on the batch and experimental site, i.e. pre-fattening lasted for 14–15 weeks for batch B1, 12–13 weeks for batch B2, and 10–11 weeks for batch B3.

2.4. Water sampling

The water quality was monitored approximately every two weeks during the experiment, depending on the weather conditions, with a total of 10 sampling events. Water temperature (°C), dissolved oxygen (ppm, saturation %), total dissolved solids (ppm), and salinity (‰) were assessed using a portable multiparameter probe (HI 9829, Hanna Instruments, Padova, Italy). Chlorophyll was evaluated using a fluorescence detector (HHLD Fluorescence-Chlorophyll, Turner Designs, USA). Data were recorded between 09:00 and 11:30 A.M. at three different locations and depths (0.50 m, 1.20 m, and 1.90 m) at each farming site and then registered as average values.

2.5. Collection of clam biometric data

Clam biometric traits (weight, SL, width, and thickness) were measured using a digital calliper (precision 0.001 mm). At the end of the pre-fattening period, clam weight, length, width, thickness, and survival were measured for all batches, using a sample of 60 clams per lantern, collected after emptying each lantern and mixing all specimens in a single plastic box. The shell strength of clams from the northern site (10 clams per lantern) was determined using a texture analyser (TA.XTplus, Stable Micro Systems, Godalming, UK), with a compression platen of 3 cm × 4 cm and test speed of 0.5 mm/sec. The collected data were elaborated using the TEXT EXPONENT 32 software (Stable Micro Systems).

The biometric traits and survival rate of B3 specimens were also assessed at 4 and 8 weeks of the pre-fattening period, using clams from the storeys at 0.50 m, 1.00 m, and 1.50 m depth (60 g of clams per storey).

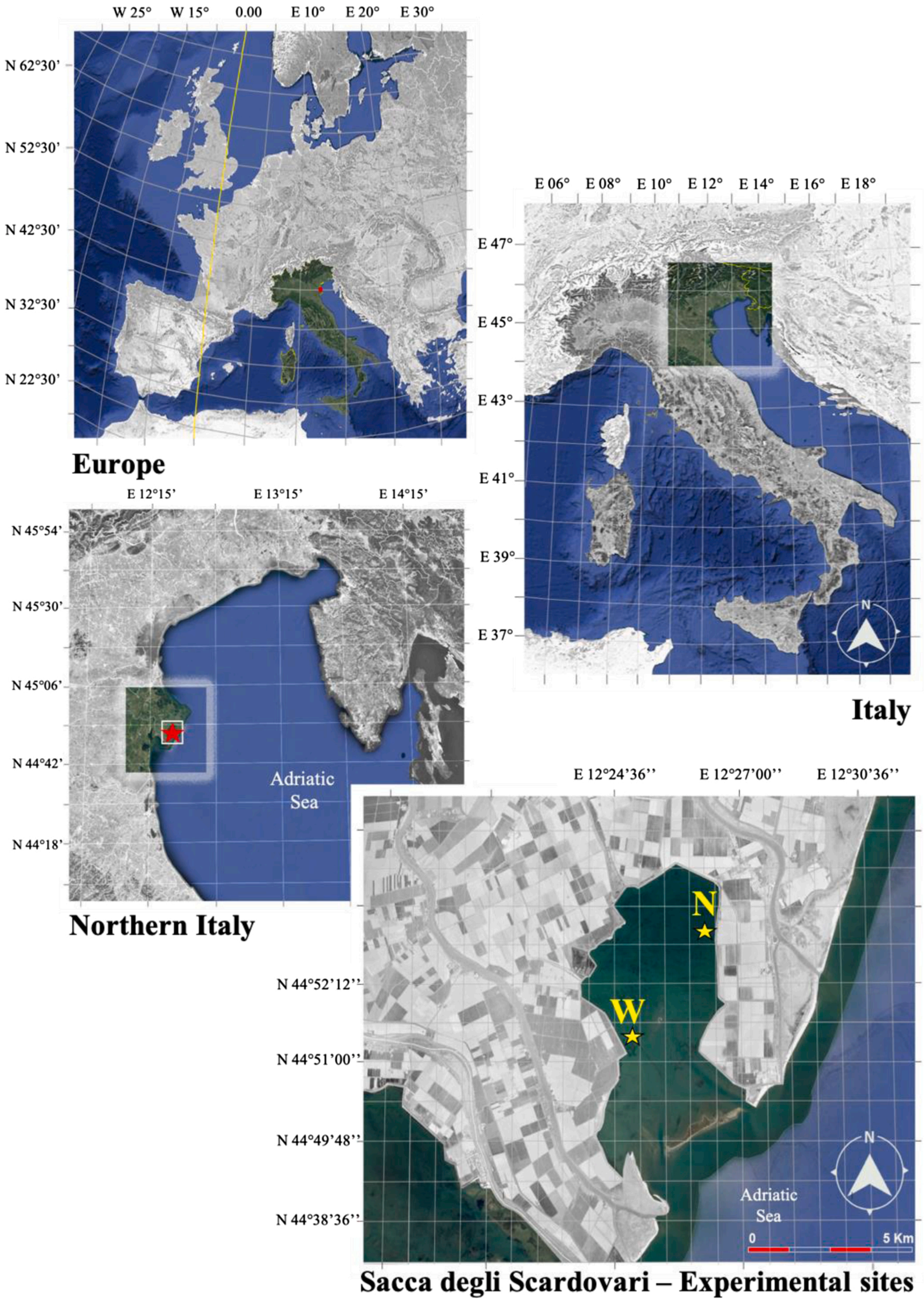

Fig. 1. Geographical location of Sacca degli Scardovari (red star) and of the two experimental sites (yellow stars; N: northern site; W: western site). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
The three batches and clam traits per batch at their arrival in Sacca degli Scardovari.

Batch	Arrival date	Batch weight (kg)	Total clams (n)	Clams/kg	Clam weight (g)
B1	15/03/2018	49.8	1,050,000	21,234	0.047
B2	29/03/2018	78.5	1,823,000	23,000	0.043
B3	13/04/2018	113.1	2,656,708	22,124	0.043

Table 2
Number of lanterns (10 storeys, 0.2 m^2/storey) with different stocking densities (EXTRA: 50,000 clams m^{-2}; HIGH: 30,000 clams m^{-2}; MEDIUM: 20,000 clams m^{-2}; LOW: 10,000 clams m^{-2}) at each experimental site (northern and western).

Batch	Number of lanterns per stocking density	
	Northern site	Western site
B1	2 EXTRA 4 HIGH 4 MEDIUM	2 EXTRA 4 HIGH 4 MEDIUM
B2	2 EXTRA 8 HIGH 4 MEDIUM	2 EXTRA 8 HIGH 8 MEDIUM
B3	7 HIGH 7 MEDIUM 7 LOW	14 HIGH 14 MEDIUM 14 LOW
Total lanterns	45	70

2.6. Sowable clams

The number of sowable clams per site, batch, and stocking density was calculated as the number of individuals suitable for sowing obtained per m^2 of the lantern after pre-fattening, using the following formula, modified from Palazzi (2015):

$$S_c = d_0 * (1 - m) * f_s$$

where

S_c= number of sowable clams produced in a given period (i.e. weeks of pre-fattening)

d_0 = initial stocking density (clams m^{-2})

m = measured mortality for each lantern (%)

f_s = fraction of clams that reached the minimum sowing length (% of total clams)

The estimated minimum sowing length (11.2 mm) was calculated based on the minimum sowing weight of 0.3 g, using weight-length regression equations calculated from B3 data (Claus et al., 1983; Dethier et al., 2019a; Palazzi, 2015). The estimated optimal sowing length (13.7 mm) was calculated based on an optimal sowing weight of 0.5 g (Claus et al., 1983; Dethier et al., 2019a; Palazzi, 2015). Then, the percentage of clams that reached the minimum and optimal lengths for sowing (f_s) was determined for each site, batch, and stocking density.

2.7. Statistical analyses

Regression analyses between clam biometric traits (weight, SL, thickness, and width) were performed using Microsoft Excel 2018 (Microsoft Corporation, 2018). Clam traits were subjected to analysis of variance, using the PROC MIXED procedure in SAS (SAS Institute Inc., 2013), with stocking density as the main factor of variability and lantern as a random effect. The data collected from the two farming sites were analysed separately due to the different pre-fattening periods, which ended a week earlier at the northern site. Clam survival was analysed using the PROC GLIMMIX procedure of SAS, with stocking density as the main effect. The Bonferroni *t*-test was used to compare the least squares means among the experimental groups. Differences were considered statistically significant at $P \leq 0.05$.

3. Results

3.1. Water quality

The water temperature varied according to the season, with minimum and maximum temperatures of 18.7 °C in late April and 27.2 °C in June, respectively, at both sites (Fig. 2). The dissolved oxygen content ranged from 3.6 ppm to 8.2 ppm, with higher values in April than in May and June, and it exhibited a sharp decrease when the water temperature increased in June. A similar trend was observed for oxygen saturation (%). The salinity varied during the experimental period, depending on the weather conditions and freshwater inflow from the terminal branches of the Po River, where the bay is located (ARPAV, 2019). The average salinity was 23‰, with a peak recorded during the first week of May (28‰, average of the two sites), followed by low values in late May and early June, consistent with the patterns observed in the lagoon (ARPAV, 2019). The amount of total dissolved solids ranged from a maximum of 22.9 ppm to a minimum of 16.0 ppm.

3.2. Correlations between clam biometric traits

Regression analyses (second-degree polynomials) between the biometric traits (SL, width, and thickness) and the weight of B3 clams at the two sites at 4, 8, and 10–11 weeks of pre-fattening yielded high determination coefficients (R^2>0.93) (Table 3). This was also observed for regressions between SL or shell width and thickness.

3.3. Clam development and survival during the pre-fattening period

Both B1 and B2 clams presented an average weight of 0.043 g at the beginning of the trial.

At the northern site, the SL decreased with increasing stocking density for both B1 clams after 14 weeks of pre-fattening (14.0 mm vs. 13.4 mm vs. 12.0 mm at MEDIUM vs. HIGH vs. EXTRA density, respectively; $P < 0.001$) and B2 clams after 12 weeks of pre-fattening (14.6 mm vs. 12.7 mm vs. 13.1 mm at MEDIUM vs. HIGH vs. EXTRA density; $P < 0.001$). In contrast, the survival rate was not affected by the stocking density (on average 96.2 % and 96.1 % for B1 and B2, respectively). The shell strength of B1 clams remained unaltered (32.9 N, on average), whereas it decreased when the stocking density of batch B2 increased from MEDIUM to HIGH (35.9 N vs. 28.1 N, respectively; $P < 0.01$), with intermediate values recorded in EXTRA density lanterns (32.3 N) (Table 4).

At the western site, clams were collected one week later than at the northern site which implied 15 and 13 weeks of pre-fattening for B1 and B2 clams respectively. The SL of B1 clams was the highest in HIGH density lanterns, followed by the MEDIUM and EXTRA density lanterns (14.2 mm vs. 13.6 mm vs. 12.9 mm, respectively; $P < 0.001$) (Table 4). The SL of B2 clams was the highest in MEDIUM density lanterns, followed by HIGH and EXTRA density lanterns (14.9 mm vs. 13.6 mm vs. 12.5 mm, respectively; $P < 0.001$). Furthermore, the survival rate decreased when the stocking density increased from MEDIUM and HIGH to EXTRA, for both B1 (84.8 % and 85.4 % vs. 52.8 %, respectively; $P < 0.05$) and B2 (92.5 % and 87.6 % vs. 67.0 %, respectively; $P < 0.01$) clams (Table 5).

The average weight of B3 clams was recorded as 0.045 g at the beginning of the pre-fattening period. Clam biometric variables (weight, thickness, width, and length) then decreased with increasing stocking density at both sites, for both 4 and 8-week samples (Tables 6 and 7). This trend was confirmed at the end of the pre-fattening period at the northern (SL: 15.6 mm vs. 13.9 mm vs. 13.2 mm, respectively; $P < 0.001$) and western (SL: 16.1 mm vs. 14.3 mm vs. 12.7 mm at LOW vs. MEDIUM vs. HIGH density, respectively; $P < 0.001$) sites. The shell

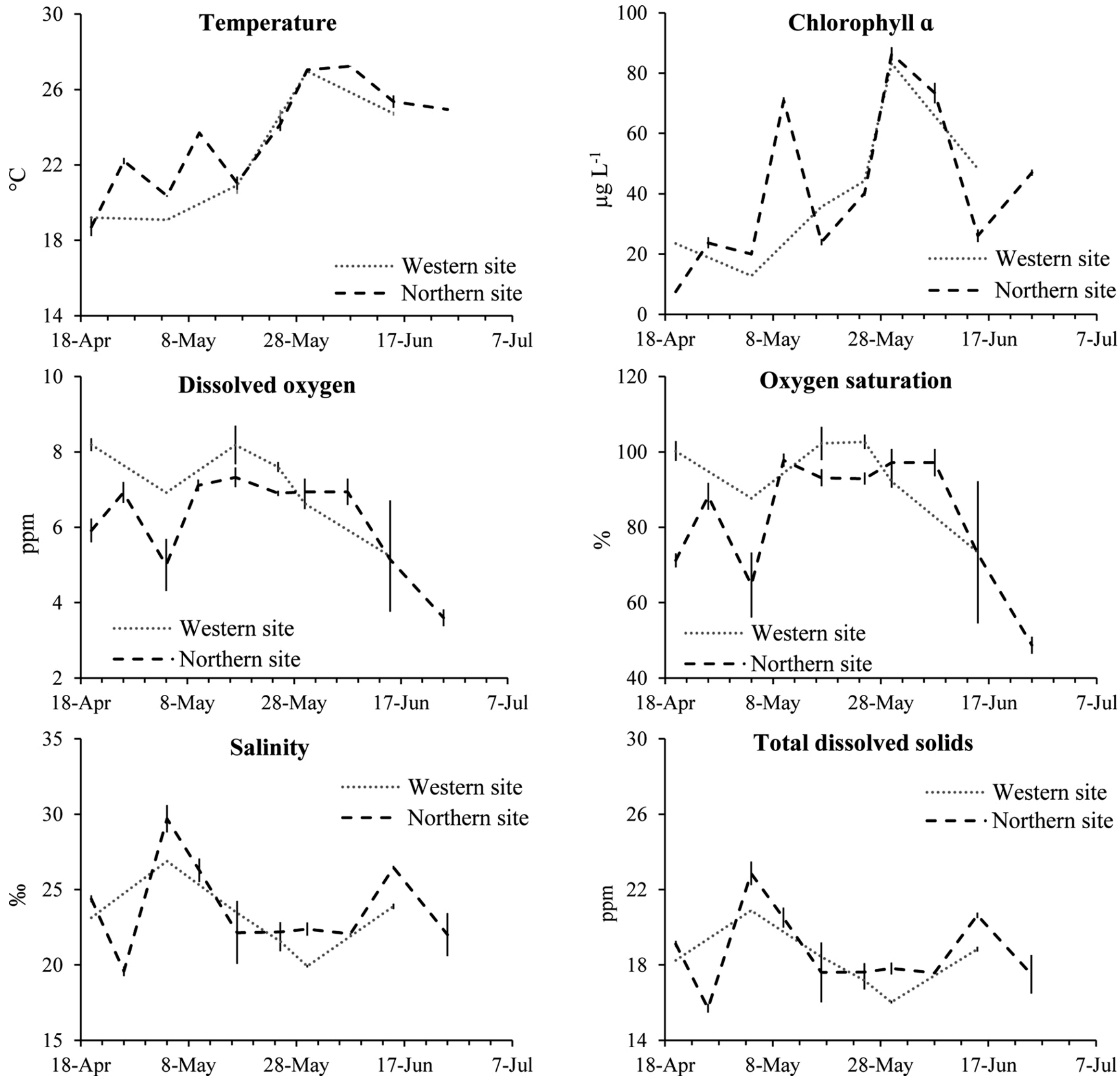

Fig. 2. Physical and chemical characteristics of water recorded at the western and northern sites in which clams were farmed in Sacca degli Scardovari during the study from mid-April to late-June (measurements taken between 9:00 and 11:30 A.M.).

strength also decreased with increasing stocking density (41.2 N vs. 35.7 N vs. 29.7 N, respectively; P < 0.01) (Table 6). No mortalities were recorded at 4 and 8 weeks at any of the sites; however, at the end of pre-fattening, the survival rates averaged 98.1 % and 94.7 % at the northern and western sites, respectively, without any differences among the stocking densities (Tables 6 and 7).

3.4. Sowable clams

The percentages of sowable clams (% total clams) were calculated for the minimum (SL: 11.2 mm) and optimal (SL: 13.7 mm) sowing size for each site, batch, and stocking density.

At the northern site, the percentage of sowable clams with the minimum size increased with decreasing stocking density from EXTRA to HIGH to MEDIUM for B1 (64.2 % vs. 67.2 % vs. 86.6 %, respectively), B2 (49.6 % vs. 73.4 % vs. 79.7 %, respectively), and from HIGH to MEDIUM to LOW for B3 (74.4 % vs. 86.9 % vs. 94.5 %, respectively) clams (Table 8). A similar pattern was observed at the western site, where the percentage of sowable clams increased with decreasing stocking density of B1 (40.1 % vs. 74.0 % vs. 78.1 % of total clams, respectively) and B2 (53.6 % vs. 75.1 % vs. 89.0 %, respectively) and of B3 (68.6 % vs. 91.1 % vs. 99.0 %, respectively) clams (Table 8).

4. Discussion

A supply of juvenile Manila clams from industrial hatcheries may support the Italian clam sector, assuring a constant supply of seed, in terms of quantity, hygiene and sanitary quality, and size uniformity (Boscolo Brusà et al., 2011; Palazzi, 2015). However, hatcheries usually provide small-sized seeds (<3.5 mm) (Claus et al., 1983; Marshall and Dunham, 2013; Zhang and Yan, 2006), which require a pre-fattening phase to attain a sowing SL of 8–15 mm (Dethier et al., 2019a; Palazzi, 2015), to improve survival and seed growth during the subsequent grow-out phase (Cigarría and Fernández, 1998, 2000; Epelbaum et al., 2011).

During the fattening phase, clams are usually farmed on the seabed, as they are a burrowing species inhabiting sandy or muddy substrates (Boscolo et al., 2003). Nevertheless, suspended cultures efficiently utilize resources through the use of a three-dimensional space in the water column, and allow for efficient harvesting and reduced ecological footprint (Dunham and Marshall, 2012; Marshall and Dunham, 2013). Furthermore, suspended systems have been shown to improve clam body conditions (Boscolo et al., 2003; Hasegawa et al., 2015; Marshall and Dunham, 2013), increase clam survival, and accelerate growth (Boscolo et al., 2003; Hasegawa et al., 2015; Marshall and Dunham,

Table 3
Regression equations and coefficients of determination (between parentheses) related to relationships between biometric measurements (clam weight; shell length, thickness and width) in batch B3 at the northern and western sites for clams sampled at 4, 8, and 10 weeks of pre-fattening.

Northern site	Weight	Length	Thickness	Width
Weight	–	$y = 0.0006x^{2.5673}$ (0.95)	$y = 0.0054x^{2.6104}$ (0.93)	$y = 0.0006x^{2.9346}$ (0.94)
Length		–	$y = 0.4841x^{0.914}$ (0.91)	$y = 1.0334x^{0.8493}$ (0.95)
Thickness			–	$y = 0.4945x^{1.07}$ (0.91)
Width				–
Western site	**Weight**	**Length**	**Thickness**	**Width**
Weight	–	$y = 0.0005x^{2.6495}$ (0.97)	$y = 0.0058x^{2.5665}$ (0.96)	$y = 0.0008x^{2.821}$ (0.96)
Length		–	$y = 0.4257x^{0.9904}$ (0.93)	$y = 0.890\ x^{0.9123}$ (0.95)
Thickness			–	$y = 0.5103x^{1.0597}$ (0.93)
Width				–

Table 4
Effect of stocking density (MEDIUM: 20,000 clams m^{-2}; HIGH: 30,000 clams m^{-2}; EXTRA: 50,000 clams m$^{-2)}$ on the biometrics, survival, and shell strength of clams farmed at the northern site in suspended lanterns (10 storeys, 0.2 m^2/storey). Data correspond to batches B1 and B2 and were collected at the end of the pre-fattening period (14 weeks for B1 and 12 weeks for B2). Values are expressed as LS means ± standard error.

	Stocking density			P-value
	MEDIUM	HIGH	EXTRA	
Batch B1				
Clams, n	400	319	159	
Weight, g	$0.561^{b} \pm 0.016$	$0.524^{b} \pm 0.016$	$0.382^{a} \pm 0.020$	<0.001
Thickness, mm	$5.82^{c} \pm 0.06$	$5.59^{b} \pm 0.07$	$4.95^{a} \pm 0.09$	<0.001
Width, mm	$9.91^{b} \pm 0.09$	$9.61^{b} \pm 0.10$	$8.56^{a} \pm 0.15$	<0.001
Length, mm	$14.0^{c} \pm 0.15$	$13.4^{b} \pm 0.16$	$12.0^{a} \pm 0.24$	<0.001
Survival, %	96.6 ± 0.75	98.2 ± 0.68	93.8 ± 1.59	0.073
Shell strength, N	33.4 ± 1.73	36.4 ± 1.51	29.0 ± 2.57	0.056
Batch B2				
Clams, n	320	520	160	
Weight, g	$0.621^{b} \pm 0.017$	$0.450^{a} \pm 0.011$	$0.497^{a} \pm 0.024$	<0.001
Thickness, mm	$5.95^{c} \pm 0.07$	$5.29^{a} \pm 0.05$	$5.57^{b} \pm 0.10$	<0.001
Width, mm	$10.1^{b} \pm 0.09$	$9.14^{a} \pm 0.07$	$9.48^{a} \pm 0.14$	<0.001
Length, mm	$14.6^{b} \pm 0.15$	$12.7^{a} \pm 0.12$	$13.1^{a} \pm 0.24$	<0.001
Survival, %	97.4 ± 1.50	95.4 ± 1.58	95.4 ± 3.64	0.636
Shell strength, N	$35.9^{b} \pm 1.49$	$28.1^{a} \pm 1.48$	$32.3^{ab} \pm 2.29$	<0.01

2013; Ramón et al., 2005).

Different types of suspended systems have been described in literature, including trays or cages suspended from rafts or longlines (Marshall and Dunham, 2013), nylon bags (Chessa et al., 2005), Teflon tubes (Dethier et al., 2019a), *poches*, and floating up-welling systems (FLUPSY) (Boscolo Brusà et al., 2011). In North Italian lagoons, suspended lanterns, such as those used in the present study, or FLUPSY are the recommended pre-fattening systems for small seeds (initial SL 2–3 mm), whereas nets, *poches* (on the seabed or in suspension), and boxes are recommended for larger seeds (initial SL 8–10 mm) (Boscolo Brusà et al., 2011).

Table 5
Effect of stocking density (MEDIUM: 20,000 clams m^{-2}; HIGH: 30,000 clams m^{-2}; EXTRA: 50,000 clams m^{-2}) on the biometrics and survival of clams farmed at the western site in suspended lanterns (10 storeys, 0.2 m^2/storey). Data correspond to batches B1 and B2 and were collected at the end of the pre-fattening period (15 weeks for B1 and 13 weeks for B2). Values are expressed as LS means ± standard error.

	Stocking density			P-value
	MEDIUM	HIGH	EXTRA	
Batch B1				
Clams, n	240	240	120	
Weight, g	$0.561^{b} \pm 0.017$	$0.624^{c} \pm 0.019$	$0.476^{a} \pm 0.022$	<0.001
Thickness, mm	$5.69^{a} \pm 0.07$	$6.10^{b} \pm 0.08$	$5.90^{ab} \pm 0.12$	<0.001
Width, mm	$9.69^{b} \pm 0.09$	$10.1^{c} \pm 0.11$	$9.25^{a} \pm 0.14$	<0.001
Length, mm	$13.6^{b} \pm 0.15$	$14.2^{c} \pm 0.17$	$12.9^{a} \pm 0.23$	<0.001
Survival, %	$84.8^{b} \pm 4.94$	$85.4^{b} \pm 4.83$	$52.8^{a} \pm 10.2$	<0.05
Batch B2				
Clams, n	480	480	120	
Weight, g	$0.702^{c} \pm 0.014$	$0.546^{b} \pm 0.012$	$0.424^{a} \pm 0.017$	<0.001
Thickness, mm	$6.28^{c} \pm 0.05$	$5.75^{b} \pm 0.05$	$5.31^{a} \pm 0.08$	<0.001
Width, mm	$10.4^{c} \pm 0.08$	$9.68^{b} \pm 0.07$	$9.03^{a} \pm 0.12$	<0.001
Length, mm	$14.9^{c} \pm 0.12$	$13.6^{b} \pm 0.11$	$12.5^{a} \pm 0.19$	<0.001
Survival, %	$92.5^{b} \pm 9.25$	$87.6^{b} \pm 2.61$	$67.0^{a} \pm 7.69$	<0.01

Table 6
Effect of stocking density (LOW: 10,000 clams m^{-2}; MEDIUM: 20,000 clams m^{-2}; HIGH: 30,000 clams m^{-2}) on the biometrics, survival, and shell strength of clams farmed at the northern site in suspended lanterns (10 storeys, 0.2 m^2/storey). Data correspond to batch B3 and were collected at different intervals during the pre-fattening period (at 4, 8, and 10 weeks of pre-fattening). Values are expressed as LS means ± standard error.

	Stocking density			P-value
	LOW	MEDIUM	HIGH	
4 weeks				
Clams, n	630	630	630	
Weight, g	$0.239^{c} \pm 0.002$	$0.211^{b} \pm 0.002$	$0.187^{a} \pm 0.002$	<0.001
Thickness, mm	$4.37^{c} \pm 0.02$	$4.17^{b} \pm 0.02$	$3.93^{a} \pm 0.02$	<0.001
Width, mm	$7.60^{c} \pm 0.03$	$7.35^{b} \pm 0.03$	$6.98^{a} \pm 0.03$	<0.001
Length, mm	$10.6^{c} \pm 0.04$	$10.1^{b} \pm 0.04$	$9.48^{a} \pm 0.04$	<0.001
8 weeks				
Clams, n	420	570	627	
Weight, g	$0.690^{c} \pm 0.010$	$0.482^{b} \pm 0.008$	$0.390^{a} \pm 0.008$	<0.001
Thickness, mm	$6.24^{c} \pm 0.04$	$5.36^{b} \pm 0.04$	$5.00^{a} \pm 0.04$	<0.001
Width, mm	$10.6^{c} \pm 0.06$	$9.34^{b} \pm 0.05$	$8.74^{a} \pm 0.06$	<0.001
Length, mm	$15.6^{c} \pm 0.09$	$13.4^{b} \pm 0.09$	$12.4^{a} \pm 0.10$	<0.001
Harvest (10 weeks)				
Clams, n	395	640	640	
Weight, g	$0.747^{c} \pm 0.015$	$0.548^{b} \pm 0.009$	$0.474^{a} \pm 0.009$	<0.001
Thickness, mm	$6.35^{c} \pm 0.05$	$5.61^{b} \pm 0.04$	$5.42^{a} \pm 0.04$	<0.001
Width, mm	$10.8^{c} \pm 0.06$	$9.66^{b} \pm 0.06$	$9.18^{a} \pm 0.06$	<0.001
Length, mm	$15.6^{c} \pm 0.12$	$13.9^{b} \pm 0.10$	$13.2^{a} \pm 0.10$	<0.001
Survival, %	97.7 ± 0.52	98.4 ± 0.47	98.3 ± 0.40	0.489
Shell strength, N	$41.2^{c} \pm 1.38$	$35.7^{b} \pm 1.47$	$29.7^{a} \pm 1.07$	<0.001

The choice of the pre-fattening system depends on the biological characteristics of the species, with the final goal of maximizing the yield per unit space, time, and capital investment (Epelbaum et al., 2011). Stocking density is a key factor affecting the survival and growth of clam seeds during development (Zhang and Yan, 2006). In the present study, the clam weight and length decreased with increasing stocking density after 4 and 8 weeks of pre-fattening in batch B3 and at the end of the pre-fattening period in all batches when the lowest densities were compared to the highest densities. Nevertheless, in B1 and B2 batches in

Table 7
Effect of stocking density (LOW: 10,000 clams m^{-2}; MEDIUM: 20,000 clams m^{-2}; HIGH: 30,000 clams m^{-2}) on the biometrics and survival of clams farmed at the western site in suspended lanterns (10 storeys, 0.2 m^2/storey). Data correspond to batch B3 and were collected at different intervals during the pre-fattening period (at 4, 8, and 11 weeks of pre-fattening). Values are expressed as LS means ± standard error.

	Stocking density			P-value
	LOW	MEDIUM	HIGH	
4 weeks				
Clams, n	420	420	420	
Weight, g	$0.215^c \pm 0.003$	$0.173^b \pm 0.003$	$0.140^a \pm 0.002$	<0.001
Thickness, mm	$4.13^c \pm 0.02$	$3.76^b \pm 0.02$	$3.52^a \pm 0.02$	<0.001
Width, mm	$7.17^c \pm 0.04$	$6.61^b \pm 0.04$	$6.14^a \pm 0.04$	<0.001
Length, mm	$9.90^c \pm 0.05$	$9.11^b \pm 0.06$	$8.39^a \pm 0.06$	<0.001
8 weeks				
Clams, n	420	420	420	
Weight, g	$0.579^c \pm 0.011$	$0.402^b \pm 0.008$	$0.302^a \pm 0.007$	<0.001
Thickness, mm	$5.79^c \pm 0.05$	$5.06^b \pm 0.04$	$4.52^a \pm 0.04$	<0.001
Width, mm	$9.89^c \pm 0.07$	$8.73^b \pm 0.06$	$7.87^a \pm 0.07$	<0.001
Length, mm	$14.2^c \pm 0.11$	$12.4^b \pm 0.10$	$11.0^a \pm 0.11$	<0.001
Harvest (11 weeks)				
Clams, n	360	420	420	
Weight, g	$0.874^c \pm 0.017$	$0.622^b \pm 0.012$	$0.448^a \pm 0.010$	<0.001
Thickness, mm	$6.89^c \pm 0.06$	$6.10^b \pm 0.05$	$5.32^a \pm 0.05$	<0.001
Width, mm	$11.5^c \pm 0.07$	$10.2^b \pm 0.08$	$9.23^a \pm 0.07$	<0.001
Length, mm	$16.1^c \pm 0.11$	$14.3^b \pm 0.10$	$12.7^a \pm 0.10$	<0.001
Survival, %	96.7 ± 9.67	95.5 ± 9.55	92.0 ± 9.20	0.179

Table 8
Sowable clams (% of total clams) with the minimum (11.2 mm, weight 0.3 g) (*in italics*) and optimal (13.7 mm, weight 0.5 g) (**in bold**) sowing sizes at the end of the pre-fattening period in suspended lanterns (10 storeys, 0.2 m^2/storey). Data refer to clams of the three batches (B1, B2, and B3) farmed at the northern and western sites at different stocking densities.

		Northern site		
Batch		B1	B2	B3
Arrival		15/03/2018	29/03/2018	13/04/2018
Harvest		18/06/2018	18/06/2018	18/06/2018
EXTRA	*Minimum size, (%)*	64.2 %	49.6 %	–
(50,000 clams m^{-2})	**Optimal size, (%)**	41.4 %	30.4 %	
HIGH	*Minimum size, (%)*	67.2 %	73.4 %	74.4 %
(30,000 clams m^{-2})	**Optimal size, (%)**	32.6 %	41.7 %	36.7 %
MEDIUM	*Minimum size, (%)*	86.6 %	79.7 %	89.6 %
(20,000 clams m^{-2})	**Optimal size, (%)**	55.4 %	47.2 %	52.3 %
LOW	*Minimum size, (%)*	–	–	94.5 %
(10,000 clams m^{-2})	**Optimal size, (%)**			78.1 %
		Western site		
Batch		B1	B2	B3
Arrival		15/03/2018	29/03/2018	13/04/2018
Harvest		25/06/2018	25/06/2018	25/06/2018
EXTRA	*Minimum size, (%)*	40.1 %	53.6 %	–
(50,000 clams m^{-2})	**Optimal size, (%)**	20.3 %	16.6 %	
HIGH	*Minimum size, (%)*	74.0 %	75.1 %	68.6 %
(30,000 clams m^{-2})	**Optimal size, (%)**	49.5 %	43.8 %	29.7 %
MEDIUM	*Minimum size, (%)*	78.1 %	89.0 %	91.1 %
(20,000 clams m^{-2})	**Optimal size, (%)**	43.2 %	68.7 %	61.9 %
LOW	*Minimum size, (%)*	–	–	99.0 %
(10,000 clams m^{-2})	**Optimal size, (%)**			92.1 %

the two sites, clams kept at the intermediate stocking density (i.e. HIGH) were sometimes similar to those kept at the lowest stocking density (i.e. MEDIUM), other times similar to those kept at the highest stocking density (i.e. EXTRA). Possibly, water conditions at the start of pre-fattening affected the adaptation stress of clams when transferred in the lagoon from the hatchery, thus increasing the individual variability in growth response in these batches. Differences in water quality, with special reference to temperature, between the hatchery and the lagoon were likely higher for B1 and B2 batches compared to B3 clams. In fact, during the first week after the arrival of clams, water temperature averaged at 9.78 ± 0.30 °C (min 8.02 °C, max 10.8 °C) in the northern site and 8.77 ± 0.39 °C (min 7.82 °C, max 9.23 °C) in the western site for batch B1; 13.0 ± 0.31 °C (min 12.1 °C, max 13.9 °C) in the northern site and 11.2 ± 0.80 °C (min 10.4 °C, max 12.4 °C) in the western site for batch B2; whereas water temperature was 18.4 ± 0.29 °C (min 16.7 °C, max 20.9 °C) in the northern site and 16.6 ± 0.76 °C (min 14.6 °C, max 19.2 °C) in the western site for batch B3. These data are averages of 24-h recordings every 30 min collected by ARPAV (2018) in Sacca degli Scardovari by its probes located in the proximity of the farming sites.

Similar decrease in clam size were observed for Manila clam D-larvae stocked at 5, 10, 15, and 20 larvae mL^{-1} (Yan et al., 2006) and juveniles (initial SL 8–9 mm, final SL 13 mm) farmed in suspended plastic trays for 4 months and stocked at 3,019, 9,057, and 15,094 clams m^{-2} (Marshall and Dunhan, 2013) or in *poches* at approximately 6,600, 13,300, and 19, 900 individuals m^{-2} for 3 months (Boscolo Brusà et al., 2011). High stocking density has been shown to negatively affect shell length and weight in other clam species, such as basket cockles (*Clinocardium nuttallii*) (initial SL 2.9 mm, initial weight 49 mg) farmed on the seabed over a 4-week period at 6060 and 11,800 individuals m^{-2} (Epelbaum et al., 2011) and razor clams (*Ensis arcuatus*) (initial SL 8 mm) farmed in suspended plastic bottles and PVC cylinders at 10,000 and 20,000 individuals m^{-2} for 85 days (da Costa et al., 2015). Growth and survival might decline with increasing stocking density, primarily due to competition for resources such as space or food (Beal and Kraus, 2002; Cigarría and Fernandez, 1998; Epelbaum et al., 2011) and direct contact between individuals, which might lead to shell damage and feeding inhibition (Dunham and Marshall, 2012; Holliday et al., 1991).

The shell strength of juvenile Manila clams (SL < 14 mm) in the present study was consistent with that reported for other marine and freshwater bivalve species with SL < 50 mm (1–88 N) (reviewed in Vasconcelos et al., 2011), and smaller clams from lanterns with high stocking density presented lower shell strength. In general, clam size is related to its shell strength (Grefsrud and Strand, 2006; Mu et al., 2018; Vasconcelos et al., 2011), which is important for reducing the risk of predation by crustaceans, fish, and birds (Vasconcelos et al., 2011) and susceptibility to shell breakage during harvesting operations (Gaspar et al., 2002).

With regard to survival, no mortality was recorded in the sampled clams up to 8 weeks of pre-fattening. At the end of pre-fattening, no differences were detected among clams farmed at the northern site. In contrast, B1 and B2 clams farmed at the western site at the highest stocking density (i.e. 50,000 individuals m^{-2}) presented lower survival rates than those at other stocking densities (30,000 and 20,000 individuals m^{-2}) at the end of the experiment (average survival rate 52.8

% vs. 85.1 % for B1 and 67.0 % vs. 90.1 % for B2; recorded one week later than at the northern site). Such contrasting results have previously been reported in literature. Some authors observed a significant effect of stocking density on the survival of juvenile Manila (Boscolo Brusà et al., 2011) and razor clams (da Costa et al., 2015), whereas others did not report any differences in the survival rates of clam species farmed at different stocking densities (Epelbaum et al., 2011; Marshall and Dunham, 2013; Yan et al., 2006).

In our study, the lower survival rates and differences between the stocking densities observed at the western site in comparison with the northern site might be attributed to the 1-week delay in clam harvest. This delay increased the clam size in the three batches (average of the different stocking densities) in the western compared to the northern site, but also caused mortality of most susceptible clams because the water conditions, i.e. temperature and especially dissolved oxygen, turned unfavourable (Fig. 2). In fact, during this week, data of ARPAV (2018) confirm that in the western site the average of daily recordings of dissolved oxygen was low and variable (7.62 ± 1.38 mg/L), and reached critical values for Manila clams survival (3.20–4.16 mg/L) during the 24-h in 4 out of 7 days. In the same period, daily recordings of water temperature averaged at 25.4 ± 0.70 °C with extreme values (≥27 °C) during the 24-h in 4 out of 7 days. The higher percentage of clams with minimum and optimal sizes observed at the western site in comparison with the northern site at the end of the pre-fattening period could be attributed to a higher mortality of smaller clams at the highest stocking density during the last pre-fattening stages (Cigarría and Fernández, 1998). Some authors observed that smaller clams are more likely to succumb to abiotic stressors, such as high temperatures and low dissolved oxygen (Dethier et al., 2019b; Tezuka et al., 2012), because they require proportionally more oxygen than larger individuals (Bougrier et al., 1995).

In the present study, regardless of the stocking density, Manila clams with an SL of approximately 3 mm sown in early April attained an average SL of 14.3 mm after 10–11 weeks. The pre-fattening duration and clam growth depend highly on the season in which juveniles are settled, regardless of the technology adopted (Boscolo Brusà et al., 2011; Soudant et al., 2004).

Considering the conditions prevalent in North Italian lagoons, it is advantageous to initiate shell farming activities at the beginning of spring and no later than April (Boscolo Brusà et al., 2011). Previous studies in the Venice Lagoon (Italy) showed that Manila clams (initial SL 7.5 mm) sown in late November reached an SL of 10 mm after 6 months of pre-fattening (late May) (Boscolo Brusà et al., 2011), whereas clams with an initial SL of 6.7 mm sown in August reached 8.7 mm after 1 month of pre-fattening. In a Mediterranean lagoon (Calich, Italy), grooved carpet shell (*Ruditapes decussatus*) juveniles (initial SL 7.9 mm) sown in late March reached an SL of 14–16 mm after 2 months of pre-fattening (Chessa et al., 2005).

The growth performance of clam seeds is highly dependent on the physico-chemical (i.e. temperature and salinity) (Matsuda et al., 2008; Xu et al., 2015) and trophic (i.e. phytoplankton availability and species) (Yan et al., 2006) characteristics of the rearing site (Boscolo Brusà et al., 2011; Dethier et al., 2019a) in addition to its hydrodynamism (i.e. wave energy and sea currents) (Dethier et al., 2019a; Hunt and Mullineaux, 2002; Takeuchi et al., 2015). In our trial, differences in water quality during the farming period (Fig. 2) does not support any differences in growth between the clams in the two sites. Data collected by ARPAV (2018) during the farming period (March 15-June 31, 2018) show that in the northern site, water temperature averaged at 19.8 ± 6.1 °C (min: 7.3 °C; max 28.4 °C) and dissolved oxygen at 8.9 ± 2.2 mg/L (min: 3.2 mg/L ; 18.9 mg/L); in the western site, temperature stand at 18.5 ± 5.6 °C (min: 6.9 °C; max 28.4 °C) and dissolved oxygen at 8.9 ± 1.7 mg/L (min: 2.9 mg/L; max: 15.8 mg/L). The water temperature from April onwards was within the suitable range for Manila clam larvae and juveniles (i.e. 18–28 °C) (Han et al., 2008; Huo et al., 2018; Shin and Lim, 2003), and it was higher than the optimal values for growth (20–23 °C) (Huo et al., 2018; Kang et al., 2016; Kim et al., 2017b) only during the last month of the study (average of daily recordings in June until harvesting; northern site: 26.4 ± 1.00 °C; western site: 24.8 ± 1.0 °C; ARPAV, 2018). In addition, extremely high level of chlorophyll *a* was recorded during late May-early June (approximately 86 μg L^{-1} at both sites) in comparison with the average yearly values for the lagoon during late spring-early summer (12 μg L^{-1}) (ARPAV, 2019). In June 2018, abnormal phytoplankton growth and a massive macroalgal bloom, primarily of *Cladophora*, *Ulva*, and *Pilifera* spp., was reported in Sacca degli Scardovari (ARPAV, 2019). This eutrophication phenomenon, coupled with the increase in the water temperature during the last month of the study, likely decreased the dissolved oxygen levels to the lowest recommended values for Manila clams (3.5 mg L^{-1}) (Kim et al., 2017a; Shin et al., 2001).

The findings of the present study provide new information for the standardization of molluscs farming in suspended net lanterns. In Sacca degli Scardovari, this farming technique was successful also in juveniles of a burrowing species, such as Manila clam, and using very high stocking densities (30,000 and 50,000 clams/m^2) compared to what previously tested and reported in literature (until 20,000 clams/m^2). Nevertheless, the increase of the stocking density reduced the clam size and the percentage of clams with a sowable size at harvest, whereas negative effects on survival were relevant only under challenging water conditions at the highest stocking density (as it was in the western site before harvesting). Thus, a stocking density of 30,000 clams/m^2 could be recommended which guarantees good percentage of sowable clams (67–75 %) and low susceptibility to challenging water conditions around harvest compared to a density of 50,000 clams/m^2. Indeed, the choice of the best stocking density should consider the cost of hatchery-produced seed and the price of clams at commercial size, the labour costs associated to the management of lanterns during pre-fattening, as well as the expected duration of the pre-fattening period. In the tested area, pre-fattening can start in April and clams can reach a suitable sowing size (SL 8−10 mm) in 10 weeks if water conditions are within suitable ranges (as it was for batch B3). Further investigations about the physiological and stress response of clams due to adaptation at the start of pre-fattening would be useful to optimize the management of hatchery-produced seeds. Surely, the longer the pre-fattening period under suitable conditions the higher the percentage of sowable clams, but harvesting time in the field must be carefully adjusted according to the specific climatic conditions of the year. In fact, extreme and quick climatic changes, with values below and above the expected yearly averages, have been recorded during the last decade, which can compromise a production cycle in few days if not properly monitored and managed.

Authors contribution

Francesco Bordignon: performed the trial, collected the experimental data, collected and prepared samples for biometric measurements; performed the statistical analyses, analysed and interpreted the data, and wrote the first draft of the manuscript; critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

Angela Trocino: conceived, designed and supervised the experiment; acquired the financial support for the project; performed the trial, collected the experimental data, collected and prepared samples for biometric measurements; performed the statistical analyses, analysed and interpreted the data, and wrote the first draft of the manuscript; critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

Emanuele Rossetti: performed the trial, collected the experimental data; critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

Cristina Zomeño: performed the trial, collected the experimental data, collected and prepared samples for biometric measurements; critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

Anton Pascual: performed the trial, collected the experimental data, collected and prepared samples for biometric measurements; critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

Marco Birolo: performed the trial, collected the experimental data, collected and prepared samples for biometric measurements; critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

Silvia Martines Llorens: critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

Gerolamo Xiccato: conceived, designed and supervised the experiment; critically reviewed the manuscript for intellectual content and gave final approval of the version to be published.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

ARPAV, 2018. Dati ambientali: Acque di transizione – Boe Delta del Po (Accessed 26 March 2021). https://www.arpa.veneto.it/dati-ambientali/open-data/idrosfera/acque-di-transizione/acque-di-transizione-boe-delta-del-po.

ARPAV, 2019. Monitoraggio delle acque di transizione della Regione Veneto. Available at: https://www.arpa.veneto.it/temi-ambientali/acqua/file-e-allegati/documenti/acque-di-transizione/rapporti-finali-e-documenti-di-classificazione-altre-lagune/Rapporto_Acque_Transizione_2018.pdf/view (Accessed 29 October 2020).

Beal, B.F., Kraus, M.G., 2002. Interactive effects of initial size, stocking density, and type of predator deterrent netting on survival and growth of cultured juveniles of the soft-shell clam, *Mya arenaria* L., in eastern Maine. Aquaculture 208, 81–111. https://doi.org/10.1016/S0044-8486(01)00900-0.

Boscolo, R., Cornello, M., Giovanardi, O., 2003. Condition index and air survival time to compare three kinds of Manila clam *Tapes philippinarum* (Adams & Reeve) farming systems. Aquac. Int. 11, 243–254. https://doi.org/10.1023/A:1024888608791.

Boscolo Brusà, R., Ponis, E., Cacciatore, F., 2011. Dalla pesca all'allevamento della vongola filippina in Laguna di Venezia: "Il preingrasso". Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA). Quaderni Ricerca Marina, 2/2011. ISBN 978-88-448-0488-6. Available at: https://www.isprambiente.gov.it/it/pubblicazioni/quaderni/ricerca-marina/dalla-pesca-allallevamento-della-vongola-filippina (accessed 29 October 2020).

Boscolo Brusà, R., Cacciatore, F., Ponis, E., Molin, E., Delaney, E., 2013. Clam culture in the Venice lagoon: stock assessment of Manila clam (*Venerupis philippinarum*) populations at a nursery site and management proposals to increase clam farming sustainability. Aquat. Living Resour. 26, 1–10. https://doi.org/10.1051/alr/2013042.

Bougrier, S., Geairon, P., Deslous-Paoli, J.M., Bacher, C., Jonquières, G., 1995. Allometric relationships and effects of temperature on clearance and oxygen consumption rates of *Crassostrea gigas* (Thunberg). Aquaculture 134, 143–154. https://doi.org/10.1016/0044-8486(95)00036-2.

Castagna, M., 2001. Aquaculture of the hard clam, *Mercenaria Mercenaria*. Chapter 15. In: Kraeuter, J.N., Castagna, M. (Eds.), Biology of the Hard Clam Developments in Aquaculture and Fisheries Science, Vol. 31. Elsevier Publication, New York, NY, pp. 675–699.

Chessa, L.A., Paesanti, F., Pais, A., Scardi, M., Serra, S., Vitale, L., 2005. Perspectives for development of low impact aquaculture in a Western Mediterranean lagoon: the case of the carpet clam *Tapes decussatus*. Aquac. Int. 13, 147–155. https://doi.org/10.1007/s10499-004-9022-6.

Cigarría, J., Fernández, J., 1998. Manila clam (Ruditapes philippinarum) culture in oyster bags: influence of density on survival, growth and biometric relationships. J. Mar. Biol. Assoc. U.K. 78, 551–560.

Cigarría, J., Fernández, J., 2000. Management of Manila clam beds: I. Influence of seed size, type of substratum and protection on initial mortality. Aquaculture 182, 173–182. https://doi.org/10.1016/S0044-8486(99)00257-4.

Claus, C., Maeckelberghe, H., de Pauw, N., 1983. Onshore nursery rearing of bivalve molluscs in Belgium. J. Aquac. Eng. Fish. Res. 2, 13–26. https://doi.org/10.1016/0144-8609(83)90003-1.

Cordero, D., Delgado, M., Liu, B., Ruesink, J., Saavedra, C., 2017. Population genetics of the Manila clam (*Ruditapes philippinarum*) introduced in North America and Europe. Sci. Rep. 7, 39745. https://doi.org/10.1038/srep39745.

Da Costa, F., Barreiro, B., Ojea, J., Nóvoa, S., Martínez-Patiño, D., 2015. Effects of stocking density on intermediate culture of the razor clam *Ensis arcuatus* (Pharidae: Bivalvia). Aquac. Res. 46, 1858–1865. https://doi.org/10.1111/are.12341.

Dethier, M.N., Dobkowskia, K., Noreenb, A., Yunc, M., Moosmillerd, A., 2019a. Vulnerability of juvenile clams to predation by shore crabs. Aquaculture 506, 350–354. https://doi.org/10.1016/j.aquaculture.2019.03.070.

Dethier, M.N., Kobelt, J., Yiu, D., Wentzel, L., Ruesink, J.L., 2019b. Context-dependence of abiotic and biotic factors influencing performance of juvenile clams. Estuar. Coast. Shelf Sci. 219, 201–209. https://doi.org/10.1016/j.ecss.2019.02.013.

Dunham, A., Marshall, R.D., 2012. Using stocking density modifications and novel growth medium to control shell deformities and biofouling in suspended culture of bivalves. Aquaculture 324–325, 234–241. https://doi.org/10.1016/j.aquaculture.2011.10.047.

Epelbaum, A., Pearce, C.M., Yuan, S., Plamondon, N., Gurney-Smith, H., 2011. Effect of stocking density and substratum on the survival, burrowing behaviour and shell morphology of juvenile basket cockle, *Clinocardium nuttalii*: implications for nursery seed production and field outplanting. Aquac. Res. 42, 975–986. https://doi.org/10.1111/j.1365-2109.2010.02680.x.

FAO, 2020. Fishery Statistical Collections (Accessed 29 October 2020). http://www.fao.org/fishery/statistics/global-aquaculture-production/en.

Gaspar, M.B., Leitão, F., Santos, M.N., Sobral, M., Chícharo, L., Chícharo, A., Monteiro, C. C., 2002. Influence of mesh size and tooth spacing on the proportion of damaged organisms in the catches of the Portuguese clam dredge fishery. J. Mar. Sci. 59, 1228–1236. https://doi.org/10.1006/jmsc.2002.1310.

Grefsrud, E.F., Strand, Ø., 2006. Comparison of shell length in wild and cultured scallops (*Pecten maximus*). Aquaculture 251, 306–313. https://doi.org/10.1016/j.aquaculture.2005.06.004.

Han, K.N., Lee, S.W., Wang, S.Y., 2008. The effect of temperature on the energy budget of the Manila clam, Ruditapes philippinarum. Aquac. Int. 16, 143–152. https://doi.org/10.1007/s10499-007-9133-y.

Hasegawa, N., Higano, J., Fujioka, Y., Ishihi, Y., Mizuno, T., Morita, K., Yamaguchi, M., Imai, H., Asao, D., Ozaki, Y., Yamamoto, Y., 2015. Culture substrate for suspended culturing of the Manila clam *Ruditapes philippinarum*. J. Aquac. Feed. Sci. Nutr. 63, 9–16. https://doi.org/10.11233/aquaculturesci.63.9.

Holliday, J.E., Maguire, G.B., Nell, J.A., 1991. Optimum stocking density for nursery culture of Sydney rock oysters (*Saccostrea commercialis*). Aquaculture 96, 7–16. https://doi.org/10.1016/0044-8486(91)90135-T.

Hunt, H.L., Mullineaux, L.S., 2002. The roles of predation and postlarval transport in recruitment of the soft shell clam (*Mya arenaria*). Limnol. Oceanogr. 47, 151–164. https://doi.org/10.4319/lo.2002.47.1.0151.

Huo, Z., Li, Y., Rbbani, M.G., Wu, Q., Yan, X., 2018. Temperature challenge on larvae and juveniles of the Manila clam *Ruditapes philippinarum*. Aquac. Res. 49, 1727–1731. https://doi.org/10.1111/are.13600.

Jara-Jara, R., Pazos, A.J., Abad, M., García-Martín, L.O., Sánchez, J.L., 1997. Growth of clam seed (*Ruditapes decussatus*) reared in the wastewater effluent from a fish farm in Galicia (N.W. Spain). Aquaculture 158, 247–262. https://doi.org/10.1016/S0044-8486(97)00196-8.

Kang, H.Y., Lee, Y.-J., Choi, K.-S., Park, H.J., Yun, S.-G., Kang, C.-K., 2016. Combined effects of temperature and seston concentration on the physiological energetics of the Manila clam *Ruditapes philippinarum*. PLoS One 11, e0152427. https://doi.org/10.1371/journal.pone.0152427.

Kim, S.L., Kwon, S.H., Lee, H.-G., Yu, O.H., 2017a. Effects of environmental and biological conditions on the recruitment and growth of the Manila Clam *Ruditapes philippinarum* on the West Coast of Korea. Ocean Sci. J. 52, 91–101. https://doi.org/10.1007/s12601-017-0003-x.

Kim, T.W., Park, S., Sin, E., 2017b. At the tipping point: differential influences of warming and deoxygenation on the survival, emergence, and respiration of cosmopolitan clams. Ecol. Evol. 8, 4860–4866. https://doi.org/10.1002/ece3.4041.

Marshall, R.D., Dunham, A., 2013. Effects of culture media and stocking density on biofouling, shell shape, growth, and survival of the Pacific oyster (*Crassostrea gigas*) and the Manila clam (*Venerupis philippinarum*) in suspended culture. Aquaculture 406, 68–78. https://doi.org/10.1016/j.aquaculture.2013.05.003.

Matsuda, M., Shinagawa, A., Higano, J., Fujii, A., Hirano, K., Ishimatsu, A., 2008. Effects of low salinity on survival, hemolymph osmolality and tissue water content of the manila clam *Ruditapes philippinarum*. J. Aquac. Feed. Sci. Nutr. 56, 127–136.

Microsoft Corporation, 2018. Microsoft Corporation, One Microsoft Way, Redmond, Washington, USA.

Mistri, M., Borja, A., Aleffi, I.F., Lardicci, C., Tagliapietra, D., Munari, C., 2018. Assessing the ecological status of Italian lagoons using a biomass-based index. Mar. Pollut. Bull. 126, 600–605. https://doi.org/10.1016/j.marpolbul.2017.09.048.

Mu, G., Duan, F., Zhang, G., Li, X., Ding, X., Zhang, L., 2018. Microstructure and mechanical property of *Ruditapes philippinarum* shell. J. Mech. Behav. Biomed. Mater. 85, 209–217. https://doi.org/10.1016/j.jmbbm.2018.06.012.

Munari, C., Rossetti, E., Mistri, M., 2013. Shell formation in cultivated bivalves cannot be part of carbon trading systems: a study case with *Mytilus galloprovincialis*. Mar. Environ. Res. 92, 264–267. https://doi.org/10.1016/j.marenvres.2013.10.006.

Munroe, D., McKinley, R.S., 2007. Commercial Manila clam (*Tapes philippinarum*) culture in British Columbia, Canada: the effects of predator netting on intertidal sediment characteristics. Estuar. Coast. Shelf Sci. 72, 319–328. https://doi.org/10.1016/j.ecss.2006.10.025.

Natali, C., Bianchini, G., 2018. Natural vs anthropogenic components in sediments from the Po River delta coastal lagoons (NE Italy). Environ. Sci. Pollut. Res. 25, 2981–2991. https://doi.org/10.1007/s11356-017-0986-y.

Palazzi, R., 2015. Ottimizzazione del preingrasso lagunare della vongola verace. Veneto Agricoltura, Legnaro, Italy. Available at: https://www.venetoagricoltura.org/upload/File/acquacoltura/RELAZIONE%20FINALE%20VONGOLA%20FEP.pdf (Accessed 29 October 2020).

Parisi, G., Centoducati, G., Gasco, L., Gatta, P.P., Moretti, V.M., Piccolo, G., Roncarati, A., Terova, G., Pais, A., 2012. Molluscs and echinoderms aquaculture: biological aspects, current status, technical progress and future perspectives for the most promising species in Italy. Ital. J. Anim. Sci. 11, 397–413. https://doi.org/10.4081/ijas.2012.e72.

Pellizzato, M., 1990. Acclimatization of the *Tapes philippinarum* species and the first experimental rearing basins in Italy. Tapes philippinarum, biologia e sperimentazione. Ente Sviluppo Agricolo del Veneto, Venice, Italy, pp. 157–170.

Pellizzato, M., Da Ros, L., 2005. Clam farming quality as a management tool: a proposal based on recent studies in Northern Adriatic lagoons. Aquac. Int. 13, 57–66. https://doi.org/10.1007/s10499-004-9030-6.

Ponti, M., Castellini, A., Ragazzoni, A., Gamba, E., Ceccherelli, V.U., Abbiati, M., 2017. Decline of the Manila clams stock in the northern Adriatic lagoons: a survey on ecological and socio-economic aspects. Acta Adriat. 58, 89–104. https://doi.org/10.32582/aa.58.1.7.

Ramón, M., Cano, J., Peña, J.B., Campos, M.J., 2005. Current status and perspectives of mollusc (bivalves and gastropods) culture in the Spanish Mediterranean. Bol. Inst. Esp. Oceanogr. 21, 361–373.

Royo, A., Ruiz-Azcona, P., Navajas, R., 2005a. Estimación de la mortalidad por siembra en cultivos de almeja japonesa *Ruditapes philippinarum* (Adams and Reeve, 1850) en la zona intermareal. Bol. Inst. Esp. Oceanogr. 21, 425–429.

Royo, A., Ruiz-Azcona, P., Navajas, R., 2005b. Incidencia de la red de protección en el cultivo de la almeja japonesa *Ruditapes philippinarum* (Adams and Reeve, 1850) en la zona intermareal. Bol. Inst. Esp. Oceanogr. 21, 441–446.

SAS Institute, 2013. The SAS/STAT 9.4 User's Guide. SAS Inst. Inc., Cary, NC, USA.

Shin, H.C., Lim, K.H., 2003. The influence of water temperature and salinity on the filtration rates of the short-necked clam *Ruditapes philippinarum*. Korean J. Malacol. 19, 1–8. ISSN:1225–3480.

Shin, Y.-K., Kim, Y., Chung, E.-Y., Hur, S.-B., 2001. Effects of the dissolved oxygen concentration on the physiology of the Manila clam, *Ruditapes philippinarum*. Korean J. Fish. Aquat. Sci. 34, 190–193.

Soudant, P., Paillard, C., Choquet, G., Lambert, C., Reid, H.I., Marhic, A., Donaghy, L., Birkbeck, T.H., 2004. Impact of season and rearing site on the physiological and immunological parameters of the Manila clam *Venerupis* (=*Tapes*, =*Ruditapes*) *philippinarum*. Aquaculture 229, 401–418. https://doi.org/10.1016/S0044-8486(03)00352-1.

Takeuchi, S., Yamada, F., Shirozu, H., Ohashi, H., Tamaki, A., 2015. Burrowing ability as a key trait in the establishment of infaunal bivalve populations following competitive release on an extensive intertidal sandflat. J. Exp. Mar. Bio. Ecol. 466, 9–23. https://doi.org/10.1016/j.jembe.2015.01.011.

Tezuka, N., Kamimura, S., Hamaguchi, M., Saito, H., Iwano, H., Egashira, J., Fukuda, Y., Tawaratsumida, T., Nagamoto, A., Nakagawa, K., 2012. Settlement, mortality and growth of the asari clam (*Ruditapes philippinarum*) for a collapsed population on a tidal flat in Nakatsu, Japan. J. Exp. Mar. Biol. Ecol. 439, 108–112. https://doi.org/10.1016/j.seares.2012.01.001.

Turolla, E., 2008. La venericoltura in Italia. In: Lovatelli, A., Farías, A., Uriarte, I. (Eds.), Estado actual del cultivo y manejo de moluscos bivalvos y su proyección futura: factores que afectan su sustentabilidad en América Latina. Taller Técnico Regional de la FAO. 20–24/08/2007, Puerto Montt, Chile. FAO Actas de Pesca y Acuicultura. No. 12. Roma, FAO, pp. 177–188.

Vasconcelos, P., Morgado-André, A., Morgado-André, C., Gaspar, M.B., 2011. Shell strength and fishing damage to the smooth clam (*Callista chione*): simulating impacts caused by bivalve dredging. J. Mar. Sci. 68, 32–42. https://doi.org/10.1093/icesjms/fsq149.

Veneto Agricoltura, 2020. La pesca in Veneto. Anno 2019. Available at: https://www.venetoagricoltura.org/wp-content/uploads/2020/09/La-pesca-in-Veneto-2019.pdf (Accessed 26 November 2020).

Whetstone, J.M., Sturmer, L.N., Oesterling, M.J., 2005. Biology and Culture of the Hard Clam (SRAC Publication No. 433). Southern Regional Aquaculture Center, USA, Stoneville, MS. Available at: https://agrilifecdn.tamu.edu/fisheries2/files/2013/09/SRAC-Publication-No.-433-Biology-and-Culture-of-the-Hard-Clam-Mercenaria-mercenaria.pdf (Accessed 13 October 2020).

Xu, X., Yang, F., Zhu, H., Zhang, Y., Liu, L., Zhang, Y., Zhao, L., 2015. Effects of temperature on oxygen consumption rate, ammonia excretion rate of juvenile Manila clam *Ruditapes philippinarum* with three color shells. J. Dalian Fish. Univ. 30, 627–633.

Yan, X., Zhang, G., Yang, F., 2006. Effects of diet, stocking density, and environmental factors on growth, survival, and metamorphosis of Manila clam *Ruditapes philippinarum* larvae. Aquaculture 253, 350–358. https://doi.org/10.1016/j.aquaculture.2005.07.030.

Zhang, G., Yan, X., 2006. A new three-phase culture method for Manila clam, *Ruditapes philippinarum*, farming in northern China. Aquaculture 258, 452–461. https://doi.org/10.1016/j.aquaculture.2006.04.046.