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Soft-Shell Production of the Invasive Atlantic Blue Crab *Callinectes sapidus* in the Lesina Lagoon (SE Italy): A First Assessment

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Abstract: The current invasion of the Mediterranean Sea by the Atlantic blue crab *Callinectes sapidus* requires the implementation of effective strategies of control and management. In native areas, the species is highly appreciated as a hard- and soft-shell seafood, and hard-shell fisheries are developing in a number of invaded countries. Here, to verify alternative approaches for enhancing the commercial value of the species, we carried out a pilot experiment to test a flow-through system for the production of soft-shell blue crabs. Fifty crabs were collected in the Lesina lagoon (Adriatic Sea, Italy) and inspected for the coloration of the line on the distal edge of the fifth pereiopod. Accordingly, they were grouped into three groups showing no, white, and red lines, symptomatic of the progression of the pre-molt phase, and maintained for 31 days in a flow-through pond system. The overall mortality rate determined during the trial was similar across the three groups and ranged between 10 and 20%, indicating that the flow-through system was effective for rearing the crabs. In addition, the molting rate was 10%, 65%, and 85% for individuals at an early, intermediate, and advanced pre-molt stage, confirming that the line coloration can be used as an effective indicator of the advancement of the molting process. Our study provides the first contribution towards the introduction in the Mediterranean fishery sector of novel procedures and practices for the production of soft-shell blue crabs, which may represent a cost-effective strategy to enhance the capture and commercialization of this invasive species as a high-quality and valuable shellfish product.

Keywords: Portunidae; seafood; invasive species; functional eradication; Mediterranean Sea

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1. Introduction

Marine bioinvasions are to date recognized as a global threat to the integrity of natural ecosystems and the activities of human populations; accordingly, they are acknowledged as one of the top conservation concerns worldwide [1]. Increasing efforts are devoted to horizon scanning exercises, allowing an early detection of potentially invasive alien species (IAS hereafter) and, in turn, the implementation of rapid eradication procedures [2–5]. Indeed, the effective eradication of marine IAS has only been achieved when species were detected early, and management responded rapidly [6]. However, mitigating the effect of persistent bioinvasions through complete eradication is problematic in marine habitats due to their high environmental connectivity [7]. In contrast, functional eradication, i.e., the reduction in population densities below levels in which impacts on the native ecosystem are considered acceptable, is presently acknowledged as an appropriate and effective control strategy to manage marine IAS and to mitigate the negative effects on the delivery of goods and services of invaded ecosystems [8,9]. This is particularly true when bioinvaders are species of economic interest, so functional eradication can be combined with commercial exploitation [10–12].

The Atlantic blue crab *Callinectes sapidus* (Rathbum, 1896) (Decapoda, Brachyura, Portunidae) is native to the western coasts of the Atlantic Ocean with a distribution ranging from the Gulf of Maine south to northern Argentina [13–15]. The species appeared in European waters at the start of the 20th century, probably introduced by ballast waters, while in the Mediterranean Sea, it was first detected in 1947 [16]; to date, the species can be found ubiquitously throughout the Mediterranean and Black Seas, as well as along the European and African Atlantic coasts [15,17]. The invasive success of *C. sapidus* relates to its r-selected life history traits of high fecundity, dispersal capacity, and fast growth, together with its broad environmental tolerance, large body size, and aggressive behavior [16]. Noticeably, negative impacts on fishing activities have been repeatedly highlighted due to, e.g., the consumption of commercial fish caught in traps and net damage, especially where established populations of *C. sapidus* are present [18,19]. Moreover, long-term impacts in invaded areas have been observed, related to declines in abundance of native species, suggesting that enduring negative effects may also occur at relatively low blue crab densities [20].

In native areas, the blue crab is a valuable shellfish product, supporting important commercial and recreational fisheries [21,22]. Noticeably, since the beginning of the nineteenth century, in the eastern United States, hard-shell blue crab production has been paralleled by aquaculture activities dedicated to soft-shell crabs [23]. The term soft-shell crab (SSC hereafter) refers to the state of any crab that has just completed the ecdysis, replacing the exoskeleton with a new decalcified and soft one. Hard-shelled crabs are characterized by a low meat yield (generally between 20 and 25%), while SCC can be consumed whole with an almost 100% meat yield, making them a much-appreciated and highly valued delicacy [23–25]. In the Mediterranean Sea, hard-shell commercial fisheries have also developed in Turkey [26] and northern Greece [27,28], increasing the economic interest towards this species across invaded Mediterranean countries. On the other hand, artisanal fisheries along the Adriatic coasts are currently struggling to find a solution to cope with the presence of established populations of the crab (https://www.catchupfish.it/prodotti-tecnici/, accessed on 9 January 2024).

A possible solution to increase the commercial interest in blue crabs from invaded areas and ensure their sustainable use is by generating higher added value and diversifying the market demand by developing SSC production [29]. Here, we focused on the blue crab population in the Lesina Lagoon (Apulia Region, SE Italy), where the occurrence of the species was first recorded in 2007 and repeatedly confirmed in the following years [30,31]. To date, blue crabs represent a predominant component of the by-catch in the basin and the species is perceived as a nuisance by fishers, given its low market price and limited interest from local processing companies. Specifically, the main objectives of the present study were the following:

(i) To explore for the first time in Europe the suitability of a pond system to produce SSC; to this end, the flow-through or recirculating cultivation facilities in use in native areas to rear pre-molt individuals of *C. sapidus* (locally known as peelers) were taken as a model [24];
(ii) To investigate the molting process in blue crabs from the Lesina Lagoon and verify if the population is characterized by a predictable molting pattern similar to that observed in native areas.

Indeed, in recent years, a number of investigations have focused on the biology and ecology of *C. sapidus* in Mediterranean waters, in terms of distribution [15,18,32], population structure and dynamics [33], and trophic habits [34–37]. Noticeably, no efforts have been made to provide detailed information on this crucial phase of the biological cycle of the species in invaded areas.

2. Materials and Methods

2.1. Study Area

The Lesina Lagoon (41.88° N and 15.45° E; Figure 1) is a microtidal shallow (0.7–1.5 m) lagoon located along the south-western Adriatic coasts of Italy. It has a total water surface of 51.4 km^2 separated from the Adriatic Sea by a sandy isthmus about 18 km and connected to it by two tidal channels: Acquarotta to the West and Schiapparo to the East [31,38].

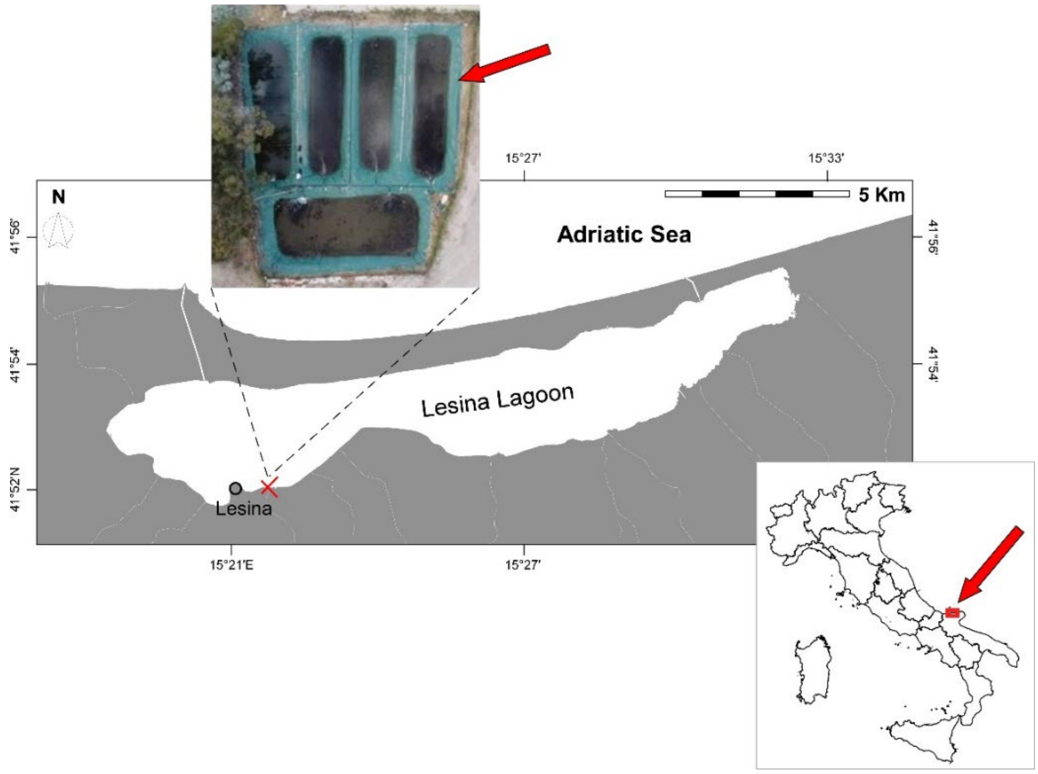

Figure 1. The Lesina Lagoon, and location of the semi-enclosed facility where this study was performed (indicated by the red cross). An image of the experimental system where crabs were reared is included at the top of the figure. The location of the pond used in the trial is indicated by a red arrow.

The lagoon's main hydrological features, temperature and salinity, follow a seasonal pattern with minimum values in winter and maximum values in summer [39]. Fishers operate in the lagoon using mainly fixed nets, known locally as "paranze". These are nylon seines with a 6 mm mesh placed perpendicularly to the shores, which convey the prey into fyke-nets with a 4–6 mm mesh installed at regular intervals along them [40].

2.2. Experimental Setup

The trial was conducted in a pond located in a semi-enclosed facility located on the southern banks of the Lesina Lagoon close to the IRBIM-CNR Institute. The facility included a system of four interconnected artificial ponds (Figure 1) waterproofed by polyethylene sheets with an average area of 100 m^2 and a depth of 1.5 m (Figure 1). The water circulation was open, and directly connected to the lagoon by means of a flow-through system connected by an underwater electric pump (Wilo-Emu FA). Following established protocols of

SSC farming in the Americas and Asia [24], a floating-box system was constructed inside of the experimental pond. These floating-box systems prevent cannibalism, as each crab was kept in an individual polyethylene cage (30 cm × 40 cm × 30 cm) immersed close to the water surface and supported by a floating system anchored to the side of the pond (Figure 2).

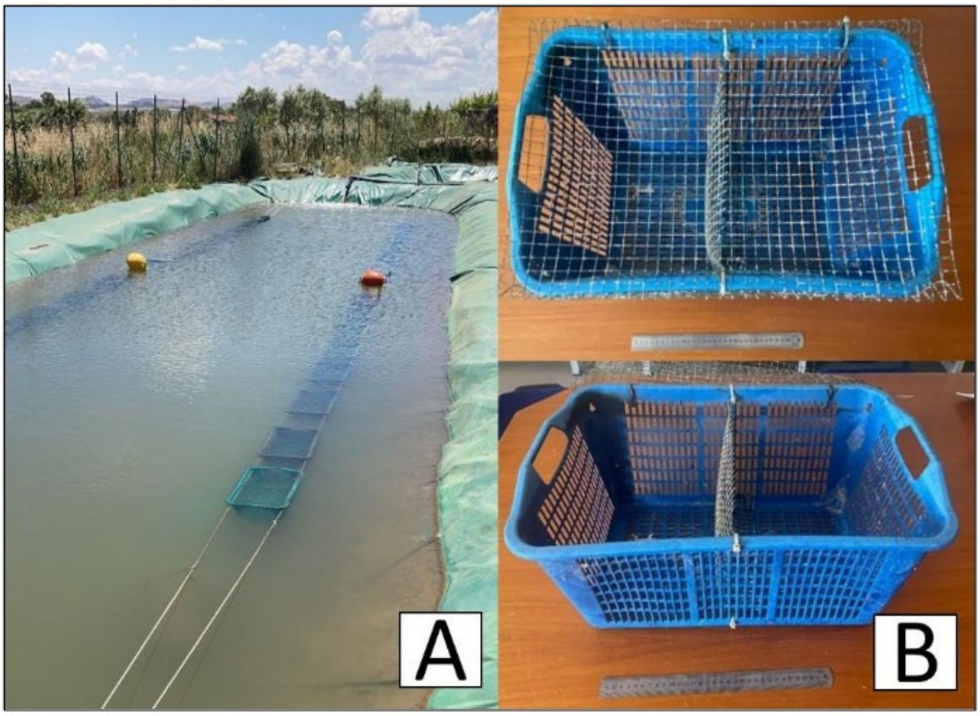

Figure 2. Floating-box system anchored to the side of the pond (**A**) and detail (**B**) of the polyethylene cages deployed to retain the individuals of *Callinectes sapidus*.

The trial took place in July 2020 as preliminary studies on blue crab's population dynamics in the basin showed abundance peaks of catches during warmer months, as well as a higher abundance of individuals in the pre-molt phase (Cilenti, personal observation). A total of 80 randomly chosen specimens of *C. sapidus* captured using fyke-nets and gillnets by local fishers were transferred by aerated tanks to the laboratories of the IRBIM-CNR Institute of Lesina, where they were inspected. Only 50 specimens showed intact appendages; these individuals were selected and divided into three groups according to pre-molt signs. Although there are several macroscopic indicators of the pre-molt phase [41], selection was carried out by inspecting the coloration of the line along the inside edge of the last flattened section of the pereiopods, where the new shell formation is more visible. This is the most reliable and widely used method to identify the pre-molt stage [41]. A white line indicates that the specimen will molt in two weeks; as molting approaches, the color gradually turns pink, which indicates that the crab will molt within one week, and then to red, indicating the crab will molt within 1–3 days [41,42] (Figure 3). Accordingly, three groups were identified: (1) 20 individuals showing the presence of a white line (W group hereafter), (2) 20 individuals showing the presence of a pink to red line (R group), and (3) the control group of 10 individuals showing no line along the edge of the flattened pereiopods (C group). The sex of all individuals was determined by examination of the apron shape; their carapace width (CW) and carapace length (CL) were measured using a caliper (to the nearest 0.1 cm), while their wet weight (WW) was measured using a digital balance (to the nearest 0.1 g). Subsequently, individual crabs were randomly introduced into the polyethylene cages.

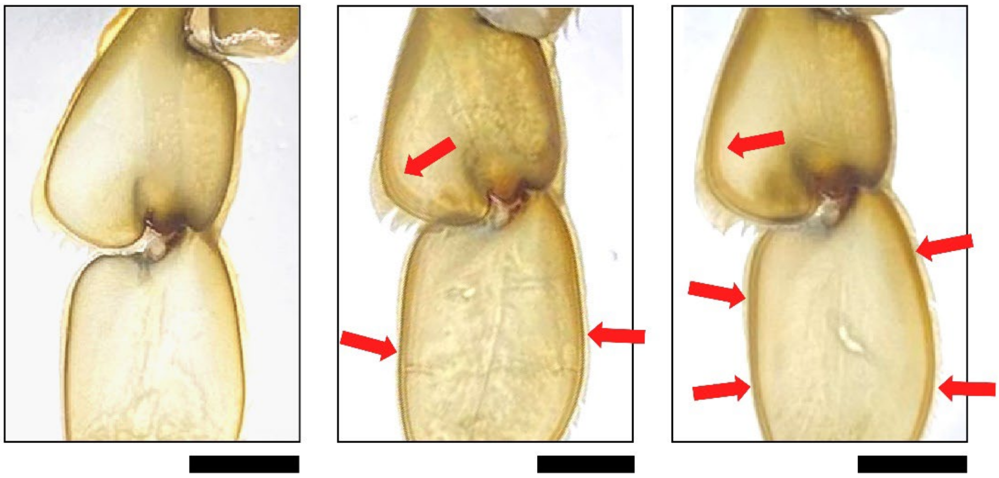

Figure 3. Absence (**A**) and presence (**A**, **B**) of the macroscopic sign indicators of the pre-molt phase in the inside edge of the last flattened sections of the pereiopod. Specifically, the pereiopod of a specimen lacking any pre-molt sign is shown in (**A**) (control **C** group); in (**B**), the arrow indicates the white line characteristic of an early pre-molt stage (W group); in (**C**), the arrow indicates the reddish line indicative of an advance pre-molt stage (R group) and the proximity of ecdysis. Black bars = 5 mm.

The trial lasted 31 days. During this study, water circulation into and out of the system was kept continuously active, ensuring an optimal water exchange between the lagoon and the pond. No food was provided, as crabs in the pre-molt stage do not feed [41]. Cages were inspected three times a day (07:00, 14:00, and 21:00) to record molting or mortality cases. Once molting was observed, crabs were removed from the cage. Pond water temperature (°C), salinity (PSU), dissolved oxygen (mg L^{-1}), and percentage of oxygen saturation (%) were monitored weekly in triplicate using a multi-parametric probe (Hydrolab DS5).

2.3. Data Analysis

Values in the text are expressed as mean $\pm$ 1 SD if not otherwise specified. Data were preliminarily checked for normality (Shapiro–Wilk test) and homoscedasticity (Cochran's C-test); when necessary, they were square root- or log(x + 1)-transformed to meet the required assumptions. Two-way ANOVAs with "sex" (two levels) and "treatment" (three levels) as orthogonal fixed factors were performed to test the differences in biometric parameters (CW, CL, and WW) among the three groups of crabs as affected by sex. Mortality and molting rates were compared using "N-1" χ-square tests as generally recommended [43,44]. Given the peculiar characteristics of the data (see Section 3—Results), a Type III (partial sum of squares) one-way permutational multivariate analysis of variance (PERMANOVA; Anderson, 2005 [45]) with 9.999 unrestricted permutations of raw data was subsequently used to test for intra-group differences in the number of days taken by specimens to molt.

3. Results

3.1. Crab Sex Ratio and Biometry at the Start of the Trial

Of the 50 crab specimens involved in the trial, 68% were females. The percentage of females in the control C group was 70%, while in the R and W group, it was, respectively, 55% and 80% (Table 1); however, no significant differences across the three treatments were determined (max χ^2 = 2.76, p = 0.11, 1 d.f. estimated for the comparison of W group vs. R group).

Table 1. Mean individual carapace width (CW), carapace length (CL), and wet weight (WW) of the crab specimens included in the different treatments at the start of the trial. Standard deviations are reported in parentheses, while the number of specimens for each combination treatment/sex is in brackets.

Treatment	Sex	CW (mm)	CL (mm)	WW (g)
C group	F (7)	108.29 (12.13)	53.86 (4.85)	100.54 (27.83)
	M (3)	114.33 (19.35)	52.01 (4.01)	132.71 (58.39)
W group	F (16)	113.44 (11.87)	55.06 (4.99)	108.64 (28.05)
	M (4)	102.01 (12.08)	53.01 (4.76)	92.39 (34.97)
R group	F (11)	102.45 (11.94)	50.18 (4.85)	84.51 (23.59)
	M (9)	103.22 (14.78)	52.89 (7.15)	97.36 (47.08)

The overall mean ($\pm$1 SD) individual carapace width, length, and wet weight of the specimens from the three treatments were 107.6 $\pm$ 13.3 mm, 53.1 $\pm$ 5.4 mm, and 100.3 $\pm$ 34.3 g. The crabs in the C group had a mean CW, CL, and WW of 110.1 $\pm$ 13.8 mm (88–126 mm min–max range), 53.3 $\pm$ 4.5 mm (46–60 mm min–max range), and 110.2 $\pm$ 38.9 g (58.1–168.1 g min–max range), respectively. In the W group, specimens had a mean CW, CL, and WW of 111.15 $\pm$ 15.5 mm (90–127 mm min–max range), 54.7 $\pm$ 4.9 mm (45–63 mm min–max range), and 105.4 $\pm$ 29.3 g (60.9–152.1 g min–max range), respectively. Finally, the CW of crabs in group R ranged between 84 and 128 mm and averaged 102.8 $\pm$ 12.9 mm, with a CL ranging between 45 and 66 mm (51.4 $\pm$ 6 mm, mean $\pm$ 1SD), and a WW ranging between 50.6 and 187.4 g (90.3 $\pm$ 35.6 g, mean $\pm$ 1SD). In addition, sex-related differences in the body size of crabs in each treatment were observed (Table 1); yet, in general, no significant effects were observed for either the factor "treatment" or "sex" on the variation in body size of the crabs (Table 2).

Table 2. Summary of two-way factorial ANOVA testing for the effect of treatment and sex on the mean carapace width (CW), carapace length (CL), and wet weight (WW) of crab specimens at the start of trial. F values are reported, with the corresponding *p* values in parentheses.

Factor	Degree of Freedom *	F_{CW}	F_{CL}	F_{WW}
Sex (1)	1	0.13 (0.72)	0.05 (0.82)	0.73 (0.39)
Treatment (2)	2	1.41 (0.25)	0.87 (0.43)	1.72 (0.19)
1 $\times$ 2	2	1.37 (0.26)	0.98 (0.38)	1.41 (0.25)

* Error: n = 44.

3.2. Pond Water Parameters

During the trial, the temperature of the water in the pond increased from 25.8 to 27.7 °C (Figure A1 in Appendix A), paralleled by a decrease in the concentration of the dissolved oxygen concentration from 7.7 to 5.8. Salinity decreased in the first week of the trail from an initial value of 28.5 PSU to 27.9, and then increased up to 28.9 PSU. Despite the observed variations, all water parameters showed mean values in the ranges suggested for optimal maintenance conditions of peelers (Table 3). Only the temperature in the second half of the trial values was >27 °C, thus exceeding those suggested by the literature.

Table 3. Mean temperature, salinity, and dissolved oxygen concentration (mean $\pm$ standard deviation) measured in the experimental pond during the trial. Water parameters suggested in the literature [46] for optimal maintenance conditions are included.

Parameter	Pond	Optimal Conditions
Temperature (°C)	26.9 $\pm$ 0.9	21.1–26.7
Salinity (PSU)	28.4 $\pm$ 0.4	$\pm$5 PSU of harvesting water
Dissolved Oxygen (mg/L)	6.5 $\pm$ 0.8	>5

3.3. Crab Mortality and Molting during the Trial

In total, seven specimens died during the trial (Table 4), with an overall mortality rate of 14%. No significant differences in mortality rates were observed across groups (max $\chi^2 = 0.04$, $P = 0.84$, 1 d.f. for the comparison of Group W vs. Group R). Noticeably, the majority of the mortality events (5) occurred during the first 24 h, and one crab from the R group died during the process of molting after 48 h.

Table 4. Mortality and molting rates of the individuals held in the floating-box system.

Treatment	Dead	Molting	Non-Molting	Days to Molting
C group	2 (20%)	1 (10%)	7 (70%)	17
W group	2 (10%)	13 (65%)	5 (25%)	13.07 ± 6.91
R group	3 (15%)	17 (85%)	0	4 ± 1.27

The lowest frequency of molting events occurred in the C group, where only one crab molted after 17 days from the beginning of the trial. Conversely, the highest molting frequencies occurred in crabs belonging to the W and R groups, with molt rates of 65.0% and 85%, respectively (Table 4).

Negligible differences were observed between them ($\chi^2 = 2.08$, $p = 0.14$, 1 d.f.); yet, both treatments showed frequencies significantly higher than that observed in the control group (W group vs. C group: $\chi^2 = 7.83$, $p = 0.005$; R group vs. C group: $\chi^2 = 15.09$, $p = 0.0001$; 1 d.f. for both tests). In addition, the number of days taken by specimens to molt varied significantly across treatments (one-way PERMANOVA, pseudo-$F_{2,30} = 25.07$, $P_{perm} = 0.0001$). On average, crabs in the R group molted after 4 days, significantly earlier than that observed for both the C and W groups (Table 4; post hoc bivariate comparisons: minimum $t = 4.46$, $p = 0.032$ for the comparison of group R vs. group C). In contrast, no significant differences were observed between groups C and W ($t = 0.71$, $p = 0.78$).

4. Discussion

Our study indicated that for *Callinectes sapidus* from the Lesina Lagoon, the color of the propodus and dactylus of the fifth pereiopod can be successfully used as an indicator of the progress of the molting process. A visual inspection allowed the identification of peelers, and accordingly, the separation of individuals in the pre-molt stage from those in the inter-molt stage as previously described [42]. These results are comparable to those observed in a study on molting success conducted in Maryland in a floating system, where the time to molt of peelers showing either a white or red line on their pereiopod took 3–10 days and 1–3 days, respectively [42]. The macroscopic sign observed during the initial selection of individuals, as well as the progressive change in the line's color from white to red, were similar to those previously reported by studies on the determination of the pre-molt phase of *C. sapidus* [42], as well as of *C. arcuatus* and *C. ornatus* [47,48]. Furthermore, significant differences between the days to molt allowed the differentiation of individuals in the very early phase of the pre-molt stage compared to those in the advanced phase.

Information on Asian and American soft-shell crab enterprises are rare and data on peeler mortality rates are difficult to obtain [24,49]. Nevertheless, the overall mortality rate recorded in this study was lower than that reported by Spitznagel et al. [50] for eight different culture facilities in the Chesapeake Bay region, where the mean mortality was 21.7 ± 2.8%. Interestingly, our results were closer to that reported for recirculating systems (16.4 ± 3.1%) than for flow-through facilities (32.9 ± 4.3%). Experiments conducted in the continuous-flow system similar to that used in the present study on *C. danae* and *C. exasperatus* [51] reported mortality rates ranging from 37.5 to 60%. A study on the North Carolina (USA) soft-shell crab industry [52] estimated that approximately 23% of the crabs placed in shedding systems die within 5 days, highlighting that the stress caused by capture and transport is one of the causes of peeler mortality. Therefore, it is likely that the death rate observed here within the first 24 h might have been due to the stress induced by capture, handling, and transport from the Lesina Lagoon. The common practice of fishers to keep crabs in waterless containers increases the probability of injuries and physiological stress and may result in immediate or delayed effects on the captured blue crabs, including mortality [53]. However, excess stress and the consequent mortality can be avoided by keeping the crabs cool and moistened during transport, as highlighted elsewhere [52].

Notably, we found that only the advanced pre-molt stage demonstrated by the reddening of the line on the paddles can be used as an effective indicator, since crabs characterized by a white line (W group) did not show significant differences from the control group in terms of days taken to molt. This lack of differentiation is likely to have been determined by the relatively low number of specimens involved in the trial, and by the duration of the trial itself. The experiment had to be terminated after one month when the variations observed in the chemical–physical characteristics of the pond water changed to summer conditions, where temperatures reached values no longer suitable for optimal crab rearing (Table 3). The high survival and molting success documented in this experiment, together with the water parameters in line with those suggested by Malone and Burden [46], indicated that the semi-closed system equipped with floating boxes can represent a viable semi-culture technology to keep individuals of *C. sapidus* under controlled conditions and allow for the completion of the molting process. It is likely that future experiments performed in larger ponds with a higher number of specimens and over a longer duration may demonstrate crabs showing no pre-molt signs in control groups, concluding the molting process, and with the appropriate statistical power to validate the results of the present study. Therefore, we are confident that the results from this investigation provide a preliminary yet robust confirmation that the procedures generally in use to identify and rear *Callinectes sapidus* for the production of soft-shell crabs in native areas can also be successfully used in the Mediterranean context.

Our study clearly suggests that the commercial interest in the blue crab of invaded areas may be increased, promoting the development of a product similar to the soft-shell crabs provided in the American and Asian markets, where the price of soft-shell crabs can exceed that of hard-shelled ones by 300–400% [29]. To our knowledge, the only example of soft-shell crab production in the Mediterranean Sea is represented by the "moleche" industry in Venice Lagoon, where males of the Mediterranean crab *Carcinus aestuarii* are seasonally harvested and held in submerged wooden or plastic containers (vieri) until molting [54,55]. Moleche represents an important source of income for the fishermen, given that their market price may exceed 80 EUR/kg [56]. Taking these already-established circumstances as a model, the introduction in the Mediterranean fishery sector of novel technical procedures and practices for the production of soft-shell blue crabs may represent a cost-effective strategy to encourage the capture and marketing of this invasive species as a high-quality and valuable shellfish product, while minimizing control costs and impacts.

5. Conclusions

Invasive alien species are undesired organisms for which the eradication and/or prevention of expansion is considered as the main management objective. However, complete eradication can be costly or unfeasible, and, in the case it is successfully achieved, even produce counterintuitive effects on the integrity of ecosystems [57]. Functional eradication and the implementation of effective strategies of the valorization of IAS as seafood may represent a cost-effective and sustainable strategy of control and mitigation. In this context, the Atlantic blue crab *Callinectes sapidus* provides an illustrative example, as it represents a highly invasive species impacting biodiversity and ecosystem services and threatening the socio-economic sector of invaded region fishing, even though that in native areas is a commercially important species [10,18]. Established blue crab populations in the Mediterranean regions need to be controlled to satisfy the principles of sustainable development that guarantee environmental protection and conservation while ensuring the economic growth of local communities. The present study provided a clear indication that the capture of *C. sapidus* specimens in the pre-molt phase together with the development of techniques and practices for their rearing could represent an additional seasonal activity, allowing professional fishers to improve their profit while contributing to mitigate the impact on invaded ecosystems.

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Institutional Review Board Statement: No ethical issues related to the use of animals in the performed analyses were involved.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data used in this study are available upon request from the corresponding author. The data are not publicly available, due to ongoing comparative analyses.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

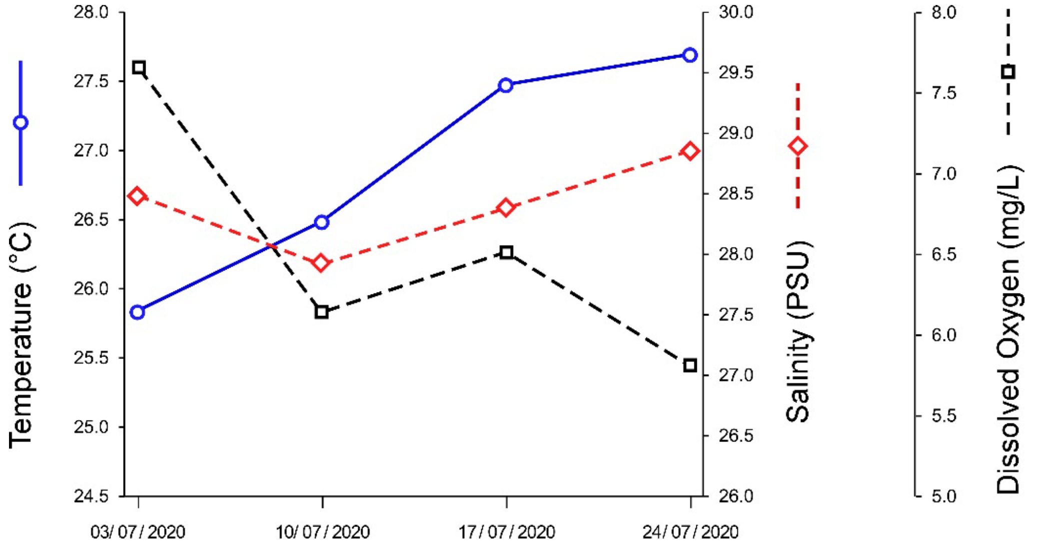

Figure A1. Variation in mean temperature, salinity, and dissolved oxygen concentration of the pond water during the trial. The standard deviation of the estimations is not included, for the sake of clarity.

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