Scientific Papers. Series D. Animal Science. Vol. LXIV, No. 1, 2021
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

LABORATORY TESTING OF THE AMERICAN BLUE CRAB'S (*CALLINECTES SAPIDUS* RATHBUN, 1896) CAPACITY OF ADAPTATION TO AQUACULTURE SYSTEMS AT THE ROMANIAN COAST

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Abstract

The blue crab, Callinectes_sapidus (Rathbun, 1896), is native to the Western Atlantic, supporting extensive fisheries and more recently aquaculture pursuits. It has become established as a non-native species in the Mediterranean, while in the Black Sea it was first reported decades ago, near the Bulgarian coast. A first specimen was collected at the Romanian coast in 1998 and, since then, individuals of this species have been reported several times. Due to its high market value and potential for aquaculture, we investigated the adaptation of the blue crab to captivity conditions. One adult was caught in fishing nets in the Mamaia Bay and transported to NIMRD's aquaculture laboratory. The crab was sexed (male) and measured (carapace width = 205 mm; carapace length = 100 mm; biomass = 537.58 g), and subsequently placed in a small PAS (pump-ashore system). Live mussels were introduced in the tank and the C. sapidus specimen was immediately observed feeding actively with the mollusks. Additionally, small fish were offered, being rapidly consumed. The overall observed behavior in captivity encourages aquaculture endeavors for this valuable species.

Key words: *aquaculture, Black Sea, captivity, crustaceans, pump-ashore system (PAS).*

INTRODUCTION

The American blue crab, *Callinectes sapidus* Rathbun, 1896, is of major interest to fisheries in the tropical and subtropical waters of the Western Atlantic. It supports large valuable commercial and recreational fisheries in the temperate areas of the Atlantic and Gulf coasts of the United States. It is the most widely harvested and consumed crab in the US, which is also the world's main producer of blue crab (Millikin & Williams, 1984). The Chesapeake Bay has supported an abundant blue crab population with an intense fishery, which currently supplies over one-third of all US commercial blue crab landings (Miller et al., 2011).

C. sapidus is a decapod crustacean, belonging to the family Portunidae. The carapace is more than twice as broad as long; it has nine blunt to acuminate teeth (outer orbital tooth and strong lateral spine included) on arched anterolateral margin. The pincers are strong, dissimilar and ridged longitudinally; the fifth legs are flattened in the form of paddles. The colour is greyish, bluish, or brownish green of varying shades and tints are present dorsally on carapace and chelipeds (Tavares, 2002).

Widely tolerant of salinity and temperature limits, the American blue crab is found in shallow waters near the coasts, especially at the mouths of rivers and streams, on sedimentary, sandy or muddy bottoms (Skolka & Gomoiu, 2004). Metabolic activity is slowed at temperatures below 20°C and blue crabs tend to become less active. When air temperatures drop below 10°C, adult crabs leave shallow, inshore waters and seek deeper areas where they bury themselves and remain in a state of torpor throughout the winter (Rome et al., 2005).

C. sapidus is native to the Western Atlantic (Chesapeake Bay), from Nova Scotia, Maine and northern Massachusetts to Argentina, including Bermuda and the Antilles (Tavares, 2002). It has also been successfully introduced, accidentally or intentionally, into both Asia and Europe. Accidental introductions have been attributed to larval transport via ship ballast water (Skolka & Gomoiu, 2004). It was introduced in Europe (Denmark, Netherlands, and adjacent North Sea, France, Golfo di Genoa); northern Adriatic; Aegean, western Black and eastern Mediterranean Seas. It has also been introduced to Japan. It is now rather

abundant in parts of the northern and eastern Mediterranean Sea and Japan (Skolka & Gomoiu, 2004).
The blue crab has become established as a non-native species in the Mediterranean basin (Holthuis, 1961). The blue crab was first recorded in the Mediterranean in Egyptian waters in the 1940s (Banoub, 1963). Subsequently, it has been reported in coastal waters off Italy (Giordani-Soika, 1951, as *Neptunus pelagicus*), Israel (Holthuis & Gottlieb, 1955), Greece (Kinzelbach, 1965) and Turkey (Kocataş & Katağan, 1983). Most recently, it has been reported in the Bay of Biscay, along the northwestern coast of Spain (Cabal et al., 2006) and the Sacca di Goro lagoon, an area located in the northern part of Italy (Manfrin et al., 2015).
In the Black Sea it was reported decades ago, near the Bulgarian coast (1968), as isolated specimens (Müller, 1986). To date, there are approximately fifteen records of occurrences of *C. sapidus* in the Black Sea (including brackish-water areas such as the Dnieper-Bug estuary) and the Sea of Azov (Snigirev et al., 2020). The first-time findings of *C. sapidus* on the north-western Black Sea shelf are in line with earlier assumptions on the hydrological characteristics of this area: relatively low salinity (compared to the Mediterranean) and a muddy substrate, an appropriate environment for *C. sapidus* proliferation (Snigirev et al., 2020). An increased number of *C. sapidus* findings in the Black Sea region during the last decade suggests the species to be naturalised in the area and widespread in the coastal waters of the Black Sea (Tokarev & Shulman, 2007).
A first specimen was collected at the Romanian coast in the summer of 1998 - a large male (85 mm long shell, 196 mm wide between the two lateral spines), and, a year later, a female would be collected in the same area - southern Romanian coast (Skolka & Gomoiu, 2004). Subsequently, specimens of this species have been reported several times in the southern part of the coast (Micu & Abaza, 2004; Nicolaev et al., 2004); one of them, captured alive, was kept in captivity for several months at the Aquarium in Constanţa. In all probability, in the southern part of the Romanian coast there is already a population of American blue crab, but its numbers are small (Skolka & Gomoiu, 2004). In recent years, isolated individuals have been reported (in 2016), accidentally caught by fishermen in the southern part of the littoral (*verbal information*). The latest records of *C. sapidus* were reported in 2020: one individual was caught in the Mamaia Bay (September 2020), one in Agigea (October 2020) and another one in Costinesti (November 2020). Production of soft-shell blue crabs represents one of the oldest aquaculture industries in the United States. The industry is dependent upon the capture of pre-molt (peeler) crabs from the wild fishery which are held in shedding trays until they molt (Oesterling, 1995). Commercial exploitation of the blue crab is rapidly increasing worldwide. One possible way to overcome the dependence on natural stocks for soft-shell crab industry is to rely on the development of technologies for reproduction, larval rearing, and cultivation of crabs in captivity (Zohar et al., 2008).
In this context, the purpose of this research was to investigate the species' capacity of adaptation to aquaculture systems at the Romanian coast.

MATERIALS AND METHODS

On 9 September 2020, one *C. sapidus* individual was accidentally caught by fishermen in the Mamaia Bay (trap net) and subsequently transferred to NIMRD's aquaculture laboratory (Figure 1).

Figure 1. *C. sapidus* specimen caught in fishing nets in the Mamaia Bay (Original photos)

After accurate identification of the species, the crab was sexed, based on the shape of the abdomen, as an adult male (Figure 2).

Figure 2. Sex determination of the *C. sapidus* specimen (adult male) (Original photo)

Initial biometric measurements of the individual were performed: carapace width (CW) = 205 mm, carapace length (CL) = 100 mm (Figure 3) and biomass = 537.58 g (Figure 4).

Figure 3. Carapace width and length measurements of the *C. sapidus* individual (Original photos)

Figure 4. Biomass of the *C. sapidus* individual (Original photo)

After one week of acclimation in a fiberglass tank, during which the animal refused to feed (Figure 5 up), it was transferred to a small pump-ashore system (PAS), provided with a natural substrate (rocks and sand) (Figure 5 down).

Figure 5. *C. sapidus* during the first week of acclimation (up) and after transfer to the PAS tank (down) (Original photos)

Land-based pump-ashore systems (PAS) are a type of flow-through system constructed on land adjacent to natural water bodies from which water is diverted or pumped (Jeffery et al., 2015). Flow-through land-based systems are used to rear all sizes of fish and invertebrates in tanks/aquaria, raceways or earth ponds, having the advantage of higher stocking densities due to the greater water exchange compared to recirculating aquaculture systems (RAS) (Jeffery et al., 2015). The water intake to NIMRD's PAS is pumped from the Black Sea and, before entering the aquarium system, it is stored in a covered settlement tank, for sedimentation and reduction of suspended solids. Additional aeration pumps were used to increase the oxygen content of the water.

Salinity and temperature in the PAS were maintained similar to the environment (average salinity 14-15‰). However, when temperature dropped below 20°C and the blue crab started to become less active, a heater was introduced into the tank in order to keep the temperature at a constant value of 22.5°C.

Live mussels were introduced in the PAS and the *C. sapidus* specimen was immediately observed feeding actively with the mollusks. Additionally, small fish (Black Sea shad, sprat, anchovy and whiting) were offered as food, being rapidly consumed.

RESULTS AND DISCUSSIONS

Overall adaptation

The *C. sapidus* specimen did not display any indication of stress in captivity, showing good perspectives for adaptation.

Feeding behavior

During daily observations, the blue crab individual was noticed feeding regularly with live Black Sea mussels - *Mytilus galloprovincialis* Lamarck, 1819 - in the tank, by detaching them from the substrate, crushing the shell with its pincers and picking the flesh (Figure 6). Among all the fish species offered as food, the blue crab preferred Black Sea shad - *Alosa tanaica* (Grimm, 1901), while the least preferred species was whiting - *Merlangius merlangu*s (Linnaeus, 1758). Anchovy - *Engraulis encrasicolus* (Linnaeus, 1758) - and sprat - *Sprattus sprattus* (Linnaeus, 1758) - were also accepted and consumed (Figure 7).

Figure 6. *C. sapidus* feeding on mussels (Original photo)

Figure 7. *C. sapidus* feeding on fish (Original photo)

Temperature and salinity

Blue crab growth is regulated by water temperature. Growth occurs when water temperatures are above 15°C (Brylawski & Miller, 2006). Water temperature above 33°C is lethal (Bauer & Miller, 2010). As the specimen was collected at the beginning of the cold season, water temperature in the PAS tank started to drop, the crab displaying an inactive behavior below 20°C. In order to stimulate growth and prevent torpor throughout the winter (Rome et al., 2005), the water in the tank was permanently heated at 22.5°C. Under such circumstances, the crab resumed its normal behavior, moving constantly and feeding regularly.

Water salinity is also important, but requirements vary by life stage. Generally, the optimum for adult blue crabs is 3-15 PSU (Rome et al., 2005), in line with Black Sea normal salinity. Regarding the pH, the tolerance range is 6-8, with less than 6 being lethal (Rome et al., 2005). Throughout the entire experimental period, salinity varied between 12-15 PSU, while pH was constant around 7.

Growth and molting

Growth and development of the blue crab, as in other crustaceans, consist of a series of larval, juvenile, and adult stages during which a variety of morphological, behavioral, and physiological changes occur. These changes are most dramatic when the animal molts (sheds its rigid exoskeleton) permitting growth and changes in body shape (Costlow & Bookhout, 1959). Sexual maturity is reached after 18 to 20 postlarval molts, at the age of 1 to 1½ years. Males continue to molt and grow after they reach sexual maturity. It is generally accepted that females cease to molt and grow (terminal molt) when they mature and mate (Mangum, 1992).

Blue crab molting (ecdysis) is a spectacular phenomenon. The molt cycle is divided into four main stages: inter-molt - when the exoskeleton is fully elaborated, pre-molt, ecdysis and post-molt (Roer & Dillaman, 2018). The entire molting process lasts about four to eight weeks; within 3 hours after the molt, the initiation of calcification takes place. Over the next 9 to 12 hours, the shell has a leathery feel. The crab then becomes stiff and brittle during the next 12 to 24 hours, and the shell becomes hard after 72 hours (Roer & Dillaman, 2018).

The first signs of ecdysis are represented by a faint outline of the second exoskeleton or new skin forming underneath the old as molting approaches ("white sign"). It usually appears about eight weeks prior to molting. Subsequently, the "pink sign" develops - a pink mark that appears on the crab's backfin, which indicates that it will molt in less than a week. This marks the appearance of the new shell underneath its present hard shell. Finally, the "red sign" develops - indicating a hard crab which will molt in less than two days (Shelley & Lovatelli, 2011).

After 6 months of captivity conditions in NIMRD's PAS, the blue crab specimen started to show the first evidence of ecdysis, namely the "white sign", which indicates that molting would occur in a matter of weeks (Figure 8).

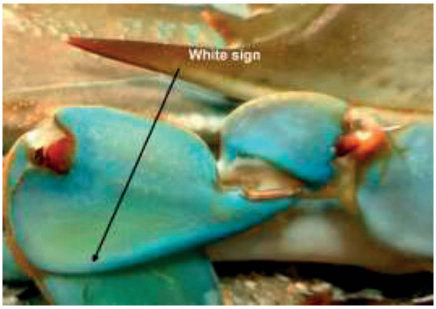

Figure 8. The *C. sapidus* specimen displaying the first mark of ecdysis ("white sign") (Original photo)

Thus, the laboratory conditions under which the blue crab was kept proved to be favorable for a proper development.

Blue crab aquaculture prospects

In recent years, the fishery production of soft-shell crabs has suffered from frequent and significant fluctuations, mainly due to irregular crab fishery landings. About 73.5% of commercial-scale blue crab production originates from wild-caught animals, resulting in large variations in seasonal supply and commercialization of individual specimen size (FAO, 2016). Natural stocks are very vulnerable to climatic and environmental changes and, particularly, to commercial fishing pressure. Regardless of the fishing method, either trawling or trapping, the crab industry dependence of natural stocks is deemed unsustainable in the medium- and long-term (Hungria et al., 2017). Uncontrolled fishing and environmental degradation were reported as the main causes behind the 70% reduction recorded in the *C. sapidus* populations in the Chesapeake Bay (US), once one of the most productive crab fishing areas in North America (Zohar et al., 2008). In 2002, experiments in Chesapeake Bay (USA) were conducted to study the feasibility of blue crab stock enhancement. During 4 years of work, over 290,000 cultured crabs were experimentally released into the bay's nursery habitats, and increased local populations at release sites by 50-250% (Zohar et al., 2008).

The only solution to overcome the dependence on natural stocks for blue crab industry is aquaculture. Commercial crab aquaculture is practiced only in the US and Asia (Hungria et al., 2017). The first experiments to keep crabs in captivity were conducted in America more than 150 years ago, using a system of cages (Perry et al., 1982). In southwestern Asia, the first trials began 50 years later, involving animals in enclosures (Keenan & Blackshaw, 1997). In more recent decades, closed systems using water recirculation (RAS) were developed for crab culture, both in the US and Asia (Hungria et al., 2017). Despite the higher costs associated with the installation and operation of closed systems, they allow greater control over environmental factors, facilitate animal handling and restraint, and minimize mortality losses (Perry et al., 1982).

Large-scale production of larvae and juveniles of *C. sapidus* in captivity is technically possible, although the final survivorship rates and overall results of the process are still unsatisfactory. The main obstacles are the excessive losses due to dietary and nutritional problems, as well as the high rates of cannibalism (Zmora et al., 2005).

At present, the major common points among the main systems currently used for blue crab farming are the confinement of the animals in the pre-molt stage and the requirement that the place used to keep the animals allows an easy monitoring of the ecdysis, as well as fast removal of the recently molted animals. Based on these common features, soft-shell blue crab farming systems can be divided into three groups: *open systems*, carried out in continuous coastal areas such as bays, coves or lagoons; *semi-closed systems*, undertaken in ponds, similar to those used for fish and shrimp farming; and *closed systems*, carried out in sheltered places and under strict control of environmental conditions (dos Santos Tavares et al., 2017). Open and semi-closed systems represent a more traditional form of cultivation and still widely used to produce *C. sapidus* crabs in the US (Oesterling, 1995). However, in recent years, industrial scale swimming crab production has focused on closed production systems (Gaudé & Anderson, 2011).

Open systems

Enclosure farming represents the most primitive and least technical method to obtain soft-shell swimming crabs, among the systems currently used. Initially, the enclosures used in the production of crabs were circular shaped and constructed with vertically arranged stakes or thin plates of wood and nailed together to prevent the crabs from escaping. A more recent development of this traditional enclosure system has been the installation of individual floating boxes or cages to protect the swimming crabs from cannibalism and predator action (dos Santos Tavares et al., 2017).

Production on an industrial scale requires the installation of thousands of floating cages, which end up occupying a large area (Oesterling, 1955). The difficulty of access seems to be the greatest disadvantage, as the management requires the use of boats, generally involving labor discomfort associated with the handling of the cages (Figure 9) (Gaude & Anderson, 2011).

Figure 9. Open system for blue crab farming: routine work of the identification of ecdysis occurrence (after dos Santos Tavares et al., 2017)

Semi-closed systems

Few changes occurred in the production systems until 1950, when a new system was developed. The floating cages were placed inside aquaculture ponds built on land, filled with water pumped from an adjacent brackish or saltwater source and returned to the environment after use (Oesterling, 1995). The ponds currently used are rectangular, with an average area of 100-200 m^2, with the bottom covered with a layer of mud or sand and mud (Figure 10). The animals are kept in small individual cages supported on floating systems, similar to those used in open systems (Oesterling, 1995). The cages are installed in long and narrow floating structures arranged side by side. To ease the management of the cages and the identification of molt, a walkway structure similar to a bridge, usually built of wood, is installed (dos Santos Tavares et al., 2017). Despite advances in water quality control, the system still depends on the existence of salty/brackish water in conditions close to ideal. Moreover, compared with open systems, the ponds involve higher construction and operational activity costs (dos Santos Tavares et al., 2017).

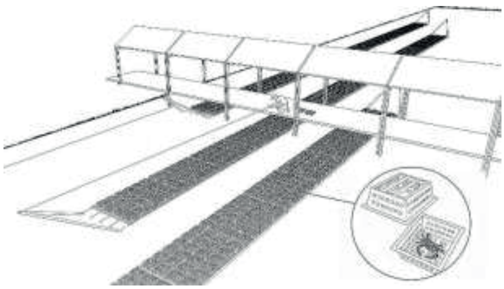

Figure 10. Semi-closed system of the production of soft-shell swimming crabs (after dos Santos Tavares et al., 2017)

Closed systems

The closed system represents the most modern form of blue crab production. The main characteristic is the use of recirculation systems, where water flows through the animal maintenance structures and then through filtration equipment or structures (mechanical, biological and chemical) before returning to the production system (dos Santos Tavares et al., 2017).

The maintenance structures used in closed systems can be communal or individual (cell compartments). Several types of tanks built of wood, concrete, polyethylene or fiberglass can be used as communal structure (Oesterling, 1995). Cell structures, in turn, involve water circulation through overlapping boxes, cages or drawers (Figure 11) (Shelley & Lovatelli, 2011). This type of production system offers several advantages over the traditional methods above mentioned, such as ensuring a greater control over environmental and operational variables; significantly increasing the availability of locations for the installation of production units; allowing the adoption of high stocking densities; and enabling a better monitoring of the occurrence of ecdysis, aside from allowing several forms of automation (Gaude & Anderson, 2011; Shelley & Lovatelli, 2011).

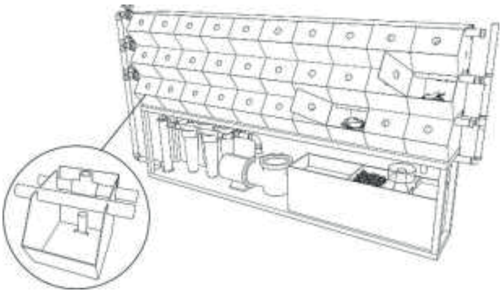

Figure 11. Closed system of cell compartment type for the cultivation of soft-shell swimming crabs (after dos Santos Tavares et al., 2017)

However, closed systems are more complex, requiring more skilled labour and greater investment and production costs (Oesterling, 1995). For instance, there are currently on the market several equipments for recirculating water indoors, including some complete cell systems specific for the production of soft-shell swimming crabs. A system with a capacity for 100 animals can be purchased, directly from specialized online sites, at prices between 10000 and 15000 US dollars (Zhongkehai, 2020).

The animals used in the production of soft-shell swimming crabs are mainly obtained through the capture of individuals in the pre-molt stage in the natural environment and then kept in captivity until molting (dos Santos Tavares et al., 2017). The decrease in the fishing supply of swimming crabs has motivated the research aiming the development of breeding techniques, larviculture and fattening of crabs, under controlled environmental conditions in recent years (Zohar et al., 2008). Large-scale production of larvae and juveniles of *C. sapidus* in captivity is technically possible, although the final survivorship rates and overall results of the process are still to be investigated. The first successful attempt at mass producing juvenile blue crabs was completed in 2005, by researchers at the Center for Biotechnology, University of Maryland Biotechnology Institute (Zmora et al., 2005). Although larvae and juveniles commercial production is still in its incipient stage, blue crab aquaculture is definitely a development to be investigated in the future.

Given the good adaptability to laboratory conditions of the *C. sapidus* specimen, further research of its aquaculture potential at the Romanian Black Sea coast is a promising prospective activity for species diversification.

CONCLUSIONS

The overall behavior of the *C. sapidus* specimen kept in NIMRD's aquaculture laboratory did not indicate signs of stress, showing good perspectives for adaptation. The blue crab individual was observed feeding regularly with live Black Sea mussels. Moreover, small fish (Black Sea shad, anchovy, sprat etc.) were accepted as food. In order to stimulate growth and prevent torpor throughout the winter, the water in the PAS tank was permanently heated at 22.5°C, the crab moving constantly and feeding regularly. As a follow-up, after 6 months under controlled conditions in the PAS tank, the crab started to show the first mark of ecdysis, namely the "white sign", which indicates that molting would occur in a matter of weeks. As such, we can conclude that the laboratory conditions under which the *C. sapidus* specimen was kept proved to be favourable for a proper development. The blue crab individual shall be further monitored, in order to accurately document the molting process.

However, given that soft-shell crab aquaculture is a costly (high prices for equipment, manpower, utilities etc.) and technologically demanding endeavour (temperature control, appropriate feed provision, skilled staff for ecdysis monitoring etc.), a socio-economic and feasibility study should be performed, in order to assess its applicability to the Romanian Black Sea area. This study must take into account that the revenues generated by selling wholesale soft-shell crabs can reach more than 20 US dollars/kg. Moreover, with a proper marketing of the blue crab as a luxury dish in restaurants, it can be advertised as a niche product, which would certainly be accepted and generate income.

Additionally, whereas our research has highlighted the most critical aspects for *C. sapidus* rearing in captivity (temperature, salinity, feeding preferences etc.), the blue crab can be an excellent candidate for the aquarium business (either public, for educational purposes, or for marine aquaria hobbyists). Records indicate that adult caught crabs survived in captivity for more than 1 year, thus, under proper culture conditions, they can be an attractive species.

In the future we intend to create a pilot system to raise blue crabs.

ACKNOWLEDGEMENTS

This study has been carried out with financial support from the NUCLEU INTELMAR Programme, funded by the Romanian Ministry of Education and Research, project no. PN 19260301, and the GFCM, through the Shellfish Aquaculture Demonstrative Center (S-ADC).

The authors would also like to thank Mr. Aurel Amzaru for kindly providing the blue crab specimen.

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