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Research Article

First record of ovigerous *Callinectes sapidus* (Rathbun, 1896) in the Gargano Lagoons (south-west Adriatic Sea)

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Abstract

The blue crab *Callinectes sapidus* (Rathbun, 1896), a native to western Atlantic coasts, is widely recorded in various Mediterranean regions, including the Gargano lagoons in the Adriatic Sea. This study investigated selected biological traits of *Callinectes sapidus* females in the Lesina and Varano lagoons during their breeding migration to more saline waters. In total, 19 individuals of *Callinectes sapidus*, 15 females and four males, were collected in the lagoons near the mouths of the seaward channels. The specimens' Carapace Width ranged from 115 to 230 mm, classifying them as medium to large crabs. In Varano lagoon in April and May 2014, two ovigerous females with egg masses of 63.39 g and 11.65 g respectively were captured. This represents the first record of ovigerous females of *C. sapidus* in this lagoon. The average Gonado-Somatic Index was 2.91±1.82 and 1.65 ± 0.65 for the specimens captured in Varano and Lesina lagoons, respectively. The average Hepato-Somatic Index was 3.88±0.80 and 4.22±0.31 for specimens captured in Varano and Lesina, respectively. Egg diameter ranged between 0.23 and 0.35 mm. Histological analysis of gonads showed stages from III to V, with individuals in emission or post-issuance stages. This study presents evidence of the establishment of *C. sapidus* populations in Varano lagoon in the south-west Adriatic Sea and provides additional information on this species in the Lesina and Varano lagoons.

Key words: blue crab, ovigerous females, GSI, HSI, non-indigenous species, Varano lagoon

Introduction

In its native range, the blue crab *Callinectes sapidus* (Rathbun, 1986) is a euryhaline and eurythermal species that inhabits estuaries and shallow coastal areas to depths of 90 m along much of the east coast of North and South America, including the Gulf of Mexico. In Europe it is considered an IAS (Invasive Alien Species) (Streftaris and Zenetos 2006). The first record in Europe was from Portugal in the early twentieth century and since then it has been widely recorded in Mediterranean coastal waters (Galil 2011), where it was first recorded in Grado lagoon in 1949 (Giordani Soika 1951). The *C. sapidus* population of the Lesina and Varano lagoons has increased since the first record (Florio et al. 2008). The mechanisms potentially responsible for the presence of blue crabs along the Mediterranean coastline include multiple introduction events as well as natural dispersion from primary introduction locations. Transport in ballast waters is considered the most probable initial vector (Nehring 2011).

The life cycle of *C. sapidus* unfolds in a variety of coastal habitats of differing salinity. In low-salinity habitats, juveniles of both sexes predominate. Mature males tend to stay in the upper and middle parts of lagoons, characterised by lower salinities, while adult and ovigerous females prefer environments with higher salinity (Aguilar et al. 2005). In warm months, ovigerous and mature females in the reproductive stage migrate to the sea, travelling long distances (sometimes > 100 km) (Carr et al. 2004). The reproductive pattern of *C. sapidus* in temperate regions is seasonal. In tropical regions, reproduction occurs year-round although there can be seasonal peaks in activity (Pinheiro and Franzoso 2002; Rasheed and Mustaquim 2010). The environmental factors that most influence sexual maturity are temperature and salinity. Salinity is an important

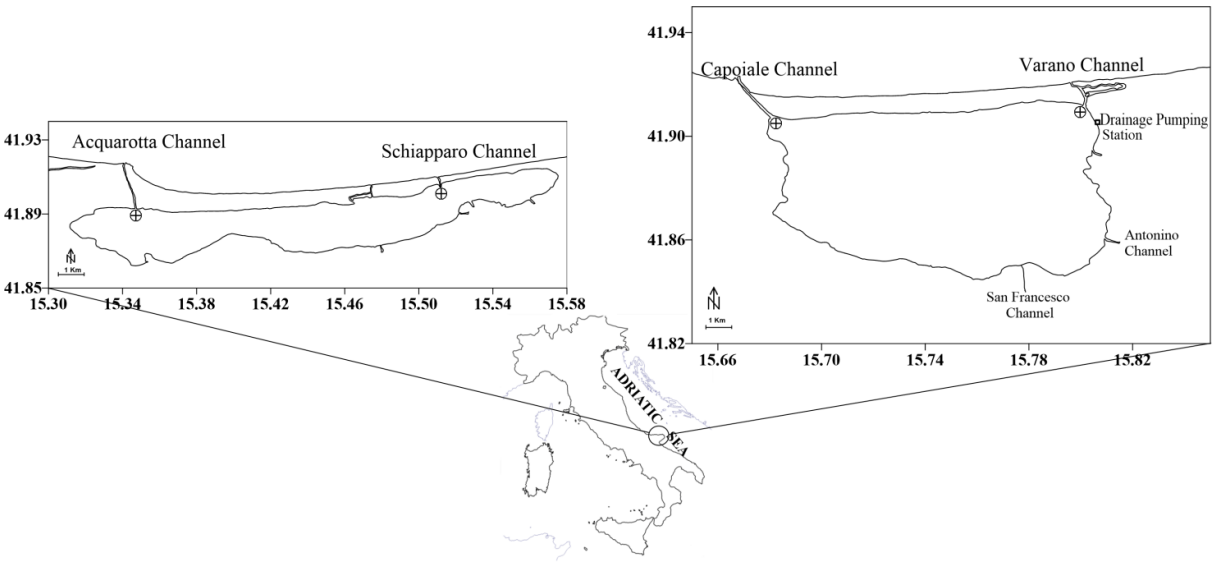

Figure 1. Map of Gargano lagoons. Sampling sites: Acquarotta and Schiapparo channels for Lesina lagoon; Capojale and Varano channels for Varano lagoon.

factor in the hatching of blue crab eggs and the survival of the larvae, which require salinities > 22.

The Lesina and Varano lagoons are a key economic resource, being used for fishing and aquaculture, although these activities may be responsible for invasion by non-indigenous species. Here we report the occurrence and establishment of the blue crab, *Callinectes sapidus* in the Lesina and Varano lagoons. The specific objectives of this study were to verify the presence of ovigerous females of *Callinectes sapidus* during migration to more saline waters and to measure selected biological indices including the hepato-somatic index (HSI) and gonado-somatic index (GSI).

Methods

Study areas

The Lesina and Varano lagoons are located on the north side of the Gargano peninsula on the southern Adriatic coast of Italy. They are connected to the Adriatic Sea by means of artificial channels located on the western and eastern sides of each lagoon (Figure 1). Due to the low tidal range and low exchange with the adjacent coastal area, the estimated water residence time is very long in both systems (Ferrarin et al. 2014; Manini et al. 2005 a, b). In Lesina lagoon, the main freshwater inputs are found along the southern shore and include two rivers, six intermittent streams and two drainage pumping stations (Lauro and Pilla) (D'Adamo et al. 2014). Varano lagoon receives freshwater inputs from groundwater springs on the south-western side, while urban wastewaters and agricultural drainage are discharged into the lagoon on the south eastern side (Specchiulli et al. 2008). The two lagoons are relatively small and shallow, and are subject to wide variations in water temperature, salinity and nutrients (Roselli et al. 2009, 2013; Specchiulli et al. 2008).

Water temperature in Lesina lagoon ranges from 10°C in winter to 27°C in summer, and salinity from 11 to 29 psu. Water temperature in Varano lagoon ranges from 10°C in winter to 29°C in summer and salinity from 24 to 29 (Table 1). Aquaculture in Varano lagoon involves mainly Mediterranean mussels *Mytilus galloprovincialis* Lamark, 1819, clams (*Ruditapes* spp.) and Japanese oysters *Crassostrea gigas* (Thunberg, 1793) (Cilenti 2007). In Lesina lagoon there are three large aquaculture plants near the shore on the western and eastern sides.

Sampling methods

Specimens of *Callinectes sapidus* were caught in gillnets and traps by local fishermen in four sites near the channels connecting the lagoons with the sea (Figure 1). These areas were chosen due

Table 1. Selected hydrological and physiographic characteristics of Lesina and Varano lagoons.

	Lesina lagoon	Varano lagoon
Location	41.88°N; 15.35°E	41.51°N; 15.47°E
Catchment land use	National Park, urban, agricultural and fisheries	National Park, urban, agricultural and mussel farming
Catchment area (km^2)	400	357
Surface area (km^2)	51	65
Mean depth (m)	0.8	4.0
Maximal depth (m)	1.5	5.8
Residence time (days)	50–250	1032
Total length from east to west (km)	22.4	11.0
Maximum width (km)	3.4	7.2
Dominant wind direction	North-northwest	North-northwest
Temperature range (°C)	10–27	10–29
Salinity range	11–29	24–29

Table 2. Carapace widths (CW), carapace length (CL), and wet weights (WW) of male and female *Callinectes sapidus* from Lesina and Varano lagoons.

		CW (mm)			CL (mm)			WW (g)			
		Mean±SD	min	max	Mean±SD	min	max	Mean±SD	min	max	N° Ind.
Lesina	male	161.7±47.5	115	210	68.7±15.0	53	83	275.5±190.1	104.9	480.5	3
	female	207.8±15	190	230	82.2±3.3	77	85	294.6±47.1	246.9	352	5
Varano	male	115	-	-	57	-	-	115.07	-	-	1
	female	199.4±10.5	180	214	82.8±5.9	76	95	304.4±43.5	219.2	369	10

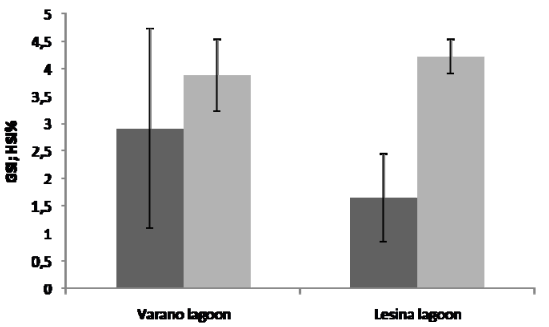

Figure 2. Mean (±SD) gonado-somatic (dark grey bar) and hepato-somatic (light grey bar) indices for female blue crabs from Varano (n = 10) and Lesina (n = 5) lagoons.

to the greater likelihood of collecting adults, and ovigerous females in particular. The sampling period was from October 2013 to July 2014.

In the laboratory, the specimens' sex was determined with reference to the shape of the abdomen apron. Carapace width (CW to 0.1 mm), carapace length (CL to 0.1 mm), and wet weight (WW to 0.1 g) were recorded for each specimen. For females, the hepatopancreas, gonads, and egg masses (if present) were removed and their wet weight (to 0.1 g) recorded. The hepato-somatic index (HSI) and gonado-somatic index (GSI) were calculated as:

GSI = Gonad wet weight × 100 / crab body wet weight

HSI = Hepatopancreas wet weight × 100 / crab body wet weight

After the egg masses were carefully removed from the pleopods and weighed, their approximate age was assigned on the basis of colour (Bland and Amerson 1974). The extracted eggs were measured (length and width to 0.01 mm) with an ocular micrometer using a binocular microscope and the degree of ripeness was estimated. The morphological characteristics of the ovaries were determined before fixation (Hard 1942). Small portions of the gonads of mature females, captured in spring, were fixed in 10% formalin and embedded in paraffin; thin sections were obtained by microtome and placed on slides. Ten slides for each gonad were randomly selected and stained with Mayer's haemalum and eosin (five slides) and Masson trichrome (five slides). The slides were examined microscopically and each was assigned a reproductive stage (based on Hard 1942).

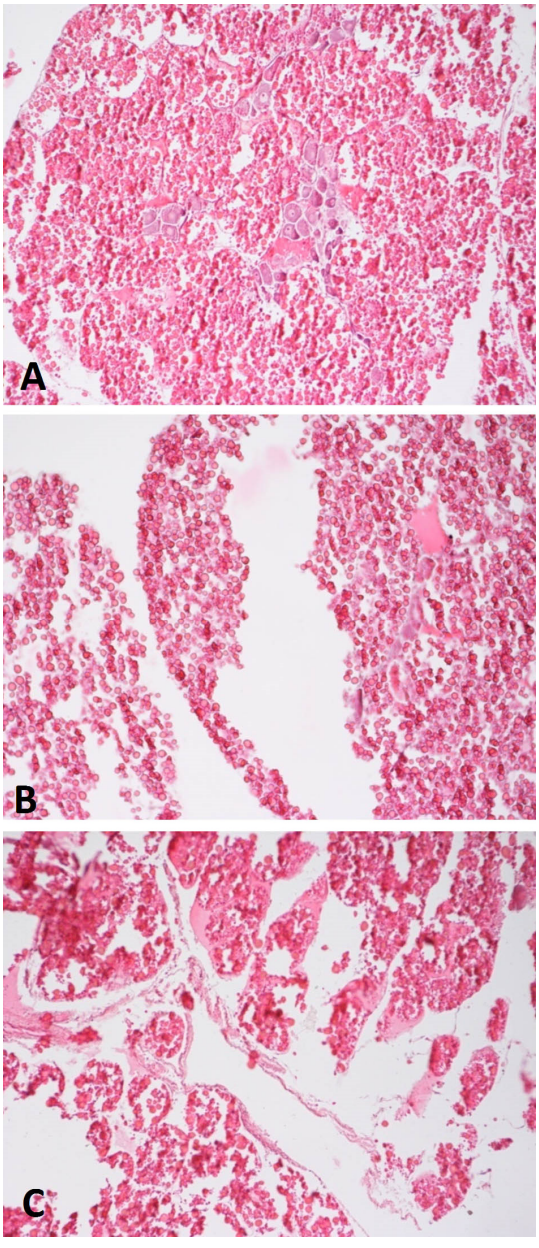

Figure 3. Histological sections of gonads of mature and ovigerous female blue crabs. A- specimen with highest egg mass value; B- specimen with highest gonado-somatic index; C- specimen with lowest egg mass value (Photomicrographs by L. Cilenti and A. Fabbrocini).

Results

During the sampling period, blue crab specimens were collected near the mouths of the Acquarotta and Varano channels (Figure 1). We collected 19 individuals, including 15 females (five in Lesina lagoon and 10 in Varano lagoon) and four males (three near the Acquarotta channel and one near the Varano channel). Twelve females and one male were caught in October: 7 females near the Varano channel, five females and one male near the Acquarotta channel. In April-May, three females (two with external egg masses) and one male were caught near the Varano channel, and two males were caught near the Acquarotta channel. The size of the males ranged between 115 and 210 mm CW and the females between 180 and 230 mm CW (Table 2). The two ovigerous females were not included in the somatic indices analysis. The hepato-somatic and gonado-somatic indices were calculated separately for each lagoon. The average GSI of individuals captured in Varano lagoon was 2.91 compared to 1.65 in Lesina lagoon (Figure 2); however, the two means did not differ significantly (*t*-test; $P > 0.05$). The mean HSI of individuals caught in Lesina lagoon was 4.22 compared to 3.88 in Varano lagoon. The two means did not differ significantly (t-test; $P > 0.05$).

Macroscopic evaluation of gonads

The ovaries of *C. sapidus* individuals captured in October consisted of pale orange lobules (Stages III-IV). In contrast, the ovaries of crabs caught in spring were orange to dark orange and occupied most of the abdominal cavity (stage IV). The ovary of the specimen with the highest ovarian index, captured in spring, appeared to have a looser consistency (stage V). The egg masses of the ovigerous females caught in April and May weighed 63.4 g and 11.4 g, respectively. The yellow-orange colour of the eggs indicates that extrusion of the egg masses had occurred one to seven days previously (Bland and Amerson 1974). Two kinds of eggs were observed: rounded and translucent or ellipsoidal and dark-brown, in both cases firmly attached to the pleopods. The eggs' major axis ranged from 0.23 to 0.35 mm, with modes of 0.25 and 0.31 mm.

Histology

Based on histological analysis, the specimen with higher egg masses was found to have a compact gonad with few but prominent atretic oocytes (stages III-IV). The specimen with the highest GSI was found to have large yolk globules throughout the cytoplasm (stage IV). The specimen with lower egg masses was found to have compact follicular cells and large yolk globules throughout the cytoplasm (stage V) (Figure 3).

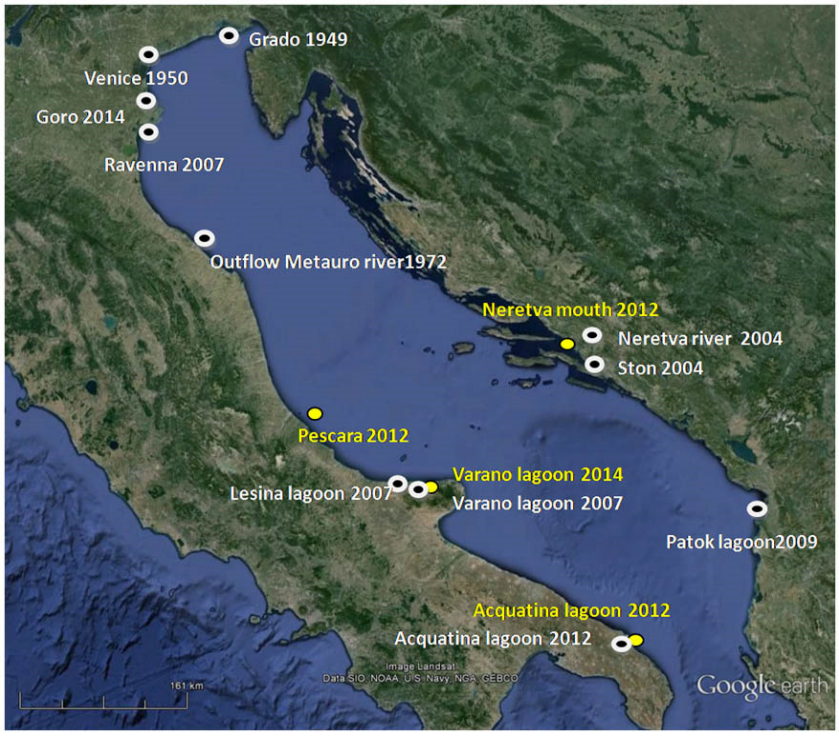

Figure 4. Locations and years (white spots with black centres) of first records of blue crab in the Adriatic Sea and sites where ovigerous females have been located (yellow spots). Map: Google Earth (modified).

Discussion

The marine environments most subject to invasion are coastal and estuarine regions, with decapod crustaceans being commonly reported as non-native invertebrates (Ruiz et al. 2000). *Callinectes sapidus* is a large decapod first recorded in the North Adriatic Sea in 1949, then spreading southwards along both coasts (Froglia 1972; Scaravelli and Mordenti 2007; Florio et al. 2008; Onofri et al. 2008; Bequiraj et al. 2010; Dulčić et al. 2011; Castriota et al. 2012; Giansante et al. 2012; Mancinelli et al. 2013; Stasolla et al. 2014; Manfrin et al. 2015) (Figure 4). The few ovigerous females recorded have been found in the middle and southern Adriatic Sea, but not in the north Adriatic Sea. This study describes for the first time the presence of ovigerous females in Varano lagoon. The specimens captured in both lagoons were of medium and large size (based on Harding 2003). The occurrence of blue crabs in the Lesina and Varano lagoons might be the result of aquaculture or natural migration from the surrounding marine areas. Since its first detection in 2007 (Florio et al. 2008), local fishermen have caught increasing quantities of adult *C. sapidus* specimens (≥120 mm large) and have now begun to market them as a product in their own right.

The morphometric analysis showed that the specimens caught in this study were on average larger than those caught in Croatia (Dulčić et al. 2011). In this study the greater size and higher proportion of females was area-dependent because we chose sampling sites near tidal channels characterised by high salinity. Indeed, the predominance of adult and ovigerous females near the mouths of the seaward channels of the Varano and Lesina lagoons characterises the sampling sites as spawning areas, to which mature and ovigerous females move in search of higher salinity waters as this favours gonad maturation during the reproduction period.

The gonado-somatic index (GSI) is a good indicator of ovarian development in crustaceans (Wu et al. 2007). During ovarian maturation in Portunidae crabs, the GSI increases while the HSI decreases (Liu et al. 2014). In this study we caught only adults in the reproductive period, and the GSI and HSI were comparable with those reported for other species belonging to the Portunidae family (Liu et al. 2014). The capture of the two ovigerous females in April and May suggests that egg extrusion occurs during spring but samples were not available for other periods. The blue crabs in Lesina lagoon do not, as yet, appear to complete the maturation cycle because

local fishermen claim to have never caught ovigerous females.

The eggs of *C. sapidus* are usually about 0.25 mm in diameter (Millikin 1984). In this study, egg diameters varied, possibly due to eggs at more than one stage of development being present at the same time. Their colour changes due to absorption of the yellow yolk and development of dark pigment in the eggs and the body of the embryos, as described by Guillory and Hein (1997). The presence of mature and egg-bearing females suggests a breeding population may have become established, especially as a single female crab can produce 2 to 8 million eggs per spawning event (Jivoff et al. 2007). Our results suggest *C. sapidus* has adapted to the Lesina and Varano lagoons, particularly the latter, which has higher average salinity. The Lesina and Varano lagoons may represent a suitable habitat for the establishment of *C. sapidus* but the potential effect of this on the existing community of the lagoons is largely unknown. Given the trophic position of the blue crab, the potential impact on native crab assemblages is likely to be negative (Carrozzo et al. 2014), but this remains to be studied, assuming the blue crab population continues to increase.

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