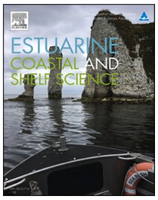
Estuarine, Coastal and Shelf Science 296 (2024) 108593

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss



Early life stages of the invasive Atlantic blue crab *Callinectes sapidus* in the Western Mediterranean Sea

A. Gil-Fernández [a,*], M. Rodilla [a], P. Prado [b,c], S. Falco [a]

[a] *Instituto de Investigación para la Gestión Integrada de las Zonas Costeras (IGIC), Universitat Politècnica de València, Carrer del Paranimf, 1, 46730 Gandia, Spain*
[b] *Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Carretera C-59, Km. 12, 1 [BV-1424, Km. 0] - Torre Marimon, 08140 Caldes de Montbui, Spain*
[c] *Institute of Environment and Marine Science Research (IMEDMAR-UCV), Universidad Católica de Valencia SVM, C/Explanada del Puerto S/n, 03710 Calpe, Alicante, Spain*

ARTICLE INFO

Keywords:
Invasive species
Zoeae
Megalopae
Larval settlement
Plankton surveys
Collectors

ABSTRACT

The early life cycle of the Atlantic blue crab *Callinectes sapidus* includes seven zoeal stages that develop in coastal waters, and a later megalopal stage that colonizes estuaries. Although these stages have been largely studied in native ecosystems, very little is known about larval patterns in smaller-scale, invaded, estuarine environments such as those available in the Mediterranean Sea. The general aim of this study is to assess the distribution and abundance of early life stages of *C. sapidus* in the communication channels of the Albufera lagoon with the Mediterranean Sea, and in adjacent coastal waters. Different sampling methods were employed to target each life stage. (1) For *zoeae*, planktonic tows were conducted using a 300 μm manta net along 200 m transects parallel to the coastline at distances of 300, 1000 and 3000 m from the shore. (2) A small and manageable passive collector model was used to capture *megalopae* and juveniles in estuarine areas. This system was first tested from 2020 to 2021 in a single channel, with a weekly sampling frequency, and two more channels were added in 2022. Additionally, intensive daily sampling was conducted in one of the channels from September to November 2022 during the settlement peak. Results showed that *zoeae* of *C. sapidus* were more abundant in August 2021, at 1000 m from the coast, and appear to have a disjoint temporal distribution with *zoeae* of other crab species. *Megalopae* and juveniles were more abundant from September to November at all sampling sites and times. In 2022, collectors featured a dominance of *megalopae* at two of the sites (ca. 73%), while the site with the lowest salinity showed a dominance of juveniles (84%). For *megalopae*, comparative results from the intensive and the regular sampling campaigns showed that time of residence within collectors is just a single day. For juveniles, higher numbers were detected with increased deployment time, suggesting a need for enhanced habitat complexity.

1. Introduction

Invasive species are an increasing problem in an ever more interconnected world. Recent data shows that this issue is even more pressing in the Mediterranean Sea, where the number of alien species is growing steadily (Zenetos et al., 2022). Globally, Arthropoda and ballast waters are the most common combination of phylum and vector achieving successful invasion (Bailey et al., 2020). The Atlantic blue crab *Callinectes sapidus* (Rathbun, 1896) falls into both categories and displays an array of characteristics that makes it a successful invader in estuarine and coastal environments. These include high fecundity (up to 2 million eggs per brood), aggressive behavior, dietary plasticity, and euryhaline and eurythermal tolerances (Millikin and Williams, 1984; Nehring, 2011). Unsurprisingly, it has been pointed as one of the most damaging invasive alien species in the Mediterranean Sea (Streftaris and Zenetos, 2006).

The native range of *C. sapidus* comprises a broad area from Northern Argentina to Nova Scotia, Canada (Millikin and Williams, 1984), but permanent population establishment from Masachussets northward did not occur until the 2010s due to global warming (Johnson, 2015). Currently, the spread of the species covers an extensive array of regions, but the invasion has been particularly successful in the Mediterranean Sea (Ragkousis et al., 2023). The presence of *C. sapidus* was first confirmed in 1949 in the Northern Adriatic Sea (Nehring, 2011), and then across the Mediterranean Sea in the following decades, although generally remained in low numbers until the 2000s, when it became

---

* Corresponding author.
E-mail address: algifer1@upv.edu.es (A. Gil-Fernández).

https://doi.org/10.1016/j.ecss.2023.108593
Received 16 June 2023; Received in revised form 31 October 2023; Accepted 7 December 2023

established in Albania, Italy and Greece (Nehring, 2011). The first record in the Spanish Mediterranean coast occurred in 2012 near the Tancada lagoon in the Ebro Delta (Castejón and Guerao, 2013). It promptly expanded southward reaching the Albufera coastal lagoon (ca. 170 km) in 2014, the Mar Menor coastal lagoon (ca. 360 km) in 2015, crossed the Strait of Gibraltar and established in the Atlantic coast of Portugal by 2016 (Casalduero et al., 2016; Encarnação et al., 2021; González-Ortegón et al., 2022; Mancinelli et al., 2017; Morais et al., 2019).

The life cycle of *C. sapidus* involves the use of several distinct environments. Mating generally occurs in inner parts of estuaries, females then to migrate to the mouth of the estuary and adjacent coastal waters for hatching (Epifanio, 2019). This reproductive migration is triggered by *zoeae* requirements of a minimum salinity of 18 to achieve a successful hatch (Millikin and Williams, 1984). Larval dispersion is mainly driven by surface water circulation. Vertical distribution of larvae is influenced not only by their developmental stage, but also by environmental factors such as salinity, pressure and light (Epifanio, 2019). The larval development includes seven zoea stages, followed by a single megalopal stage that returns to estuarine areas. On average, the duration of the zoeal stage ranges from 30 to 50 days, and the duration of the megalopal stage is estimated to last for another 10–15 days, which can be altered by temperature and salinity (Costlow, 1967; Costlow and Bookhout, 1959). Chemical cues may also play a role in the duration of the megalopal stage, and signalize the pathway for entering estuaries (Forward et al., 1997, Forward et al., 2003). Metamorphosis into juveniles usually occurs in nursery habitats like submerged vegetation, and from there they start dispersing upstream (Epifanio, 2019).

Although significant information on the life cycle of *C. sapidus* is available from its native range, the Mediterranean Sea offers a contrasting environment. The Mid-Atlantic Bight (MAB), which extends from Massachusetts to North Carolina, is one of the native regions where the life cycle of *C. sapidus* has been extensively studied (Epifanio et al., 1984; Jones and Epifanio, 1995; Tilburg et al., 2009). In this area, important estuaries such as the Chesapeake Bay or the Delaware Bay are classified as partially mixed and vertically mixed respectively, whereas Mediterranean estuaries are classified as salt-wedge estuaries due to the small tidal range (Biggs, 1978; Ibañez et al., 1997). In Mediterranean estuaries the combination of strong and clearly marked haloclines, smaller scale, and higher average seawater salinity creates a system where brackish waters are confined to very small areas (Ibañez et al., 1997; Valjarević et al., 2020). Therefore, *C. sapidus* overcomes drastic variations in salinity (freshwater to seawater) in the spatial scale of hundreds of meters, particularly during reproductive migration or larval ingress.

Standardized passive collectors, sometimes referred as Artificial Substrate Units (ASUs), were employed in several Atlantic studies as a method to capture early life stages of *C. sapidus* (Bishop et al., 2010; Forward et al., 2004; Metcalf et al., 1995). The most common design consists of a plastic cylinder (30–40 cm long) covered with *hog's air*, an air-conditioning filter material. Given that Western Mediterranean estuaries are characterized by shallow depths and heavy stratification, the use of large collectors might obscure the association between physicochemical variables (i.e., salinity) and the number of recovered individuals. Alternative small collectors previously obtained good results in studies for cryptofauna and meiofauna (Costa et al., 2016; Myers and Southgate, 1980).

In this context, several sampling campaigns were carried out to obtain insights about the distribution of early life stages of *C. sapidus* in an invaded Mediterranean region. Specific targeted questions were: 1) what is the spatio-temporal distribution of *zoeae* of *C. sapidus* in coastal waters adjacent to the Albufera lagoon? 2) can small collectors efficiently capture early benthic life stages of *C. sapidus* in Mediterranean estuarine areas? and 3) what is the settlement and abundance pattern of early benthic life stages of *C. sapidus* in Mediterranean estuarine areas?

2. Materials and methods

2.1. Study area

The Gulf of Valencia stretches 400 km from the southern edge of the Ebro shelf to the promontory of Cap La Nao, forming a Tertiary basin in the northwestern part of the Mediterranean Sea. The coastline is dominated by beaches, usually exposed and with fine or medium grain size sand. Coastal hydrodynamics is characterized by a low tidal range (average 0.24 m), a daily tidal regime, and southward sediment transport along the coastline (Morales, 2018; *Puertos del Estado, 2022*; Ribó et al., 2015). The seabed is composed of well-sorted fine sands.

The Albufera of Valencia Natural Park, including the Albufera coastal lagoon and surrounding wetlands, is a protected environment located ca. 10 km south of the city of Valencia, close to the middle of the Gulf of Valencia. The lagoon occupies an area of 28 km^2, with a 0.5–2 m depth range, and the surrounding wetlands occupy 223 km^2. The area is connected to the Mediterranean Sea by three main channels ("golas"), equipped with floodgates. North to South these channels are Gola de Pujol (Pu), Gola de Perellonet (Pt), and Gola de Perelló (P) (Morales, 2018) (Fig. 1).

Each channel exhibits distinctive characteristics, and their floodgates can be managed independently from the others. The Gola de Pujol and Gola de Perellonet are primarily natural channels, whereas the Gola de Perelló serves as a recreational and commercial fishing harbor. The necessary freshwater levels for rice cultivation in the wetlands surrounding the Albufera lagoon are managed through the floodgates. This regulation results in decreased salinity in the coastal waters adjacent to the lagoon when irrigation water is released into the Mediterranean Sea.

The Albufera Natural Park is facing significant anthropogenic pressure because of its proximity to a densely populated area. The lagoon features a hypereutrophic state due to the excessive inflow of organic materials and inorganic nutrients, mainly nitrogen and phosphorus (Soria, 2006).

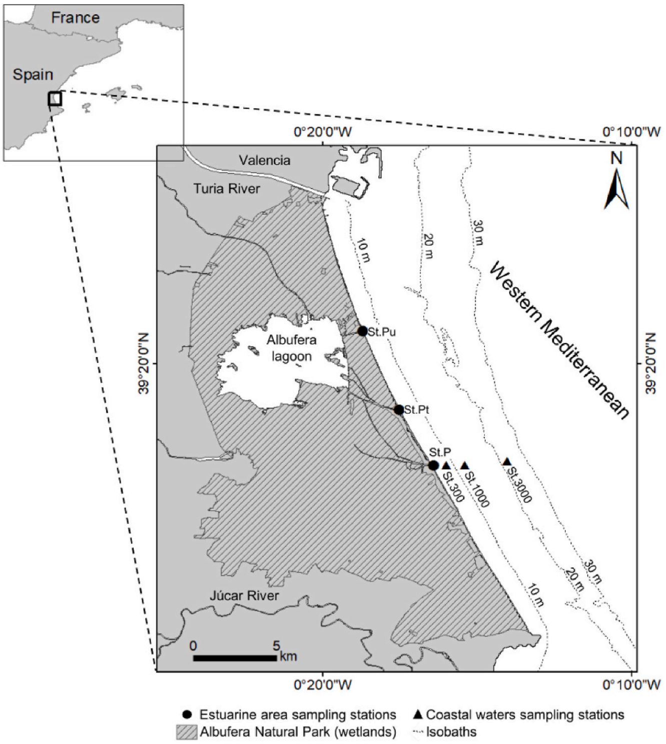


Fig. 1. Study area overview showing the location of sampling sites. Estuarine waters (circles) were sampled with passive collectors and coastal waters (triangles) through planktonic tows.

The local fisher's guild from El Palmar operating in the Albufera lagoon captured the first *C. sapidus* in the fall of 2014. Landings reached a maximum of 29 t in 2021, and more than 15 t in 2022.

2.2. Coastal area sampling

From May to November 2021, ten zooplankton samplings were conducted to collect *zoeae* of *C. sapidus*. At first, we aimed for a sampling frequency of 15–20 d, however, due to logistical problems and adverse weather conditions, it ranged from 15 to 37 d. Samples were obtained by trawling a manta net (0.28 m^2 entrance area, 300 μm mesh size) in transects parallel to the coastline (C1 sampling campaign). Each sampling event comprised three transects located at 300 (6 m depth), 1000 (13 m depth), and 3000 m (22 m depth) from the shore (St.300, St.1000, and St.3000 respectively) (Fig. 1). Each transect consisted of a 200 m track parallel to the coastline covered at 3 knots, filtering an approximate volume of 56 m^3 from the layer located between 0.6 and 1 m deep, where the largest number of *zoeae* are concentrated (Epifanio, 1988). All transects were carried out on calm weather days (Beaufort scale 2 or lower), from 9 to 11am. Transect depth was adjusted using buoys attached to the net frame and subsequently verified with a dive computer affixed to the same frame. Salinity and temperature were measured using a WTW 3320 probe at the northernmost point of each transect at 1 m depth.

At the end of each transect the manta net was thoroughly rinsed with seawater from the outside, using a low-pressure water pump to accumulate all the material into the cod end. The cod end was then detached, the sample preserved in 80% ethanol, and a new cod end replaced the used one. In the laboratory all materials were extracted from the cod end by carefully rinsing it and preserved in ethanol 80%. Samples were processed in two steps. First, *zoeae* were separated using a stereoscope (Leica Wild M8), then examined using an inverted microscope (Leica DM IL LED). *Zoeae* were identified by their morphological characteristics (Castellani and Edwards, 2017; Costlow and Bookhout, 1959; Mantelatto et al., 2014).

In addition, genetic sequencing was used to confirm the species identity of multiple specimens belonging to different samples. Mitochondrial DNA from the enzyme cytochrome oxidase subunit 1 (COI) was isolated from whole *zoeae* using a modified Puregene method (Gentra Systems) at the University of Regensburg, Germany (Schubart et al., 2022). In cases of suboptimal COI sequencing results, 16 S rRNA sequencing was used as an alternative. PCR products were sequenced by Macrogen Europe, and the nucleotide sequences were tested using Blast for species identification (Supplementary Material S1-S4).

2.3. Estuarine area sampling

Nylon mesh "*shower puffs*" of 12–13 cm in diameter were used as collectors to sample *megalopae* and juveniles of *C. sapidus*. Each collector is composed of a single nylon mesh and a weighted retrieval line. Final mesh position was 10 cm above the substrate. Collectors were located at the mouths of the three connecting channels between the Albufera lagoon and the Mediterranean Sea, at the Pujol (St. Pu, 2 m depth), Perellonet (St. Pt, 1.5 m depth) and Perelló (St. P, 1.5 m depth) sites (Fig. 1). Collectors were spaced between 1 and 2 m, and 4–5 replicas per site.

Three sampling campaigns were carried out in the estuarine (E) area. The E1 sampling campaign was conducted weekly (7-d) between August 13, 2020 and September 03, 2021 and took place only at St. P using four collectors. In the E2 sampling campaign, St. Pt and St. Pu were added, and five collectors were simultaneously placed at each site. The E2 also had a weekly (7-d) sampling frequency and ranged from September 10, 2021 to December 18, 2022. The E3 sampling campaign was aimed at capturing the peak of *megalopae* and juveniles arrival to estuarine areas and was therefore shorter and more intensive, with a daily (1-d) sampling frequency between September 22, 2022 to November 22, 2022 at St. P (Table 1). The E3 sampling campaign also added a sixth collector deployed 10 cm under the water surface for additional data about vertical distribution of *megalopae* inside the estuary. The surface collector was deployed using the same system previously described for bottom collectors but with a shorter line, directly above one of the bottom collectors. E3 bottom collectors were located adjacent to E2 collectors. Tide related data during E3 campaign was obtained from the "Valencia 3" tide gauge installed in the Port of Valencia (*Puertos del Estado, 2022*), ca. 17 km northwards of St. P. To evaluate the effectiveness of the collectors in capturing the early life stages of *C. sapidus* over time, we compared the data collected from the weekly (E2) and daily (E3) sampling campaigns. Additionally, we generated a new weekly data series from E3 data to refer to the 'theoretical weekly accumulation' to further compare the efficiency of replacement periods. The theoretical weekly accumulation is a dummy value obtained from the addition of average daily values up to a whole week. From September 25, 2022 to November 22, 2022 a conductivity/salinity logger (HOBO U24-002-C) was also deployed at the same depth as the bottom collectors, with a 30-min logging interval. At each sampling, salinity and temperature were measured close to the collectors using a WTW 3320 probe.

Collectors were replaced by clean ones on every sampling occasion. In the following hours, the fauna present in the collectors was extracted using a standardized procedure, modified from van Montfrans et al. (1990). Briefly, collectors were soaked in 3 L of freshwater for 20 min, then rinsed with an additional 3 L of freshwater three consecutive times. Water was decanted through a 300 μm mesh-sieve, and *megalopae* and juveniles were counted and classified. This process was 98% effective for *megalopae* and 100% for juveniles as determined by an analysis of the cumulative number of individuals found in six successive 3 L rinses per collector (N = 5). *Megalopae* and juveniles were identified by morphological characteristics (Costlow and Bookhout, 1959; Pessani et al., 2004).

Using the same process than in the coastal area sampling, genetic sequencing was used to confirm the identification of multiple specimens belonging to different samples, encompassing *megalopae* and juveniles (Supplementary Material S1-S4).

2.4. Laboratory trial with megalopae

To gain further insights into the time to metamorphosis from *megalopae* to juveniles, an experiment was conducted with individuals obtained during the E3 sampling campaign. A total of 67 *megalopae* were kept alive in six plastic trays with aerated seawater. The plastic trays were covered with transparent glass to prevent evaporation and allow a circadian rhythm. *Megalopae* were fed *ad libitum* with small pieces of commercial fish food until all of them turned into juveniles. *Megalopae* were monitored daily and maintained at a salinity of 36 and 20–23 °C, to simulate conditions in the natural environment.

2.5. Data analysis

Data are described using the average and the standard deviation as a measure of data dispersion. The Wilkoxon signed rank test (R 4.2.3) with $\alpha = 5\%$ was used to assess similarities in the average number of colonizers (i.e., *megalopae* and juveniles) in E3 (daily) and E2 (weekly) sampling campaigns, and to compare the theoretical weekly accumulation against real weekly rates. For E3, only data from dates overlapping with E2 were included in the analysis.

Mann-Whitney W test (STATGRAPHICS Centurion 18 Version 18.1.13) with $\alpha = 5\%$ was used to assess the magnitude of differences between bottom and surface collectors.

Table 1
Data summary (minimum, maximum and average values) of the different sampling campaigns targeting early life stages of *Callinectes sapidus*. The coastal campaign targeted zoeae and megalopae, whereas estuarine campaigns targeted megalopae and juveniles. Site name (St) and dates, in dd/mm/yy format, are indicated in brackets. Dash means not applicable.

Sampling campaign		E1-Estuarine 13/08/20-03/09/21	C1-Coastal 01/05/21–22/11/21	E2-Estuarine 10/09/21-18/12/22	E3-Estuarine 22/09/22-22/11/22
Salinity	Min (St, date)	0.6 (St. P, 03/09/21)	33 (St.300, 26/08/21)	0.5 (St. P, 25/03/22)	0.6 (St. P, 27/09&08/10/22)
	Max (St, date)	36.6 (St. P, 06/08/21)	38 (St.3000, 26/09/21)	38.3 (St. Pt, 19/11/21)	38.2 (St. P, 16/11/22)
	Average	15.1	37.3	12.5	24.3
Temp [°C]	Min (St, date)	5.2 (St. P, 12/01/21)	16.7 (St.300, 01/05/21)	8 (St. P, 20/01/22)	16 (St. P, 22/11/22)
	Max (St, date)	29.1 (St. P, 06/08/21)	27.5 (St.300, 26/08/21)	30.1 (St. Pt, 26/07/22)	26.6 (St. P, 18/10/22)
	Average	20.0	23.0	20.2	22.1
Collectors [n]		4	–	5	5
Sampling frequency [d]		7	*15-37	7	1
Zoeae [n • m^{-3}]	Min (St, date)	–	0	–	–
	Max (St, date)	–	3.64 (St.1000, 04/08/21)	–	–
	Average	–	0.43	–	–
Megalopae [n • $collector^{-1}$]	Min (St, date)	0	0	0	0
	Max (St, date)	141 (St. P, 25/09/20)	0	97 (St. P, 18/10/22)	150 (St. P, 19/10/22)
	Average	1.8	0	5.4	9.1
Juveniles [n • $collector^{-1}$]	Min (St, date)	0	–	0	0
	Max (St, date)	19 (St. P, 14/10/20)	–	25 (St. P, 25/10/22)	3 (St. P, 20&23/10/22)
	Average	1.2	–	1.4	0.2

3. Results

3.1. Coastal area sampling

During the C1 sampling campaign, a total of 721 *zoeae* of *C. sapidus* were collected and identified. The density of *zoeae* ranged from 0 to 3.64 *zoeae* • m^{-3} (Table 1) and displayed a consistent pattern of variation across sampling times and shore distances.

Temporally, no *zoeae* were found in May 2021, but a gradual increase in density was observed from June to August 2021. The peak abundance was detected on the August 4, 2021 at both St.300 and St.1000, and on the August 26, 2021 at St.3000. On the August 4, 2021, the survey recorded 54.5% of the total *zoeae*, while previous and subsequent samplings accounted for 14.8% and 9.1%, respectively, hence framing the period between 18th of July and the August 26, 2021 as the one of greatest abundance. From late August to October 2021, a general decreasing trend in *zoeae* density was observed across all sites, with no *zoeae* found in November (Fig. 2).

The vast majority of the collected *zoeae* of *C. sapidus* were at the stage 1 (>95%), and only a small number of them where at the stage 2 (<5%). No further zoeal stages were found. The St.1000 had the highest abundance of *zoeae*, accounting for 66.3% of the total *zoeae*, followed by the St.300 with 33.7%, while the St.3000 exhibited a negligible representation (<0.1%) of the total *zoeae*. The St.1000 exhibited the highest *zoeae* density in 6 out of 7 samplings where *zoeae* were present.

The relative abundance of *zoeae* of *C. sapidus* in relation to the total number of *zoeae* reached a maximum of 98.9% on the August 4, 2021 (Fig. 3). The total number of *zoeae* of other decapod species was 2011, meaning 26.4% of the total number of *zoeae* collected across the entire C1 sampling campaign were *C. sapidus*. Other species identified by genetic sequencing included *Achelous hastatus* (Linnaeus, 1767)*, Herbstia condyliata* (Fabricius, 1787)*, Liocarcinus vernalis* (Risso, 1827)*, Maja crispata* (Risso, 1827)*, Pachygrapsus marmoratus* (Fabricius, 1787)*, Portumnus latipes* (Pennant, 1777) and *Sirpus zariquieyi* (Gordon, 1953). Additionally, although no *megalopae* of *C. sapidus* were found in the coastal waters during the C1 sampling campaign (Table 1), 13 *megalopae* of *P. marmoratus* were collected.

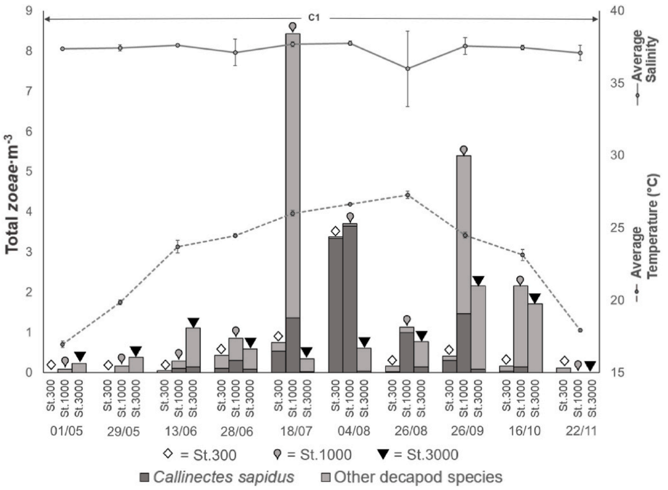


Fig. 2. Density of zoeae per m^3 at each sampling site for *C. sapidus* (dark grey) and other decapods (light grey) in 2021. Sampling stations were located at 300, 1000 and 3000 m from the coast (St.300, St.1000 and St.3000 respectively). Right axis: average ± SD salinity and temperature (°C) at each sampling time. Data from the coastal area sampling campaign (C1).

3.2. Estuarine area sampling

A total of 1777 *megalopae* and 586 juveniles of *C. sapidus* were collected at St. P along the 2 years and 4 months period that included the E1 and E2 sampling campaigns (Table 1; Fig. 4). From August 2020 to December 2022, the majority of the *megalopae* of each year (average 84.2 ± 8.7 %) were consistently collected during the months of September and October, whereas for juveniles the collection period was October and November (average 64.4 ± 29%). In 2020, September was

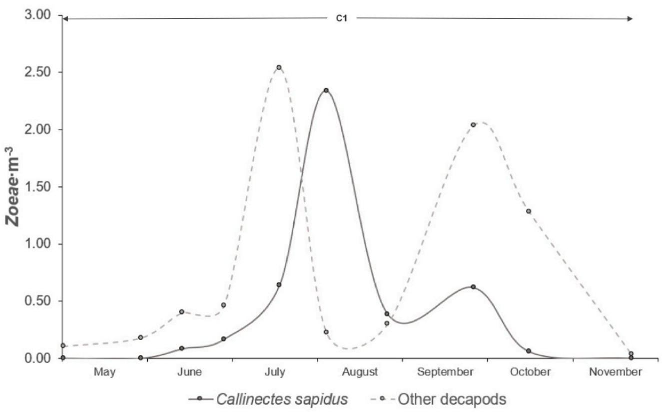


Fig. 3. Average density of zoeae of *C. sapidus* (solid lined) and other decapod species (dashed line) in 2021. Data from the coastal area sampling campaign (C1).

the month with the highest number of *megalopae* (71.3%), also featuring an early peak of juveniles (34.1%). During 2021 and 2022, the settlement peak was in October, with 71.1% of the *megalopae* and 35.4% of the juveniles in 2021 and 73% and 63.4% respectively for 2022 (Table 1). These results frame the settlement peak of *megalopae* and juveniles in St. P between September and November. Considering the 3 sampling sites during 2022, the months of September, October, and November accounted for over 98 ± 2.9 % of the *megalopae* and over 96 ± 0.6 % of the juveniles of the whole year. A total of 2017 *megalopae* and 1114 juveniles were captured during this period. This trends further confirms that the settlement peak occurs between September and November (Fig. 5).

From September to November 2022, St. Pu had the highest abundance of *megalopae*, accounting for 61.8% of the total, followed by St. P with 34.2% and St. Pt with only 4%. Juveniles showed a more even distribution across sites than *megalopae*. St. Pt accounted for 38.8% of the juveniles, followed closely by St. Pu with 38.2% and St. P with 22.9% (Fig. 5). Salinity showed high variability across the different sampling sites with a value of 14.4 ± 13.6, whereas temperature displayed relatively consistent values of 21.2 ± 1.1 °C. The average salinity at St. P was 29.6 ± 12.5, 3 ± 3.1 at St. Pt and 11.3 ± 10.9 at St. Pu.

A comparative between daily (E3) and weekly (E2) data (Table 1) is provided for *megalopae* (Fig. 6) and juveniles (Fig. 7), focusing on St. P records from 23/09/22 to 22/11/22. During that period a total of 4003 *megalopae* and 320 juveniles were collected. For *megalopae*, the results of 1-d vs. 7-d deployments were analogous (p-value = 1, 12 ± 19.2 and 12.9 ± 22.4 respectively), thus evidencing that the theoretical weekly accumulation was much higher than the observed 7-d deployment values (p-value <0.01, 72.5 ± 82.4 vs. 12.9 ± 22.4 respectively). In contrast, substantially more juveniles were captured in 7-d vs. 1-d deployments (p-value <0.05, 5.5 ± 5.1 vs. 0.13 ± 0.17, respectively). The amounts of juveniles captured during 7-d deployments were even significantly higher than theoretical weekly accumulations (p-value = 0.05, 5.5 ± 5.1 vs. 1.4 ± 1.5 respectively).

The mayority of the juveniles found through all estuarine campaigns (Table 1) were small juveniles, measuring under 2 cm carapace width (including the lateral spines). The presence of other decapods was also confirmed in the area, the most common being *P. marmoratus* in both megalopal and juvenile stages, although juveniles of other species like *Xantho poressa* (Olivi, 1792) or *Brachynotus sexdentatus* (Risso, 1827) were also found occasionally.

Comparing the total *megalopae* obtained from the surface collector (SC) to those obtained from another collector located directly beneath it but 10 cm over the bottom (BC), we found rates of *megalopae* that were one order of magnitude higher in the bottom (p-value <0.001). Even when comparing the *megalopae* obtained in the SC to the lowest of 5 daily replicates in BC (MinBC), still 3 times more *megalopae* were found at the bottom (p-value <0.05, Table 2). Only on a few occasions, the numbers of *megalopae* in SC exceeded those in BC and in MinBC (9.8% and 16.4%, respectively), and concurred with low availability of individuals (less than 20 per collector).

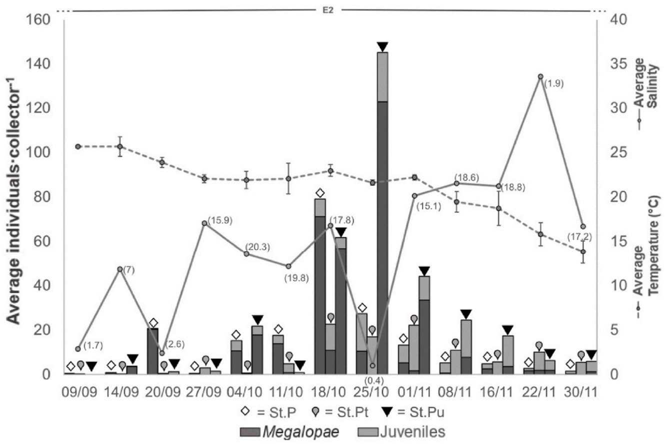


Fig. 5. Average number of megalopae (dark grey) and juveniles (light grey) of *Callinectes sapidus* per collector, grouped per sampling site (St. P, St. Pt and St. Pu) in September, October and November of 2022 (dd/mm date format). Right axis: average ± SD temperature (°C) at each sampling time. SD values for salinity were too high to be represented with error bars, so a value between brackets has been added instead close to each marker. Data from the E2 sampling campaign.

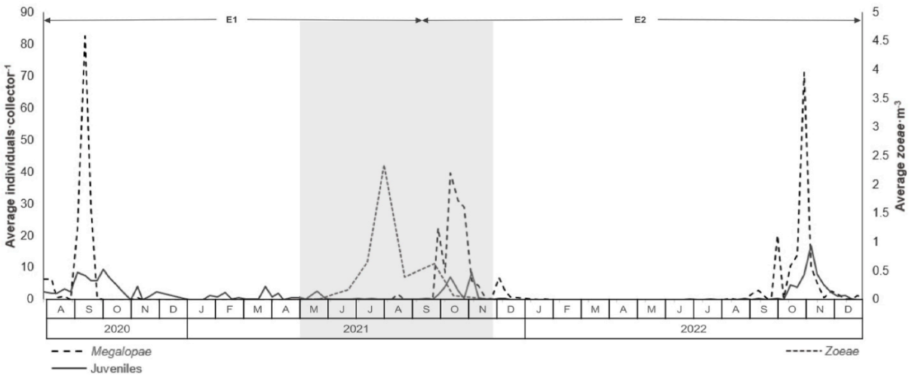


Fig. 4. Average number of megalopae (dashed line) and juveniles (solid line) of *Callinectes sapidus* at the mouth of the Perelló channel (St. P) from 2020 to 2022. Data from the two estuarine sampling campaigns (E1 and E2). Right axis: Average number of zoeae of *C. sapidus* (dotted line) captured in coastal waters, adjacent to the mouth of the channel, from 2021. Data from the coastal area sampling campaign (C1) are shown shaded in grey.

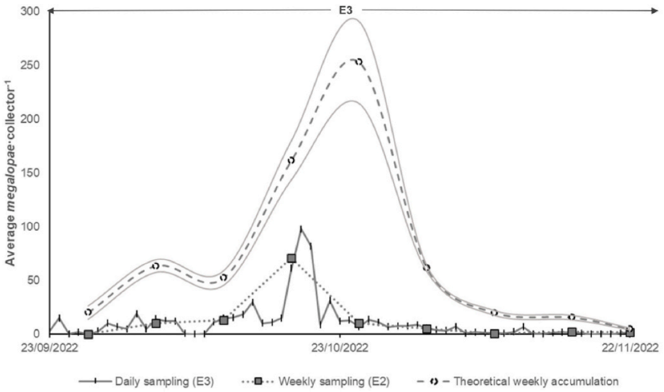


Fig. 6. Comparative of the average number of megalopae of *Callinectes sapidus* per collector. Daily sampling data (solid line) and weekly sampling data (dotted line) obtained at the mouth of the Perelló channel (St. P). The theoretical weekly accumulation (dashed line) is a dummy value obtained from the addition of the averages of daily samplings until a weekly sampling occasion (average ± SD).

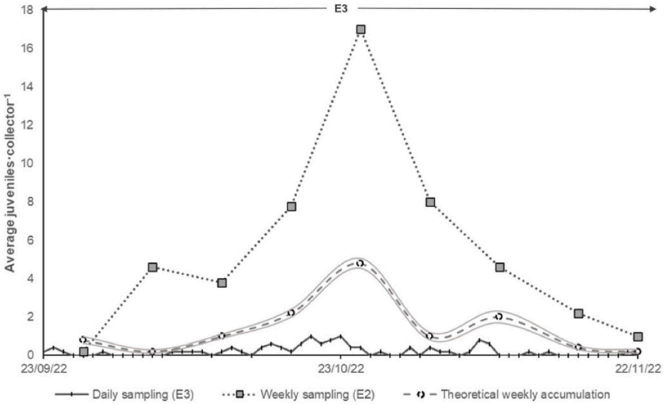


Fig. 7. Comparative of the average number of juveniles of *Callinectes sapidus* per collector. Daily sampling data (solid line) and weekly sampling data (dotted line) obtained at the mouth of the Perelló channel (St. P). The theoretical weekly accumulation (dashed line) is a dummy value obtained from the addition of the averages of daily samplings until a weekly sampling occasion (average ± SD).

Table 2
Comparative abundance of megalopae of *Callinectes sapidus* in the collector located 10 cm under the water surface (Surface), in the bottom collector located directly below it (Bottom) and the minimum value of all the bottom collectors per day (Min. Bottom), from September to November 2022 at the mouth of the Perelló channel (St. P). Number of replicate (days) between brackets.

Collector	Surface(61)	Bottom(61)	Min. Bottom(61)
Min *megalopae*	0	0	0
Max *megalopae*	4	101	34
Average *megalopae*	0.8	11.2	3.4
Cumulative *megalopae*	49	686	205

3.3. *Laboratory trial with megalopae*

Additional information related to the time to metamorphosis of *megalopae* into juveniles is presented in Table 3. The 6 replicate trials provided consistent data of the time required for this process with an average duration of 6.50 ± 1.05 d. Laboratory conditions were fixed at similar values than those in the field during the E3 campaign (mean salinity and temperature of 36.4 and 22.8 °C, respectively) supporting similar possible duration in the field under analogous *ad libitum* conditions.

Table 3
Data summary of the time to metamorphosis from megalopae to juveniles of *Callinectes sapidus* at a salinity 36 and 20–23 °C. Data from 67 megalopae distributed in 6 replicas.

	Min	Max	Average ± SD
Initial *megalopae* [n]	10	12	11.17 ± 0.75
Survival ratio [%]	66.67	100	82.27 ± 10.63
Time to ≥ 50% *megalopae* to juveniles [d]	3	5	4.33 ± 0.82
Time to 100% *megalopae* to juveniles [d]	5	8	6.50 ± 1.05

4. Discussion

4.1. *Spatio-temporal distribution of zoeae*

To our knowledge there is no previously documented information on the abundance or distribution of *zoeae* in the Mediterranean Sea. The maximum density found in this study (3.64 *zoeae* • m^{-3}) was lower than in similar surveys conducted in the native range of *C. sapidus*. Epifanio et al. (1984) found densities up to 7.7 ± 3.2 *zoeae* • m^{-3} in the inner part of the mouth of Delaware Bay, which are higher but within the same order of magnitude. A previous study in Delaware Bay found much lower densities (0.55 ± 0.89 *zoeae* • m^{-3}), probably related to adverse weather conditions (Dittel and Epifanio, 1982). However, Tilburg et al. (2009) sampled in the coastal area adjacent to Delaware Bay and found that the most common densities were of hundreds *zoeae* • m^{-3}, and densities up to thousands *zoeae* • m^{-3} were also frequent. Mesh size and sampling depth were very similar among the three studies. Convergent circulation in Delaware Bay (Kahn and Helser, 2005) coupled with the high local abundance of *C. sapidus* might be factors contributing to such high larval densities, whereas in our study area there is a predominant southward water circulation along the coast (Morales, 2018; Ribó et al., 2015). Regarding abundances of different zoeal stages, predominantly first *zoeae* were found in this study, similarly to Epifanio et al. (1984). In contrast, Tilburg et al. (2009) found every zoeal stage, but those in advanced stages were limited to the farthest sampling points, more than 10 km from the coast. This distribution pattern was also observed in the Gulf of Mexico (Rabalais et al., 1995) but the location of advanced zoeal stages in the Mediterranean Sea remains to be found.

4.2. *Larval development*

The effect of salinity and temperature conditions on hatching, molting, survival, and development time of *zoeae* of *C. sapidus* has been only tested to a maximum salinity of 32–33 (Costlow and Bookhout, 1959; Sandoz and Rogers, 1944). In fact, although temperatures reported for Delaware Bay are within the range of temperatures in our study area, salinities are higher in the Mediterranean (37–38 vs. 28–32) (Epifanio et al., 1984; Tilburg et al., 2009). Optimal salinity for hatching was found to be between 23 and 28, with the most active feeding for *zoeae* observed at a salinity of 24 (Sandoz and Rogers, 1944). Based on this information, the high salinities in the Mediterranean Sea might not be particularly suitable for *zoeae*, although no specific information on possible effects for larvae development is available. Yet, the successful colonization of the Mediterranean Sea by *C. sapidus* suggests significant advantages in other biological aspects at larval or later life stages. This is further supported by the fact that *C. sapidus* in the Mediterranean Sea exhibits the founder effect (González-Ortegón et al., 2022).

The observed peak of *zoeae* in August 2021 with a secondary lower peak in September 2021 (Fig. 3), aligns with the findings of Epifanio et al. (1984). The *zoeae* peak abundance in August is also found in the native range, but there overlaps with the period of greatest abundance of at least other 5 decapod species (Dittel and Epifanio, 1982). However, obtained data shows that maximum *zoeae* abundance is temporally offset with maximum abundance of the rest of the decapod community. This mismatch in the Mediterranean Sea could potentially provide an advantage to *zoeae*, allowing them to thrive in an environment with fewer competitors.

4.3. Influence of collector deployment time

For *megalopae* data analysis from overlapping E3 and E2 sampling occasions suggest that the time of residence inside collectors is as much as one day. This is potentially linked to the daily tidal regime. The usual mechanism of estuarine colonization by *megalopae* is called 'flood tide transport' and involves ascending through the water column during nocturnal flood tides to be carried inwards the estuary (DeVries et al., 1994; Olmi, 1994; Welch and Forward, 2001). Tankersley et al. (1995) documented that slight increases in salinity of only 0.3 to 0.5 can trigger the ascension of *megalopae* across the water column. In our study area, comparable or even bigger salinity increases occurred in St. P in 28 out of 58 monitored days (48.3%) during nocturnal flood tides during the E3 campaign. Turbulence is another factor that induces sustained swimming in *megalopae* of *C. sapidus* (Welch et al., 1999). Although no related data is available for the present study, localized turbulence increases associated to the tidal phase might be possible due to canalization through narrow channels (golas). Maximum tide range was under 13 cm during that period. In addition, *megalopae* can adjust their swimming behavior in response to the presence of odors from potential nursery habitats and predators, further shaping their estuarine ingress (Forward et al., 2003). Potential nursery habitats in the native range are typically characterized by plant species such as *Zostera marina* or *Spartina alterniflora*, but no submerged vegetation was available in St. P. Yet, the entire Albufera lagoon can be classified as a *Phragmites* and *Typha* marsh, which is also a suitable habitat for juveniles (Jivoff and Able, 2003; Long et al., 2011). Regarding potential local predators and/or competitors (e.g., *P. marmoratus*, *Eriphia verrucosa* (Forskål, 1775), *X. poressa*), which are not present in the native range, the possible mediating effect of predator chemical cues remains to be tested. Time to metamorphosis for *megalopae* during E3 sampling campaign was too large (ca. 6–7 d) to be considered a shaping factor, even if the presence of other chemical cues such as humic acids or ammonium might also accelerate or delay it (Forward et al., 1997). Overall, the combination of turbulence and the presence of humic acids within local channels is possibly enough to stimulate daily *megalopae* migration upstream, while the importance of other mechanisms such as flood tide transport and predator cues is unclear.

Conversely, for juvenile abundances, data evidence an increase in the attraction along the seven days of deployment. Such increase is likely related to the development of a biofouling community, from biofilms to primary and secondary colonizers. Another factor that might be playing a role is the amount of organic material trapped by collectors. This material is mostly comprised of marsh plant debris and provides both a food resource and enhanced habitat complexity for developing juveniles (Hyman et al., 2023; Rakocinski et al., 2003).

Standardization of the number of individuals collected must be handled carefully, since settlement rate might largely vary with deployment time. For example, Bishop et al. (2010), deployed collectors for more than one day, and divided the total abundance by the number of deployment days, leading an underestimation of *megalopae*. Tankersley et al. (2002) tested hourly vs. all-night collectors and observed that the cumulative settlement of *megalopae* in hourly collectors was higher than in the all-night collectors. Therefore, to obtain accurate measures of maximum *megalopae* settlement in collectors, factors that should be taken into consideration include not only deployment time but also night tidal phases, the size of the collector and the availability of chemical cues. In contrast, for juveniles, this standardization would lead to an overestimation of daily captures, as an increase in sampling frequency reduces the total amount of juveniles captured. It's worth noting that these affirmations might be true only for small size collectors, and that larger ones such as those used by Bishop et al. (2010) might accumulate *megalopae* for more than a day or be perceived as suitable hideouts for juveniles quicker.

4.4. Vertical distribution of megalopae

In the estuarine area *megalopae* showed higher preference for bottom than for surface collectors, which is coherent with patterns reported by Bishop et al. (2010). Metcalf et al. (1995) generally found more *megalopae* at the bottom, even though on some occasions surface collectors outperformed those at the bottom. In contrast, van Montfrans et al. (1990) indicated a preliminary study where more *megalopae* were found just below the water surface, but no data is available. Olmi (1994) documented that photic inhibition was even very intense at shallow estuarine areas, which would prevent any type of daily movement in our estuarine area. On the continental shelf, previous evidence shows *megalopae* being very abundant during the day in the surface layer (Johnson, 1985), while inconclusive results are reported for open estuarine waters (Epifanio et al., 1984). In this study, average salinities of 37 across coastal sites evidence that they fall outside the area of estuarine influence, thus suggesting the undergoing of other factors. There is a precedent for capturing 2 *megalopae* in open water surveys in 2005 and 2011 in a region close to the study area, but no precise information about the depth of capture is available (Png-Gonzalez et al., 2021).

4.5. Megalopae settlement pattern

The settlement pattern of *megalopae* observed over three years in St. P shows a consistent peak in September and October. A similar settlement pattern was observed in Turkey, but with the peak occurring in August and September (Yesilyurt and Tureli, 2016). The detected settlement pattern is similar to those in the native range, but with less interannual variability and a narrower temporal distribution (Jones and Epifanio, 1995; Ogburn et al., 2009). This might be a consequence of the water regime regulation by floodgates in our three estuarine sampling sites, in two main ways. First, they can force the grouping of females moving into the sea for hatching, prompting a very time-restricted reproductive migration event. Second, floodgates might also hold the entrance of *megalopae* until enough water from the lagoon, containing the necessary chemical cues, is released. Considering the position of our sites within the estuarine system, our scenario is more similar to that investigated by Epifanio et al. (1984), also showing a clear peak of *megalopae* in September. Epifanio et al. (1984) also evidences a ca. 5–6 week delay for capturing fifty percent of total *zoeae* and *megalopae*, which in our case is extended to ca. 9–10 weeks. Two factors might be affecting this delay; first the Mediterranean physicochemical conditions that can alter the duration of the planktonic life stages (Costlow, 1967; Costlow and Bookhout, 1959). Second, the oceanographic conditions, which in Delaware Bay promote high larval retention that can prompt *megalopae* and juveniles to return to their native estuary (Tilburg et al., 2005, 2009). In our study region, the currents along the coastline persistently feature a southward direction (Morales, 2018; Ribó et al., 2015), that might favor the colonization of southern estuarine areas, as happened during *C. sapidus* colonization along the Spanish Mediterranean coast.

Two different proportions of early colonizers have been identified among the 3 estuarine sampling sites. St. Pu and St. P exhibited similar proportions of *C. sapidus* early life stages, with a dominance of *megalopae* over juveniles (74% vs. 26% and 73% vs. 27% respectively). In contrast, St. Pt exhibited a very different pattern, with a clear dominance of juveniles (16% *megalopae* vs. 84% juveniles). Temperature was very similar among all sampling sites (21.2 ± 1.1 °C), while salinity showed high variability (11.3 ± 10.9, 3 ± 3.1 and 29.6 ± 12.5 at St. Pu, St. Pt and St. P respectively). The main difference between St. Pt and the other two sampling sites is the lower salinity, which has been proved to impact the survival of *megalopae* metamorphosing into juveniles in laboratory studies (Costlow, 1967). Specifically, the combination of salinity and temperature in St. Pt would cause 100% failure of metamorphosis, while in St. Pu this rate falls to 30%. Juveniles have better osmoregulation and are able to thrive in the low salinity present in St. Pt (Millikin and Williams, 1984). Overall, our results suggest that average estuarine salinity is an important factor shaping the colonization of early life stages of *C. sapidus*, which matches previous results from South Carolina (Mense and Wenner, 1989).

5. Conclusions

In the study area, the peak of *C. sapidus zoeae* was recorded in mid-summer in surface coastal waters 1 km offshore, and appears to be temporally disjoint with that of the *zoeae* assemblage of other crab species. *Megalopae* and juveniles, caught using passive collectors in the estuarine areas (connecting channels between the Albufera lagoon and the Mediterranean Sea), were more abundant in late summer-early autumn.

Our research has proved the validity of these new nylon mesh collectors, different from those used in previous studies in the western Atlantic. Furthermore, by comparing daily with weekly sampling campaigns, the residence time within collectors has been established in just a single day for *megalopae*, while there is an accumulation of juveniles over time suggest their need for enhanced habitat complexity.

Although much remains to be known about the effects of *C. sapidus* in the Mediterranean Sea, this study offers the first insights into the distribution and abundance of early life stages of *C. sapidus*. Knowledge of this crucial phase can help when undertaking management actions, in sites where *C. sapidus* is considered a menace for local biodiversity or a fishing resource. The proposed sampling protocol with collectors is easily manageable and requires low investment, thus providing a useful tool for obtaining information about colonization patterns of *C. sapidus* in small Mediterranean estuaries.

CRediT authorship contribution statement

A. Gil-Fernández: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Rodilla:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **P. Prado:** Writing – review & editing, Writing – original draft, Funding acquisition. **S. Falco:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Acknowledgements

We want to thank Professor Christoph D. Schubart for his support and training in molecular analysis to A. Gil-Fernández, whose recent passing has left a big hole in the crab world. May his soul rest in peace.

This project was funded by the Ministry of Science and Innovation (Grant PID 2020-118476RR-C21 by MCIN/AEI/10.13039/501100011033) and by the Universitat Politècnica de València (PAID-01-18, CRUE), to which we are very thankful.

We would also like to thank the anonymous reviewers for the comments and suggestions that improved the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2023.108593.

References

Bailey, S.A., Brown, L., Campbell, M.L., Canning-Clode, J., Carlton, J.T., Castro, N., Chainho, P., Chan, F.T., Creed, J.C., Curd, A., 2020. Trends in the detection of aquatic non-indigenous species across global marine, estuarine and freshwater ecosystems: a 50-year perspective. Divers. Distrib. 26, 1780–1797. https://doi.org/10.1111/ddi.13167.

Biggs, R.B., 1978. Coastal bays. Coastal sedimentary environments 69–99. https://doi.org/10.1007/978-1-4684-0056-4_3.

Bishop, T.D., Miller, H.L., Walker, R.L., Hurley, D.H., Menken, T., Tilburg, C.E., 2010. Blue crab (*Callinectes sapidus* Rathbun, 1896) settlement at three Georgia (USA) estuarine sites. Estuar. Coast 33, 688–698. https://doi.org/10.1007/s12237-009-9259-4.

Casalduero, F.G., Esplá, A.R., Muñoz, A.I., Castillo, F.G., Hernández, F.M., González-Carrión, F., 2016. Alloctonous marine invertebrates in the Mar Menor lagoon, Mar Menor: una laguna singular y sensible. Evaluación científica de su estado. Instituto Español de Oceanografía, Ministerio de Economía y Competitividad, pp. 157–178.

Castejón, D., Guerao, G., 2013. A new record of the American blue crab, *Callinectes sapidus* Rathbun, 1896 (Decapoda: Brachyura: portunidae), from the Mediterranean coast of the Iberian Peninsula. BioInvasions Records 2, 141–143. https://doi.org/10.3391/bir.2013.2.2.08.

Castellani, C., Edwards, M., 2017. Marine Plankton: A Practical Guide to Ecology, Methodology, and Taxonomy. Oxford University Press.

Costa, A.B.H.P., Valença, A.P.M.C., Dos Santos, P.J.P., 2016. Is meiofauna community structure in Artificial Substrate Units a good tool to assess anthropogenic impact in estuaries? Mar. Pollut. Bull. 110, 354–361. https://doi.org/10.1016/j.marpolbul.2016.06.041.

Costlow, J.D., 1967. The effect of salinity and temperature on survival and metamorphosis of megalops of the blue crab *Callinectes sapidus*. Helgol. Wiss. Meeresunters. 15, 84–97. https://doi.org/10.1007/BF01618611.

Costlow, J.D., Bookhout, C.G., 1959. The larval development of *Callinectes sapidus* Rathbun reared in the laboratory. Biol. Bull. 116, 373–396. https://doi.org/10.2307/1538947.

del Estado, Puertos, 2022. REDMAR. Informe Anual 2022 de Valencia 3. https://bancodatos.puertos.es//BD/informes/anuales/3/3651N22.pdf.

DeVries, M.C., Tankersley, R.A., Forward, R.B., Kirby-Smith, W.W., Luettich, R.A., 1994. Abundance of estuarine crab larvae is associated with tidal hydrologic variables. Mar. Biol. 118, 403–413. https://doi.org/10.1007/BF00350297.

Dittel, A.I., Epifanio, C.E., 1982. Seasonal abundance and vertical distribution of crab larvae in Delaware Bay. Estuaries 5, 197–202. https://doi.org/10.2307/1351835.

Encarnação, J., Baptista, V., Teodósio, M.A., Morais, P., 2021. Low-cost citizen science effectively monitors the rapid expansion of a marine invasive species. Front. Environ. Sci. 9, 752705 https://doi.org/10.3389/fenvs.2021.752705.

Epifanio, C.E., 1988. Dispersal strategies of two species of swimming crab on the continental shelf adjacent to Delaware Bay. Mar. Ecol. Prog. Ser. 243–248.

Epifanio, C.E., 2019. Early life history of the blue crab *Callinectes sapidus*: a review. J. Shellfish Res. 38, 1–22. https://doi.org/10.2983/035.038.0101.

Epifanio, C., Valenti, C., Pembroke, A., 1984. Dispersal and recruitment of blue crab larvae in Delaware Bay, USA. Estuarine. Coastal and Shelf Science 18, 1–12. https://doi.org/10.1016/0272-7714(84)90002-7.

Forward, R.B., Tankersley, R.A., Blondel, D., Rittschof, D., 1997. Metamorphosis of the blue crab *Callinectes sapidus*: effects of humic acids and ammonium. Mar. Ecol. Prog. Ser. 157, 277–286. https://doi.org/10.3354/meps157277.

Forward, R.B., Tankersley, R., Smith, K., Welch, J., 2003. Effects of chemical cues on orientation of blue crab, *Callinectes sapidus, megalopae* in flow: implications for location of nursery areas. Mar. Biol. 142, 747–756. https://doi.org/10.1007/s00227-002-0966-7.

Forward, R.B., Cohen, J.H., Irvine, R.D., Lax, J.L., Mitchell, R., Schick, A.M., Smith, M.M., Thompson, J.M., Venezia, J.I., 2004. Settlement of blue crab *Callinectes sapidus megalopae* in a North Carolina estuary. Mar. Ecol. Prog. Ser. 269, 237–247. https://doi.org/10.3354/meps269237.

González-Ortegón, E., Berger, S., Encarnação, J., Chairi, H., Morais, P., Teodosio, M.A., Oliva-Paterna, F.J., Schubart, C.D., Cuesta, J.A., 2022. Free pass through the pillars of hercules? Genetic and historical insights into the recent expansion of the atlantic blue crab *Callinectes sapidus* to the west and the east of the Strait of Gibraltar. Front. Mar. Sci. 1121 https://doi.org/10.3389/fmars.2022.918026.

Hyman, A., Miller, C., Shi, D., Lipcius, R., 2023. Effects of structural complexity and water depth on the juvenile blue crab *Callinectes sapidus* in a simulated salt marsh mesocosm. bioRxiv, 524977. https://doi.org/10.1101/2023.01.23.524977, 2023.2001. 2023.

Ibañez, C., Pont, D., Prat, N., 1997. Characterization of the Ebre and Rhone estuaries: a basis for defining and classifying salt-wedge estuaries. Limnol. Oceanogr. 42, 89–101. https://doi.org/10.4319/lo.1997.42.1.0089.

Jivoff, P.R., Able, K.W., 2003. Blue crab, *Callinectes sapidus*, response to the invasive common reed, *Phragmites australis*: abundance, size, sex ratio, and molting frequency. Estuaries 26, 587–595. https://doi.org/10.1007/BF02823733.

Johnson, D.F., 1985. The distribution of brachyuran crustacean *megalopae* in the waters of the York River, lower Chesapeake Bay and adjacent shelf: implications for recruitment. Estuar. Coast Shelf Sci. 20, 693–705. https://doi.org/10.1016/0272-7714(85)90026-5.

Johnson, D.S., 2015. The savory swimmer swims north: a northern range extension of the blue crab *Callinectes sapidus*? J. Crustac Biol. 35, 105–110. https://doi.org/10.1163/1937240X-00002293.

Jones, M., Epifanio, C., 1995. Settlement of brachyuran *megalopae* in Delaware Bay: an analysis of time series data. Mar. Ecol. Prog. Ser. 125, 67–76. https://doi.org/10.3354/meps125067.

Kahn, D.M., Helser, T.E., 2005. Abundance, dynamics and mortality rates of the Delaware Bay stock of blue crabs, *Callinectes sapidus*. J. Shellfish Res. 24, 269–284. https://doi.org/10.2983/0730-8000(2005)24[269:ADAMRO]2.0.CO;2.

Long, W.C., Grow, J.N., Majoris, J.E., Hines, A.H., 2011. Effects of anthropogenic shoreline hardening and invasion by *Phragmites australis* on habitat quality for juvenile blue crabs (*Callinectes sapidus*). J. Exp. Mar. Biol. Ecol. 409, 215–222. https://doi.org/10.1016/j.jembe.2011.08.024.

Mancinelli, G., Chainho, P., Cilenti, L., Falco, S., Kapiris, K., Katselis, G., Ribeiro, F., 2017. The Atlantic blue crab *Callinectes sapidus* in southern European coastal waters: distribution, impact and prospective invasion management strategies. Mar. Pollut. Bull. 119, 5–11. https://doi.org/10.1016/j.marpolbul.2017.02.050.

Mantelatto, F.L., Reigada, A.L., Gatti, A.C., Cuesta, J.A., 2014. Morphology of the first zoeal stages of five species of the portunid genus *Callinectes* (Decapoda, Brachyura) hatched at the laboratory. An Acad. Bras Ciências 86, 755–768. https://doi.org/10.1590/0001-3765201420130030.

Mense, D.J., Wenner, E.L., 1989. Distribution and abundance of early life history stages of the blue crab, *Callinectes sapidus*, in tidal marsh creeks near Charleston, South Carolina. Estuaries 12, 157–168. https://doi.org/10.2307/1351820.

Metcalf, K.S., Van Montfrans, J., Lipcius, R.N., Orth, R.J., 1995. Settlement indices for blue crab megalopae in the York River, Virginia: temporal relationships and statistical efficiency. Bull. Mar. Sci. 57, 781–792.

Millikin, M.R., Williams, A.B., 1984. Synopsis of Biological Data on the Blue Crab, *Callinectes sapidus* Rathbun. NOAA Technical Report NMFS 1. FAO Fisheries Synopsis 138.

Morais, P., Gaspar, M., Garel, E., Baptista, V., Cruz, J., Cerveira, I., Leitao, F., Teodosio, M., 2019. The Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 expands its non-native distribution into the Ria Formosa lagoon and the Guadiana estuary (SW-Iberian Peninsula, Europe). BioInvasions Records 8, 123–133. https://doi.org/10.3391/bir.2019.8.1.14.

Morales, J.A., 2018. The Spanish Coastal Systems: Dynamic Processes, Sediments and Management. Springer. https://doi.org/10.1007/978-3-319-93169-2.

Myers, A., Southgate, T., 1980. Artificial substrates as a means of monitoring rocky shore cryptofauna. J. Mar. Biol. Assoc. U. K. 60, 963–975. https://doi.org/10.1017/S0025315400042028.

Nehring, S., 2011. Invasion history and success of the American blue crab *Callinectes sapidus* in European and adjacent waters. In: In the Wrong Place-Alien Marine Crustaceans: Distribution, Biology and Impacts, pp. 607–624. https://doi.org/10.1007/978-94-007-0591-3_21.

Ogburn, M., Diaz, H., Forward, R.B., 2009. Mechanisms regulating estuarine ingress of blue crab *Callinectes sapidus megalopae*. Mar. Ecol. Prog. Ser. 389, 181–192. https://doi.org/10.3354/meps08139.

Olmi, E.J., 1994. Vertical migration of blue crab *Callinectes sapidus megalopae*: implications for transport in estuaries. Mar. Ecol. Prog. Ser. 113, 39. https://doi.org/10.3354/meps113039.

Pessani, D., Tirelli, T., Flagella, S., 2004. Key for the identification of Mediterranean brachyuran *megalopae*. Mediterr. Mar. Sci. 5, 53–64. https://doi.org/10.12681/mms.203.

Png-Gonzalez, L., Papiol, V., Balbín, R., Cartes, J.E., Carbonell, A., 2021. Larvae of the blue crab *Callinectes sapidus* rathbun, 1896 (Decapoda: Brachyura: portunidae) in the balearic archipelago (NW Mediterranean Sea). Marine Biodiversity Records 14, 1–5. https://doi.org/10.1186/s41200-021-00217-5.

Rabalais, N.N., Burditt Jr., R.F., Coen, L.D., Cole, B.E., Eleuterius, C., Heck Jr., K.L., McTigue, T.A., Morgan, S.G., Perry, H.M., Truesdale, F.M., 1995. Settlement of *Callinectes sapidus megalopae* on artificial collectors in four Gulf of Mexico estuaries. Bull. Mar. Sci. 57, 855–876.

Ragkousis, M., Zenetos, A., Souissi, J.B., Hoffman, R., Ghanem, R., Taşkın, E., Muresan, M., Karpova, E., Slynko, E., Dağlı, E., 2023. Unpublished Mediterranean and Black Sea Records of Marine Alien, Cryptogenic, and Neonative Species. https://doi.org/10.3391/bir.2023.12.2.01.

Rakocinski, C.F., Perry, H.M., Abney, M.A., Larsen, K.M., 2003. Soft-sediment recruitment dynamics of early blue crab stages in Mississippi Sound. Bull. Mar. Sci. 72, 393–408.

Ribó, M., Puig, P., van Haren, H., 2015. Hydrodynamics over the Gulf of Valencia continental slope and their role in sediment transport. Deep Sea Res. Oceanogr. Res. Pap. 95, 54–66. https://doi.org/10.1016/j.dsr.2014.10.004.

Sandoz, M., Rogers, R., 1944. The effect of environmental factors on hatching, moulting, and survival of zoea larvae of the blue crab *Callinectes sapidus* Rathbun. Ecology 25, 216–228. https://doi.org/10.2307/1930693.

Schubart, C.D., Deli, T., Mancinelli, G., Cilenti, L., Gil-Fernández, A., Falco, S., Berger, S., 2022. Phylogeography of the atlantic blue crab *Callinectes sapidus* (Brachyura: portunidae) in the americas versus the Mediterranean Sea: determining origins and genetic connectivity of a large-scale invasion. Biology 12, 35. https://doi.org/10.3390/biology12010035.

Soria, J.M., 2006. Past, present and future of la Albufera of Valencia natural Park. Limnética 25, 135–142. https://doi.org/10.23818/limn.25.10.

Streftaris, N., Zenetos, A., 2006. Alien marine species in the Mediterranean-the 100 'Worst Invasives' and their impact. Mediterr. Mar. Sci. 7, 87–118. https://doi.org/10.12681/mms.180.

Tankersley, R., McKelvey, L., Forward, R.B., 1995. Responses of estuarine crab *megalopae* to pressure, salinity and light: implications for flood-tide transport. Mar. Biol. 122, 391–400. https://doi.org/10.1007/BF00350871.

Tankersley, R., Welch, J., Forward, R.B., 2002. Settlement times of blue crab (*Callinectes sapidus) megalopae* during flood-tide transport. Mar. Biol. 141, 863–875. https://doi.org/10.1007/s00227-002-0896-4.

Tilburg, C.E., Reager, J.T., Whitney, M.M., 2005. The physics of blue crab larval recruitment in Delaware Bay: a model study. J. Mar. Res. 63, 471–495.

Tilburg, C.E., Dittel, A.I., Epifanio, C.E., 2009. High concentrations of blue crab (*Callinectes sapidus*) larvae along the offshore edge of a coastal current: effects of convergent circulation. Fish. Oceanogr. 18, 135–146. https://doi.org/10.1111/j.1365-2419.2009.00502.x.

Valjarević, A., Filipović, D., Milanović, M., Valjarević, D., 2020. New updated World maps of sea-surface salinity. Pure Appl. Geophys. 177, 2977–2992. https://doi.org/10.1007/s00024-019-02404-z.

van Montfrans, J., Peery, C.A., Orth, R.J., 1990. Daily, monthly and annual settlement patterns by *Callinectes sapidus* and *Neopanope sayi megalopae* on artificial collectors deployed in the York River, Virginia: 1985–1988. Bull. Mar. Sci. 46, 214–229.

Welch, J., Forward, R.B., 2001. Flood tide transport of blue crab, *Callinectes sapidus*, postlarvae: behavioral responses to salinity and turbulence. Mar. Biol. 139, 911–918. https://doi.org/10.1007/s002270100649.

Welch, J.M., Forward, R.B., Howd, P.A., 1999. Behavioral responses of blue crab *Callinectes sapidus* postlarvae to turbulence: implications for selective tidal stream transport. Mar. Ecol. Prog. Ser. 179, 135–143. https://doi.org/10.3354/meps179135.

Yesilyurt, I.N., Tureli, C., 2016. Settlement of blue crab (*Callinectes sapidus) megalopae* in the north shore of Yumurtalik Cove (Turkey). Fresenius Environ. Bull. 25, 1007–1018.

Zenetos, A., Tsiamis, K., Galanidi, M., Carvalho, N., Bartilotti, C., Canning-Clode, J., Castriota, L., Chainho, P., Comas-Gonzalez, R., Costa, A.C., 2022. Status and trends in the rate of introduction of marine non-indigenous species in European seas. Diversity 14, 1077. https://doi.org/10.3390/d14121077.