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Escuela de Doctorado

Doctoral Thesis

Ecology of the non-native crab *Cronius ruber* (Lamarck, 1818) (Decapoda, Brachyura, Portunidae) in Gran Canaria waters

José Antonio Martín García

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Las Palmas de Gran Canaria

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D. JESÚS GARCÍA RUBIANO COORDINADOR DEL PROGRAMA DE DOCTORADO EN CALIDAD AMBIENTAL Y RECURSOS NATURALES (DOCARNA) DE LA UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA.

INFORMA,

Que la Comisión Académica del Programa de Doctorado, en su sesión de fecha................ tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada .$^{\mathrm{Ec}}$ología del cangrejo no-nativo *Cronius ruber* (Lamarck, 1818)(Decapoda, Brachyura, Portunidae) en Gran Canaria: implicaciones para su gestión y control."presentada por el doctorando D. José Antonio Martín García y dirigida por el Doctor Raül Triay Portella.

Y para que así conste, y a efectos de lo previsto en el Art$^{\underline{o}}$ 11 del Reglamento de Estudios de Doctorado (BOULPGC 7/10/2016) de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a...... de dos mil veintitres

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Doctoral Thesis

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Doctoral Thesis presented by M Sc. José Antonio Martín García

Directed by Dr. Raül Triay Portella

Director	**Doctoral Candidate**
(signature)	(signature)

Las Palmas de Gran Canaria, November 2024

El mar dará a cada hombre
una nueva esperanza,
como el dormir le da
sueños

Cristóbal Colón
(1451-1506)

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Thesis Preview

This Doctoral Thesis, directed by Dr. Raül Triay Portella and supervised by Dr. José Mario González Pajuelo, is entitled "Ecology of the non-native crab *Cronius ruber* (Lamarck, 1818) (Decapoda, Brachyura, Portunidae) in Gran Canaria waters: Implications for its management and control." It originated from a detailed study on the invasive species *C. ruber*, carried out under the COINVA project of the Institute of Environmental Studies and Natural Resources (i-UNAT) at the University of Las Palmas de Gran Canaria (ULPGC), integrated into the Doctoral Program of Environmental Quality and Natural Resources (DOCARNA).

The information gathered in this project represents a crucial step towards deepening our understanding of the invasive crab *C. ruber* and collecting data on its populations in the coastal waters of the Canary Islands. The Doctoral thesis includes three original research articles, published in peer-reviewed scientific journals (Journal Citation Report), which focus on the feeding, reproduction, and growth of *C. ruber*. These studies contribute to a better understanding of *C. ruber* and its impact on Canarian coastal ecosystems, which will allow effective management and control of the populations.

In addition to the proposed objectives, the dissemination of knowledge about the invasive species *Cronius ruber* was expanded through oral and poster presentations at international congresses. Understanding the population dynamics and life history and of invasive species is crucial for addressing their environmental impacts. However, research on the life cycle and basic population parameters of *C. ruber* is limited in both its native and non-native ranges.

This thesis follows the compendium format outlined in Regulation 1/2023 on doctoral studies at the University of Las Palmas de Gran Canaria, approved by the University's Governing Council on January 26, 2023 (BOULPGC No. 2). It begins with a general introduction addressing key questions, objectives, and the study's hypothesis. Subsequent chapters present scientific contributions supporting the hypothesis, culminating in the main conclusions.

Summary/Resumen

El propósito central de esta tesis es realizar un estudio de la ecología de *Cronius ruber* (Lamarck, 1818), un crustáceo decápodo:portunido nadador que fue documentado en Canarias por primera vez en 2010. La rápida y sorprendente expansiónn de esta especie aguas del archipiélago canario ha generado considerable interés científico, destacando la necesidad de investigar meticulosamente su ecología y ciclo vital. Curiosamente, *Cronius ruber* ha suscitado poco interés en sus regiones de origen, lo que ha supuesto la ausencia de información biológica y de datos sobre esta especie.

Gracias a los proyectos ProId2017010083 (COINVA) financiado por el Gobierno de Canarias y los fondos FEDER de la UE y el proyecto CEI2019-06 (ICRAC) de la Agencia Canaria de Investigación Innovación y Sociedad de la Información (ACIISI), se definieron objetivos claros y específicos para investigar a *C. ruber*, centrándose en aspectos fundamentales de su ciclo vital y ecología como la alimentación, reproducción y crecimiento. Este enfoque contribuyó significativamente a mitigar las lagunas en el conocimiento existente sobre esta especie. Además, durante el desarrollo de la tesis se pudo alcanzar metas adicionales, como evaluar la dispersión y abundancia de *C. ruber*. En el marco de esos estudios se llevaron a cabo observaciones sistemáticas para determinar la presencia o ausencia de *C. ruber* a lo largo del litoral de Gran Canaria, lo cual fue fundamental para averiguar su expansión geográfica. Asimismo, se evaluó la efectividad de diversas técnicas de muestreo para controlar la propagación del cangrejo invasor *C. ruber*. La investigación sobre *C. ruber* es vital no solo para comprender las dinámicas biológicas de la especie, sino también para valorar su impacto potencial sobre las comunidades biológicas autóctonas y los ecosistemas de áreas no nativas donde se ha establecido.

Los resultados de este estudio ofrecen perspectivas valiosas sobre la interacción de *C. ruber* con los ecosistemas que invade, lo cual es clave para la gestión y conservación de los ecosistemas y de su biodiversidad en las regiones afectadas por especies invasoras.

Para lograr las metas marcadas en esta tesis, se realizaron diversas actividades de campo y laboratorio. Se recolectaron y analizaron un total

de 523 ejemplares de *C. ruber* para los aspectos de reproducción y crecimiento. Adicionalmente, se examinaron 278 individuos con el fin de analizar detalladamente el contenido estomacal y evaluar la ingesta diaria. Las actividades de captura se efectuaron manualmente a lo largo de un periodo comprendido entre enero de 2018 y febrero de 2020. Durante ese tiempo, se realizaron inmersiones a profundidades que variaban entre 1 y 8 metros, seleccionando zonas que ofrecían la mayor probabilidad de encontrar a estos crustáceos en su hábitat natural.

El análisis de la composición de la dieta de *C. ruber* resulta fundamental para comprender y evaluar sus potenciales impactos en los ecosistemas donde se ha introducido. La dieta se analizó tanto desde una perspectiva demográfica como estacional. Para ello se examinaron los contenidos estomacales de 278 cangrejos, de los cuales se identificaron 716 muestras de presas, correspondientes a 52 taxones distintos, incluyendo tejidos y restos que no pudieron ser identificados. Estos especímenes se clasificaron en tres grupos según su tamaño: 87 juveniles, con un ancho del caparazón (CW) de entre 17,51 y 55,50 mm; 128 adultos, con un CW de 55,60 a 75,50 mm; y 73 adultos viejos, con un CW de 75,60 a 91,44 mm. Se observó que más del 18% de los cangrejos analizados presentaban estómagos vacíos.

La frecuencia de aparición, expresada como porcentaje (% Of), se estimó como la tasa entre el número total de estómagos examinados y el número total de estómagos que contenían presas, analizados por grupo y sexo (macho/hembra). Las presas más comúnmente encontradas fueron los cangrejos branquiuros, representando el 51,54% del total, seguidos de los poliquetos con un 34,36%. Los equinoideos fueron observados en el 22,47% de los casos, los gasterópodos en el 21,15% y los perciformes en el 20,70%. Además, las investigaciones realizadas durante muestreos nocturnos revelaron la presencia de presas que no se habían detectado en los análisis de contenido estomacal diurno.

En cuanto a las tasas de ingestión diarias, basadas en el consumo de poliquetos, se observó un patrón de mayor consumo entre los juveniles (< 55 mm CW) y los cangrejos adultos (entre 55 mm y 75 mm CW), en comparación con los adultos mayores (> 75 mm CW). Este patrón de consumo estuvo en consonancia con el número y tipo de presas encontradas en los estómagos, que mostraron variaciones según las estaciones del año y los grupos ontogénicos de los cangrejos. Además, las observaciones visuales nocturnas demostraron que los depredadores nativos se alimentaban del cangrejo invasor, incluyendo meros, pulpos y elasmobranquios.

El estudio sobre la dieta permitió entender mejor cómo *C. ruber* puede afectar a las poblaciones nativas. La diversidad en la dieta de esta especie sugiere una capacidad de adaptación que podría conferirle una ventaja competitiva sobre las especies autóctonas, además sugiere que *C. ruber* sigue una dieta generalista, la información sobre las diferencias en la dieta entre los diferentes grupos difería significativamente entre las estaciones, grupo ontogénico y el sexo, proporciona datos valiosos sobre el desarrollo y comportamiento alimentario a lo largo de su ciclo de vida. Esta investigación es crucial para desarrollar estrategias alimenticias de *C. ruber* y como estas pueden variar en función del CW, lo que a su vez influye en su interacción con el ecosistema y las especies invasoras.

Este portunidio asume el papel de mesodepredador generalista, como se puede observar en la gran diversidad y variedad de presas identificadas en su contenido estomacal, y las observadas durante las inmersiones nocturnas. Es destacable que varias especies amenazadas o de importancia comercial constituyen los componentes principales de su dieta.

Las características reproductoras de *C. ruber* fueron examinadas, abarcando aspectos como la estructura de sexos, la madurez sexual, la fecundidad y el desarrollo de los huevos. El proceso de desarrollo de los huevos se detalló y clasificó en cuatro etapas claramente diferenciadas, proporcionando una visión detallada de su ciclo reproductor. Las hembras fueron significativamente más abundantes que los machos con una proporción de cangrejos macho de (1:1.44). Además, las hembras fueron significativamente más abundantes que los machos en los tamaños de 50-65 mm de ancho de caparazón (CW) (1:>1.84), sin embargo, los machos fueron significativamente dominantes en las clases de tamaño mayores de 70 mm CW, particularmente sobre los 85 mm CW, en los que solo se observaron machos.

La reproducción de *C. ruber* tiene lugare durante todo el año, con oviposiciones sucesivas inmediatamente después del periodo de incubación de los huevos. Este proceso puede repetirse hasta seis veces en las hembras debido a la cantidad de esperma transferido durante la fecundación y almacenado en la hembra. La talla a la cual alcanza la madurez sexual para las hembras se estimó en 49,17 mm CW, mientras que la madurez sexual en los machos se estimó en 52,19 mm CW. Aunque se observó durante todos los meses hembras con ovarios maduros estas fueron más abundante en los meses de julio a noviembre, mientras que los machos con testículos maduros también fueron observados durante todo el año.

Las características reproductoras de *C. ruber* explican la rápida expansión y colonización de esta especie más allá de su área de distribución nativa. Esta especie es un desovador múltiple con una nueva oviposición inmediatamente después de finalizar el periodo de incubación de los huevos. El periodo de desarrollo de los huevos dura algo menos de 1 mes. Este proceso puede ser realizado por las hembras hasta seis veces gracias al esperma almacenado en la espermateca. La actividad de desove se extiende a lo largo de todo el año mostrando un potencial reproductor que alcanza el millón de huevos por oviposición.

La mayor presencia de hembras en rangos de tamaño más comunes indica una estrategia reproductora enfocada en maximizar la producción de descendientes, lo que potencialmente acelera el aumento de la población y su extensión en áreas no nativas. Por otro lado, la presencia de machos en tamaños mayores podría relacionarse con la competencia por parejas o con un desarrollo diferencial que favorece la supervivencia y el éxito reproductor en etapas más avanzadas de su vida.

El patrón de crecimiento de *C. ruber* se describió utilizando el modelo de von Bertalanffy. El crecimiento relativo mostró variaciones significativas a lo largo de la ontogenia de la especie, con cambios notables entre los 54,5 y 57,8 mm CW en los machos y a los 49,9 mm de CW en las hembras. Además, la alometría del crecimiento, que estudia cómo las diferentes partes del cuerpo cambian en proporción respecto al tamaño general, reveló un incremento positivo tanto en las muelas de los machos como en el abdomen de las hembras. Los parámetros de crecimiento de von Bertalanffy se estimaron utilizando datos de frecuencia de longitud. *C. ruber* demostró ser una especie de crecimiento rápido, alcanzando la fase de crecimiento postpuberal (madurez) entre 0,5 y 1 año de edad para los machos, y entre 0,4 y 0,8 años para las hembras, con un aumento de más del 638% en los machos y un 443% en las hembras en términos de edad. Se observaron 13 mudas en la primera fase de la vida (hasta 30 mm de CW), seguidas por seis mudas desde esta fase hasta el final de la vida, además de una muda terminal final.

Los depredadores de *C. ruber* están recogidos por la UICN en su la lista roja o son objeto de actividades extractivas, lo que destaca la importancia de entender los patrones de dieta asociados a los rasgos demográficos de esta especie. La rápida tasa de crecimiento y las características reproductoras de *C. ruber* explican su expansión y colonización más allá de su área de distribución nativa. Los resultados también proporcionan información clave para las directrices de control y gestión de esta especie invasora, subrayando la importancia de considerar las características de su reproducción al diseñar medidas efectivas para mitigar su impacto en ecosistemas vulnerables. Los resultados proporcionan una línea de base crucial para investigaciones futuras sobre los posibles impactos de esta especie no nativa, sino que también podrían respaldar la inclusión de *C. ruber* en la lista de especies exóticas invasoras de la Unión Europea.

Chapter 1. General Introduction

1.1. Preamble

The growing threat of invasive alien species to the world's marine ecosystems is one of the most important challenges for communities and ecosystem conservation. Recent research (Gallardo et al., 2016; Seebens et al., 2017; Diagne et al., 2020) highlights the particularly severe impact of these species on marine biodiversity, with a special focus on Oceanic Islands (Couchamp et al., 2003; Gien et al., 2023). Within this issue, crustaceans emerge as one of the most harmful groups. Their ability generate high negative impacts on ecosystems through interactions with autochthonous communities is noteworthy (Walton et al., 2002; Hánfing, 2011).

Invasive crustaceans, in particular, have a great capacity for adaptation and dispersion, significantly altering native communities through their effects as efficient predators (Hollebone and Hay, 2008). These organisms have not only been identified as some of the most successful invasive species (Galil, 2011) but have also demonstrated the ability to growt to high population densities, competing aggressively with native species for resources such as shelter and food (Brockerhoff and McLay, 2011). Within the crustacean family, predatory brachyurans stand out for their successful adaptation and dispersion, leading to a significant impact on population parameters over time (Rato et al., 2021). There are various species known for this behavior, such as the green crab *Hemigrapsus sanguineus* (de Haan, 1835), the blue crab *Callinectes sapidus* Rathbun, 1896, the european green crab *Carcinus maenas* (Linnaeus, 1758), and the mangrove tree crab *Aratus pisonii* (H. Milne Edwards, 1837). These species exhibit different feeding behaviors, but all have a significant threat to the invaded ecosystems (Griffen, 2014). Additionally, they can have negative consequences on artesanal fisheries as they compete for the same resources (Márquez et al., 2024).

This Doctoral Thesis focuses on the crab *Cronius ruber* (Larmarck, 1818), a pantropical invasive non-indigenous species introduced to the Canary Islands. Although the specific vector of its introduction is unknown, invasive species like *C. ruber* often capitalize on human infrastructure for their dispersion. Specifically, offshore oil platforms have emerged as critical vectors in introducing invasive species to the Canary Islands, including fish and corals (Brito et al., 2017; González et al., 2017; Espino et al., 2018; Rodríguez et al., 2020; López et al., 2022). The port of Las Palmas (Gran Canaria), considered an international center for naval repairs of oil platforms since 2011 (López et al., 2019), appears to have a critical role in this dynamic. The correlation between the arrival of these platforms from the coasts of West Africa and the appearance of *C. ruber* in Gran Canaria suggests a significant connection (Pajuelo et al., 2016; González et al., 2017). Furthermore, marine port infrastructures, such as these platforms, provide an ideal environment for invasive species to establish and spread to nearby areas (Castro et al., 2011), supporting the idea that human activities in harbour, ports and oil platforms significantly contribute to the dispersion of invasive alien species like *C. ruber*.

1.2. Crustaceans: Feeding patterns and diet composition

There is evidence suggesting that the shape and size of the crabs' carapace, as well as the size of their intestine, may be evolutionarily determined by their dietary needs. The quality of the diet they consume can influence the width of their intestine (Villa et al., 2023).

These crustaceans have a versatile diet as they feed on both plants and animals. Their feeding habits can vary depending on the species and the environment they inhabit. The feeding of brachyurans is closely linked to their ability to adapt to different environments, enabling them to tap into a wide variety of food resources (Kraemer et al., 2007; Garbary et al., 2014) . These marine organisms are generalist consumers (Weis, 2010; Kotta et al., 2018), meaning they are omnivores and feed on a wide range of preys.

In the studies carried out with *Cronius ruber*, it has been demonstrated through the analysis of its stomach contents that it has a wide variety of prey in its diet, which characterizes it as a generalist predator. During night dives, prey consumption and cannibalistic behavior was observed *in situ* (Figure 1.1), feeding on the shells of *C. ruber* during molting; this behavior was observed in all ontogenetic groups. Variability was identified in the feeding preference of *C. ruber* among different ontogenetic groups and seasons, evidencing fluctuations in prey choices according to ontogeny and seasonal period. As for daily ingestion rates based on polychaetes, they indicated higher prey consumption by juvenile crabs.

1.3. Body size, relative growth and sexual dimorphism: Key factors in the reproduction of brachyuran crustaceans

The body size of female brachyuran crabs plays a crucial role in their reproductive success. Although some brachyuran families face allometric constraints due to the limited space within their carapace, most crab species avoid this limitation by increasing the number of embryos produced annually. This strategy involves distributing reproductive allocation over time, rather than confining it to the limited space of the carapace (Hines, 1982). This adaptation is essential to maximize reproductive efficiency within the physical constraints imposed by their morphology.

Moreover, positive allometric growth in brachyuran crabs plays a vital role in reproduction. Positive allometric growth means that as the crab grows, its shell increases in width proportionally with length, providing more surface area to carry a larger number of embryos during hatching (Fazhan et al., 2021). This morphological adaptation is particularly relevant in the study of *Cronius ruber*, where positive allometry has been observed in the relationship between carapace length (CL) and carapace width (CW). This indicates that the carapace widens in proportion to its length as the crab increases in size.

Males of *C. ruber*, a species of brachyuran crabs, have a larger carapace tan females. This is a common trait in brachyuran crabs, evidencing sexual dimorphism in these species (Marochi et al., 2013). During copulation, the male positions himself behind and above the female (Shinozaki-Mendes et al., 2020), but this tactic could also be to protect her during mating, using her larger carapace as a protective shield.

The morphological and behavioral characteristics of brachyuran crabs are intrinsically linked to their reproductive success and survival strategies in their environment. Invasive alien species that exhibit differential morphological traits may possess ecological advantages that would increase their likelihood of stability (Ferré et al., 2021).

1.4. Key Factors in the growth of Brachyuran crustaceans

Understanding the ecology and dynamics of populations requires a

Figura 1.1. Foraging behaviour of *Cronius ruber* under natural conditions(a-h) as a predator and (h) as prey. Different prey devoured at night: (a) *Eurythoe complanata* (Polychaeta), (b) *Pilumnus villosissimus* (Brachyura), (c) head of *Similiparma lurida* (Fish), (d) *Haliotis tuberculata coccinea* (Gastropoda), (e) *Aplysia dactylomela* (Gastropoda), (f) *Bulla mabuler* (Gastropoda), (g) *Mactra stultorum* (Bivalvia) and (h) *Octopus vulgaris* (Cephalopoda) preying on *C. ruber*.

deep understanding of one of their most crucial life processes: growth (Chang et al., 2012). The growth rate in crustaceans varies significantly, with differences observed within the same species and between species (Hartnoll, 1982).

Lifetime growth in crustaceans is governed by two key elements: (a) the molting process, which involves the physiological accumulation of molt reserves; and (b) the molt pattern. Each of these components is influenced by multiple factors. While the initiation of molt is often regulated by hormones, it constitutes an intricate physiological process, (Aiken, 1980; Hartnoll, 2001).

The molting of crabs is a mechanism of adaptation and growth. This process allows them not only to grow, but also to combat mechanical aging. Decapods can molt their exoskeleton more than twenty times during their life (Skinner, 1985). Molting is essential for their growth, which can be determinate or indeterminate (Hartnoll 1982; Hartnoll, 2001).

The growth of crabs, whether determinate or indeterminate, is intrinsically linked to their molt cycles. Species with determinate growth, the size they can reach is limited, whereas in species with indeterminate growth, they can continue to grow throughout their lives, provided conditions are favorable and they can successfully molt (Vogt, 2012).

The growth pattern of crabs is developed by a dynamic interplay of extrinsic factors, as temperature, salinity and food availability, and intrinsic factors, as hormonal control. These elements, in conjunction with strategies inherent to their biological cycle, generate a remarkable diversity in their growth patterns (Chang et al., 2012). These elements can operate in isolation or together, significantly influencing both the rate and nature of growth in crustaceans.

Diet is a crucial factor in the growth of crabs. Diet and food availability have a major impact on various aspects of their development. The quality and quantity of available food directly influence their growth rate, although this depends on particular conditions (Gencer and Aguilar, 2023).

Both the growth and reproduction of crustaceans involve a high energetic cost. There are obvious trade-offs between these two critical processes in the life history of organisms, (Abrams and Rowe, 1996). The brachyuran *Carcinus maenas* stops its growth through a process known as terminal molt, an evolutionary strategy designed to maximize its reproductive efficiency (Styrishave et al., 2008).

In the environment, especially in factors such as temperature, has a critical role in the hormonal control of organisms, which in turn affects the sensitivity of their molting cycle to environmental variations. Specifically, in the case of juvenile crabs of the species *Portunus pelagicus* (Linnaeus, 1758), environmental conditions such as temperature and salinity have been observed to influence their growth rate. However, recent studies, such as those conducted by (Liu et al., 2022; Apriliano et al., 2023), have shown that this impact on growth rate is not significant.

The crab *Cronius ruber* exhibits a positive allometry in its relationship between carapace length (CL) and carapace width (CW), indicating that carapace width increases proportionally more than carapace length as the crab grows. In terms of growth, males experience a greater than 638% increase in size, while females grow by 443%. During the the early stages of his life, crabs go through 13 molts until they reach a size of 30 mm carapace width (CW). After this point, and until the end of their life, only 6 additional molts occur, including a terminal molt (Figure 1.2). The life expectancy of the crab until the terminal molt is approximately 2.5 years, and the survival period following this terminal molt exceeds 1.5 years.

Figure 1.2. Moulting of *Cronius ruber* in one of the experimental tanks.

1.5. Main objetives

The main goal of this dissertation thesis is to explore the knowledge of *Cronius ruber*, an invasive species for which there is limited information. This study focuses on understanding its life cycle and ecology, especially in the coastal ecosystems of the Canary Islands (central-northeast Atlantic).

Specifically, this thesis addresses in one of its chapters the feeding, diet and food habits of *C. ruber*, exploring its role as a predator in the ecosystem. Another chapter investigates the reproductive aspects of *C. ruber*, focusing on maturity, development and fecundity, thus contributing to the understanding of its population dynamics. Finally, a chapter deals with the growth of the species, providing crucial data on its development and maturation. These findings are crucial to develop strategies for the management and population control of *C. ruber* in its invasive habitats.

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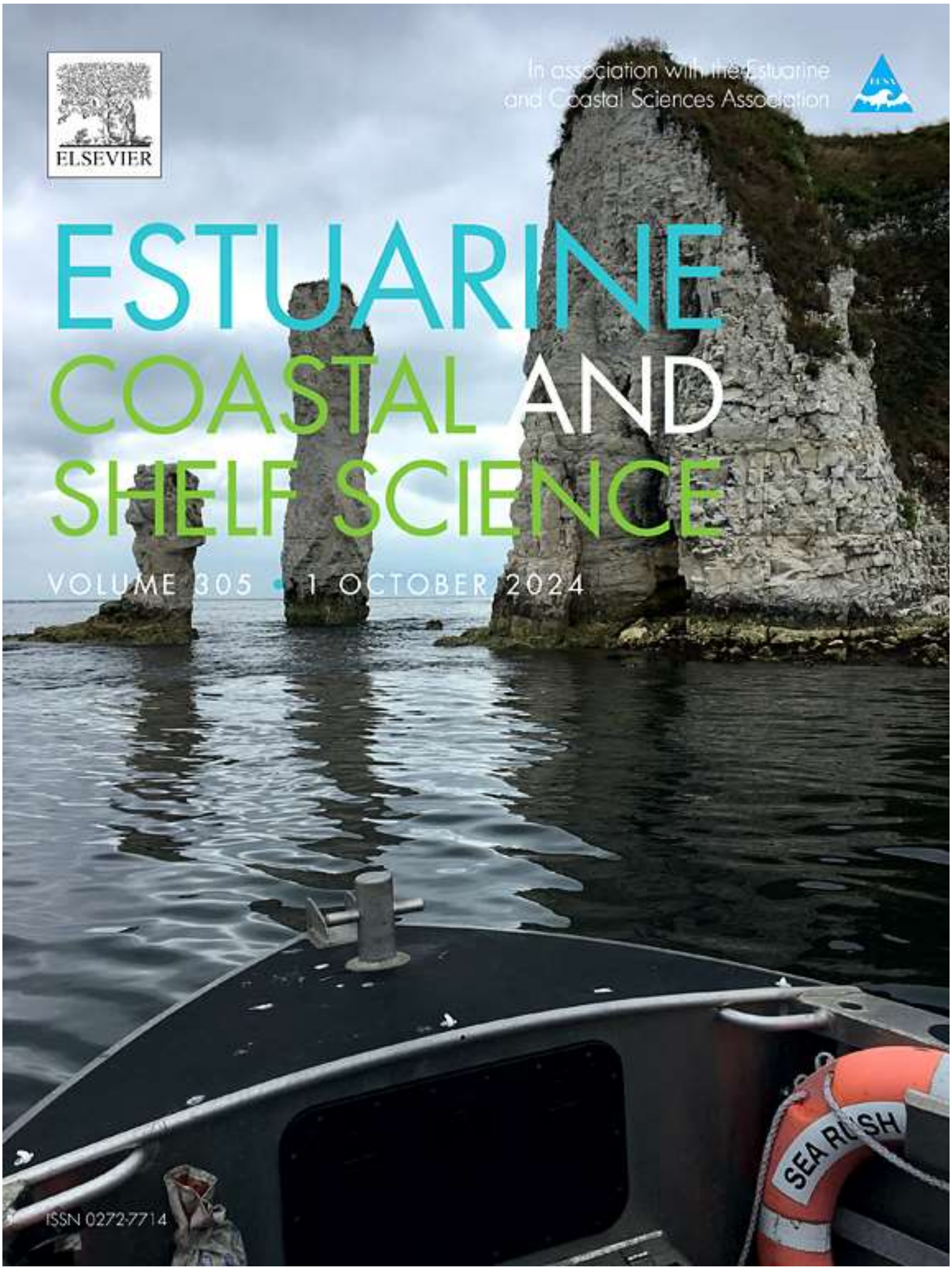
Chapter 2. Relevance of feeding ecology in the management of invasive species: Prey variability in a novel invasive crab



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Relevance of feeding ecology in the management of invasive species: Prey variability in a novel invasive crab

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ABSTRACT

The diet composition of non-indigenous species (NIS) provides essential information to recognise potential impacts on ecosystems. This study examined the feeding ecology of the novel invasive crab *Cronius ruber* from demographic and seasonal perspectives. It identified 52 prey items in crab gut contents (n = 278), and more than 18% of the studied specimens had empty guts. The high-frequency prey belonged to Brachyuran (51.54%) and Polychaete (34.36%), followed by Echinidea (22.47%), Gastropoda (21.15%) and Perciformes (20.70%). Additionally, the night sampling showed prey that were not observed in the examined stomach contents. The daily ingestion rates based on polychaeta indicated more prey consumption by juveniles (<55 mm carapace width (CW)) and adults crabs (55 mm–75 mm CW) than the old adults (>75 mm CW). This falls in line with the number of prey items retained in individuals' guts, which changed seasonally and in ontogenic groups. Moreover, the visual night observations showed that native predators foraged on the invasive crab. These predators were groupers, octopus and elasmobranchs. The seasonal and ontogenic differences observed in diet through the stomach content analysis and daily ingestion rates suggest that *C. ruber* eats a generalist diet. The dissimilarity analysis suggested possible resource partitioning in ontogenic groups. Our results could represent the baseline for future studies into the possible impacts of this invasive NIS, as well as some arguments to include *C. ruber* on the list of invasive alien species of European Union concern.

1. Introduction

Invasive non-indigenous species (NIS) may cause significant impacts and are a major cause of biodiversity loss worldwide (Courchamp et al., 2017). On oceanic islands, human overpopulation increases the impact of these threats, which results in higher biodiversity losses (Riera et al., 2014). This is consistent with other studies which have suggested that invasive NIS exert their strongest impact on islands (Courchamp et al., 2003; Glen et al., 2013). In this scenario, dispersion of invasive NISs could be favoured by intensified human activity, e.g. oil rig translocations (Pajuelo et al., 2016) or maritime traffic (Castro et al., 2020), coupled with ongoing climate change (Pyšek et al., 2020; Bennett et al., 2021).

Understanding and quantifying the impacts that invasive NIS inflict on communities and ecosystems are crucial for targeting the limited resources available for their management (Parker et al., 1999; Keller et al., 2011). Once invasive NIS have been successfully established in marine habitats, eradication is not expected (Thresher and Kuris, 2004). Notwithstanding, some studies have pointed out that interception and pathway removal are effective strategies for reducing future impacts (Carlton et al., 2005). In line with this, a functional eradication framework addresses the urgent need for conservation action in situations involving high-priority invaders (Green and Grosholz, 2021). Many invasive NIS are simultaneously recognised by others to provide valuable ecosystem services or cultural benefits, or to be of intrinsic worth (McNeely, 2001; Schlaepfer et al., 2011).

Invaders can decrease native species' abundance by predation or via several strategies, which drive more predation pressure than homologous native predators (Noonburg and Byers, 2005; Salo et al., 2007) and reach higher densities (Parker et al., 2013), and all this with a stronger per capita effect on prey (Diamond et al., 1989) or more successfully captured prey (Bollache et al., 2008; Haddaway et al., 2012; Dick et al.,

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2013). Therefore, invasive species have been associated with falloffs in species diversity and ecosystem resilience in recipient habitats (Baber and Babbitt, 2003; Brown et al., 2002). Knowledge of diet-specific components is especially relevant for invasive NIS management. This is particularly true if prey components are threatened species or species that provide economic, social, and environmental benefits.

Crustaceans are a highly invasive group of marine organisms (Hänfling et al., 2011) that have had adverse impacts on numerous habitats around the globe (Galil et al., 2011). Brachyuran crabs are particularly interesting crustaceans. They are considered a successful invasive group (Brockerhoff and McLay, 2011) linked with significant ecological (Kraemer et al., 2007; Garbary et al., 2014) and socio-economic impacts (Edgell and Hollander, 2011). Invasive non-indigenous crabs quickly develop high densities and show competitive advantages over native fauna (Brockerhoff and McLay, 2011) because they play a key role in the local trophic web given their wide food strategy that allows them to interact with many species that belong to separate trophic levels (Weis, 2010; Kotta et al., 2018). Our study focuses on a novel invasive NIS, the blackpoint sculling crab *Cronius ruber* (Lamarck, 1818). This crab was first observed on the Canary Islands in June 2010 (COINVA, 2019) and was reported by community-based science in 2016 (González et al., 2017). Prior to its observation, *C. ruber* spread rapidly around the Canary Islands archipelago, and even to the nearest northern archipelago of Madeira (see Schäfer et al., 2019). Although the introduction vector of *C. ruber* in the Webbnesia region remains unknown, current temperature trends in the region suggest that its establishment is linked with the ongoing tropicalisation process (Schäfer et al., 2019).

The life cycle and ecology of *C. ruber* remain unknown in native areas, and only the first zoeal stage has been described (Fransozo et al., 2002). This could be explained by the role it plays within its natural range because it is not regarded as either a dominant species or one of commercial interest in native areas (Mantelatto and Fransozo, 2000; Beneditto et al., 2010). In fact West Atlantic populations can be threatened by *Charybdis hellerii,* another invasive NIS portunid crab species (Sant'Anna et al., 2012; Ferry et al., 2017). The main goal of the present study is to describe the diet and ingestion rate of a marine invasive NIS in the Webbnesia region.

2. Materials and methods

2.1. Study area and sampling procedure

The present study was conducted between January 2018 and February 2020 in two localities on the Gran Canaria Island (Canary Islands, central-east Atlantic): Playa de las Nieves (Agaete) (28°05′58.9"N 15°42′37.4"W) in the northern part and Santa Agueda Bay (El Pajar) in the southern part (27°45′02.4"N 15°40′13.4"W) (Fig. 1). Crabs were collected by hand using artificial lights at depths from 1 m to 7 m on a rocky/sandy bottom next to artificial harbours. Sampling took place in winter (January–February 2018, 2019 and 2020) and summer (July–August 2018 and 2019). Locations were selected because they had the highest known *C. ruber* densities recorded in the archipelago (0.22 ± 0.04 crabs/m^2; COINVA, 2019). Each sampling was conducted after sunset and before midnight. This period is considered the major peak activity for decapods in general due to circadian rhythms (Bauer, 1985; Kronfeld-Schor and Dayan, 2003). This period was also observed for *C. ruber* (Triay-Portella et al., 2018). Samples were stored in a frozen container to avoid regurgitation or digestion of stomach contents immediately after collection (Williams, 1981). Live predation and prey retention were recorded whenever possible during 24 underwater exploratory surveys conducted in rocky/sandy habitats.

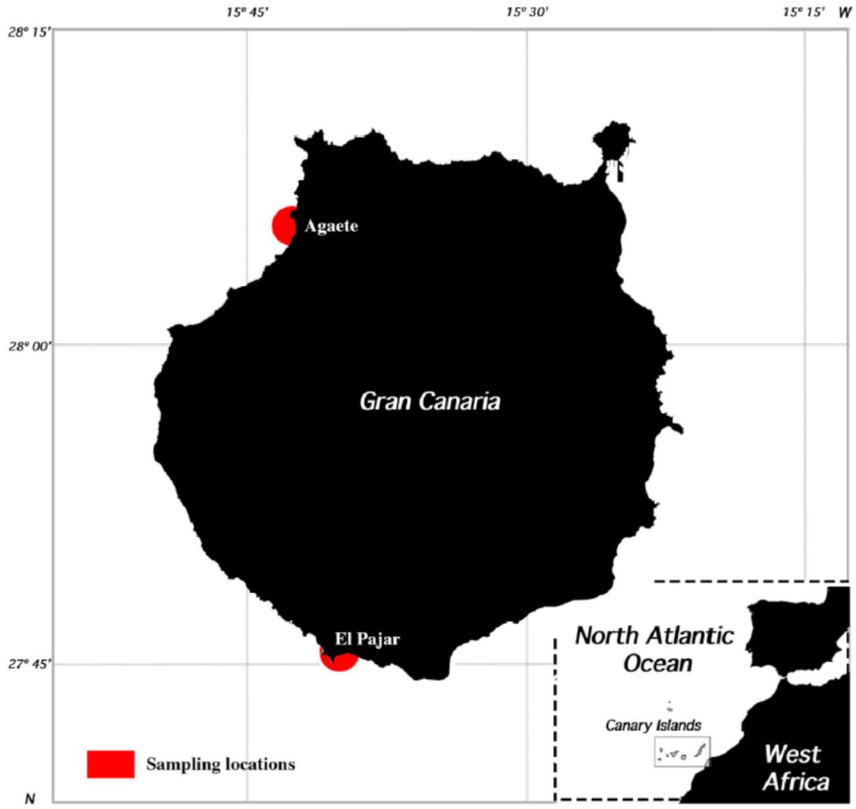

Fig. 1. Location of the two study (collecting) sites on the Gran Canaria Island coast (Canary Islands, central-east Atlantic). Playa de las Nieves (Agaete) (28°05′58.9"N 15°42′37.4"W) in the northern part and Santa Agueda Bay (El Pajar) in the southern part (27°45′02.4"N 15°40′13.4"W) of this island.

All the crabs were measured for carapace width (CW) by a digital calliper (0.01 mm precision). Ontogeny categories were based on size upon first maturity and the biggest-sized crab recorded. “Juveniles” were assigned to individuals as having <55 mm CW. The “adults” category comprised those crabs with CW ranging from 55 mm to 75 mm “Old adults” were designated as individuals with >75 mm CW. This “old adults” fraction represents the largest sizes of the studied population. The largest captured crab had a CW of 91.5 mm (COINVA, 2019).

2.2. *Taxonomic identification of prey and stomach analyses*

Stomach items (prey) were classified according to morphological characteristics. The pertinent taxonomic reference literature helped to classify items (prey) to a high taxonomic degree (i.e., species or genus), and to a low taxonomic level (i.e., family or above) if this was not possible (Fauchald, 1977; Holthuis, 1993; Ingle, 1993, 1997; Smaldon et al., 1993). A collection of biological specimens (ULPGC collection) was consulted during the prey identification process.

Diet composition was determined following the revised methodology proposed for stomach analyses by da Silveira et al. (2020). Frequency of occurrence (% O_f) was expressed as a percentage between the total number of examined stomachs and the total number of stomachs with prey assessed per group. It was calculated for all the populations by sex (male/female) and ontogenic groups (juveniles/adults/old adults). Diet composition was analysed at two taxonomic levels: the genus/species level; the order/infraorder level in broad taxonomic groups. The first approach was followed for identification purposes to identify the prey species of *C. ruber*. This latter approach aimed to favour the comparison made between other diet studies in relation to invasive or native species.

2.3. *Daily ingestion rate*

An experiment was designed to investigate the differences in the daily ingestion rate (*dIR*) in each ontogenic group (juveniles, adults, old adults). A sample of 12 individuals was captured by hand. Then six crabs in each category were weighed and set up in tanks. The tank volume was 80 L. Based on the *C. ruber* diet (see the Results section), polychaeta *Hermodice carunculata* was selected as the model prey due to its ubiquity in the ontogenic group diet, and for its high abundance and good availability in the environment. The live prey items were dried and weighed on precision balances (0.001 g). The *dIR* was calculated as the ratio between the prey weight consumed over 24 h and crab weight. At 10 a.m., prey were placed inside tanks. The remaining polychaetes were removed after a 24-h period. The remaining food was weighed after a 10-min drying time. During the experiment, crabs were subjected to alternate 24-h starvation periods. Each experiment was conducted on 10 consecutive days (5 *dIR* data per crab x 6 crabs in each Group x 2 ontogenic groups). The *dIR* was determined as in (Jobling, 1997): *dIR* = (total prey weight consumed in 24 h/crab weight) x 100.

2.4. *Statistical analyses*

A permutational multivariate analysis of variance (PERMANOVA) (Anderson et al., 2008) tested whether the crab diet composition of crabs significantly differed in presence/absence terms between seasons (fixed factor with two levels), ontogenic group (fixed factor with three levels) and sex (fixed factor with two levels). Pairwise comparisons (through 9999 permutations of raw data) resolved the ontogenic group differences separately for each group. A resemblance matrix was constructed using the Bray-Curtis Similarity Index (Clarke and Gorley, 2006). A mixed model analysis and Bonferroni post hoc tests were applied to test the differences in dIR between sexes and ontogeny. The model was based on the fixed factor sex (two levels; male vs. female) and the fixed factor ontogeny (two levels; juveniles and adults vs. old adults). Five consecutive dIR measurements were considered to be repetitions of the same crab individual. The ANOSIM analysis tested the differences between ontogenic groups and seasonally as far as the mean number of prey was/items retained in gut contents were concerned. Pairwise comparisons to the ontogenic groups within seasons were applied. The similarity percentage analysis procedure, SIMPER (Clarke and Gorley, 2006), identified the main species/items that contributed (>1.5%) to the diet dissimilarity separately for both season and ontogenic groups.

3. Results

The stomachs of 278 invasive (*C. ruber*) crabs were analysed (18.35% of empty stomachs), of which 87 were juveniles (17.51–55.50 mm CW), 128 were adults (55.60–75.50 mm CW) and 73 were old adults (75.60–91.44 mm CW). They included 148 females (19.77–85.62 mm CW) and 120 males (17.51–91.44 mm CW). The analyses of stomachs resulted in 716 prey appearances, which belonged to 50 taxa (non-identified tissue and debris increased the number of items to 52). Prey were initially classified into five broad categories: Annelida (ANN), Crustacea (CRUST), Perciformes (FISH), Echinodermata (EQUI) and Mollusca (MOL). Across the five main prey categories, each prey was identified at the lowest taxonomic level. This resulted in 23 identified prey at the species level, 11 at the genus level, four species at the family level and 12 at the above family level (Table 1 and Table S1).

3.1. *Underwater visual observations*

Night underwater surveys provided additional prey and predator accounts (Fig. 2). Of the prey observed in the underwater surveys, new species of FISH (Diplodus cadenati, Labriosomus nuchipinnis, Serranus scriba, Similiparma lurida, Sparisoma cretense and Thalassoma pavo), Gastropoda (Aplysia dactylomela, Bulla mabillei and Felimare picta), CRUST (Xantho incisus) and Polychaeta (Hermodice carunculata) were components on the diet species list. These species were absent or unidentified in gut content. In addition, extant species were observed in stomach contents and underwater surveys (Fig. 3). The expected top predators were recorded to forage on C. ruber at night or by collecting opportunistic observations (sitizens-science base information) from predator gut content: Octopus vulgaris and Octopus macropus (MOL, Cephalopoda); Mycteroperca fusca and Ephinephelus marginatus (FISH, Serranidae); Aetomylaeus bovinus and Taeniura grabata (Myliobatiformes, Myliobatidae and Dasyatidae). Finally at night, dive sampling cannibalistic behaviours were observed in conjunction with the crabs from the different ontogenic groups that foraged on C. ruber carapace (i. e., juveniles foraging on large moults).

3.2. *Diet and ingestion rate*

The ratio of the identified prey at the species level differed considerably in groups (Table S1). Echinidea were identified at the species level in all cases. Conversely, Annelida, FISH and some families belonging to MOL (Gastropoda and Bivalvia) were among the lowest species identification (more than 83% of prey identified above the genus level). CRUST were generally identified in high percentages (66% and 99%) at the species level, i.e., the family Anomura was identified at the species level in all cases (Table S1). The representative structures and characteristic items of each group are presented in Table S2.

CRUST were the most frequently prey observed in *C. ruber* gut contents (62.1% O_f). Brachyura is a ubiquitous prey family in gut contents. *Xantho* accounted for the highest frequency at the genus level. However, the second most frequent item in this group was not one of the identified Brachyura. From an ontogenic perspective, old adults (i.e., dominant crabs) accounted for the highest frequency of Brachyura as prey. Annelida and MOL were the second and third most frequent groups with 36.5% and 34.8%, respectively. Polychaeta was the main family in Annelida. The appearance of Polychaeta was more frequent in females (37.9% O_f) than in males (29.5% O_f). Gastropoda was the family present in most gut contents for MOL prey (21.15% O_f). Adults and old adults

Table 1

Frequency of occurrence (% O_f) expressed as a percentage between the total number of examined stomachs and the total number of stomachs with prey assessed per group. All the populations were calculated by sex (male/female) and ontogenic group (juveniles/adults/old adults). "Juveniles" were assigned to individuals under 55 mm carapace width (CW). The "adults" category comprised crabs' CW ranging from 55 to 75 mm "Old adults" were designated as those individuals with >75 mm CW.

Group/Items	Class/Order/Infraorder	Frequency of occurrence (%O_f)					
		All the individuals	males	females	juveniles	adults	old adults
		n = 278	n = 120	n = 148	n = 87	n = 128	n = 73
TISSUE		14.1	10.53	16.67	12.28	14.81	14.52
DEBRIS		12.78	12.63	12.88	12.28	12.96	12.9
ALGAE		2.64	2.11	3.03	3.51	1.85	3.23
ANN		36.56	31.58	40.15	33.33	42.59	29.03
	Polychaeta	34.36	29.47	37.88	31.58	38.89	29.03
	Sypunculida	2.2	3.16	1.52		3.7	1.61
CRUST		62.11	63.16	61.36	68.42	58.33	62.9
	Anomura	12.78	6.32	17.42	15.79	15.74	4.84
	Axiidea	4.41	4.21	4.55	8.77	2.78	3.23
	Brachyura	51.54	54.74	49.24	52.63	48.15	56.45
	Isopoda	5.29	3.16	6.82	1.75	6.48	6.45
FISH	Perciformes	20.7	14.74	25	31.58	17.59	16.13
ECHI	Echinidea	22.47	24.21	21.21	21.33	23.15	16.13
MOL		34.8	38.95	31.82	28.07	39.81	32.26
	Bivalvia	13.66	18.95	9.85	12.28	16.67	9.68
	Gastropoda	21.15	24.21	18.94	14.04	22.22	25.81
	Polyplacophora	7.93	8.42	7.58	7.02	11.11	3.23
	No. of items	52	41	48	33	46	37
	% of empty stomach	18.35					

were the main demographic groups to forage on this species. FISH were the prey items that accounted for the widest variation between ontogenic groups. Juveniles were the group with highest frequency of fish in diet (31.6% O_f), and ate twice the amount of fish consumed by adults and old adults (17.6% and 16.1% O_f, respectively).

Ontogeny was the key factor in daily ingestion rate (*dIR*) differences. The *dIR* values showed significant variations between ontogenic groups ($F = 18.240$, $p = 0.003$) and in combined fixed factors ontogeny * sex ($F = 6.527$, $p = 0.034$). The mean *dIR* in juvenile and adult crabs (23.159 ± 1.89 day^{-1}) was higher than that in old adults (13.234 ± 1.342%, day^{-1}).

3.3. Dimensions of seasonality and ontogeny in diet

The prey composition in the *C. ruber* diet in presence/absence terms differed between summer and winter ("season", $p < 0.02$, Table 2), and also between ontogenic groups ($p < 0.04$, Table 2). The pairwise comparisons showed differences among ontogenic groups, sexes and seasons.

The mean number of prey in stomach contents revealed differences in ontogeny ($p < 0.02$, Fig. 4). An increase in the mean number of prey was observed in summer for both juveniles and adults ($p < 0.03$). In contrast, old adults displayed fewer prey in winter and retained smaller numbers of prey than adults generally did (Fig. 4).

The SIMPER routine indicated that when the juvenile diet composition was seasonally compared, all the prey items at the family level contributed to the seasonal differences, except Brachyura, which took a dominant position in both seasons (Table 3). Compared to the adult group, the taxa that contributed to the seasonal changes were Brachyura, Polychaeta, Echinidea, FISH, Gastropoda, Bivalvia, Anomura and Polyplacophora. In the old adults, the most important taxa were Polychaeta, Brachyura, Gastropoda, FISH, Echinidea and Bivalvia. For season, the older crabs' diet in summer was reduced to Brachyura and Polychaeta, unlike the youngest groups whose diet was generally more diverse (Table 3). Polychaeta was an important prey in all the demographic groups, but with a differential importance in each group for season. Juveniles and old adults displayed inverse patterns of abundance, in which small crabs consumed more Polychaeta in summer. Conversely, the presence of these prey in older crabs in winter continued. Brachyura was an important prey for all the groups, but was ubiquitous for old adults in winter. FISH was frequently observed in juveniles' diet because they are an important resource. Their importance in winter decreased through ontogeny, and they were almost absent in older adults.

4. Discussion

In the present study, *C. ruber* diet components are defined for the first time. The present results suggest that *C. ruber* is a generalist mesopredator based on the broad range of prey items and the wide variation in the carnivorous diet items identified in its gut contents and observed in underwater surveys. Here we detail our conclusions about the major questions addressed in this study, as well as future directions to evaluate the possible impacts of this and other invasive crab species.

4.1. Feeding strategies

Lack of knowledge about *C. ruber* ecology in its natural populations means that this study presents the first approach to address its foraging patterns and feeding strategies. Our results describe *C. ruber* as a possible generalist mesopredator that forages among a range of species belonging to CRUST, MOL, Annelida, Echinidea and FISH. Similar diets have been described in the most harmful global invasive portunid crabs (i.e., *Carcinus maenas*, Elner, 1981, Siegenthaler et al., 2022; *Callinectes sapidus*, Laughlin, 1982, Prado et al., 2022 or *Charybdis hellerii*, Sant'Anna et al., 2015). These species have the potential to dramatically change newly colonised ecosystems through cascade effects (Papacostas and Freestone, 2019) and have been responsible for regime shifts to degrade ecosystem statuses (Kotta et al., 2018).

We herein observed seasonal and ontogenic changes in *C. ruber* diet. Shifts in diet with ontogeny indicate that the diet of juveniles and adult crabs is diversified, and they consume more prey items per day than old crabs. These differentiated groups' activity exhibits clear shifts in summer. Old crabs consume fewer prey and become less specialised in summer. Many factors can explain these differences. Dominant males exhibit agonistic behaviour, including aggression and fights, which delimits their territories or them obtaining and retaining resources, such as food, shelter and mates (Parker, 1974; Smith et al., 1994; Romano and

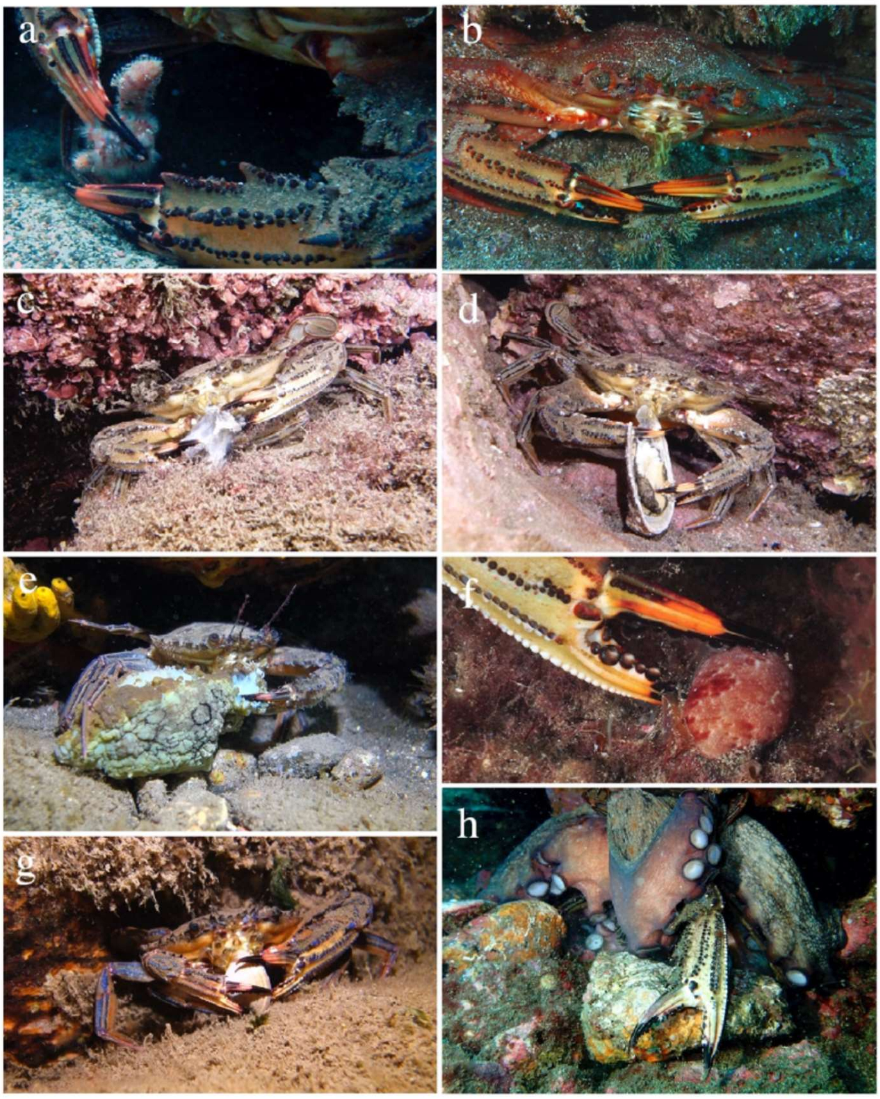

Fig. 2. Foraging behaviour of *C. ruber* under natural conditions (a–h) as a predator and (h) as prey. Different prey devoured at night: (a) *Eurythoe complanata* (Polychaeta), (b) *Pilumnus villosissimus* (Brachyura), (c) head of *Similiparma lurida* (FISH), (d) *Haliotis tuberculata coccinea* (Gastropoda), (e) *Aplysia dactylomela* (Gastropoda), (f) *Bulla mabillei* (Gastropoda), (g) *Mactra stultorum* (Bivalvia) and (h) *Octopus vulgaris* (Cephalopoda) preying on *C. ruber*.

Zeng, 2017). The impact of invasive populations is often determined by scaling up from per capita measurements made using single individuals or several individuals of the same ontogenic group or sex (e.g., Lodge et al., 1994; Rossong et al., 2006). Our results suggest that all the ontogenic groups must be represented in per capita effects or other scaling approaches. In addition, intraspecific interactions with portunid crabs usually result in cannibalism or sublethal predation (Mansour and Lipcius, 1991).

The polychaeta *Hermodice carunculata* is actually considered an invasive species in the Mediterranean Sea (Righi et al., 2020). Information about invasive species' diet habits is often used as a baseline in experimental ecology. The obtained results suggest polychaeta to be a possible prey candidate used in per capita effect experiments, daily consumption rates, among others. The ubiquity of *H. carunculata* in *C. ruber* diet and the presence of many invasive crab species in the Mediterranean Sea (Katsanevakis et al., 2014; Stasolla et al., 2021) allow us to propose this, or a similar polychaete, as a prey candidate in invasive crab experimental ecology.

4.2. Invasive crab demography

The results confirm that juveniles and adults have much more negative impacts on the ecosystem than old adult crabs. This observation is demonstrated by groups' diet showing greater diversification, a high *dIR* and major prey retention in their stomachs than old and dominant adult crabs. Juveniles obtained a higher ingestion rate than old adults because they have high energy requirements for growth. Fishery activity is a source of selective mortality for wild populations because it changes the structure of the population under exploitation (Stevens et al., 2020). This is the case of most crab fisheries, which typically select large dominant males (Carver et al., 2005). Some studies conclude that invasive crab populations possess self-regulation mechanisms based on dominancy, cannibalism and agonistic interactions (Dittel et al., 1995; Lovrich and Sainte-Marie, 1997; Moksnes, 2004). Future studies should clarify the impact of extractive activity on invasive crab populations.

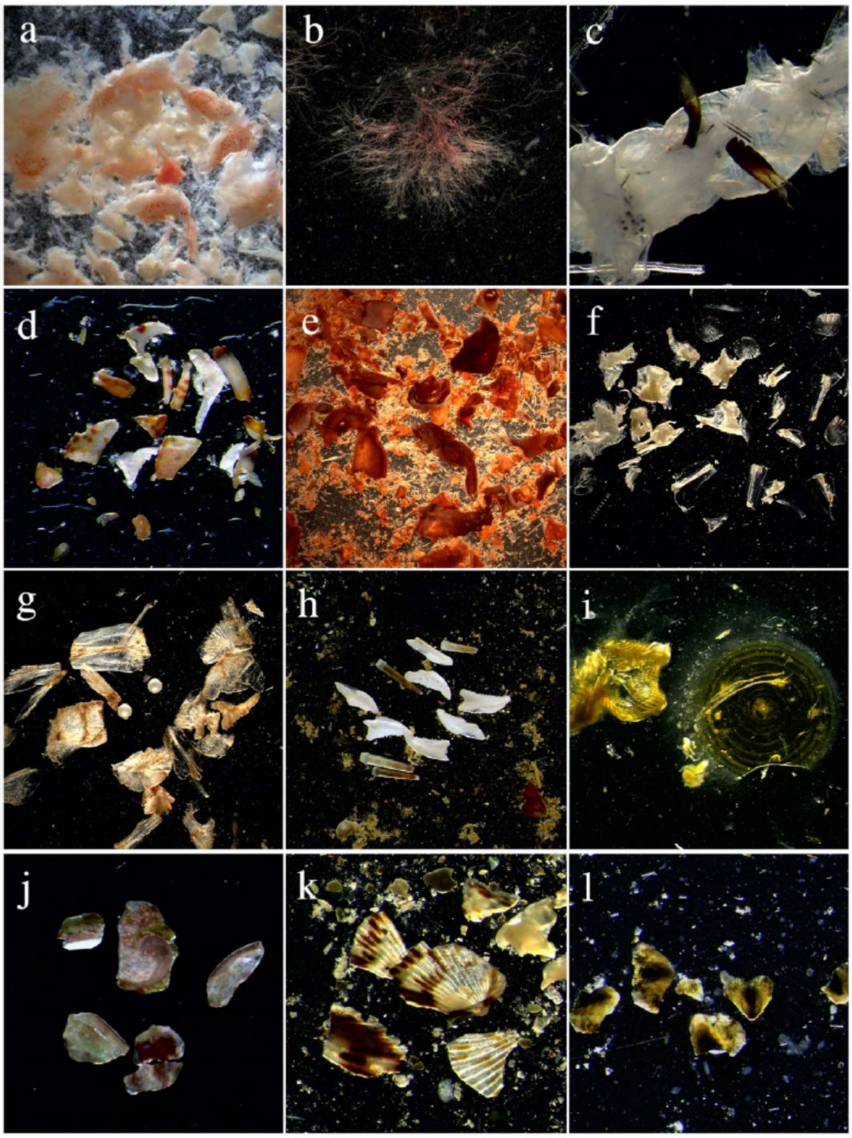

Fig. 3. Examples of the separate prey that represent the main groups identified in the stomach contents of *C. ruber*. (a) Unknown tissue, (b) algae (red algae), (c) Polychaete (*Eurythoe complanata*), (d) Anomura (*Calcinus tubularis*), (e) Brachyura (*Xantho* sp.), (f) FISH (*Gobius niger*), (g) FISH (*Scorpaena* sp.), (h) Echinidea (*Paracentrotus lividus*), (i) Gastropoda (*Phorcus* sp.), (j) Gastropoda (*Haliotis tuberculata coccinea*), (k) Bivalvia (Cardiidae) and (l) Polyplacophora (*Chiton canariensis*). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2

Results of the PERMANOVA testing for diet differences in *C. ruber* in the presence/absence terms of prey in stomachs according to ontogenic groups (juveniles, adults, old adults), sex (male, female) and season (winter, summer). "Juveniles" were assigned to individuals with <55 mm CW. The "adults" category comprised those crabs whose CW ranged from 55 mm to 75 mm "Old adults" were designated as the individuals with >75 mm CW *, $p < 0.05$; **, $p < 0.01$. df, degrees of freedom; SS, sum of squares; MS, mean squares; Pseudo-F, pseudo F value; p, p value.

.	df	SS	MS	Pseudo-F	P
Sex	1	4482.5	4482.5	0.98561	0.423
Ontogenic groups	2	15595	7797.4	1.7145	0.041*
Season	1	10773	10773	2.3687	0.02*
Sex*Ontogenic groups	2	8747.6	4373.8	0.96171	0.515
Sex*Season	1	2620.7	2620.7	0.57624	0.806
Ontogenic groups*Season	2	8995.7	4497.8	0.98898	0.452
Sex*Ontogenic groups*Season	1	6505.1	6505.1	1.4303	0.199
Residuals	216	9.82E+05	4548		
Total	226	1.04E+06			

4.3. Impacts on threatened species and fishery resources

From the management perspective, foraging or diet studies into invasive species suggest which populations (prey) should be prioritised or controlled. The fact that *Haliotis coccinea canariensis* is on the diet list confirms that *C. ruber* consumes threatened species in the region. Additionally, the ubiquity of the genus *Xantho* as prey is a matter of concern because this group species represents the bait of highly prized traditional fisheries in the region (Bortone et al., 1991; Bas, 1995). To date, high pressure on this resource has been restricted to intertidal areas, but *C. ruber* intrusion could draw out this pressure to circalittoral areas.

Our underwater surveys suggest that apex predators, such as groupers, elasmobranchs and MOL (*Octopus*), are the largest *C. ruber* predators in the area. In its native area, *C. ruber* is the most important species in the diet of *Epinephelus marginatus* (Machado et al., 2008; Freitas et al., 2017). Our observations signal interactions and future directions for trophic studies to determine either strength or vulnerability against the colonisation and dispersal of invasive crabs like *C. ruber* in other areas.

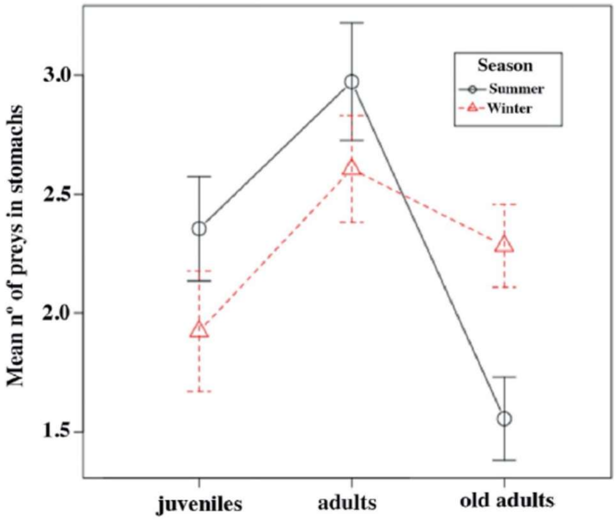

Fig. 4. Effect of ontogenic group (juveniles, adults, old adults) and season (summer and winter) on the mean number (± standard error) of prey (as items) retained in *C. ruber* stomachs. "Juveniles" were assigned to the individuals with <55 mm (CW). The "adults" category comprised the crabs with 55 and 75 mm CW. "Old adults" were designated as the individuals with >75 mm CW.

5. Conclusions

The present document is the first contribution to *C. ruber* ecology as an invasive NIS. This portunid plays a similar mesopredator role to that observed in important global invasive crabs. Several threatened or commercially important species are major components of its diet. Some *C. ruber* predators feature among IUCN Red list species or are targets of extractive activities. *C. ruber* diet patterns are associated with the demographic traits that intrinsically become important to manage them. The present results could represent the baseline for future studies on the impact of this invasive NIS, and form part of arguments to include *C. ruber* on the list of invasive alien species of European Union concern.

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Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

Author's contributions

Raül Triay-Portella: Conceptualization, investigation, sampling, formal analysis, writing – review & editing. José A. Martín: Conceptualization, Investigation, Sampling, Writing – review. Lucía Luque: Sampling, Investigation, Writing. José G. Pajuelo: Conceptualization, Investigation, Writing – review & editing.

Table 3
The SIMPER testing results for diet dissimilarity in *C. ruber* in presence/absence terms of prey in gut contents according to ontogenic groups (juveniles, adults, old adults) and season (winter, summer). *, $p < 0.05$; **, $p < 0.01$.

Juveniles (CW ≤ 55 mm)
Summer & Winter average dissimilarity = 72.58

Pres	Summer	Winter	
	Mean Appearance	Mean Appearance	C%
Brachyura	0.54	0.56	18.57
Polychaeta	0.46	0.22	15.82
Perciformes	0.17	0.44	15.29
Echinidea	0.33	0.25	12.54
Anomura	0.21	0.13	9.67
Bivalvia	0.21	0.09	8.68
Gastropoda	0.21	0.09	7.20
Polyplacophora	0.08	0.13	6.15

Adults (55 mm < CW ≤ 75 mm)
Summer & Winter average dissimilarity = 74.03

Prey	Summer	Winter	
	Mean Appearance	Mean Appearance	C%
Brachyura	0.54	0.45	19.03
Polychaeta	0.40	0.41	18.68
Echinidea	0.22	0.25	11.01
Perciformes	0.16	0.20	10.45
Gastropoda	0.24	0.20	10.40
Bivalvia	0.22	0.13	8.44
Anomura	0.14	0.18	8.42
Polyplacophora	0.12	0.11	6.36

Old adults (CW > 75 mm)
Summer & Winter average dissimilarity = 54.16

Prey	Summer	Winter	
	Mean Appearance	Mean Appearance	C%
Polychaeta	0.29	0.67	32.07
Brachyura	0.62	1.00	25.70
Gastropoda	0.29	0.00	13.07
Perciformes	0.18	0.00	9.87
Echinidea	0.18	0.00	7.48
Bivalvia	0.11	0.00	4.68

CRediT authorship contribution statement

Raül Triay-Portella: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **José A. Martín:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Lucía Luque:** Writing – review & editing, Visualization, Validation, Methodology, Data curation. **José G. Pajuelo:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Investigation, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2022.107949.

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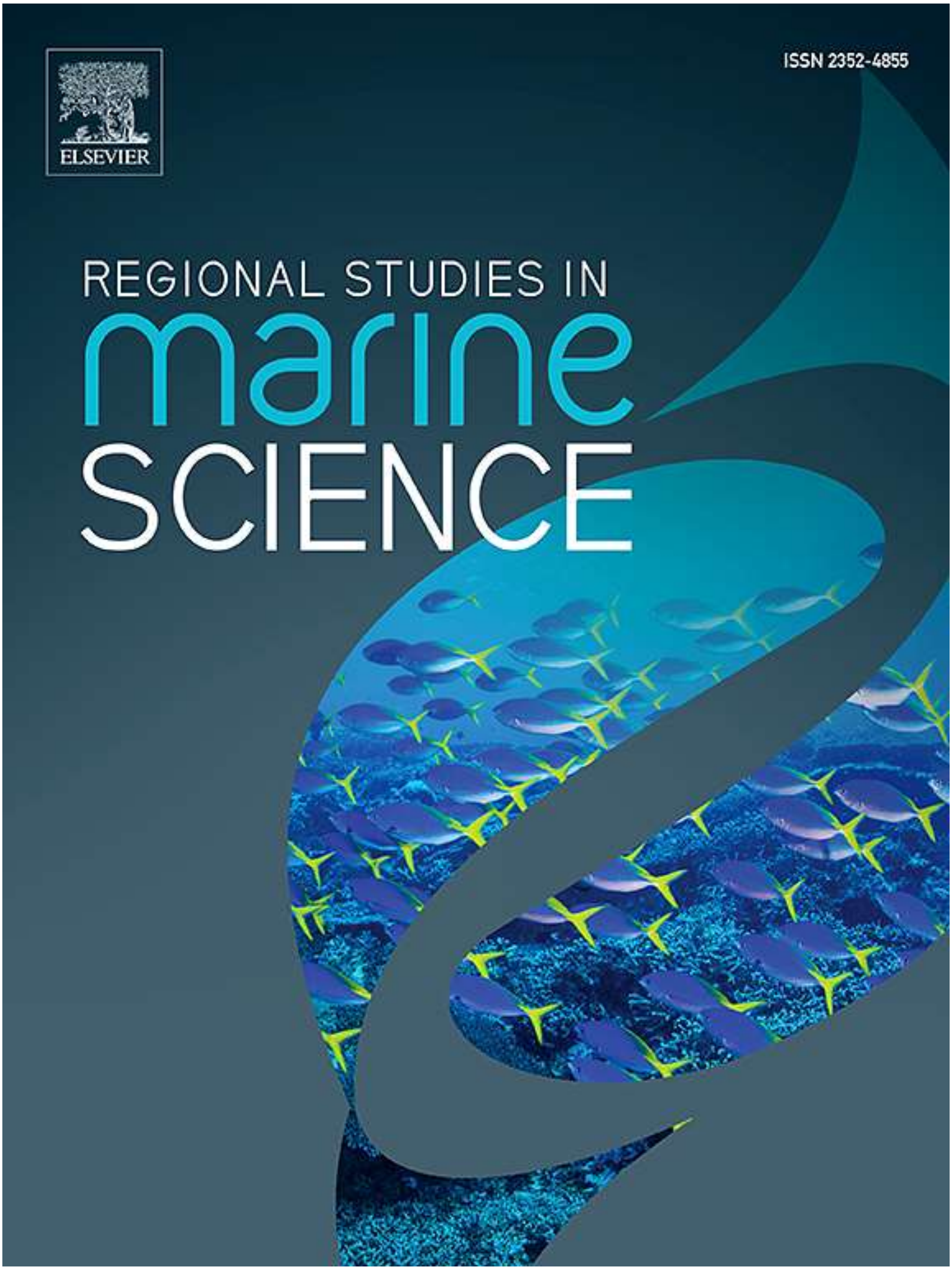
Chapter 3. Reproductive features of the crab *Cronius ruber* (Brachyura, Portunidae) on the Canary Islands (Central Eastern Atlantic, Spain



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Reproductive features of the invasive crab *Cronius ruber* (Brachyura, Portunidae) on the Canary Islands (central eastern Atlantic), Spain

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ABSTRACT

The main features of the life cycle and ecology of the invasive species *Cronius ruber* in both its native range and invaded areas are unknown. Therefore, the reproductive traits of this invasive allochthonous species were studied in the Canary Islands, including sex structure, maturity, fecundity, and egg development. Females were more abundant than males, mainly in the size range between 25 and 80 mm CW. Only males were observed in size classes above 80 mm CW. Reproduction of *C. ruber* is continuous throughout the year, with a new oviposition immediately after the end of the egg incubation period. This process can be repeated in females up to six times, without re-mating, due to the sperm stored by females in the spermathecae. Sexual maturity was estimated at a similar size in both sexes, corresponding to 57–59 % of their maximum observed size. Egg development appeared to be completely synchronous, with a development period lasting slightly less than 1 month. The mean fecundity was 657,282 eggs, with a maximum observed fecundity of 988,509 eggs. This species shows a high reproductive potential, characteristic of invasive species.

1. Introduction

Decapod species are among the most abundant invertebrates in marine habitats and play a crucial role in ecosystems (Haedrich et al., 1980; Merrett and Haedrich, 1997; Cartes and Carrassón, 2004; Fanelli et al., 2007). Biological invasions, including those by decapods, are among the major global causes of biodiversity loss. Within decapods, the infraorder Brachyura is particularly noteworthy as it includes numerous successful invasive non-indigenous species (NIS) (Brockerhoff and McLay, 2011) that have significant ecological impacts (Kraemer et al., 2007; Garbary et al., 2014) and contribute substantially to biodiversity loss in ecosystems worldwide (Courchamp et al., 2017). On oceanic islands like the Canary Islands, high human pressure on natural resources and ecosystem degradation due to human overpopulation increase the impact of invasive INS (Triay-Portella et al., 2022). Although invasive NIS eradication is not expected, colonisation and establishment in marine habitats (Thresher and Kuris, 2004), and removal, have been demonstrated as effective strategies for dwindling the population and its impacts on habitats (Triay-Portella et al., 2022).

Invasive species can decrease the biomass of native species *via* several pathways related to

species' reproductive and predation characteristics (Noonburg and Byers, 2005; Salo et al., 2007; Parker et al., 2013; Triay-Portella et al., 2022). Invasive allochthonous crabs develop high densities and show competitive advantages over autochthonous fauna (Brockerhoff and McLay, 2011). Knowledge of reproductive characteristics is one of the main objectives for INS management and assessments. Knowing the main biological characteristics is especially important because, although INS display better behaviour in the non-indigenous area, many invasive species show similar traits to native species and may be predictors of invasion success with an interaction between biological traits and environmental conditions (Parker et al., 2013).

The main objective of the present study is to describe the reproductive characteristics (oocyte development, ovarian maturation, embryonic development, histologically confirmed size upon sexual maturity, and spawning and hatching season) of an invasive marine INS in the central eastern Atlantic: the black-tip shovel crab *Cronius ruber* (Lamarck, 1818). This crab was first observed 12 years ago on the Gran

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Canaria Island (Canary Islands, Spain) (Coinva, 2019). After that observation, *C. ruber* rapidly spread throughout the Canary archipelago, and went as far as Madeira (Schäfer et al., 2019). The life cycle and ecology of *C. ruber* remain unknown in its native and invaded areas (Triay-Portella et al., 2022).

Cronius ruber is a pantropical species that lives in the Atlantic and east Pacific Oceans. In the east Pacific, it is reported from Mexico to Peru (including the Galápagos Islands). In the west Atlantic, it is found from South Carolina to Brazil. In the east Atlantic, it is observed from Mauritania to Angola, including the Cape Verde archipelago (Manning and Holthuis, 1981). *C. ruber* is a euryhaline and eurythermic crab that inhabits all types of shallow water substrata, including mud, mud and sand, fine sands, coarse shelly sands, calcareous algae, shell gravels, rocks, rocks with algae, stones and boulders (Manning and Holthuis, 1981; González et al., 2017) at depths up to 69 m, but mainly at shallower depths of < 30 m (Manning and Holthuis, 1981).

2. Materials and methods

2.1. Study area and sampling

Cronius ruber specimens were collected by hand from January to December 2019 in heavily four anthropized areas (Agaete, La Garita-El Hombre, Arinaga and El Pajar) on the Gran Canaria Island (Canary Islands, Spain) at depths from 1 m to 8 m on a rocky/sandy bottom (Fig. 1). Samplings were always conducted after sunset and before midnight, which are the major *C. ruber* activity peaks (Triay-Portella et al., 2022). Monthly, two divers collected samples by conducting two transects of 25 m long and 2 m wide (0.5 m on each side of divers) at two different depths: 3 m (1–4 m depth) and 6 m (5–8 m depth). Carapace condition (hard or soft) was also recorded in pairs of embraced males and females. Carapace width (CW), the maximum transversal diameter at the midline of the carapace, for each crab was taken following Triay-Portella et al. (2014) with a digital calliper (0.01 mm precision). CW was taken on the upper crab side in the left-right direction.

2.2. Population structure (Size and sex ratio)

Sex, ovigerous condition and the colour of external eggs on pleopods were also recorded. Total weight (TW), total egg weight (EW) and gonad weight (GW) were recorded to the nearest 0.1 g. A macroscopic six-stage maturity scale based on ovary size, shape and condition was used (Erdman and Blake, 1988; Triay-Portella et al., 2014). For males, a macroscopic three-stage maturity scale based on size, colour and shape of testes was applied (Triay-Portella et al., 2014). Ovaries were preserved in Histofix® and histologically processed to verify the macroscopically assigned maturity stage. The seminal receptacle of ovigerous females was examined histologically to assess the occurrence of multiple spawnings during the same intermoult period (Sant'Anna et al., 2012). Ovarian tissues were dehydrated in a series of growing ethanol solutions (70–100 %), cleared in Isoparafine H®, and finally embedded in paraffin Paraplast®. Tissues were sectioned at a thickness of 5 µm and were first stained with Harris haematoxylin and then with eosin (Triay-Portella et al., 2017; Bautista et al., 2024).

The ratio between males and females (sex ratio) was estimated by size intervals and for the total sample. The sex ratio was tested for deviation from the expected 1:1 ratio using the Pearson chi-square goodness-of-fit test (Sachs, 1982; Sokal and Rohlf, 2012). Differences in the mean CW and TW between males and females were tested by the Student's t-test. The Kolmogorov-Smirnov non-parametric Z-test was used to test the differences in the CW and TW frequencies between males and females

2.3. Gonadal maturity

The estimation of physiological size upon first sexual maturity was calculated as the proportion of mature females (histological stages II to VI) by size class. Sexual maturity for males was estimated as the proportion of histological mature males (stage III) by size classes. Size at sexual maturity was estimated by fitting data to the logistic equation $P=100/1+\exp(-r(CW-CW50)$ by non-linear regression, where CW50 is size at 50 % maturity, P is the proportion of morphologically or physiologically mature crabs in each size class, and r is the model parameter. The relative reproductive potential (RRP) was estimated for *C. ruber* following Biscoito et al. (2015) using the equation $RRPi= Mi\times Fi\times Ci$, where RRPi is the RRP for size class i, Mi is the proportion of mature females in size-class i, Fi is the mean individual fecundity of size-class and Ci is the proportion of size class i in the sample (Biscoito et al., 2015). The RRP estimates the size class of spawning females that makes the greatest contribution to egg production in a population (Biscoito et al., 2015; Bautista et al., 2024). The mean fecundity at size estimated following Triay-Portella et al. (2017), the maturity size and the size structure of the female population were used to calculate the RRP.

2.4. Oviposition periods (Including rearing)

In order to assess the reproductive period, the monthly relative proportion of *C. ruber* in each sexual maturity stage (both sexes) and its ovigerous condition (females) were determined. The gonadosomatic index (GSI) of specimens was also calculated by dividing GW by TW, and was referred to by month. The mean CW values were compared among maturity stages by an analysis of variance (ANOVA). Then Dunnett's T3

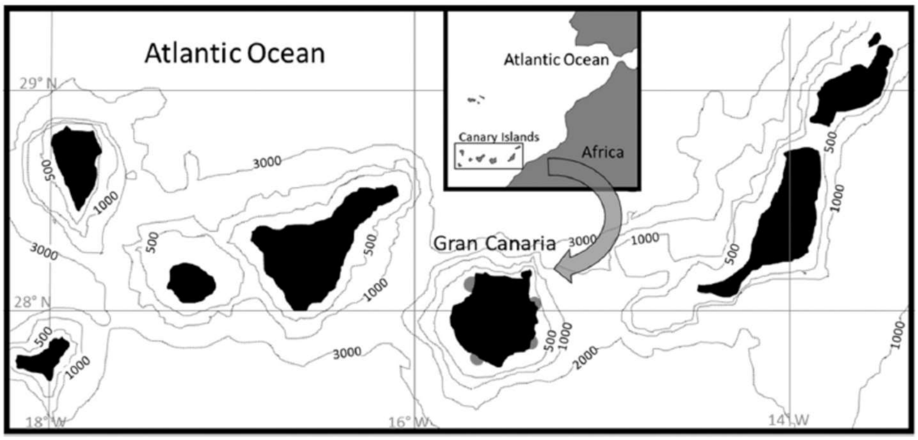

Fig. 1. Canary Islands. Sampling areas in Gran Canaria.

post hoc test was used to determine which samples differed (Sokal and Rohlf, 2012). The post hoc test identifies homogeneous subsets of means that do not differ from one another by testing the null hypothesis of equality in the mean CW with a significance level of 5 % (α=0.05) (Sokal and Rohlf, 2012). Lack of differences in the mean CW among maturity stages identified by Dunnett's T3 test was verified by an ANOVA.

Mature females (n=15) after ecdysis were kept separately in tanks with sand bottoms to facilitate egg extrusion (Fazhan et al., 2022) for 1 year to estimate the number and time that elapsed between ovipositions. Each tank had a capacity ranging from 120 to 500 liters, providing ample space for the crabs. The water temperature in tanks was maintained at the same levels as those recorded monthly at sea. The crabs were fed daily with a diet consisting of a mixture of fresh fish and polychaeta to ensure a balanced nutritional intake, providing approximately 5 % of their body weight per day. To maintain water quality, 25 % of the tank water was replaced twice a week with fresh seawater, which helped prevent the buildup of waste products and maintain appropriate salinity levels. The tanks were kept under a controlled photoperiod regime of 12 hours of light and 12 hours of darkness to simulate natural day-night cycles, using full-spectrum aquarium lights set to mimic natural sunlight. Water parameters such as pH, salinity, ammonia, nitrite, and nitrate levels were monitored weekly to ensure a stable and healthy environment for the crabs. The pH was maintained between 8.0 and 8.3, and salinity was kept at 35 ppt. Ammonia and nitrite levels were kept below 0.05 mg/L, and nitrate levels were maintained below 10 mg/L.

2.5. *Fecundity*

Ovigerous females from all the size classes were used to estimate fecundity. The eggs were removed from females, and egg masses were then placed on 100-µm mesh, washed and isolated from pleopods. After removing excess wash water, eggs were weighed (wet weight). Based on Mori (1986), only those females with eggs in the first development stage were considered for the *C. ruber* fecundity estimates. A subsample of 5 % in weight from each selected female was counted through a dissection microscope. Batch fecundity (F), defined as egg production by batch, was estimated by the gravimetric method (Triay-Portella et al., 2014). A linear function was fitted to F *versus* CW.

Egg developments were described and classified into four stages, which correlated with the egg mass colour adapted from Triay-Portella et al. (2014).

3. Results

3.1. *Size structure*

In all, 523 *C. ruber* individuals were collected. Males (n=214) ranged from 27.3 to 90.6 mm CW and from 7.5 to 233.7 g TW. Females (n=309) ranged from 26.4 to 82.3 mm CW and from 6.2 to 142.5 g TW. Significant differences in the mean CW and TW were found between males (66.7±12.3 mm and 86.5±48.5 g) and females (62.6±9.7 mm and 62.2 ±27.8 g) (t-test, CW t=4.11, $p<0.0001$; TW t=6.55, $p<0.0001$). The CW and TW ranges also differed significantly between males and females (Kolmogorov-Smirnov non-parametric test, CW Z=3.45, $p<0.0001$; TW Z=3.44, $p<0.0001$).

3.2. *Sex ratio*

Females were significantly more abundant than males with a male: female proportion of 1:1.44 (χ^2-test, $\chi^2=17.25$, $p<0.0001$). Females were more abundant than males in the 50–65 mm CW size class (1:>1.84, χ^2-test, $\chi^2>56.73$, $p<0.0001$). However, males were significantly dominant in the size classes larger than 70 mm CW (χ^2-test, $\chi^2>13.08$, $p<0.001$), particularly over 85 mm CW, for which only males were observed (Fig. 2).

3.3. *Sexual maturity*

Cronius ruber ovaries are H-shaped with the spermatheca extending ventrally to vulvae (gonopores), which open on the sixth thoracic somite. Of the different recorded ovary colours or colour shades, six categories were histologically characterized (Fig. 3).

Immature colourless ovaries (stage I) presented the germ strand surrounded by fibrous connective tissue with a very thick ovarian wall and oogonia close to it. Early developed ovaries (stage II) displayed ivory or orange shades, characterized by oocytes in different previtellogenic stages. Resting ovaries (stage III) presented various shades of grey or brown, and were characterized by oocytes in an advanced previtellogenic stage with abundant fibrous connective tissue. The

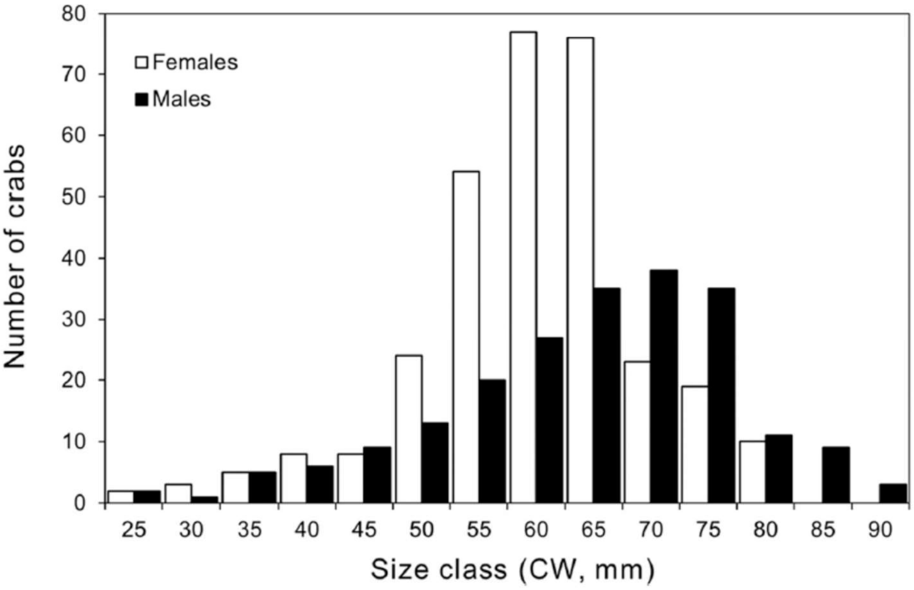

Fig. 2. Number of specimens by size class of 5 mm of carapace width for males and females of *Cronius ruber*.

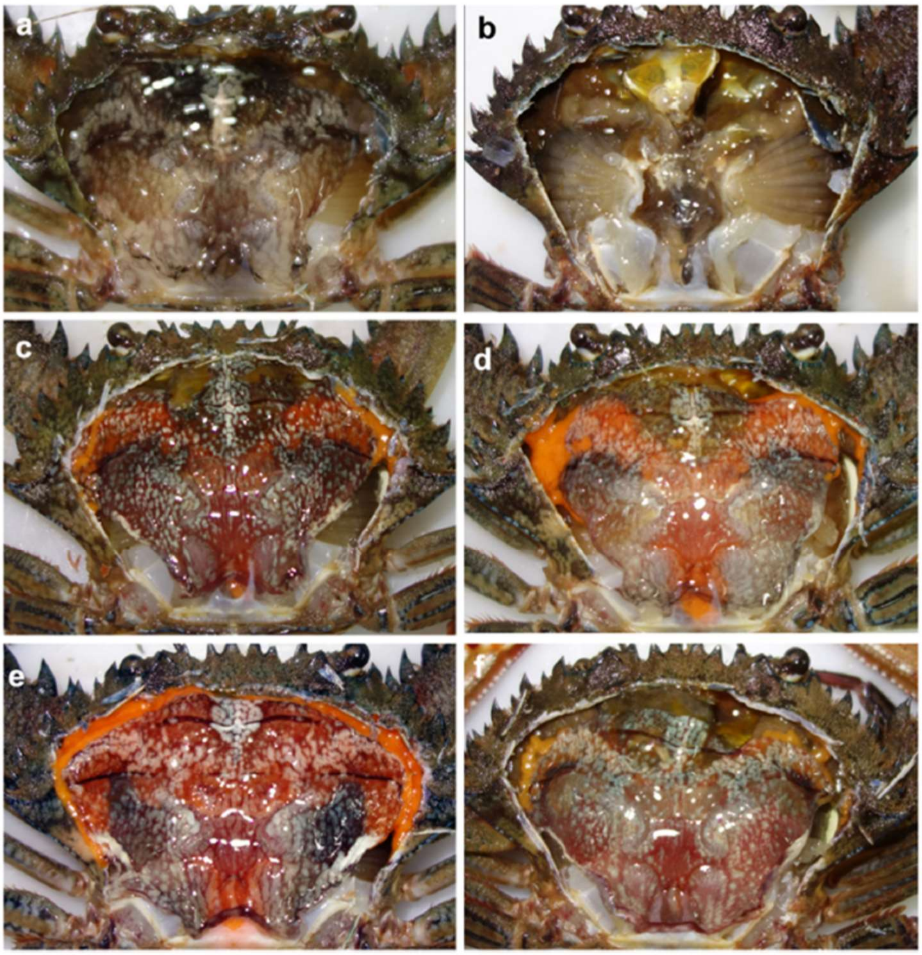

Fig. 3. Macroscopical scale of sexual maturity stages in *C ruber*. Females: (a) I, immature; (b) II, early developed; (c) III, resting; (d) IV, advance; (e) V, mature; (f) VI, post-spawning.

colour of the advanced ovaries (stage IV) varied from orange to brown, lilac, violet or grey, and were histologically characterized by oocytes in early to late vitellogenesis stages. Mature ovaries (stage V) were characterized by purple, lilac, grey or brown masses occupying nearly all the cavity, and were histologically dominated by mature oocytes with a high concentration of yolk globules of granular appearance due to the high concentration of yolk globules. Oocytes are visible to the naked eye. Postspawning ovaries (stage VI) presented a very flaccid mass of a purple, brown or grey colour with a thin ovarian wall, and the remaining unspawned eggs underwent reabsorption (Fig. 4). Spermathecae contained spermatophores inside and were in the same development stage as that observed in the mature testes of males.

The total female sample (Fig. 5) comprised 23 females with immature ovaries (mean 41.61±8.47 mm CW, range 26.40–58.82 mm CW), 45 with early developed ovaries (mean 59.92±6.70 mm CW, range 41.72–77.36 mm CW), 67 with developing/resting ovaries (mean 65.62 ±7.51 mm CW, range 44.03–82.10 mm CW), 78 with advanced ovaries (mean 65.77±8.21 mm CW, range 48.20–82.30 mm CW), 65 with mature ovaries (mean 63.36±6.84 mm CW, range 47.40–80.17 mm CW) and 31 with postspawning ovaries (mean 65.30±6.51 mm CW, range 54.70–81.7 mm CW).

For females, significant differences were observed in the mean CW among the ovarian development stages (ANOVA, $F= 43.89$ $p<0.0001$). Dunnett's T3 *post hoc* test showed one group of ovarian stages with no differences in the mean CW value among stages (maturity stages III–VI, $p>0.579$), but with significant differences between stage I and stage II, and also between each stage from III to IV ($p<0.012$). Lack of significant differences in the mean CW among females from stage III to VI was verified by an ANOVA ($F= 1493$, $p=0.217$).

For the total male sample (Fig. 6), 38 were immature (stage I) (mean 47.95±8.20 mm CW, range 27.3–60.2 mm CW), 50 were advanced (stages II) (mean 60.83±4.476 mm CW, range 44.32–65.70 mm CW) and 126 mature (stages III) (mean 74.61±6.72 mm CW, range 48.36–90.60 mm CW). Males obtained different mean CW values among the gonadal development stages (ANOVA, $F=265.6$, $p<0.0001$). Dunnett's *T3 post hoc* test showed significant differences in CW among stages ($p<0.001$).

For females, length upon sexual maturity CW_{50}, based on the histological maturity stage, was estimated as 49.17 mm CW (Fig. 7). Size upon sexual maturity onset in males (CW_{50}), based on the macroscopical analysis, was estimated at 52.19 mm CW (Fig. 8).

3.4. *Spawning period*

The females with mature ovaries were observed every month, but were more abundant from July to November. The females showing ovaries in the postspawning stage were recorded all year round (Fig. 9). A significant correlation between the gonadal maturity stage and the egg mass colour pattern was observed ($p=0.01$).

Immature males were observed throughout the year, but were more abundant mainly from March to June. The males with mature testes were recorded all year round (Fig. 10).

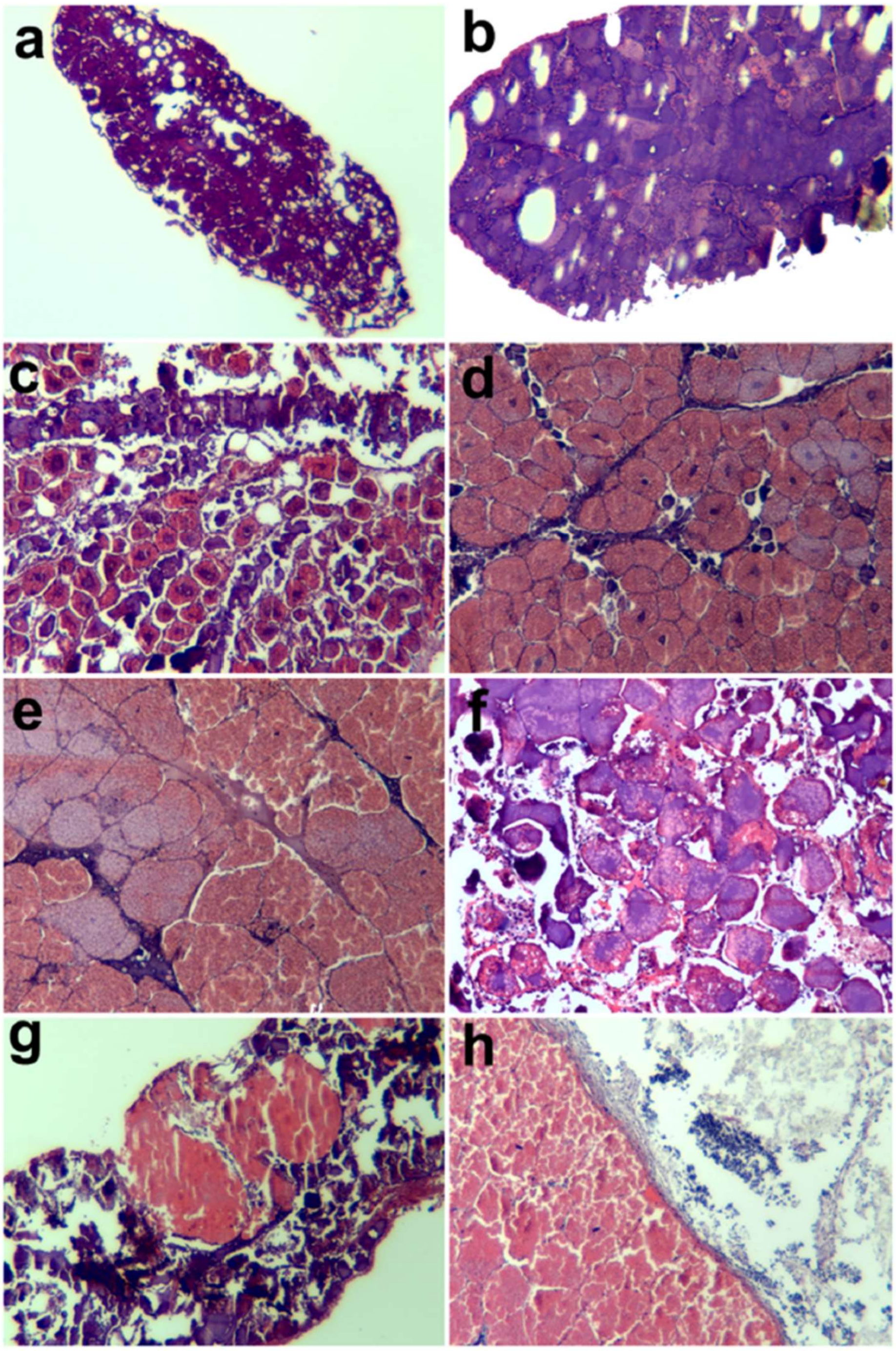

Fig. 4. Histological sections illustrating *C. ruber* ovaries at different maturity stages. (a) I, immature; germ strand surrounded by fibrous connective tissue (b) II, early developed; oocytes in different previtellogenic stage; (c) III, resting; oocytes in advanced previtellogenic stage and with abundant fibrous connective tissue (d) IV, advance; oocytes in early to late stages of vitellogenesis (e) V, mature; mature oocytes (f) VI, post-spawning. Oocytes in atresia stage (g) and spermatheca (h).

For females and males, the GSI gave high values all year round (Fig. 11). The ANOVA showed differences in the GSI among the CW size classes for females ($F=2.396$, $p<0.007$). Dunnett's *T3 post hoc* test identified two homogeneous CW size class subsets with no differences in the GSI values (25–45 mm CW; 50–80 mm CW).

Of all the sampled females, 85 were observed under the ovigerous condition (mean 66.07±6.87 mm CW; 79.29±25.84 g TW). Their sizes ranged from 50.0–81.7 mm CW, and 224 females were collected under the non-ovigerous condition (mean 61.19±10.25 mm CW; 55.50 ±25.55 g TW) and ranged from 26.4 to 82.3 mm CW (Fig. 12). The females under the non-ovigerous condition had a smaller mean CW (t-test, $t=4.82$, $p<0.0001$) and TW than the ovigerous females (t-test, $t=7.22$, $p<0.0001$). Ovigerous females were observed throughout the year although non-ovigerous females were more abundant in all the months (Fig. 13).

The RRP showed tha the females of 60–70 mm CW (57 % of females) yielded 68.9 % of the population egg production. The females smaller

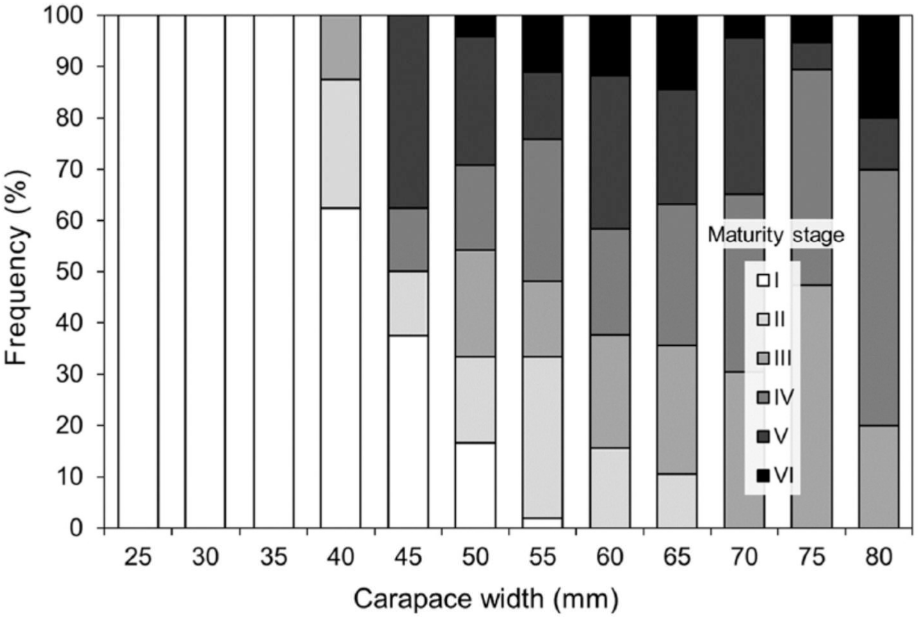

Fig. 5. Frequency of specimens by maturity stages and size class of 5 mm of carapace width for females of *Cronius ruber*.

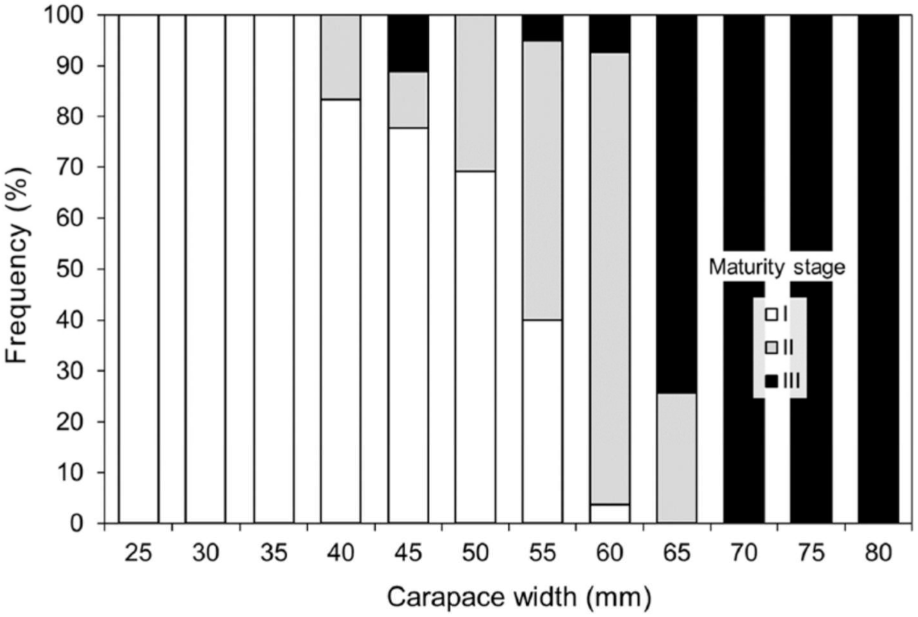

Fig. 6. Frequency of specimens by maturity stages and size class of 5 mm of carapace width for males of *Cronius ruber*.

than 60 mm CW comprised 33.7 % of the sample, but produced 7.6 % of eggs (Fig. 14).

3.5. Fecundity and egg development

Eggs fully filled with yolk and undivided (stage I) were observed in orange egg masses. In stage II, eggs showed a visible free yolk region, and they were observed in red or orange egg masses. The eggs with embryos showing slightly pigmented eyes (stage III) were observed in brown egg masses. In stage IV, embryos had some visible pigmented structures as enlarged eyes, segmented appendages and abdomen, and were found in brown egg masses (Figs. 15 and 16).

The eggs presented a spherical shape. The mean number of external eggs carried by females was calculated as being 657,282±203,688 for a range from 141,356 eggs (carried by 55.0 mm CW/39.3 g TW) to 988,509 eggs (carried by 72.6 mm CW/108.6 g TW). Regression analysis showed a significant, positive correlation between logarithmic fecundity and carapace width relationship ($\log \text{Fecundity} = -1.542 + 3.953 \log \text{CW}$, $r2 = 0.629$, $P < 0.001$) (Fig. 17).

3.6. Mating

Female fertilisation by males occurs after ecdysis. Thereafter, isolated females had up to six ovipositions during their culture in laboratory tanks, with a mean of 3.6±0.94 ovipositions. Egg extrusion events occurred from 1 to 6 months after females' isolation.

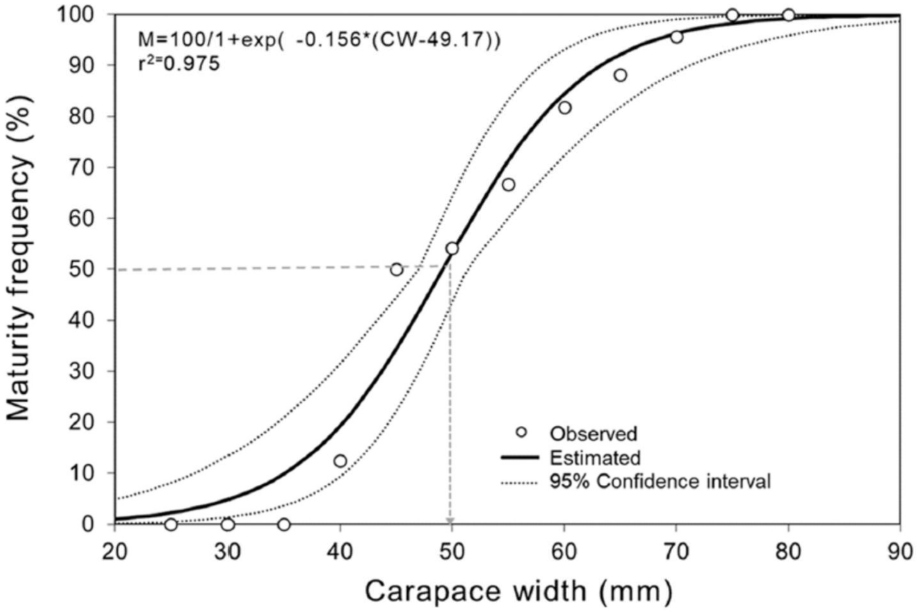

Fig. 7. Ogives for size at first maturity, based on histological maturity condition for females of *Cronius ruber*. r^2, determination coefficient.

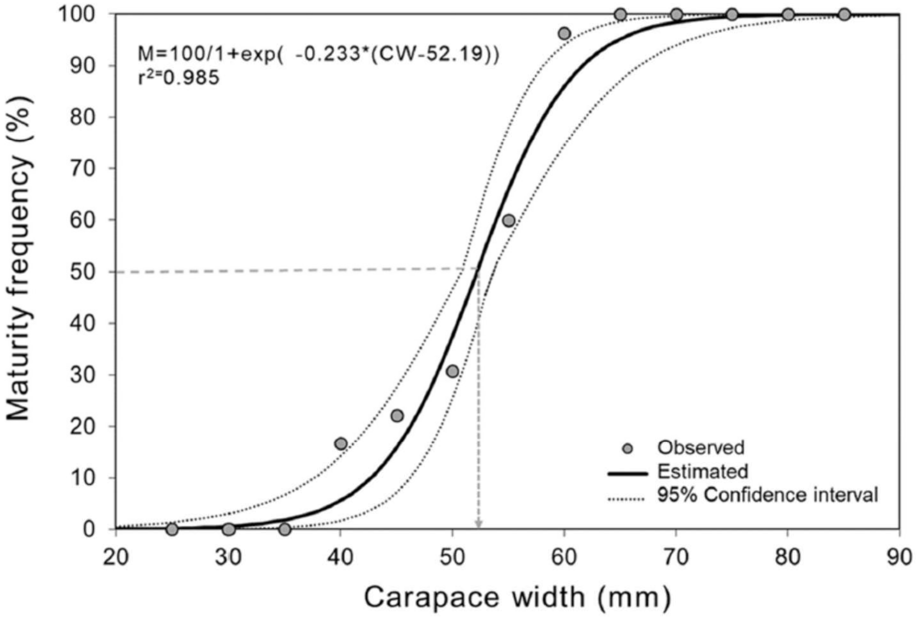

Fig. 8. Ogive for size at first maturity, based on macroscopical maturity condition (M) for males of *Cronius ruber*. r^2, determination coefficient.

4. Discussion

The *C. ruber* sex ratio was skewed towards females throughout the year. Portunid crab populations biased in favour of females are not uncommon in natural populations (Abelló et al., 1990; Lee and Hsu, 2003; Doi et al., 2008; Hosseini et al., 2014; Tureli and Yesilyurt, 2018). The sex ratio in a population varies with changes in habitat and environmental characteristics, and with migrations linked with the different habitat preferences of each sex associated with differences in physiological tolerances or involving sexual differences in longevity (survival rates) or growth rates (Baeta et al., 2005; Doi et al., 2008; Young et al., 2017; Young and Elliott, 2020). The selected sampling method, done by hand, suggests that the observed skewed sex ratio in favour of females was real and not a consequence of using traps or due to sampling methods. When traps are used, a skewed sex ratio in favour of males is observed, which is attributed to more males than females being caught with traps. This is because male portunids enter traps more because they are larger, and are more territorial than females (Aagaard et al., 1995; Young et al., 2017; Young and Elliott, 2020).

The presence of mature and ovigerous females in all the months reveals that *C. ruber* spawning activity occurs all year long in this non-indigenous area. Spawning activity throughout the year has also been observed in many portunid crabs, such as *Portunus pelagicus* (Linnaeus, 1758) (de Lestang et al., 2003), *Charybdis (Charybdis) feriata* (Linnaeus, 1758) (Pillay and Nair, 1973), *Charybdis (Gonioneptunuus) bimaculate* (Miers, 1889) (Doi et al., 2008) and *Charybdis (Charybdis) natator*

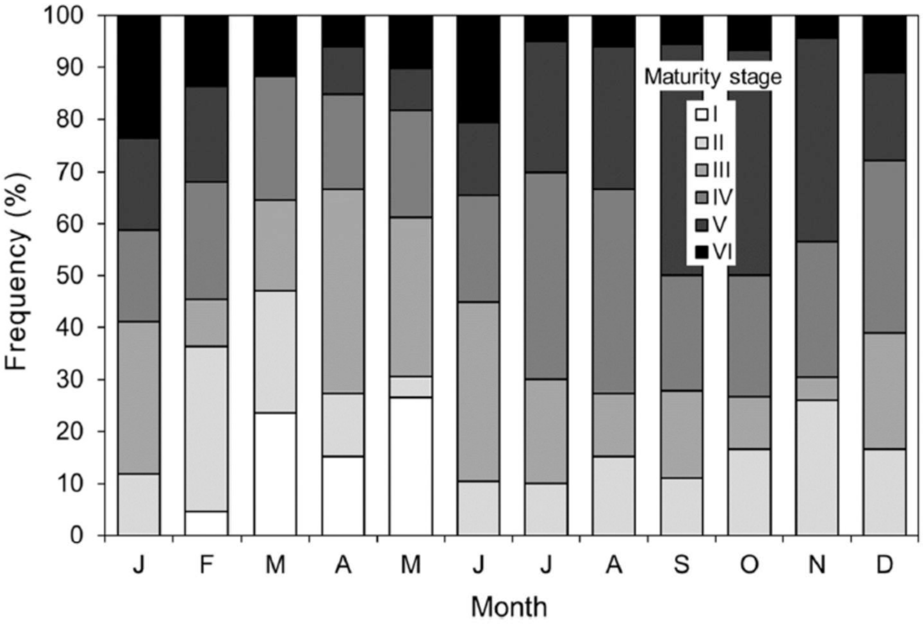

Fig. 9. Frequency of specimens by maturity stages and season for females of *Cronius ruber*.

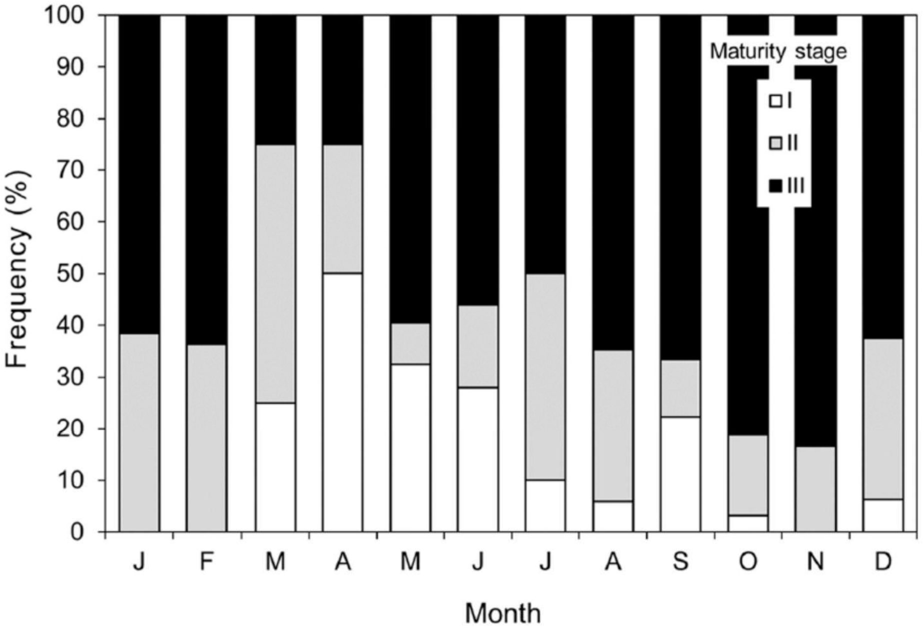

Fig. 10. Frequency of specimens by maturity stages and season for males of *Cronius ruber*.

(Herbst, 1794) (Sallam and Gab-Alla, 2010). The *C. ruber* maturity stage pattern confirms year-round spawning because, during extended spawning and breeding seasons, individuals asynchronously reproduce with some in the developing or mature stage and others in the spent stage (Giese, 1959; Sallam and Gab-Alla, 2010).

The GSI values for females remained high throughout the year. The GSI can be used as an indicator of maturity when values are independent of body size (Nawer et al., 2022). However in *C. ruber,* the GSI cannot be used as an appropriate index to assess maturity status because of the trend observed between the GSI and body size. However, the macroscopic maturity stage correlated highly with the histologically determined maturity stages, and the percentage of (macroscopic-histological) occurrence was above 98 %.

Size upon sexual maturity is reached in portunid crabs between mainly 43–56 % of their maximum size (i.e. de Lestang et al., 2003; Hall et al., 2006; Rasheed et al., 2021). In *C. ruber,* the overlap in size range between immature and mature is large. Differences in size upon maturity between individuals of the same species may be due to variation in moult increments and number of moults (Hines, 1988). *C. ruber* females reach sexual maturity earlier than males. In portunid crabs, both possibilities have been described for different species (Young and Elliott, 2020). Early sexual maturity for females has been observed i.e. by Abelló et al. (1990) in *Plybius depurator* (Linnaeus, 1758) and *Macropipus tuberculatus* (Roux, 1830), and by de Lestang et al. (2003) in *Portunus*

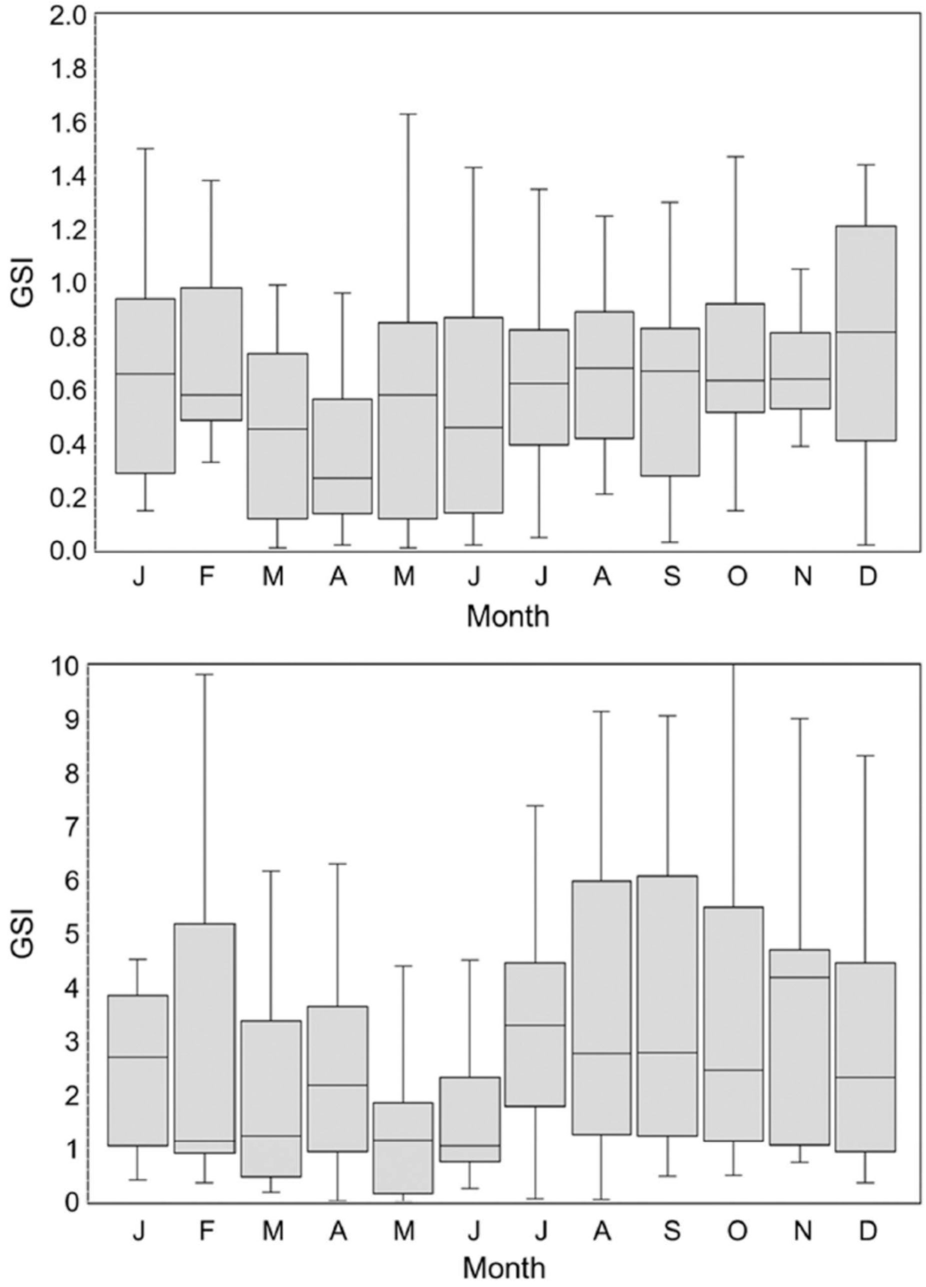

Fig. 11. GSI values (minimum, first quartile, median, third quartile, and maximum) by month for males (up) and females (down) of *Cronius ruber*.

pelagicus.

Cronius ruber shows a correlation between body size and fecundity. This correlation has been observed in size and weight for many Portunid crabs by other authors (Mantelatto and Fransozo, 1997; Cházaro-Olvera et al., 2000; Doi et al., 2008; Pereira et al., 2009; Rodríguez-Félix et al., 2018; Young and Elliott, 2020). Of Portunidae crabs, fecundity varies between genera and species mainly between one and four million due to body size, food, abiotic conditions, season and latitude (Mantelatto and Fransozo, 1997; Cházaro-Olvera et al., 2000; Darnell et al., 2009, 2010; Rodríguez-Félix et al., 2018). For similar sized portunid crabs individual (80 mm CW), fecundity is between one and two million eggs, which confirms that *C. ruber* has a high reproductive potential, as do other portunid crabs that are exposed to the marked larval mortality characteristic of portunid crabs (Rodríguez-Félix et al., 2018; Young and Elliott, 2020). The large *C. ruber* individuals obtained the highest fecundity values due to a longer intermoult period than the small crabs because they have more time to accumulate the energy reserves needed to produce eggs before ecdysis (de Lestang et al., 2003; Tureli and Yesilyurt, 2018).

Sperm transfer occurs in *C. ruber* through the typical portunid sperm plug (Hartnoll, 1968, 1969; Sant'Anna et al., 2012). Ovigerous *C. ruber* females with mature gonads had sperm stored in the seminal receptacle. However, ovigerous females with resting ovaries had an empty seminal receptacle. This feature confirmed multiple spawning, with a new oviposition immediately after the egg incubation period. This strategy increases larval production (Dineen et al., 2001) and such a pattern has also been described in other portunid crabs (Costa and Negreiros-Fransozo, 1998; Dineen et al., 2001; Sallam and Gab-Alla,

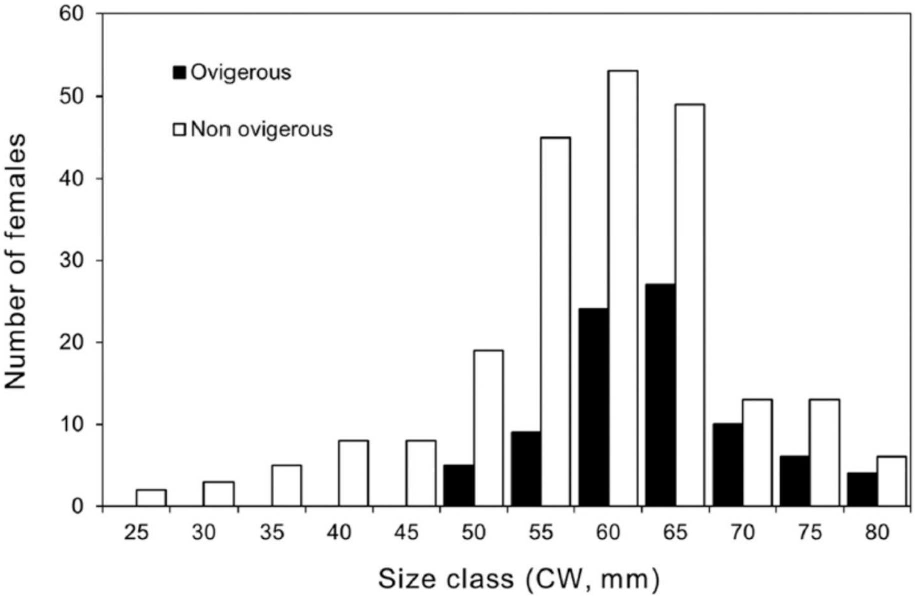

Fig. 12. Number of females (by ovigerous or non-ovigerous condition) by size class of 5 mm of *Cronius ruber.*

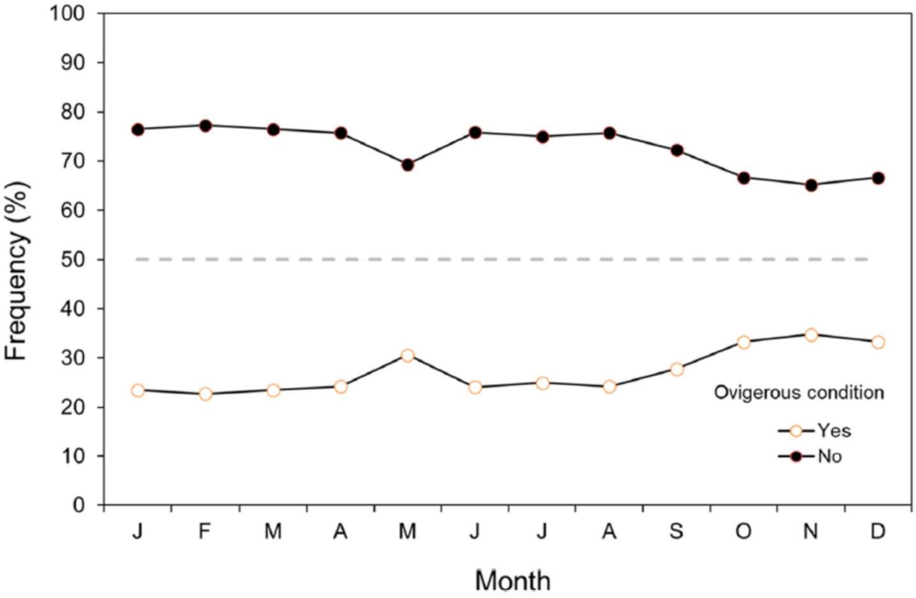

Fig. 13. Frequency of females (by ovigerous or non-ovigerous condition) by months of *Cronius ruber.*

2010; Sant'Anna et al., 2012). This reproductive strategy may be responsible partly for the colonisation success of portunid invasive species (Dineen et al., 2001; Sant'Anna et al., 2012).

Isolated females had up to six ovipositions during their laboratory rearing in tanks. Females' ability to have two ovipositions or more (broods) and to be ovigerous twice or more times is due to the fact that the amount of sperm transferred during fertilisation is sufficient for females to fertilise several clutches. Similar results have been reported by Haddon and Wear (1993) in *Ovalipes catharus* (White in White & Doubleday, 1843) (up to five clutches), Norman (1996) in *Thalamita sima* H. Mile Edwards, 1834 (up to seven consecutive clutches), Ryan (1967) in *Portunus sanguinolentus* (Herbst, 1783) (three clutches) and Darnell et al. (2010) in *Callinectes sapidus* Rathbun, 1896 (up to seven egg masses per year from a single mating).

In *C. ruber,* the number of eggs per oviparous female may vary between different geographic areas because, besides being correlated with female size, it depends on food availability and seawater temperature (Gehrels et al., 2016; Young and Elliott, 2020). The time that elapsed between two egg extrusion processes for *C. ruber* is 24±5 days. The timing of the release of female portunid crabs may vary because females have the ability to release eggs only when environmental conditions are optimal (Young and Elliott, 2020).

It is likely that the F estimated for *C. ruber* is higher in other areas because the number of extruded eggs per female varies with size, food availability and water temperature (Gehrels et al., 2016; Young and Elliott, 2020). Therefore, the circumstance of larger females in warmer waters than those of the Canary Islands, and with high food availability compared to the oligotrophic character of the Canary Islands, will be

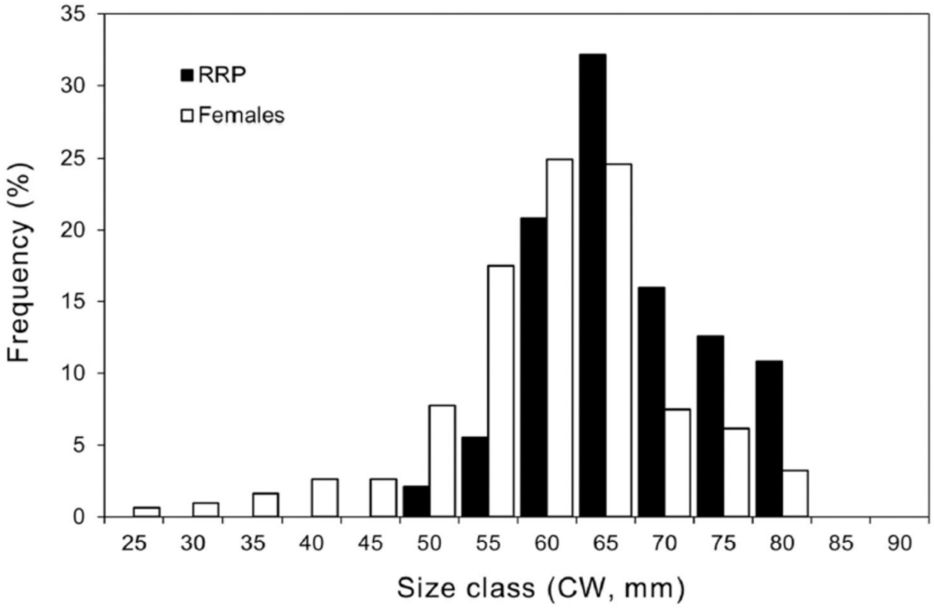

Fig. 14. Relative reproductive potential (RRP) values by size class of 5 mm of carapace width for females of *Cronius ruber*.

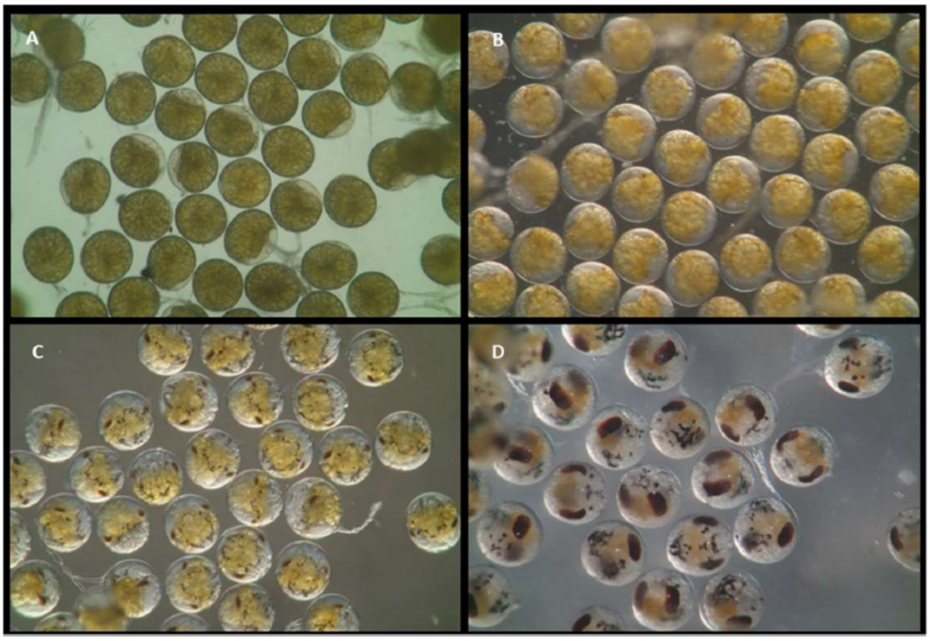

Fig. 15. Egg morphology in *Cronius ruber*: (a) eggs undivided and fully filled with yolk, (b) eggs with a free region of yolk visible, (c) embryos pigmented, with eyes as enlarging crescents, (d) embryos with visible pigmented structures, enlarging eyes, segmented appendages and abdomen appearing.

Fig. 16. Colour pattern of egg masses attached to pleopods of *Cronius ruber*: (a) orange, (b) red, and (c) brown.

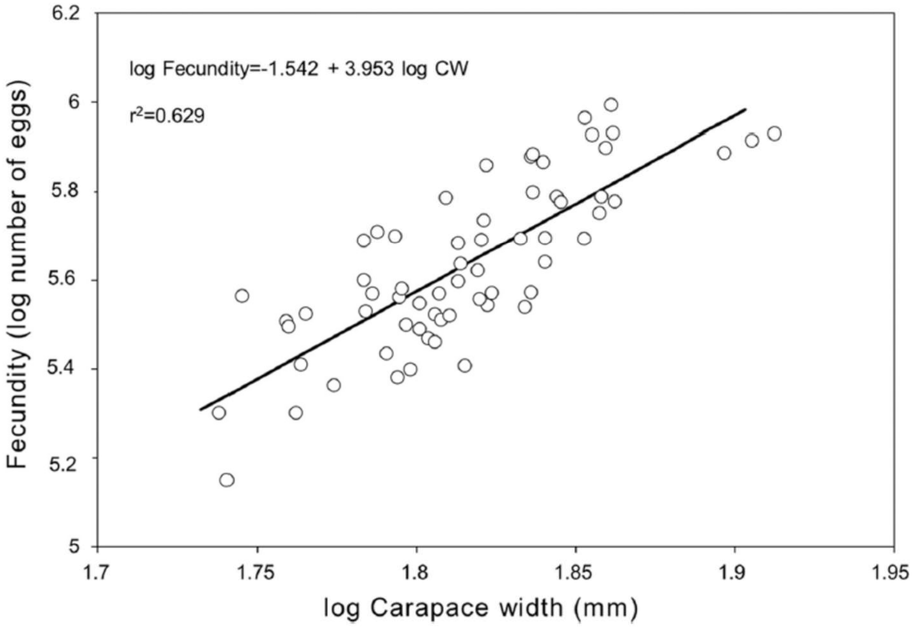

Fig. 17. Fecundity estimates by carapace width for *Cronius ruber*. r^2, determination coefficient. Line fitted correspond to the model estimated.

where the upper F limit will be observed in this species (Audet et al., 2008; Flores et al., 2009; Young and Elliott, 2020).

The *C. ruber* females cultured in tanks had various egg extrusion events up to 6 months after female isolation. For portunid crabs, there is no predictable estimated time between mating and egg extrusion due to females' ability to store spermatophores in her spermathecae for a long period of time (Young and Elliott, 2020).

The diameter of *C. ruber* eggs ranges from 0.25 (stage I) to 0.38 mm (stage 4), and this range is common among Portunids species (Dineen et al., 2001; Doi et al., 2008). The *C. ruber* incubation period lasts less than 1 month. This short incubation period has been observed in other portunid crabs, where hatching ends 1 month after egg extrusion (Nawer et al., 2022). In portunid crabs, mating occurs shortly after the female moult (soft-female mating) with protection by males during the pre-moult and postcopulatory timing (Hartnoll, 1969), except for hard-female mating in *T. sima* (Norman, 1996).

5. Conclusions

The reproductive characteristics of *C. ruber* explain the rapid expansion and colonisation of this species beyond its native range. This species is a multiple spawner with a new oviposition immediately after ending the egg incubation period. The egg development period lasts slightly less than 1 month. This process can be performed by females up to six times thanks to stored sperm. Spawning activity extends throughout the year in this non-indigenous zone, with a high reproductive potential that reaches one million eggs per oviposition. The acquired information forms part of the baseline on this invasive NIS' impact, and comprises part of the arguments for *C. ruber* to be included on the EU list of invasive alien species.

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CRediT authorship contribution statement

José G. Pajuelo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **José A. Martín:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Raül Triay-Portella:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, 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

Data will be made available on request.

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This study has been developed in the framework of the Ph. D. proposal of MSc José Antonio Martín.

Code availability

Not applicable.

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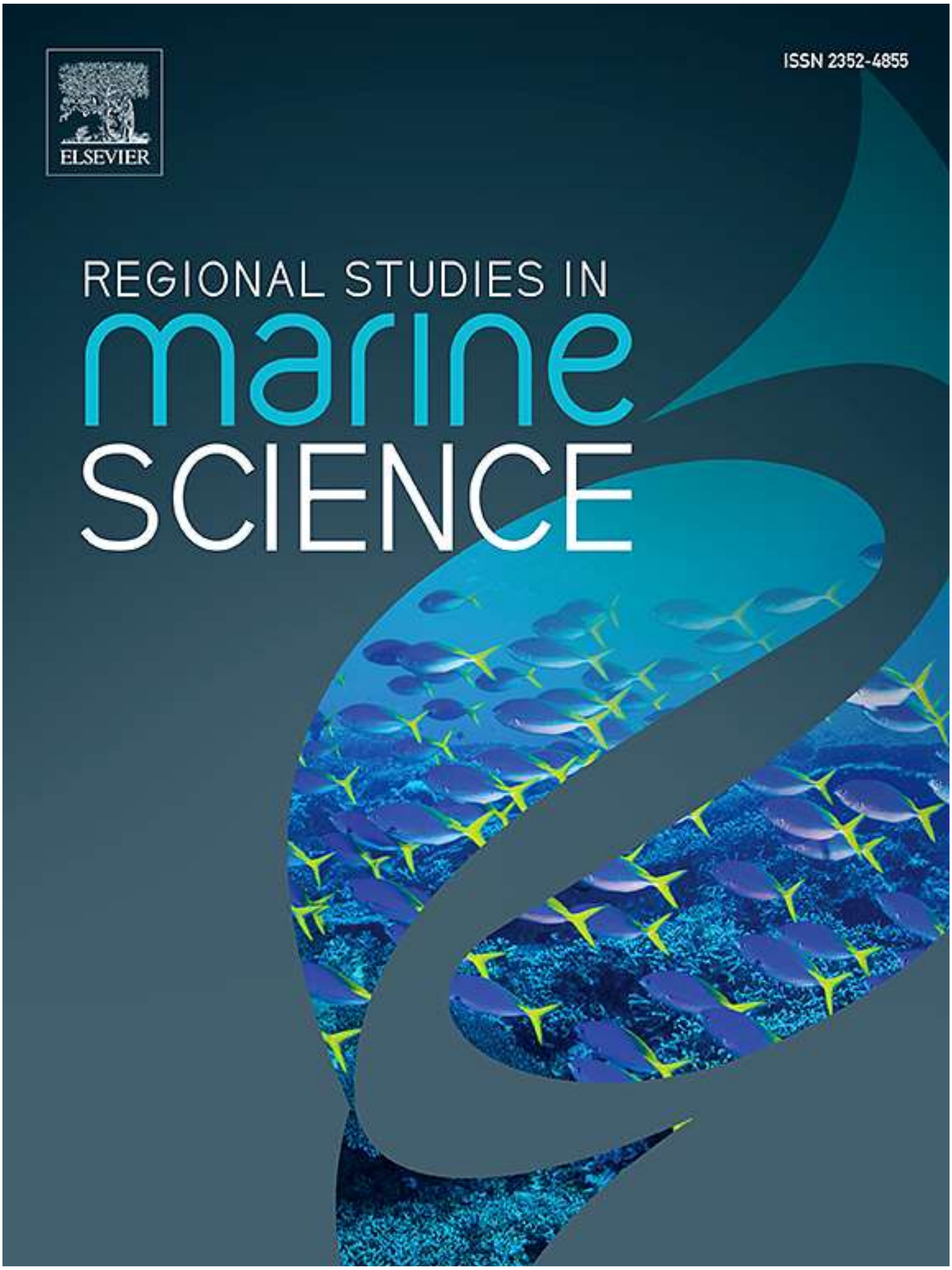
Chapter 4. Growth patterns of the invasive crab *Cronius ruber* (Brachyura, Portunideae) in the Canary Islands (Central – East Atlantic)



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Growth patterns of the invasive crab *Cronius ruber* (Brachyura, Portunidae) in the Canary Islands (Central-East Atlantic)

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ABSTRACT

The growth pattern, including the moult process, relative growth, and estimation of von Bertalanffy growth parameters of the invasive non-indigenus species (NIS) *Cronius ruber* were studied in the Canary Islands. Despite being found in both, native and invaded areas, the life cycle and ecology of this species remain unknown. The study revealed a greater abundance of females than males, with males exhibiting larger carapace sizes and weights than females. Throughout ontogeny, relative growth was observed, with males reaching carapace widths (CW) between 54.5 and 57.8 mm and females reaching 49.9 mm CW. Allometry in the growth of the males' chelae and females' abdomen was consistently positive. *Cronius ruber* was found to be a fast-growing species, reaching the postpubertal growth phase (maturity) between 0.5 and 1 year of age in males and between 0.4 and 0.8 years in females. The intermoult period increased by more than 638 % in males and 443 % in females with age. Individuals undergo 13 moults during the first phase of life (until 30 mm CW) and then reduced to 6 moults until the end of life, concluding with a final terminal moult. The lifespan until the terminal moult is approximately 2.5 years, followed by a survival time over 1.5 years. von Bertalanffy growth parameters estimated using monthly CW-frequency data for males ($L_\infty = 97.4$ mm CW, $k = 1.89$ years^{-1}, $t_0 = 0.31$ years), and for females ($L_\infty = 86.72$ mm CW, $k = 1.91$ years^{-1}, $t_0 = 0.29$ years). Similar results were obtained using growth increment data.

1. Introduction

The spread of non-native marine species into new territories is recognized as a significant environmental issue and a global challenge for ecosystems (Pajuelo et al., 2016). This phenomenon, which involves the introduction, both accidental and intentional, of various species into areas well beyond their indigenous habitats, stands as a critical concern for natural environments worldwide. Such species, which would not have been able to expand into these regions without human involvement, pose threats and challenges to the ecosystems they invade (Bolaños et al., 2012).

Cronius ruber (Lamarck, 1818) is a recently reported non-native invader species with a wide distribution in shallow warm waters along the coast of the Atlantic Ocean (González et al., 2017). This species, native to the Brazilian water, has extended its range from the eastern Atlantic coast to Canary Islands (Central East Atlantic) from the secondary area of distribution (González et al., 2017). It was first reported in the Canary Islands a decade ago, probably transported via oil ring directly from the Gulf of Senegal (González et al., 2017). Once established, these allochthonous species can cause alterations in the ecosystem of the invaded area and economic and social impacts (Carlton and Geller, 1993; Carlton and Ruiz, 2005; Geller et al., 2010; Negri et al., 2018). The impact of *C. ruber* to date is limited and it is related with his widespread carnivorous diet (Triay-Portella et al., 2022). The impact stems from its predatory behavior on native species and competition with native crabs for habitat, especially when some of these local species are endangered, such as *Haliotis coccinea canariensis* (Triay-Portella et al., 2022). In fact, *C. ruber* exhibits a high level of aggressive territorial behavior against other crabs or other species including the octopodid *Callistoctopus macropus* (Triay-Portella et al., 2022).

Cronius ruber seeks habitats in its non-native regions, thriving in areas with or without varied structures (Coinva, 2019). Observations indicate its presence in soft sediment zones, beneath rocks and debris, along breakwaters, and throughout areas significantly impacted by hu-

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man activities. The prevalence of *C. ruber* in an ecosystem often leads to decreased populations of other crab species, urchins, polychaetes, gastropods, bivalves, and small fish due to its competitive dominance (Triay-Portella et al., 2022).

Understanding the life cycle traits and population behavior of invasive organisms is crucial for mitigating their negative impacts on the environment (Bolaños et al., 2012). Yet, detailed research on the life cycle and fundamental population parameters of *C. ruber* in both, its original and introduced territories, is lacking. This study aims to elucidate the invasive crab *C. ruber*'s growth patterns in the eastern central Atlantic, encompassing aspects such as age, growth rates, lifespan, molting frequency, and morphometric development.

The findings from this investigation, in conjunction with data on reproductive behaviors, will contribute to a broad comprehension of the species' ecology. This knowledge is essential for implement effective management practices to address the broader ecological consequences instigated by the presence of this invasive species.

2. Materials and methods

2.1. Sampling and biological data collection

Specimens of *C. ruber* were collected by hand from January 2018 to December 2018 in heavily anthropized areas at depths between 1 and 8 m on rocky/sandy bottoms. Samplings were always conducted after sunset and before midnight, the major peak of activity for *C. ruber* (Triay-Portella et al., 2022).

Measurements for each crab were taken following Triay-Portella et al. (2014) using a digital calliper with 0.01 mm precision: carapace width (CW), carapace length (CL), right/left propodus length (R/L-CHL), right/left propodus height (R/L-CHW), and abdomen width of the fourth abdominal segment (AS4W) in females (Fig. 1). The measurements were specified as follows: CW is the widest transverse distance across the carapace, measured from the antepenultimate to the ultimate lateral spines, excluding the spine itself; CL represents the linear distance along the carapace's center from the diastema to its rear margin; R/L-CHW denotes the propodus's broadest point, measured at its articulation with the dactylus; R/L-CHL is measured from the palm's furthest point to the tip of the dactylus; AS4W describes the broadest part of the fourth segment of the abdomen. Measurements of CW and CL were conducted on the crab's dorsal side, oriented from side to side and front to back, respectively; R/L-CHL was measured along the outer edge of the chelae, from tip to base; AS4W measurements were taken on the ventral side, spanning from side to side. All measurements were recorded with precision to the nearest millimeter. Additionally, the sex of the crabs and their total body weight (TW, accurate to 0.1 g) were documented. Crabs showing signs of damage or with limbs in regeneration were excluded from the study (Mantelatto and Garcia, 2001).

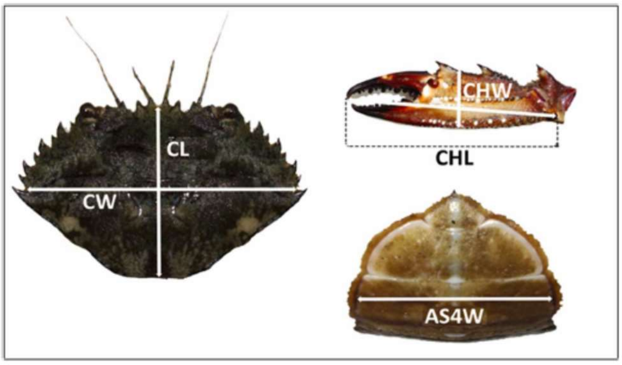

Fig. 1. Measurements taken for each specimen of *Cronius ruber*: carapace width (CW), carapace length (CL), width of chela (CHW), hand-length of chela (CHL), and maximum width of the fifth abdominal somite (AS4W).

2.2. Relationships between size and weight

The CW–TW relationship was determined for males and females by means of a power function. Differences in the CW–TW and CW–CL lineal form of relationships between males and females were evaluated by an F-test, and differences between values of b and the expected value from isometric growth were evaluated by a t-test (Sachs, 1982). Relationships between CW and CL were calculated to facilitate the comparison of data. The Kolmogorov-Smirnov non-parametric Z-test was used to analyse the differences in CW and TW ranges in males and females (Sokal and Rohlf, 2012).

2.3. Relative growth

The dimensions of the chelae, both in length and width, were compared with the CW for both genders and with the AS4W in females to examine potential shifts linked to the pubertal molt (Somerton, 1980; Attrill et al., 1991). We applied a power function to the dataset using non-linear regression for groups that could be distinctly separated, with transition points determined through iterative searches in increments of 0.1 mm CW (Somerton, 1980). To decide if a dual-model approach provided a better fit than a single unified model, we utilized an F-test that evaluates the differences in the residual sum of squares (Somerton, 1980). Differences in slopes and y-intercepts among the linear relationships, indicative of pre- and post-pubertal growth stages, were assessed using an ANCOVA test (Mantelatto and Garcia, 2001). The differential growth patterns of the chelae (considering both length and width) and the AS4W during the pre- and post-pubertal phases were analyzed using a t-test (Sachs, 1982). The nature of allometry was inferred from the regression slope: $b = 1$ indicates isometry; $b < 1$ signifies negative allometry; and $b > 1$ indicates positive allometry (Mantelatto and Garcia, 2001).

2.4. Moulting

A total of 50 individuals (25 males and 25 females) were kept separately in tanks to estimate the number of ecdysis and duration of anecdysis in this species. Specimens were selected in 10 mm CW size groups. The water temperature in the tanks was the same as that recorded at sea each month (~18–24° C). The time elapsed between the initial and final process of ecdysis was also recorded. The relationship between percentage of increment in size and carapace width at end of ecdysis was estimated using a linear regression. This equation was used to estimate the number of ecdysis during the first stage of the species.

2.5. Growth parameters

The growth increment data obtained from the cultured individuals were used to estimate the parameters of the von Bertalanffy growth function. Two methods were used to estimate the parameters, Appeldoorn (Appeldoorn, 1987; Soriano and Pauly, 1989) and Munro's (Munro, 1982) methods. Both methodologies use growth increment data to estimate the growth parameters CW_∞ (mm) and k (years^{-1}).

Appeldoorn`s method obtained the von Bertalanffy seasonal growth parameters by minimizing the function using the Marquardt's algorithm for a nonlinear fit: $SSE = \sum_i \{ CW_{i+\Delta t} - (CW_\infty - (CW - CW_i)\, exp\,[-(k\Delta t - S_t + S_{t+\Delta t})])\, \}^2$ where St is $(Ck/2\pi)\cdot\sin(2\pi(t-ts))$; $St+\Delta t$ is $(Ck/2\pi)\cdot\sin(2\pi((t+\Delta t)-ts))$; and ts is $0.5 + WP$. For $C = 0$ and $WP = 0$, the simple von Bertalanffy growth function is obtained (Appeldoorn, 1987; Soriano and Pauly, 1989).

Munro's method minimizes the coefficient of variation of the function: $\text{Ratio} = \ln[(CW_{\infty}\text{-}CW_{m})\text{-}\ln(CW_{\infty}\text{-}CW_{r})]/t_{r}$, where CW_{m} is the carapace width before the moulting process (initial carapace width), CW_{r} is the carapace width after moulting, and t_{r} is the time of the intermoult period between two consecutive moulting processes (Munro, 1982).

The monthly CW-frequency data were also used to estimate the parameters of the von Bertalanffy simple and seasonal growth functions by means of the ELEFAN I routine in the FISAT II program (Pauly and David, 1981; Pauly and Morgan, 1987; Gayanilo et al., 1996). The fitted data adhered to a growth curve of the form: $CW = CW_{\infty}(1\text{-}\exp(\text{-}k(t\text{-}t_{0}) + St_{s} + St_{0})$ where: St_{s} is $(Ck/2\pi)\cdot\sin(2\pi\ (t\text{-}t_{s}))$; St_{0} is $(Ck/2\pi)\cdot\sin(2\ \pi\ (t_{0}\text{-}t_{s}))$; and L_{t} is the length at time t, where CW_{∞} is the asymptotic carapace width, k is the intrinsic growth rate, t_0 is the age at which the carapace width of crabs is 0, C is the amplitude of seasonal growth oscillation, ts is the age at the beginning of growth oscillation, and $WP = t_s + 0.5$ is the time of year when growth is slowest. For $C = 0$ and $WP = 0$, the simple von Bertalanffy growth function is obtained.

3. Results

3.1. *Relationships between size and weight*

In this study, 523 specimens of *C. ruber* were gathered, with 214 males displaying a size range from 27.3 to 90.6 mm in CW and weighing between 7.5 and 233.7 g, and 309 females measuring from 26.4 to 82.3 mm in CW and weighing from 6.2 to 142.5 g (Fig. 2). It was observed that males were generally larger and heavier than females. Analysis revealed disparities in average CW and TW between males (66.7 ± 12.3 mm and 86.5 ± 48.5 g, respectively) and females (62.6 ± 9.7 mm and 62.2 ± 27.8 g, respectively), with statistical significance (t-test, CW $t = 4.11$, $p < 0.0001$; TW $t = 6.55$, $p < 0.0001$). Furthermore, the distribution ranges for both CW and TW significantly varied between the sexes (Kolmogorov-Smirnov non-parametric test, CW $Z = 3.45$, $p < 0.0001$; TW $Z = 3.44$, $p < 0.0001$).

The analysis of the CW–TW relationship also showed significant sex-based differences (F-test, $F = 4.32$, $p < 0.001$), with a consistently positive allometric pattern for males across all measurements (t-test, $t > 7.746$, $p < 0.001$) (Table 1). However, no significant differences were detected in the CW–CL relationship between males and females (F-test,

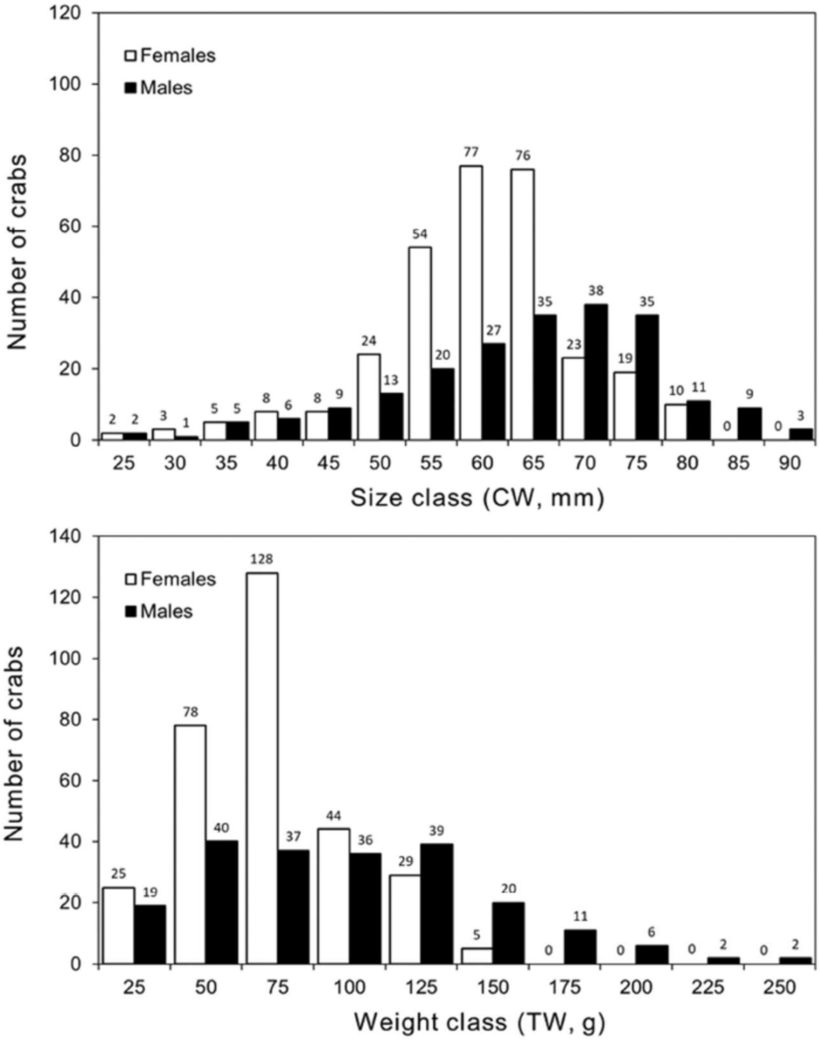

Fig. 2. Number of specimens by carapace width (CW) (top) and total weight (TW) (bottom) of males and females of *Cronius ruber*. Numbers correspond to individuals.

Table 1
Parameters of CW-TW ($TW = a \cdot CW^b$) and CW-CL relationships ($CL = a + b \cdot CW$) for males, females and all crabs of *Cronius ruber* a, intercept; b, allometric coefficient; S.E., standard error; r^2, determination coefficient; n, number of specimens; t, t-test value; *, significance at 0.05 level.

Relationship	Sex	a	b	S.E.(b)	r^2	n	t
CW-TW	Males	0.000033	3.488	0.063	0.954	214	7.746*
CW-TW	Females	0.000248	2.989	0.0704	0.880	309	0.156
CW-TW	All crabs	0.000036	3.458	0.048	0.922	523	9.541*
CL-TW	Males	0.000255	3.311	0.061	0.949	214	5.098*
CL-TW	Females	0.000945	2.937	0.071	0.874	309	0.887
CL-TW	All crabs	0.000205	3.360	0.054	0.911	523	6.501*
CL-CW	Males	0.601	1.459	0.014	0.990	214	32.78*
CL-CW	Females	1.044	1.438	0.0061	0.991	309	73.01*
CL-CW	All crabs	0.551	1.454	0.0090	0.991	523	50.44*

$F = 1.21$, $p > 0.05$), with the pattern being positively allometric in all instances (t-test, $t > 32.78$, $p < 0.001$) (Table 1).

3.2. *Relative growth*

The dimensions of chelae width (R/L-CHW) and length (R/L-CHL) in males were significantly greater and broader than those in females, as determined by ANOVA ($F > 81.15$, $p < 0.001$). No differences were found in the length or width of the right and left chelae between males and females (ANOVA, $F < 0.17$, $p > 0.68$). For females, the CW size at which a shift to allometric growth in AS4W occurs was identified at 49.9 mm (Fig. 3). The ANCOVA and F-test highlighted significant disparities between the regression lines for pre- and post-pubertal growth phases (F-test, $F > 7.13$, $p < 0.001$; ANCOVA $p < 0.001$). The allometry of AS4W growth was consistently positive across all subsets analyzed, whether before or after the pubertal phase. The analysis of females exceeding the size at which positive allometric growth begins demonstrated a notable increase in slope magnitude (t-test, $t = 5.25$, $p < 0.0001$).

The growth of hand length (CHL) and width (CHW) of the right chelae in both males and females was assessed against CW to explore potential shifts linked to the pubertal molt (Fig. 4). The growth patterns of males indicated significant changes throughout their development (F-test, $F > 9.17$, $p < 0.001$), with transitions observed at 54.5 mm CW (CHL) and 57.8 mm CW (CHW), making different growth patterns before and after these points (ANCOVA $p < 0.001$).

The allometry of chelae growth remained positively skewed (t-test, $t > 11.42$, $p < 0.001$), regardless of the chelae's width or length or the developmental phase (pre- or post-pubertal). The model for females exceeding the size at which growth allometry shifts displayed a significant increase in their growth slope. No discontinuities were detected in the relative growth of chelae in females throughout their ontogeny. The allometric growth in chelae length for females was also positively skewed (t-test, $t > 6.78$, $p < 0.001$).

3.3. *Moulting*

Of the 50 crabs reared in tanks, 21 died before their first moult in the laboratory. The maximum number of moults recorded for the same individual *C. ruber* was 5 times (Fig. 5). The minimum size reared was 37 mm CW (after the first moult in the tank), and the maximum size reached in culture after moulting was 82 mm CW.

The moulting cycle consists of an intermoult period (termed diecdysis if short and anecdysis if longer) which varied from 26 to 32 days for individuals sized between 30 and 39 mm CW, to 142–166 days for individuals in the larger size classes (70–79 mm CW) (Table 2). Ecdysis occurs in an average of 3.3 ± 0.8 hours. During the intermoult period *C. ruber* feeds actively. One male of 87.4 mm CW was reared for 438 days after ecdysis and one female of 80.6 mm CW was reared for 357 days without undergoing a new moult.

Increment in body size after shedding the old exoskeleton varied from 4.9 to 5.3 mm CW for smaller individuals (for males and females) to 10.3–9.1 mm CW for the size classes between 70 and 79 mm CW (Fig. 6). Molting was a short process (less than 2 hours) that was observed in all months of the year (Table 2). The increment in size as a percentage of carapace width at release relationship was estimated for both males and females (Fig. 7). The number of ecdysis events was also calculated for males and females (Fig. 8).

3.4. *Growth parameters*

The von Bertalanffy growth curves determined by Munro and Appeldoorn methods using growth increment data, and the estimated by ELE-

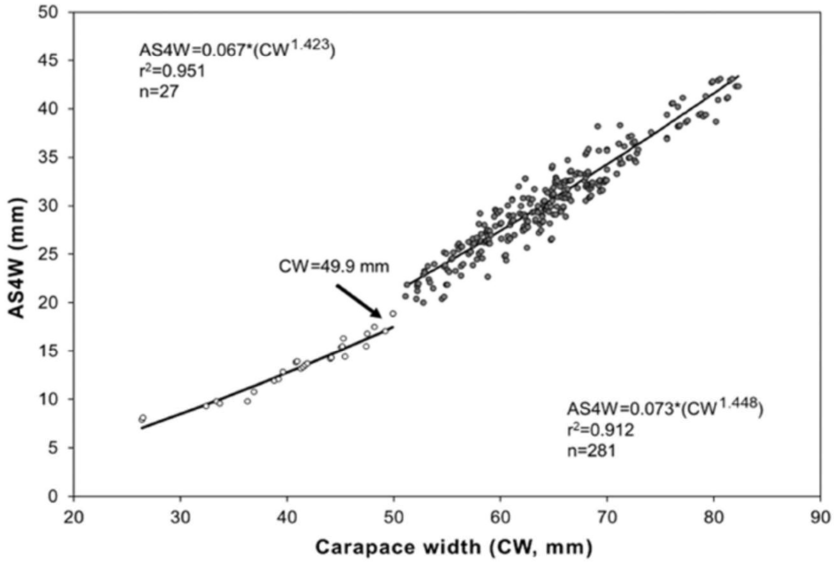

Fig. 3. Maximum width of the fourth abdominal somite (AS4W) plotted against carapace width (CW) in females of *Cronius ruber*. n, number of crabs. r^2, determination coefficient. Lines fitted correspond to each potential model estimated. Arrows indicate the transition point.

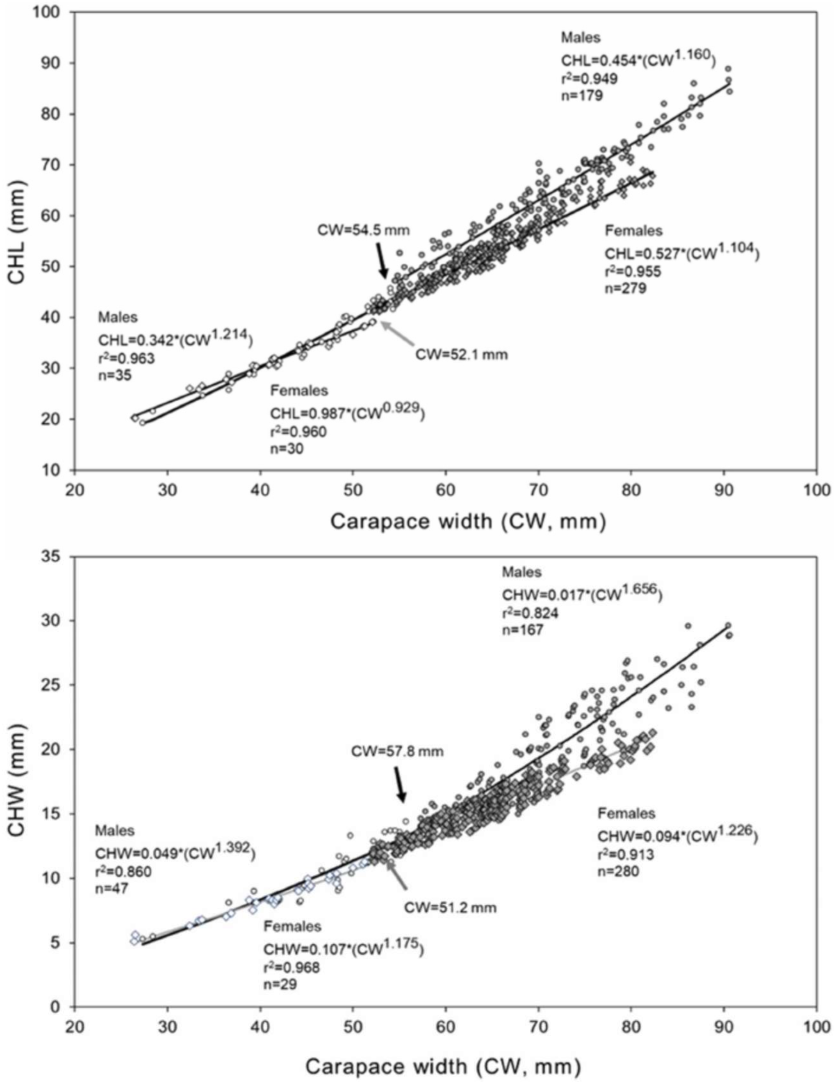

Fig. 4. Chela length (CHL) (top) and chela width (CHW) (bottom) plotted against carapace width (CW). n, number of crabs. r^2, determination coefficient. Lines fitted correspond to each potential model estimated. Arrows indicate the transition point.

FAN method using CW-frequency data are presented in Figs. 9 and 10 for males and females, respectively. Males show a higher asymptotic length than females and a similar growth rate when using the Appeldoorn and ELEFAN methods (growth increment data and monthy CW-frequency data). The asymptotic values obtained are consistent with the maximum observed length. However, Munro's method estimates a similar asymptotic length for both sexes and these values are smaller than the highest carapace width recorded.

4. Discussion

Individuals were collected from soft bottoms and under rocks; however, no individuals smaller than 25 mm were found in these habitats. It is important to consider that the failure to detect and collect crabs smaller than 25 mm CW may be due to the sampling protocol, which involved only visual detection of crabs. Smaller crabs are more difficult to detect compared to larger crabs, especially when there is limited air time during dives (Bolaños et al., 2012). Additionally, no small crabs were observed in catches using baited traps were in the same area where adults were found (Coinva, 2019). In other Portunidae species, such as *Charybdis (Charybdis) hellerii*, juvenile individuals are often located in different areas than adults, mainly on patches of algae (Mantelatto and Correa, 1996; Mantelatto and Souza-Carey, 1998; Mantelatto and Garcia, 2001). However, no small crabs were observed in algae patches using three different sampling methods (Triay-Portella et al., 2018). Portunids prefer habitats with high structural complexity (Thiel and Dernedde, 1994; Bessa et al., 2010; Young and Elliott, 2020) and avoid open sandy areas without shelter to evade predation and cannibalism (Young and Elliott, 2020). This suggests that there are likely breeding areas that correspond to habitats different from those sampled for this species.

Males reach larger sizes and weight than females. This characteristic is common in many portunid species such as *Charybdis (Charybdis) natator* (Vidhya et al., 2018), *Portunus pelagicus* (Potter et al., 1983), and *Portunus sanguinolentus* (Sukumaran et al., 1986), and it plays an important role during the mating process (Hartnoll, 1982; Abelló et al., 1990). The larger body size of males provides the necessary capacity for manipulating the female during mating (Santos et al., 1995; Sant'Anna et al., 2012). The skewed sex ratio in favor of females in the *Cronius ruber* population is an advantage for the expansion and colonization of

Fig. 5. Ecdysis in a female of *Cronius ruber* reared in tanks in the laboratory.

Table 2
Increment in carapace width in males and females *Cronius ruber* after shedding the old exoskeleton and period of anecdysis by size class interval.

	Females		Males	
Size class interval (CW, mm)	Size increment (CW, mm)	Anecdysis period (days)	Size increment (CW, mm)	Anecdysis period (days)
30–39	5.3 ± 0.53	32.3 ± 4.16	4.9	26.0
40–49	7.0 ± 0.21	51.6 ± 3.79	6.6 ± 0.93	47.5 ± 9.04
50–59	7.4 ± 0.22	62.5 ± 6.36	7.7 ± 0.14	82.5 ± 13.4
60–69	8.1 ± 0.60	94.6 ± 6.66	9.0 ± 0.35	114.1 ± 6.01
70–79	8.8 ± 0.28	142.5 ± 28.9	9.9 ± 0.32	166.0 ± 24.4
80–89	---	357	---	438

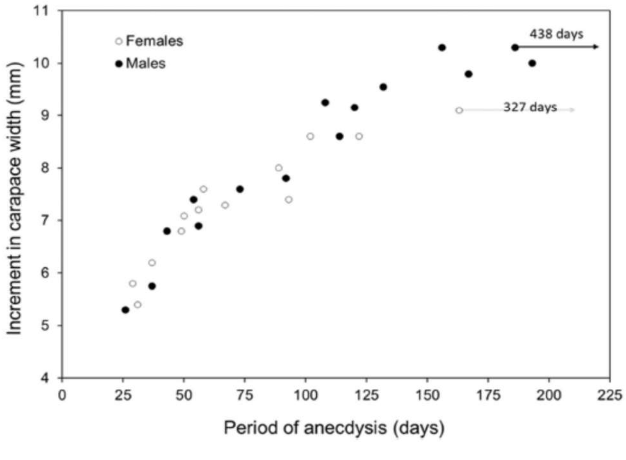

Fig. 6. Increments in carapace width in males and females of *Cronius ruber* in function of the period of time of anecdysis (in days).

new areas. This is because females can generate 5 or 6 clutches of offspring using the sperm stored in the spermathecae to fertilize without mating. On the other hand, a small number of males is sufficient to mate and fertilize all females during their progressive molt, respectively (Triay-Portella et al., 2024).

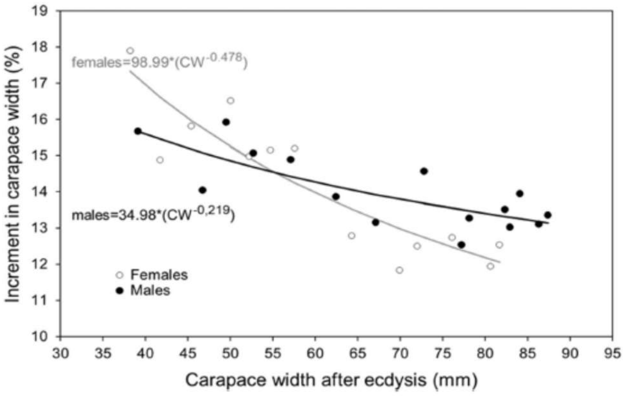

Fig. 7. Relationship between increments in carapace width in percentage and carapace width after ecdysis in males and females of *Cronius ruber*.

Cronius ruber also exhibited dimorphism in weight, with males reaching higher values. This may be due to the enlargement of male chelipeds, as reported for other portunids such as *C. (C.) natator* (Sallam and Gab-Alla, 2010), *Charybdis (Charybdis) affinis* (Chu, 1999) and *Charybdis (Gonioneptunus) bimaculata* (Doi et al., 2008).

A positive allometry was found in the CL-CW relationship, indicating that the carapace widens relative to its length as size increases. This is likely associated with the development of the gonads which occupy a large volume of the cephalothoracic cavity (Abelló, 1989a, 1989b). This type of allometry has also been observed in other portunid crabs (Davidson and Marsden, 1987; Abelló et al., 1990).

The ontogenic change in the chelae showed a reduction in growth. Portunids exhibit an evolutionary constraint against high positive allometries in the relative growth of appendages, particularly chelae, as it may negatively affect their swimming ability (Abelló et al., 1990). Portunid crabs are capable of swimming freely in the water column, and it is advantageous for them to remain as light as possible. High positive allometry in the relative growth of the chela, as seen in strictly benthic crabs, tends to reduce this ability. This is likely why allometric growth

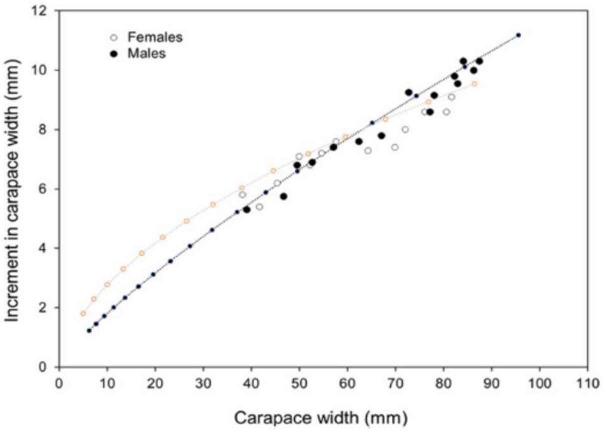

Fig. 8. Relationship between increment in carapace width (estimated from increment size in percentage) and carapace width at release in males and females of *Cronius ruber*.

parameters in most portunid crabs are lower than those shown by strictly benthic crabs (Abelló et al., 1990).

Changes observed in the growth of chelipeds in males and the width of the abdomen in females have been described as playing important roles in the behavior of other portunid species such as *C. (C.) hellerii*, *Charybdis (Charybdis) feriata*, *C. (C.) natator* and *Charybdis (Archias) smithii* (Campbell and Fielder, 1986; Sumpton, 1990; Couwelaar et al., 1997). In the family Portunidae, the abdomen is fixed in immature (prepubertal) females. However, the abdomen become movable in mature (pubertal) females (Van Engel, 1990; Haefner, 1990; Fischer and Wolff, 2006; Bolaños et al., 2012). The ontogenetic change in the growth of the abdomen in females and in the chela in males are secondary sexual character because they are related to reproductive function (Hartnoll, 1978; Yamada et al., 2005). The abdomen of mature females serves as an incubation chamber during the development of eggs, which are attached to the setose pleopods (Yamada et al., 2005) and the chela is used by males for territorial defence and combat due to their aggressive behaviour. The chela is also used during mating and courtship to transport and support females during copulation (Yamada et al., 2005). While gonopodium development is critical for female insemination and may better reflect male body size at sexual maturity (Doi et al., 2008), relative chela growth can also be used to identify sexual maturity (Yamada et al., 2005). The ontogenetic change in the relative growth of their chela or abdomen at pubertal moult makes these structures fully functional at sexual maturity (Yamada et al., 2005). The values obtained are slightly higher than those on sexual maturity reported by Triay-Portella et al. (2024), who estimate sexual maturity at 52.19 mm CW for males and 49.17 mm CW for females.

During the first phase of life (5–25 mm CW), an estimated 13 moults occur, whereas from then until the end of life, 6–7 moults occur. This is because age is inversely proportional to moult frequency (Klassen and Locke, 2007; Young and Elliott, 2020). Crabs moult much more frequently when young, with short periods of intermediate moult (diecdysis), and intermediate moult intervals becoming much longer (anecdysis) as crabs age, until the crab enters terminal anecdysis (Young and Elliott, 2020).

The longest ecdysis recorded among reared specimens was almost 200 days. Maintenance in rearing conditions for more than 400 days after the last ecdysis suggests the existence of a terminal ecdysis, indicating that they will not moult again. The existence of terminal moult has been observed in other species. Moulting occurs throughout the year in *C. ruber*, as has been observed in other Portunoidea such as the carcinid *Carcinus maenas*. Chronological intermoult times recorded for *Cronius ruber* (26–166 days; crabs 30–79 mm CW) were very similar to those of another invasive Portunidae, *Callinectes sapidus*. This species moults every 18–153 days (crabs 29–85 mm CW) in Chesapeake Bay waters maintained at 16–28 °C (Brylawski and Miller, 2006). However, Bilen and Yesilyurt (2014) observed that *Callinectes sapidus* moults faster in Turkish waters (3–67 days; crabs 14–75 mm CW). This could be due to the higher temperature recorded during culture (30.2 °C) and a shorter experimental period (133 days).

The maximum size attained by *C. ruber* appears to be 80 mm CW for females and over 90 mm CW for males. Females are smaller than males when they reach sexual maturity and begin to incubate eggs (Young and Elliott, 2020). The life-span of *C. ruber* has been estimated at 2.5 years until the terminal moult, with a subsequent survival period more than 1.5 years. These results are consistent with observations for other Portunoidea in their Atlantic populations, such as *Carcinus maenas*, which has a life expectancy of 4–7 years (Klassen and Locke, 2007; Yamada et al., 2005; Young and Elliott, 2020). Other portunid crab

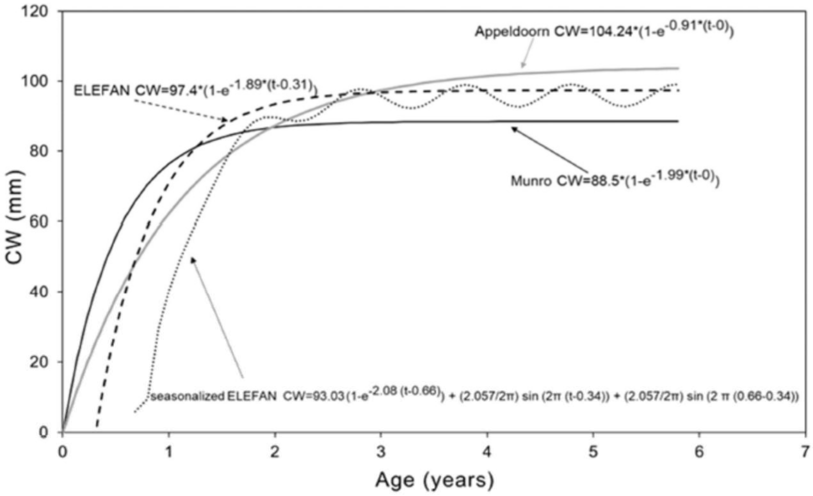

Fig. 9. The von Bertalanffy growth curves for males *Cronius ruber* determined by the Munro, and Appeldoorn methods (using growth increment data) and by the ELEFAN method (simple and seasonalized using monthy CW-frequency data).

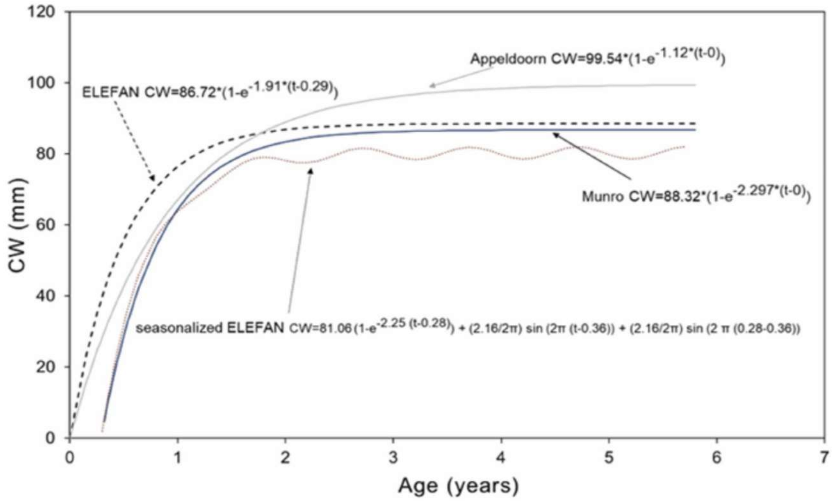

Fig. 10. The von Bertalanffy growth curves for females of *Cronius ruber* determined by Munro, and Appeldoorn methods (using growth increment data) and ELEFAN method (simple and seasonalized using monthy CW-frequency data).

species have life spans ranging from 2 to 4 years, as seen in *Charybdis (Charybdis) japonica* (Ogawa, 1997) and *Portunus trituberculatus* (Ariyama, 1993), and at least three years, as in the carcinid *Carcinus aestuarii* (Furota et al., 1999).

Differences in growth between sexes are mainly due to the greater energy invested in reproductive production by females. Upon reaching sexual maturity, growth decreases (Hartnoll, 1982) because part of the energy is diverted to reproduction, primarily by females (Lee and Hsu, 2003). As a result of greater investment in reproduction and less growth, females attain smaller size and weight than males (Cobb and Caddy, 1989; Lee and Hsu, 2003). Such differences in the amount of energy expended have been observed in other portunids such as *P. sanguinolentus*, *P. pelagicus*, and *Scylla serrata* (Jacob et al., 1990; Reeby et al., 1990; Sukumaran and Neelakantan, 1997). Size at sexual maturity is reached during their first year of life, as in other portunid species such as *P. sanguinolentus* (Lee and Hsu, 2003).

Gayanilo and Pauly (1997) proposed that asymptotic size must be approximately 5 % larger than the maximum observed size. According to this, the best estimate of asymptotic length and longevity corresponds to the value indicated by the simple von Bertalanffy growth equation estimated from the length frequency distributions.

Among the growth equations estimated for *Cronius ruber*, the equation obtained using the Munro method slightly underestimates growth. The other three equations have similar results, so the simple von Bertalanffy model estimated from CW frequency data is the most appropriate, as it is the simplest model that explains growth with a smaller number of parameters and the most widely used, allowing for comparison the parameters (Bustos et al., 2009). The reduction in growth rate with age is related to diet. Juveniles have a higher ingestion rate than older adults and a greater diversity of preys because they have high energy requirements for growth (Triay-Portella et al., 2022).

It should be noted that the estimated longevity values in the Canary Islands may be slightly lower than those of other Atlantic populations because the Canary Islands and Madeira are the northernmost areas of distribution. Crabs' populations close to the northernmost limit of distribution show slower growth compared to populations from more favourable environments (Young and Elliott, 2020).

Many portunid species that have successfully colonized new habitats have done so because they possess biological characteristics that confer adaptive advantages for invasion. These characteristics include rapid growth and maturation, high fecundity, short turnover time, the ability of females to store sperm and produce multiple offspring in a single season, a broad carnivorous diet, and the ability to utilize multiple habitats (Sant'Anna et al., 2012). *C. ruber* presents these characteristics in their non-native area (Coinva, 2019; Triay-Portella et al., 2022, 2024).

5. Conclusions

The high growth rate of *C. ruber* helps to understand the great expansion and rapid settlement of this species outside its natural area. The short period of time it takes to reach terminal molt is evidence of its great ability to compete with species in the areas it colonizes. The information obtained on the growth pattern increases the baseline on this invasive alien species, expanding the arguments for adding *C. ruber* to the EU list of invasive alien species. Eradication of non-native invasive species as *C. ruber* is practically impossible, so the removal of mature individuals has proven to be an effective strategy to reduce the population and its effects on habitats. This requires intensive fishing with selective traps for this species, especially in anthropized areas (Coinva, 2019). The development of effective management strategies to maintain the *C. ruber* population at the lowest possible levels must be accompanied by measures that lead to the recovery of the populations of their potential predators in the area overexploited by fishing (Coinva, 2019; Triay-Portella et al., 2022).

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CRediT authorship contribution statement

José G. Pajuelo: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization, Project administration. **José A. Martín:** Writing – original draft, Investigation, Formal analysis, Data curation, Writing – review & editing. **Raül Triay-Portella:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

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

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Data Availability

Data will be made available on request.

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Chapter 5. Conclusions

1. *Cronius ruber* is a generalist mesopredator that forages among a range of species belonging to Crustacea, Mollusca, Annelida, Echinidea and Teleost groups.

2. Some of the prey observed in the underwater surveys, were absent or unidentified in gut content of *Cronius ruber*.

3. Cannibalistic behaviours were observed in *Cronius ruber*.

4. Juveniles of *Cronius ruber* consume more prey items per day than old individuals.

5. Males have a larger carapace size than females, and the same goes for weight.

6. Males of *Cronius ruber* exhibit agonistic behaviour.

7. Predators observed on *Cronius ruber* were *Octopus vulgaris*, *Octopus Macropus*, *Mycteroperca fusca*, *Ephinephelus marginatus*, *Aetomylaeus bovinus* and *Taeniura grabata.*

8. Throughout ontogeny, a change in the relative growth was observed, between ≈54-58 mm carapace width for males and ≈50 mm carapace width for females.

9. Positive allometry in the growth of the males' chelaes and for the females' abdomen were recorded.

10. *Cronius ruber* is a fast-growing species that reaches the postpubertal growth phase (maturity) between 0.5 and 1 year of age in males and between 0.4 and 0.8 years in females.

11. The intermoult period increases by more than 600% in males and 400% in females with age.

12. During the first phase of life *Cronius ruber* has 13 moults (until 30 mm carapace width) and from then until the end of life is reduced to 6 moults.

13. *Cronius ruber* has a terminal moult.

14. The life span until the terminal moult is 2.5 years and the subsequent survival time is more than 1.5 years.

15. von Bertalanffy growth parameter were estimated for males: $L\infty$=97.4 mm carapace width, k=1.89 years^{-1}, to=0.31 years, and for females: $L\infty$=86.72 mm

carapace width, k=1.91 years^{-1}, to=0.29 years.

16. Females were more abundant than males, mainly in the size range between 25 and 80 mm carapace width. Only males were observed in size classes above 80 mm carapace width.

17. Reproduction of *Cronius ruber* is continuos throughout the year, with a new oviposition immediately after the end of the egg incubation period.

18. The oviposition process can be repeated in females up to six times, without re-mating, due to the sperm stored by females in the spermathecae.

19. Sexual maturity was estimated at a similar size in both sexes, corresponding to ≈57-59% of their maximum observed size.

20. Egg development appeared to be completely synchronous, with a development period lasting slightly less than 1 month.

21. The mean fecundity was approximately 600.000 eggs, with a maximum observed fecundity near to 1 million eggs.

22. This species shows a high reproductive potential, characteristic of invasive species.

23. For females, length upon sexual maturity was estimated as approximately 49 mm carapace width and for males as 52 mm carapace width.

24. Female fertilisation occurs after ecdysis.

25. Isolated females had up to six ovipositions and egg extrusion events occurred from 1 to 6 months after isolation.

26. The results represent the baseline for future studies on the impact of this invasive non-indigenous species NIS, and form part of arguments to include *Cronius ruber* on the list of invasive alien species of European Union concern.

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