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.
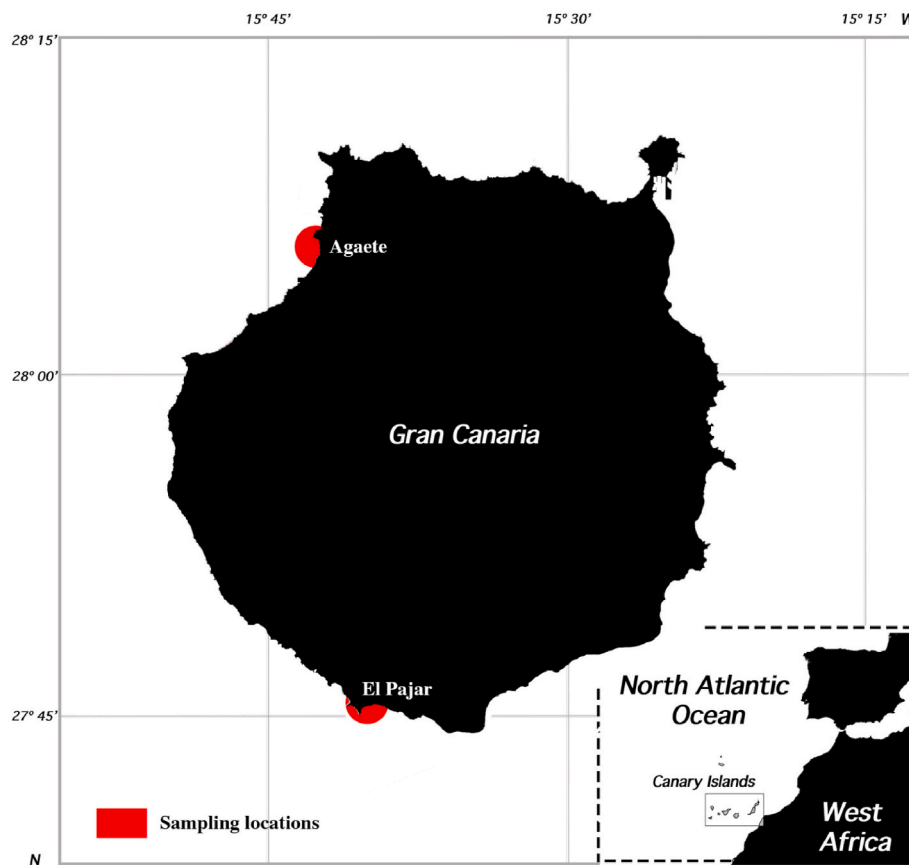

Fig. 1. Location of the two study (collecting) sites on the Gran Canaria Island coast (Canary Islands, central-east Atlantic). Playa de las Nieves (Agaete) (28°05′58.9″N 15°42′37.4″W) in the northern part and Santa Agueda Bay (El Pajar) in the southern part (27°45′02.4″N 15°40′13.4″W) of this island.

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 uising 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 un-identified 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 (Myliobati-formes, 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.
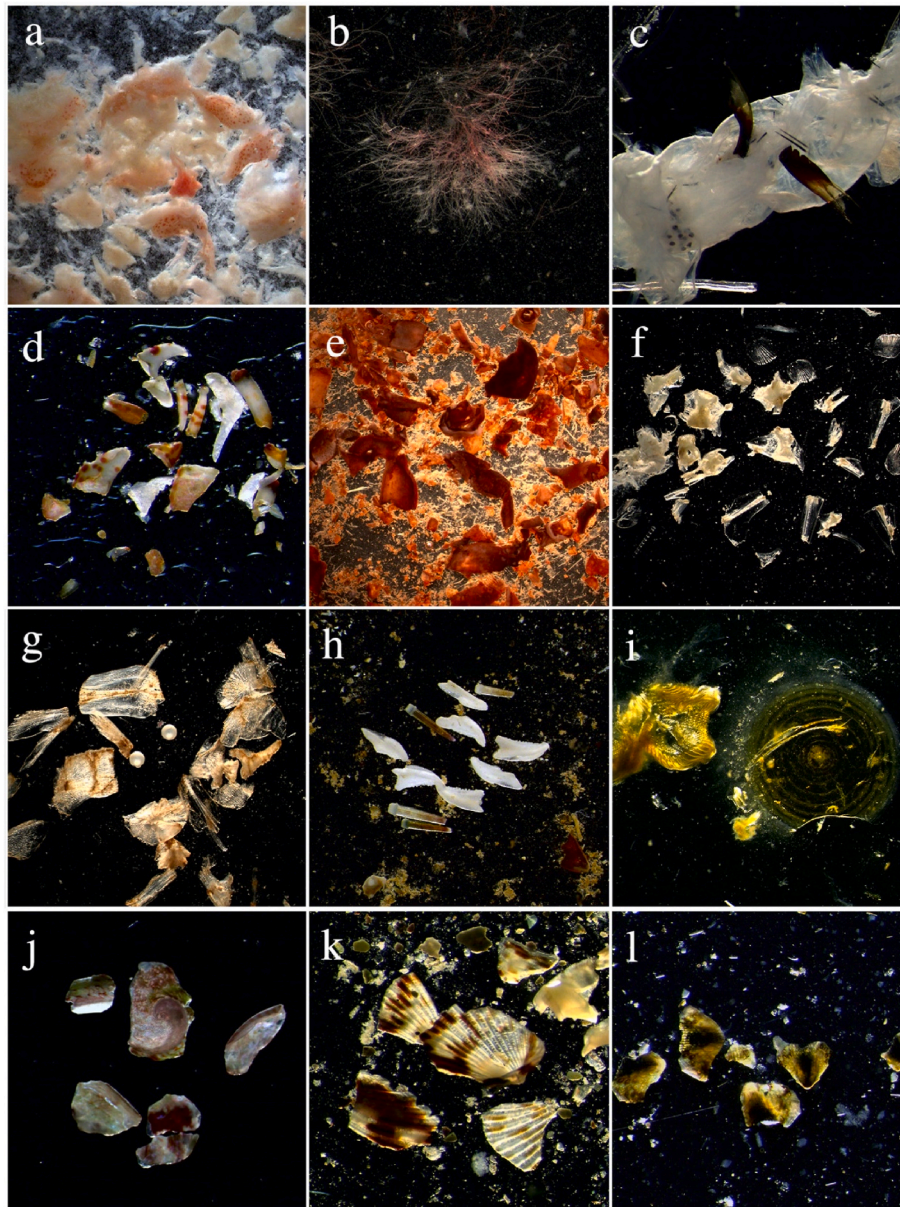
Fig. 3. Examples of the separate prey that represent the main groups identified in the stomach contents of *C. ruber*. (a) Unknown tissue, (b) algae (red algae), (c) Polychaete (*Eurythoe complanata*), (d) Anomura (*Calcinus tubularis*), (e) Brachyura (*Xantho* sp.), (f) FISH (*Gobius niger*), (g) FISH (*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.
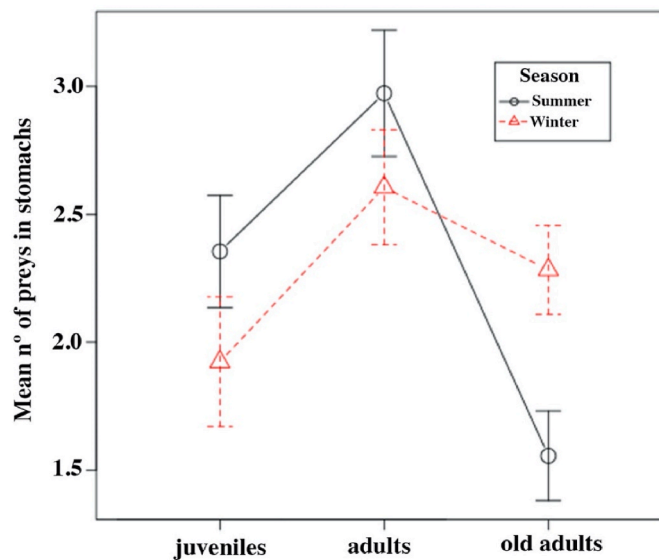
Fig. 4. Effect of ontogenic group (juveniles, adults, old adults) and season (summer and winter) on the mean number ($\pm$ standard error) of prey (as items) retained in *C. ruber* stomachs. "Juveniles" were assigned to the individuals with <55 mm (CW). The "adults" category comprised the crabs with 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.

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:

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

Conceptualization, Investigation, Sampling, Writing – review. Lucía Luque: Sampling, Investigation, Writing. José G. Pajuelo: Conceptualization, Investigation, Writing – review & editing.

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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