Prey size and species preferences in the invasive blue crab, *Callinectes sapidus*: potential effects in marine and freshwater ecosystems

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ABSTRACT

The blue crab, *Callinectes sapidus* is an invasive species in the Mediterranean region. In Ebro Delta bays, it poses an important risk for the cultivation of Mediterranean mussel (*Mytillus galloprovincialis*) and Pacific oyster (*Magallana gigas*). Besides, the species thrives in the Ebro River hosting abundant populations of apple snail (*Pomacea maculata*) and Asian clam (*Corbicula fluminea*). Food-preference experiments were conducted to assess the effect of predator and prey sizes and prey type (*M. galloprovincialis* vs. *M. gigas* and *P. maculata* vs. *C. fluminea*) in predation patterns and its possible causes. Our results show that except for the Pacific oyster, which attains protection at sizes of 50-70 mm and was little consumed (0 to 16%), the other preys are readily predated, at variable rates (mussels: 38 to 96%; apple snail: 58 to 93%, and Asian clam: 67 to 100%), depending on predator and prey sizes. Juveniles and young blue crab adults showed greater consumption of small and medium mussels and a similar trend occurred with Asian clam. In contrast, large and medium apple snails were more heavily predated by adult blue crabs. Species comparisons also showed higher predation of mussels than oysters (71 vs. 8%), and of apple snail than Asian clam (99 vs. 72%). Once the shell barrier was removed, preference for mussels was still preserved, suggesting a nutritional preference. Our results point the need of fishing blue crab in marine areas to prevent losses in mussel production and highlight the potential control exerted over undesired invasive mollusk species.

Key words: predation pressure · prey manipulability · Mediterranean mussel · Pacific oyster · apple snail · Asian clam

1. Introduction

The blue Atlantic crab, *Callinectes sapidus* Rathburn, 1896, is a decapod crustacean of the *Portunidae* family, native to a vast stretch of the western Atlantic seaboard (Hill et al*.,* 1989), from Maine to the Río de la Plata. The species is euryhaline and eurythermal and can inhabit estuaries, lagoons and other coastal habitats. It is characterized by high fecundity and aggressive behavior (Hines, 2003; Mancinelli et al*.,* 2013). The species supports large valuable commercial and recreational fisheries in the temperate areas of the Atlantic and the Gulf of Mexico and is the most widely consumed crab in the USA (Paolisso, 2007). In the Mediterranean the species was accidentally introduced in Greece in 1948 (Serbetis, 1959; Zenetos et al., 2018) and since then its abundance has been gradually increased posing a threat to native fisheries, and the overall diversity (Zenetos et al., 2005; Nehring, 2011; Mancinelli et al., 2017). Its detection in the Spanish coasts occurred much later than the first records in Greece and Italy, with a first observation in the Tancada lagoon (Ebro Delta) in 2012 (Castejón and Guerao, 2013). However, its expansion along the Mediterranean coast seems to happen fast (Izquierdo-Gómez and Izquierdo-Muñoz, 2016; González-Wangüemert and Pujol, 2016), and presently have already reached the Southern coast of Portugal (Vasconcelos et al., 2019). Previous studies in other Mediterranean countries including Albania (Beqiraj and Kashta, 2010), Italy (Mancinelli et al., 2013, 2017) and Croatia (Dulčić et al., 2011) suggest that the great outcompeting capacity of the species can alter the functioning of natural ecosystems and impact local fisheries (Nehring 2011). Currently, it is listed as one of the 100 worst invasive species in the Mediterranean (reviewed by Zenetos et al., 2005).

The blue crab is regarded as a generalist omnivorous consumer (Hill and Weissburg, 2013), capable of feeding on a variety of food resources depending on availability and stage of ontogenic development. Laughlin (1982) investigated the stomach contents of over 4,000 blue crabs and found that the main food items taken by all size classes were bivalves (35.7%), followed by fishes (11.9%), xanthid crabs (11.4%), blue crabs (9.0%), shrimps (4.9%), gastropods (4.8%) and to a lesser extent plant matter (3.9%). In particular, the consumption of bivalves was the highest (39%) for juveniles and adults (60-119 mm and ≥ 120 mm, respectively; Miller et al., 1975), whereas recruits (≤ 59 mm) ingested significantly higher proportions of plant matter (10 to 12%). This implies that the blue crab has the potential to inflict a large effect at multiple trophic levels in benthic communities (Carrozzo et al., 2014; Mancinelli et al., 2016), but bivalve populations are potentially the most vulnerable to blue crab predation, particularly juveniles and adults. Hence, determining the mechanisms involved in decisions taken by predators of different sizes are crucial to understand which prey size or species are more exposed to mortality and to reach management decisions on cultivation systems exposed to blue crab invasion. According to available information from native ecosystems, increasing shell size can provide a refuge from predation and there is a critical upper threshold of prey size from which predation is unfeasible (e.g., Seed, 1980, 1982; Hughes and Seed, 1981; Arnold, 1984; Eggleston, 1990a,b; Lin, 1991), but details on predation strategies in the Mediterranean is still limited (but see Kampouris et al., 2019). However, somehow contrasting patterns of size predation appear to occur among species, possibly associated to differences in the energetic cost of breaking each type of shell (Micheli, 1995). For instance, in the hard clam, *Mercenaria mercenaria*, individuals larger than 40 mm cannot be predated, whereas below that threshold consumption the range of sizes that can be consumed increases with predator size (Arnold, 1984; Peterson, 1990), and comparable patterns are observed in the oyster *Crassostrea virginica* (Bisker and Castagna, 1987; Eggleston, 1990a,b). In contrast, studies with the ribbed mussel, *Geukensia demisa*, point out to a larger critical upper threshold size for prey consumption (80-90 mm; Lin, 1991) and a consisting preference for small mussels (< 25 mm) that minimizes the time spent handling the prey and maximizes the net rate of energy intake (Seed, 1980, 1982; Hughes and Seed, 1981). In addition, different prey species may also have distinctive nutritional or palatability features that can play an important role during the selection process (review by Weissburg et al., 2002) and interfere with the effects of variability in shell hardness, strength of the adductor muscles, and overall manipulability associated to prey size and shape. There is evidence that crustacean predators have the capability to identify chemical mixtures characteristic of given food items and discriminate among them (e.g., Carr and Derby, 1986; Carr, 1988; Wight et al., 1990). However, even though the blue crab has been shown to effectively conduct odor-guided navigation to locate its preys thanks to receptors located at the antennules (Gleeson et al., 1996; Page et al., 2011) the possible influence of nutritional prey features in predation decisions has not yet been addressed. In addition, predation success may be further influenced by prey behavior when they are exposed to predators in terms of possible use of chemical defenses or craw-out capacity (Covich et al., 1994), and the duration of such responses.

In the Ebro Delta, the blue crab can be found both in estuarine environments (coastal lagoons, bays and Ebro River estuary) and in freshwater habitats (Ebro River and drainage channels for rice agriculture) (López and Rodon, 2018). In particular, Ebro Delta bays are considered very productive coastal areas in comparison with the adjacent open sea and their waters support important bivalve cultures of the Mediterranean mussel, *Mytilus galloprovincialis* and the Pacific oyster, *Magallana gigas* (Delgado, 1989) that constitute one of the main local economic activities. Given the great swimming capacity of the blue crab, the increasing abundance of the species within bay waters poses a major risk to bivalves cultured in suspension from fixed rafts. In the lower Ebro River and its delta, however, two highly invasive species the golden apple snail, *Pomacea maculata*, and the Asian clam, *Corbicula fluminea*, are commonly found (Oscoz et al., 2010; Nebra et al., 2011; Faria et al., 2018) and constitute a potential food resource for the growing blue crab population. In fact, a substantial decrease in the abundance of *P. maculata* has been observed during the last years, and heavy predation by blue crab is suspected (Gil Fernández, 2018). In this context, a series of aquarium experiments were designed to ascertain: (1) size preferences for marine (*M. galloprovincialis* and *M. gigas*) and freshwater preys (*P. maculata* and *C. fluminea*) by three *C. sapidus* size ages (juveniles, small adults, and large adults) which feature the highest predation on mollusks populations (Laughlin, 1982); (2) prey species preferences (*M. galloprovincialis* vs. *M. gigas*) and (*P. maculata* vs. *C. fluminea*) at each blue crab age size; and (3) the role of shell hardness (as a proxy of prey manipulability) and the nutritional characteristics of prey species in driving observed patterns of preferences. In addition, we describe the handling techniques used by *C. sapidus* to feed on the different mollusk species and we record the biomass consumed at each experimental trial in order to attain some rough knowledge on the potential ranges of biomass consumption in the wild.

2. Materials and methods

2.1. Collection of predators and prey items

2.1.1. Blue crab collection

A total of 360 individuals of blue crab were used across all food choice experiments conducted. All of them were bought alive from the fishermen's association of the Encanyissada Lagoon which operates a large trap net structure located in the main connection channel with the Alfacs Bay (Fig. 1) that allows for high fishing yields of blue crab. During the summer, males constitute the dominant sex in local estuarine waters, whereas females are mostly found in open waters (Prado, personal observation). Hence, only males were used throughout the experiments in order to avoid sex-related differences in claw morphology leading to variable functional responses (Eggleston, 1990b). Individuals were directly selected from different sections of the trap net according to their weight classes: small (juveniles: 32.4 to 95.7 g WW), medium (small adults: 114.9 to 235.6 g WW), and large (large adults: 237.5 to 404.2 g WW) (see also Table 1 for full details of average weight and length measures). These sizes included the entire commercial range of the species, which is thought to cause damage to local bivalve farms. In the case of experiments with marine preys (mussels and oysters), crabs were transported to our facilities 24 h before to each experiment, in order to allow for acclimation of individuals and ensure non-feeding conditions during that period. For experiments with freshwater preys (apple snail and Asian clam), since there is no local blue crab fishery in freshwater habitats capable of covering experimental needs, they were transported to our facilities 5 days in advance to allow for necessary acclimation from salinities of ca. 31 in the area of capture to levels of 8 (see Gleeson et al., 1996). Periods longer than 5 days would have been necessary to reach acclimation to freshwater conditions but were discarded because they might have enhanced captivity stress leading to altered responses. Thus, a salinity of 8 was chosen as a compromise for the wellbeing of both predator and preys in both with and without shell experiments (see later). This salinity is tolerated without acclimation by both apple snail (ca. 48 h exposure without any detrimental effects; Serra 2017) and Asian clam (no detectable stress at salinities below 15-20; Ferreira-Rodríguez and Pardo, 2016). Given the longer stabling period that crabs needed to achieve acclimation to lower salinity, individuals were feed with frozen mussels to prevent starvation stress until 24 h prior to each experiment. Since the blue crab is a highly invasive species, all individuals were sacrificed by freezing at -20°C at the end of each experiment.

2.1.2. Marine preys

Mussels (*M. galloprovincialis*) and oysters (*M. gigas*) were bought from the local bivalve farmers in the Alfacs Bay and maintained alive during a week at the IRTA aquaculture facilities by feeding them with a mix of three species of microalgae (*Isochrysis* aff. *galbana* (T-ISO), *Tetraselmis chuii*, and *Chaetoceros calcitrans*) produced at the IRTA's hatchery. For each prey species, three distinctive categories (N= 10 each) were considered for size preference experiments: small (0.15 to 0.33 g WW and 1.25 to 2.5 g WW), medium (0.58 to 0.93 g WW, and 2.7 to 4.8 g WW), and large (1.74 to 5.2 g WW and 6.02 to 18.9 g WW), respectively for mussels and oysters (see also Table 1 for average length and weight measures). These categories are representative of the overall ranges that are usually exposed to blue crab predation in bivalve farms, although in the case of the oysters, the "small" category was also the smallest size available during the experimental period. For prey species food choice experiments (with and without shell), large mussels and medium size oysters (N= 10 additional size measures each) featuring similar sizes were used to attain reasonable comparisons.

2.1.3. Freshwater preys

Apple snails (*P. maculata*) and Asian clams (*C. fluminea*) were collected by hand at a salinity of ca. 3 from the drainage canal network in the Ebro Delta (Fig. 1), where both species are very abundant. Animals were collected in two occasions for size class and prey choice trials in numbers that were slightly above of those required for the experiments in order to allow for some potential mortality in captivity conditions. Asian clams were also feed with the available species of phytoplankton, whereas apple snails were given lettuce until their use for experimental purposes. The three categories (N= 10) considered for size preference experiments were: small (0.61 to 1.14 g WW and 0.09 to 0.20 g WW), medium (1.97 to 3.57 g WW, and 0.28 to 0.45 g WW), and large (11.71 to 31.08 g WW and 0.81 to 1.09 g WW), respectively for apple snail and Asian clam (see also Table 1). As for marine species, these prey categories embraced the variability observed in the field during the current study and past fieldwork (Serra, 2017). For prey species food choice experiments (with and without shell), small apple snails and large Asian clams (N= 10 additional size measures each) featuring similar sizes were used for comparative purposes.

2.2. Food preference experiments

Twenty-four food preference experiments (4 prey size preference experiments x 3 blue crab sizes and 4 prey item preference experiments x 3 blue crab sizes) were conducted over a 2-month period in July-August 2019 within a greenhouse experimental facility. All experiments were conducted over a similar natural summer water temperature (27-28 °C), light photoperiod and pH (8.01 to 8.09). All tanks were aerated, and oxygen was carefully monitored throughout the experiment to ensure that it was always maintained above 80%. For salinity, as indicated previously, two different settings were conducted. For marine preys, we used sea water pumped from the Alfacs Bay, which is stored for decantation and then filtered for use within our aquaculture facilities (ca. 37 in summer). For freshwater preys (with and without shell), we decreased salinity to 8 by progressively adding freshwater over the 5 days acclimation period.

In prey size experiments, three blue crab individuals of a given size per tank (N= 5 300 L tanks; i.e., a total of 15 individuals per food assay), each deployed within a metallic mesh cage were offered three prey items of each size (i.e., 3 small, 3 medium and 3 large) and consumption monitored at 1, 2, 3, 4, 5, and 24 h after deployment (usually, from 10:00 am of one day to 10:00 am of the next day). Similarly, in prey type experiments with and without shell (ie., bivalves were provided open so the flesh of the animal was readily available to the crabs), three blue crabs per tank (N= 5 tanks) placed within individual mesh cages were offered three individuals of each prey type (mussel and oyster or apple snail and Asian clam) and consumption monitored at the same time intervals indicated above over a total period of 24 h. Results were expressed as the number of prey units of each size or species consumed at each monitoring time. For each experiment, the handling techniques used by the crabs were carefully observed and reported. In addition, average biomass values of each prey size and species were used to calculate the total biomass consumed at each experimental trial in order to attain some rough estimation of potential ranges of daily biomass ingestion at each crab size. In the case of prey type experiments without shell (i.e., death animals), an additional group of 3 individuals of each species were placed within a tray in the water above each cage to check for tissue losses over the 24 h period. Results showed an average decline of 14.1 ± 0.9% of the WW which was corrected in the infrequent cases of partially eaten items.

2.3. Estimations of shell hardness

For each of the four prey species investigated, three individuals of each size were collected and used alive for shell hardness estimations in order to avoid brittleness processes occurring after the death of the animal (Micheli, 1995). The resistance of each species and size to breakage (strength per unit surface) was investigated by compressing the animals using a TecTake hydraulic press (ref. 401669) with a nominal power of 350 N, a total capacity of 6000 kg, and a digital manometer to allow accurate readings in bars. This procedure caused a rapid death of the animals by concussion with presumably minimal suffering involved. Currently, only cephalopods are included in the Directive 2010/63/UE on the protection of animals used for scientific purposes because there is scientific evidence of their ability to experience pain, suffering, anguish, and lasting harm, whereas there is not for the other mollusk groups.

2.4. Nutritional analyses

Three individuals of similar large size (see Table 1) of each prey species were collected during the experimental period in July-August 2019, in order to prevent possible seasonal differences. All individuals were dried at 60°C for 48 h and then ground to a powder with a mortar and pestle. Total organic matter (%) was calculated by subtraction of dry samples after combustion at 500°C for 5 h and obtaining the ash-free dry weight. Total lipids (%) were extracted from dried samples by direct elution with chloroform and methanol, using the methods described by Folch et al., (1957). Total carbohydrates (%) were determined with the widely used phenol-sulfuric acid assay of Dubois et al., (1956) based on colorimetric absorbance at 490 nm. Total protein analyses were carried out by combustion at the IRTA facilities. For calorimetry, caloric content in proteins (23.6 KJ g^{-1}), lipids (39.5 KJ g^{-1}), and carbohydrates (17.2 KJ g^{-1}) was used to estimate the total energy content in each of the preys expressed in KJ per g DW.

2.5. Data analyses

2.5.1. Food preference experiments

The significance of differences among blue crab size categories (fixed factor, three levels) during the experiments was investigated with a one-way MANOVA (total width, width without spines, length and wet weight as dependent variables) in order to prevent confounding results in food choice experiments. Similarly, a one-way MANOVA (using width, length and weight for all bivalves and operculum width, length and weight for apple snail) was used to assess differences among size categories (fixed factor, three levels) of each prey species.

For each crab size (N= 15 individuals), differences in cumulative consumption among the 3 sizes of the 4 prey species (mussel, oyster, apple snail, and Asian clam) were investigated using the Friedman ANOVA by ranks (Conover, 1980) (see Cronin et al., 2002; Prado and Heck, 2011 for a similar approach) at the different evaluation times (1, 2, 3, 4, 5, and 24 h after the start of the experiment. Non-parametric post hoc comparisons (Wilcoxon matched pairs test) with the Bonferroni adjustment were used to assess differences among the three pairs of sizes, and to correct for possible increases in Type I error associated to multiple comparisons. For prey species comparisons (i.e., mussel vs. oyster and apple snail vs. Asian clam with and without shell), the Wilcoxon matched pairs test was directly used to test for differences in cumulative consumption between offered pairs of prey items at each evaluation time.

For each group of marine and freshwater prey species (mussel vs. oyster and apple snail vs. Asian clam) and shell vs. without shell effect, we assessed differences in the total daily biomass consumed (i.e., all prey sizes or offered species combined) by each size of blue crab using a two-way factorial ANOVA. Student-Newman-Keuls (SNK) post hoc comparisons were used to identify differences among sizes and patterns of biomass consumption.

2.5.2. Shell hardness and nutritional analyses

Differences in the pressure needed to break each type of shell size were investigated with a two-way nested ANOVA with species and size as fixed factors. Differences in the nutritional composition (percent proteins, carbohydrates, lipids, and ashes) of the different prey species was investigated with a one-way PERMANOVA and subsequent pair-wise tests.

For all parametric analyses, homogeneity of variances and normality assumptions were tested by Cochran's test and Kolmogorov-Smirnov distribution-fitting test of the residuals, respectively. Data were transformed when necessary to meet test assumptions. On some occasions, transformation was not possible, and the level of significance was reduced from *p*= 0.05 to *p*= 0.01. ANOVA is generally considered to be robust to such violations, especially in large experiments (i.e. total df>30; Underwood, 1997). All analyses were conducted using the Statistica V12.0 software.

3. Results

3.1. Differences among blue crab and prey sizes

Results from MANOVA showed that there were important differences among size categories of both predator and preys as a basic condition to attribute size effects in later food preference experiments (see Tables 1 and 2). Further SNK evidenced differences among the three size categories for all the dependent variables investigated, except for shell width between small and medium oysters (Table 2).

3.2. Food preference experiments

3.2.1. Size preferences

For mussels, large crabs showed no consumption preference, being equally able to feed on all the three sizes offered throughout the experimental period. Medium size crabs, however, showed a preference for medium and small mussels compared to large mussels through most of the experiment (from 3 to 24 h after). A similar pattern was also observed for small crabs, which showed higher consumption of small and medium size mussels during most of the experiment, although differences were not significant after 24 h (Table 3a, Fig. 2a-c).

For oysters, overall consumption rates were too low in all food preference trials and no differences could be detected (Table 3b, Fig 2d-f). Yet, large crabs were still able to consume 7 small and 1 medium oysters (N= 45 each), whereas medium crabs only consumed 2 small oysters, and small crabs were not able to feed.

For apple snail, large crabs tended to consume higher numbers of large individuals than medium and small ones during most of the experiment, but differences were only significant after 24 h. Medium crabs also displayed a significant preference for large individuals during most of the experiment, except after 24 h when no differences were found. In contrast, small crabs showed no preference for any size at any time of the experiment (Table 4a, Fig. 3a-c).

For Asian clam, large and medium crabs showed no size preference but small crabs tended to consume larger numbers of small individuals than of the larger sizes throughout the experiment, although the effect was only significant after 24 h (Table 4b, Fig. 3d-f).

3.2.2. Prey preferences

In marine prey preference trials with prey of similar size, large crabs consistently preferred mussels to oysters throughout the experiment, and this preference was maintained when the shell was removed, except at the end of the experimental period when the preferred item was scarce. Medium crabs showed the same patterns with whole preys, and there was also a clear trend towards higher consumption of mussels once the shell was removed, although it was only significant at 2 h after the start of the experiment. For small crabs, prey consumption with shell was low through most of the experimental period, but higher mussel consumption was observed after 24 h. Once the shell was removed, small crabs also preferred mussels to oysters during most of the experiment but showed non-significant differences at 5 and 24 h when mussels were scarce (Table 5a, Fig. 4).

For freshwater preys, preferences were strikingly higher for apple snail than Asian clam for all crab sizes and times, except 1 and 2 h after the start of the experiment with small crabs. Conversely to patterns in marine preys, once the shell was removed no differences were detected for any crab size and time. In fact, in many cases there was no variability at all in prey consumption across replicate cages and test could not be computed (Table 5b, Fig. 5).

3.2.3. Handling techniques

Mediterranean mussel: Juvenile crabs did not normally crush any size of mussel and valves were generally found intact, whereas both adult sizes could also break the weaker umbonal part or the upper part of the mussel (Fig. 6a). When shells were found intact, attacks took place by holding individuals across the dorsoventral axis, with the byssal threats looking upwards and then exerting pressure with the claw until both valves were slightly ajar. Then, the other claw was introduced between the valves and used to pull to tear them apart.

Pacific oyster: Only the smaller oyster size (ca. 50 per 70 mm) could be to some minor degree predated by the largest blue crab sizes (ca. 129 mm CW). The technique consisted in holding the individual across the flat sides and gradually eroding the edges of the shell until there was a fine space between the valves to allow the introduction of the claw (Fig. 6b). In some instances, both valves were fully detached from each other during the manipulation process.

Apple snail: Unwisely, individuals were generally observed outside the shell in the presence of the predator. When this occurred, the animal was grabbed with the claw around the posterior part of the head and pulled out of the shell. When retreated, then the shell was partly crushed, and the animal pulled out of the shell (Fig. 6c). The bright pink albumen gland containing developing eggs was not consumed.

Asian clam: All shell sizes were found open intact, and the technique was similar to that used in for opening mussels without breaking them (Fig. 6d). Yet, given the rounder anatomy of the clam, individuals were hold in any position along the shell margins in order to make pressure for separating the valves.

3.2.4. Biomass consumption

For all crab sizes investigated, the consumption of mussel biomass (all sizes included) was higher than that of oyster (8.4 ± 1.1 vs. 1.2 ± 0.8 gWW, 6.6 ± 0.6 vs. 0.3 ± 0.3 gWW, and 7.6 ± 0.7 gWW vs. null consumption, respectively for large, medium and small crabs; Table 6A). Apple snails showed the highest biomass consumption among investigated preys, with greater values than Asian clam for all crab sizes (60.6 ± 2.3 vs. 4 ± 0.1 gWW, 50.6 ± 3.3 vs. 2.9 ± 0.4 gWW, and 42.6 ± 4 gWW vs. 3.2 ± 0.4 gWW; respectively for large, medium and small crabs Table 6B). Large crabs also consumed greater biomass than medium and small crabs, although effects were due to patterns observed for apple snail, whereas biomass consumption Asian clam showed similar values for all crab sizes (i.e., significant Size and Prey x Size interactions; Table 6B).

For marine preys (large mussels and medium oysters included), the effect of shell removal increased biomass consumption by all crab sizes (11 ± 1.3 vs. 18 ± 0.9 gWW, 9.4 ± 0.7 vs. 17.8 ± 0.5 gWW, and, 3.6 ± 1.1 vs. 15.6 ± 0.9 gWW, respectively each crab size; Table 6C). In addition, a significant effect of crab size was detected due to smaller crabs being able to consume less large mussels and medium oysters with shell than medium and large crabs. In contrast, although freshwater preys (small apple snail and large Asian clam included) also showed important shell effects, the increase in biomass consumption was comparatively very small for all crab sizes (3.9 ± 0.2 vs. 4.5 ± 0 gWW, 3.8 ± 0.2 vs. 4.5 ± 0 gWW, and, 4.2 ± 0.1 vs. 4.3 ± 0.2 gWW, respectively each crab size; Table 6D).

3.3. Shell hardness and nutritional differences

3.3.1. Shell hardness

Results from two-way ANOVA showed important differences across investigated species. In particular, mussels and apple snails showed similarly low values (3.56 ± 0.38 and 3.67 ± 0.61 bars, respectively), followed by Asian clam (7.67 ± 1.17 bars) and oyster (17.94 ± 2.25 bars). In all species, increased size enhanced the pressure needed to break the shells, but differences across sizes were not always significant. In Asian clam, and particularly in apple snail, a *plateau* seems to be reached at medium sizes with no further increase in shell hardness, whereas mussel showed the smallest differences across sizes and oyster the largest (Table 7a, Fig. 7a).

3.3.2. Nutritional differences

Results from one-way PERMANOVA showed that there was a large variability in the composition of proteins, carbohydrates, lipids and ashes among investigated prey species, all of them being significantly different (Table 7b, Fig. 7b). Protein contents were similarly high in the Pacific oyster, followed by Asian clam and Apple snail, (50.5 ± 4.7%, 45.6 ± 1%, and 42.5 ± 4.72%, respectively) and lowest in the Mediterranean mussel (37.5 ± 0.5%). Levels of carbohydrates were more similar in Asian clam and mussel (34.8 ± 2.2% and 33.9 ± 1.3%, respectively) and lower in apple snail (16.6 ± 1.9%) and oyster (3.6 ± 1%). Lipids were similar in oyster, Asian clam and mussel (15.5 ± 1.6%, 13.7 ± 0.7%, and 11.3 ± 0.8%) and slightly lower in apple snail (7.5 ± 0.7%). These compositions resulted on important differences in the energy content across food items ($F_{3,8}$= 8.37; p= 0.0075). These differences were due to lower energy content in apple snail (0.78 ± 0.03 JK g^{-1}) compared to the other food items (1.16 ± 0.09 JK g^{-1}, 1.07 ± 0.07 JK g^{-1}, and 0.99 ± 0.05 JK g^{-1}, respectively for oyster, Asian clam, and mussel). The levels of ashes, was higher in apple snail and oyster (33.3 ± 3.8% and 30.3 ± 2.9%), followed by mussel (17.2 ± 2.2%) and Asian clam (5.8 ± 0.5%).

4. Discussion

4.1. Food preference patterns and driving variables

The refuge value provided by shells varied greatly among investigated preys, depending mostly on shell hardness and overall manipulability and on predator size. The Pacific oyster, *M. gigas*, featuring the hardest shell showed the greatest rates of survival, with only few individuals of the smallest size (ca. 50-70 mm) being predated by the largest size of blue crab. This size consumption pattern is slightly above the upper threshold size reported for predation on *C. virginica* (ca. 40 mm; Arnold, 1984), suggesting that the later species is slightly less vulnerable to predation. In contrast, no critical upper threshold of prey size was observed for Mediterranean mussel, apple snail, and Asian clam, with all size ranges being potentially predated by some blue crab size, although protection may occur at exceptionally large prey sizes (e.g., the ribbed mussel, *Geukensia demissa* does not get attacked at sizes of 80-90 mm; Lin, 1991). Juvenile blue crabs (and also young adults in the case of Mediterranean mussel) displayed a significant preference for the smaller prey sizes (see also Seed, 1980, 1982; Seed and Hughes, 1981), evidencing that greater prey size can decrease predation. Once large predator size is attained (150-160 mm CW), variability in energy intake across prey sizes may be equally advantageous to differences in the time spent handling the prey, thus removing size preference effects. Once the prey shell was removed, no agreement between blue crab preferences and the overall nutritional energy across prey species was observed, although differences may have been too little to trigger an effect. Nevertheless, a nutritional preference for mussel vs. oyster was detected, which could be associated to considerably higher levels of carbohydrates (by ca. 10-fold) or to higher presence of other required compounds not identified during the present study (e.g., specific amino acids, quaternary ammonium compounds, organic acids, nucleotides and related substances; Carr, 1988). Overall, discrimination between mollusk species once the shell was removed was unexpected and suggests that the blue crab might not be such a generalist consumer as previously though (e.g., Hill and Weissburg, 2013).

4.2. Effects in marine ecosystems

The results of this study are valuable for those who wish to conduct bivalve production for commercial exploitation in the Mediterranean. The seed of the Mediterranean mussel (2 to 18 mm length) is commonly trapped in collectors deployed in bays or the open sea, and then transplanted to ropes hanging from fixed raft for intensive culture during the fall (Ramón et al., 2007). Since the smallest mussel size used in this study (ca. 27 mm shell length) was still easily predated by all crab sizes, those below that size are also potentially vulnerable. According to our results, only large sizes (> 70 mm) may experience decreased predation by being targeted by a lower spectrum of crab sizes as shell hardness increases with size. Therefore, if the abundance of blue crab keeps increasing the entire production of Mediterranean mussel could be compromised by the lack of an effective escape size from predation. Yet, our experiments were conducted with detached mussels in aquarium conditions, and other factors that may affect predation rates in the field do also need to be considered. For instance, Lin (1991) conducted blue crab predation experiments with the ribbed mussel, *G. demissa*, and found that enhanced burial within the sediment, attachment strength, and group living within the mussel matrix, could minimize the predation efficiency of the blue crab and reduce mortality rates. Also, although the blue crab is an excellent swimmer, predation success may also be strongly influenced by hydrodynamic conditions (see Powers and Kittinger, 2002), thus minimizing expected patterns of prey susceptibility on floating rafts compared with benthic conditions. Besides, according to Reimer and Harms-Ringdahl (2001) mussel populations can show certain plasticity in their morphology when exposed to predators including shell thickness, adductor muscle size, and strengthening of the byssal attachment, that may decrease predation rates in the field. Yet, compared to the Mediterranean mussel, the Pacific oyster, *C. gigas*, offers higher resistance against blue crab predation, although our recommendations are limited by the large minimal size that could be obtained for the experimental purposes (ca. 50 by 70 mm). In the Ebro Delta, the spat of the Pacific oyster (ca.

10-20 mm length) is usually imported from France and then glued to ropes with cement and placed into floating rafts until they reach their market size. Given that predation on the smaller oyster size class was already very low, it could be reasonable to assume that individuals may be at risk for a period of 5 months (beginning of February to the end of June) that they need to grow from ca. 10-20 mm to a critical upper threshold of ca. 50 mm (Dàmaso et al., 2011). In temperate regions of natural distribution, the blue crab has been indicated to become lethargic when the temperature drops and to enter a period of dormancy (Rathbun, 1896; Van Engel, 1958; Sulkin and Miller, 1975; Jensen and Miller, 2005), which also agrees with decreased abundances of the species within shallow Ebro Delta bays during winter and spring (López and Rodon, 2018). Therefore, the Pacific oyster may be able to attain a refuge size prior the crab activity increases, thus minimizing predation losses. Yet, given that the Pacific oyster is documented to be among the worst invasive species in the Mediterranean Sea (Zenetos et al., 2005), enhancing its cultivation should not be regarded as a solution for the continuity of shellfish production. Instead, we advocate for assessing the viability of shifting to other native oyster species such as *Ostrea edulis*, which is also present naturally in Ebro Delta Bays and appears to coexist with the presence of the blue crab.

4.3. Effects in riverine ecosystems

The Ebro Delta has hosted a well-established, self-sustaining population of the apple snail, *P. maculata*, since 2009 (Andree and López, 2013), which poses a major threat to the native biodiversity of the Ebro River (Oscoz et al., 2010) and has cost the public administrations millions of euros in financial aids and control measures to the rice sector (e.g., see DOGC 7399 Ordre ARP/132/2017). To our knowledge, the glossy ibis, *Plegadis falcinellus* is the only apple snail predator so far confirmed in European habitats (Bertolero and Navarro, 2018), and this work constitutes the first report of confirmed predation by the blue crab under experimental conditions. This finding concurs with an important decrease of apple snail (including egg clutches) in the Ebro River and rice fields' drainage canals since blue crab abundances became increasingly high (Gil Fernández, 2018), suggesting that the species might be exerting an effective predation control. In addition, an increased predation from other species (especially birds) cannot be ruled out. Although more experimental work is necessary to determine predation rates in the field, current populations of apple snail seem to be now lower and mostly relegated to rice fields and certain drainage channels where the abundance of blue crab is null or very low (Prado, personal observation). According to our experimental results, the low shell hardness of the species (only 1.3 to 5.3 bars) provides a low refuge value that can partly account for the high predation rates on apple snail. However, conversely to mussels, apple snails displayed an active foraging behavior with long periods of time outside the shell despite the presence of blue crabs, which appear to additionally favor predation rates. This evidences that individuals were fine during the experimental period despite salinity conditions of 8 are slightly suboptimal for the species (Serra, 2017), and are infrequent in natural habitats except some for some rice field drainage channels adjacent to Ebro Delta Bays or at low river flows. Higher numbers of large and medium prey were targeted by adult crabs, presumably because an enhanced energy gain is obtained and the overall manipulability was very low (i.e., the energy maximization premise; see Elner and Hughes, 1978). Since medium and large individuals are those that are reproductively active in the population (Estoy et al., 2002), preference for these sizes in adult crabs may have also contributed to the observed decline of apple snail and egg clutches in the Ebro River, rather than a direct predation. In fact, the bright pink albumen gland containing developing eggs was rejected by the crabs, suggesting that they could detect the presence of noxious substances such as indigestible polysaccharides and toxic proteins that are generally regarded as deterrents from predation (Giglio et al., 2016). Despite the discard of these undesired parts, the biomass consumption of apple snail reached values of 42.5 to 60.6 g WW $\cdot$ d^{-1}, respectively from juvenile to adult sizes, which is ca. 7 times higher than mussel, and 15 times higher than Asian clam preys. This suggests that when predation is exerted on a prey that requires little manipulation, consumption rates can reach much higher values (e.g., Seed, 1980; this study). In all the experiments conducted, daily consumption of prey biomass was also highly increased by removing the shell.

In regards of the Asian clam, *C. fluminea*, the species has been present in the Ebro Delta and lower stretch of the Ebro River for over two decades (Oscoz et al., 2010) and the current distribution stretches over the whole river reaching densities of over 40,000 individuals per m^2 in certain points where the blue crab is not present, especially in the Aragon region (Ismael Sanz from Paleoymas, public comm.). Although the effects caused on other native fauna in the Ebro River have not been investigated, such large densities of individuals may impact the ecosystem at multiple levels including alteration of benthic substrates, outcompeting native bivalve species for food and physical space, and potential repercussions in food webs and biogeochemical cycles, among other detrimental effects (Araujo et al., 1993; Sousa et al., 2008, 2014; Oscoz et al., 2010). Besides, by actively feeding on phytoplankton the Asian clam could also increase water transparency enhancing the rapid proliferation of macrophytes (mainly *Potamogeton pectinatus*) on the riverbed, though the main driver is phosphorus decline (Ibáñez et al., 2012). However, no proper population assessment has been yet conducted in the lower stretch of the Ebro River where the species coexist with the blue crab. Compared with the apple snail, lower consumption rates of Asian clam at all crab sizes concurred with enhanced shell hardness (up to 11.2 bars) and lower exposure degree of soft body parts. Yet, the handling technique for Asian clam consisted in pulling the valves apart without breaking the shell, suggesting that adductor muscle size and strength (Reimer and Harms-Ringdahl, 2001) rather than shell hardness was the main factor involved in prey manipulability. Only juvenile crabs showed decreased capacity to open medium and large sizes of Asian clam (ca. 18 to 27 mm), although in other sites the species has been reported to reach greater sizes than those considered in this experiment (up to 50 mm; Marsh, 1985) and may attain certain protection in size. On the other hand, given the large availability of individuals in the Ebro River and the high reproduction rates of the species (Byrne et al., 2000; Sousa et al., 2008), the capacity of the population to sustain predation rates by blue crabs appears to be substantial. Our results from food preference experiments suggest that now that the local abundance of apple snail in the Ebro River is low, the blue crab population could be intensively feeding on Asian clam. This situation contrasts with other invaded areas such as the River Minho estuary where the abundant population of Asian clam is barely consumed by higher trophic levels such as birds, fishes and mammals and great part of the biomass goes directly to the detritus food-web (Sousa et al., 2008). Although the presence of the blue crab can be an effective managing tool for controlling the abundance of invasive mollusk species in the Ebro River, it may also have a negative effect on some of the last remaining populations of endangered freshwater mussels (Gómez and Araujo 2008) and other native freshwater mollusks.

5. Conclussions

Variability in the degree of prey manipulability mostly including shell hardness but also the strength of the adductor muscle and the foraging behavior of the prey appear to be key factors explaining patterns of prey size predation (Seed 1980, 1982; Hughes and Seed 1981; Lin 1981; Bisker and Castagna 1987; Eggleston 1990a,b) and preferences between species, although further research is needed to clarify certain nutritional preference for mussels. Except for the Pacific oyster, no refuge size was attained for the other species investigated, which are highly vulnerable to blue crab predation especially the apple snail which featured the weakest shell and long periods of unsheltered, active foraging. The use of blue crab to control these other undesired species should be undertaken with care, due to unknown effects at the ecosystem level. In marine habitats, given the commercial value of the blue crab (Paolisso 2007), is in our hands to establish an adequate level of fishing pressure capable of preventing losses in mussel production and controlling the spread of the species into other Mediterranean areas.

Acknowledgements

Financial support was provided by the Generalitat de Catalunya (Departament d’Agricultura, Ramaderia, Pesca i Alimentació). P. Prado was contracted under the INIA-CCAA cooperative research program for postdoctoral incorporation from the Spanish National Institute for Agricultural and Food Research and Technology (INIA). Authors are grateful to Arnau Costes for his assistance during the experimental part of the work, and to Marta Sastre for assistance with the determination of nutritional contents. We also thank Dr. Candela Marco-Méndez and the two anonymous referees for a critical review that significantly helped to improve the ms.

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Fig. 1. Map of the Ebro Delta, showing the location of the Encanyissada Lagoon, Ebro Delta Bays, and the drainage and irrigation canal network used for rice agriculture. In the Alfacs and the Fangar Bay, the approximate location of mussel and oyster farms is also indicated.

Fig. 2. Cumulative consumption of marine prey sizes (mussel and oyster) by each grab size (large, medium, and small) during food preference experiments. (a-c) Mussel (M), and (d-f) Oyster (O). Error bars are SE.

Fig. 3. Cumulative consumption of freshwater prey sizes (apple snail and Asian clam) by each crab size (large, medium, and small) during food preference experiments. (a-c) Apple snail (S), and (d-f) Asian clam (C). Error bars are SE.

Fig. 4. Cumulative consumption of marine preys (large mussels vs. medium oysters) with and without shell by each crab size (large, medium, and small) during food preference experiments. (a-c) Mussel (M) vs. oyster (O) with shell, and (d-f) Mussel vs. oyster without shell. Error bars are SE.

Fig. 5. Cumulative consumption of freshwater preys (small apple snails vs. large Asian clam) with and without shell by each grab size (large, medium, and small) during food preference experiments. (a-c) Apple snail (S) vs. Asian clam (C) with shell, and (d-f) Apple snail vs. Asian clam without shell. Error bars are SE.

Fig. 6. Handling techniques used by blue crabs to predate on the different prey species and aspect of the remaining shells. (a) Mediterranean mussel; (b) Pacific oyster; (c) Apple snail; and (d) Asian clam.

Fig. 7. (a) Pressure (in bars) needed for breaking the shells of the four prey species at each investigated size; and (b) nutritional contents (%) of proteins, carbohydrates, lipids and ash, and total energy per species.

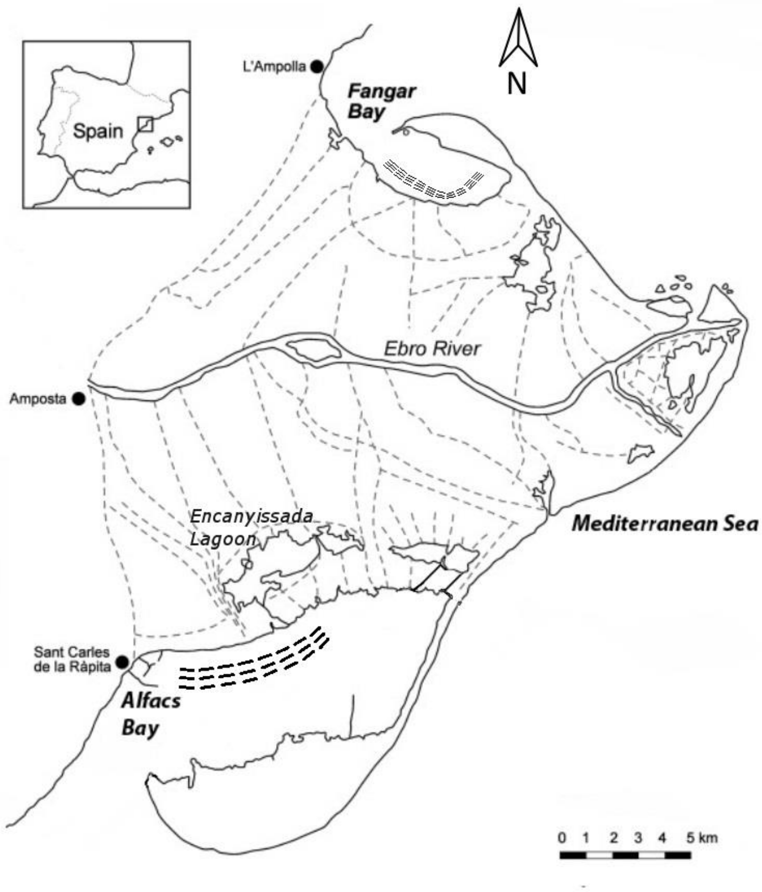
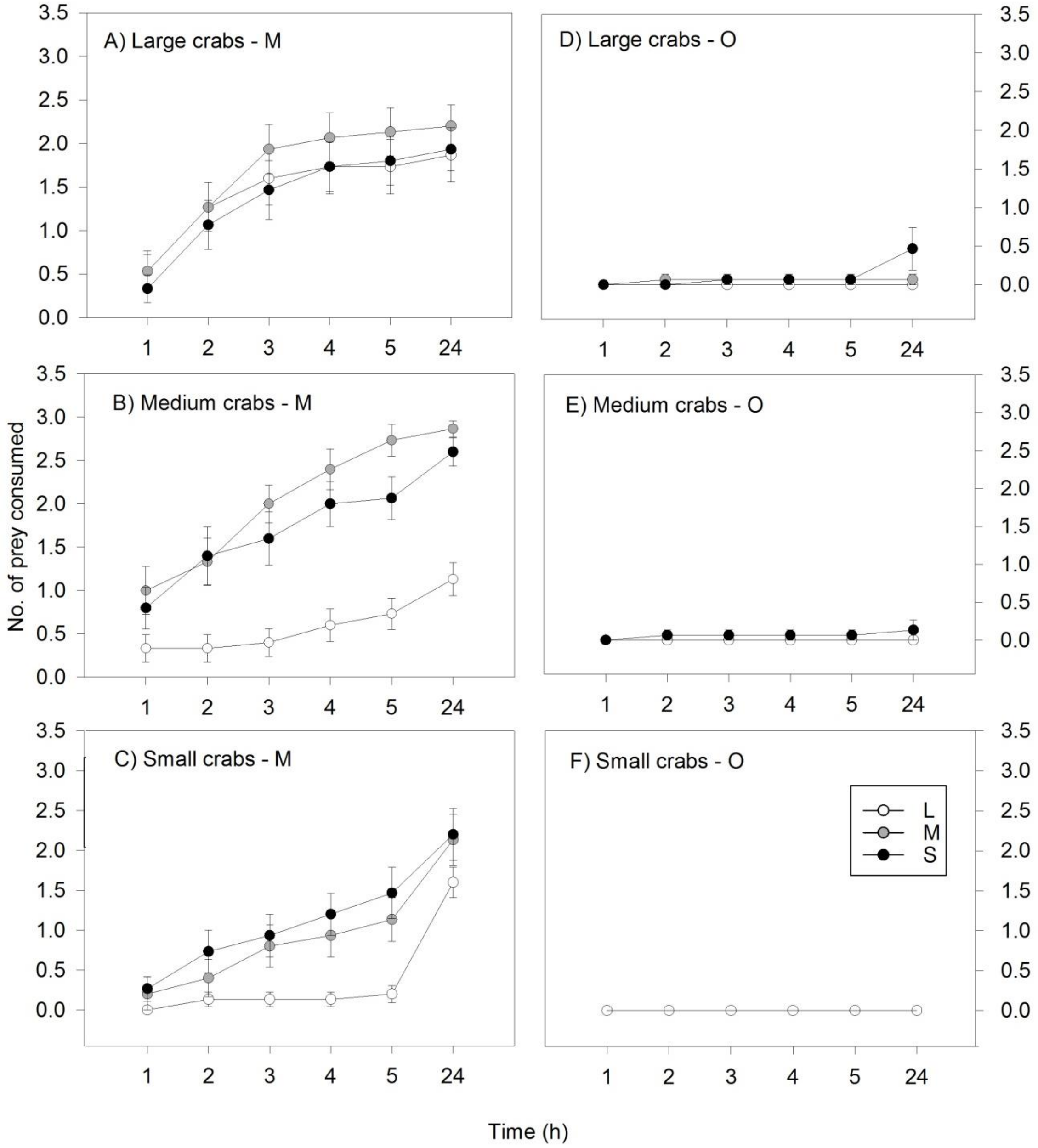

Spain
L'Ampolla
Fangar Bay
N
Ebro River
Amposta
Encanyissada Lagoon
Mediterranean Sea
Sant Carles de la Ràpita
Alfacs Bay
0 1 2 3 4 5 km

Fig. 1



A) Large crabs - M
B) Medium crabs - M
C) Small crabs - M
D) Large crabs - O
E) Medium crabs - O
F) Small crabs - O
No. of prey consumed
Time (h)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
1
2
3
4
5
24
L
M
S

Fig. 2

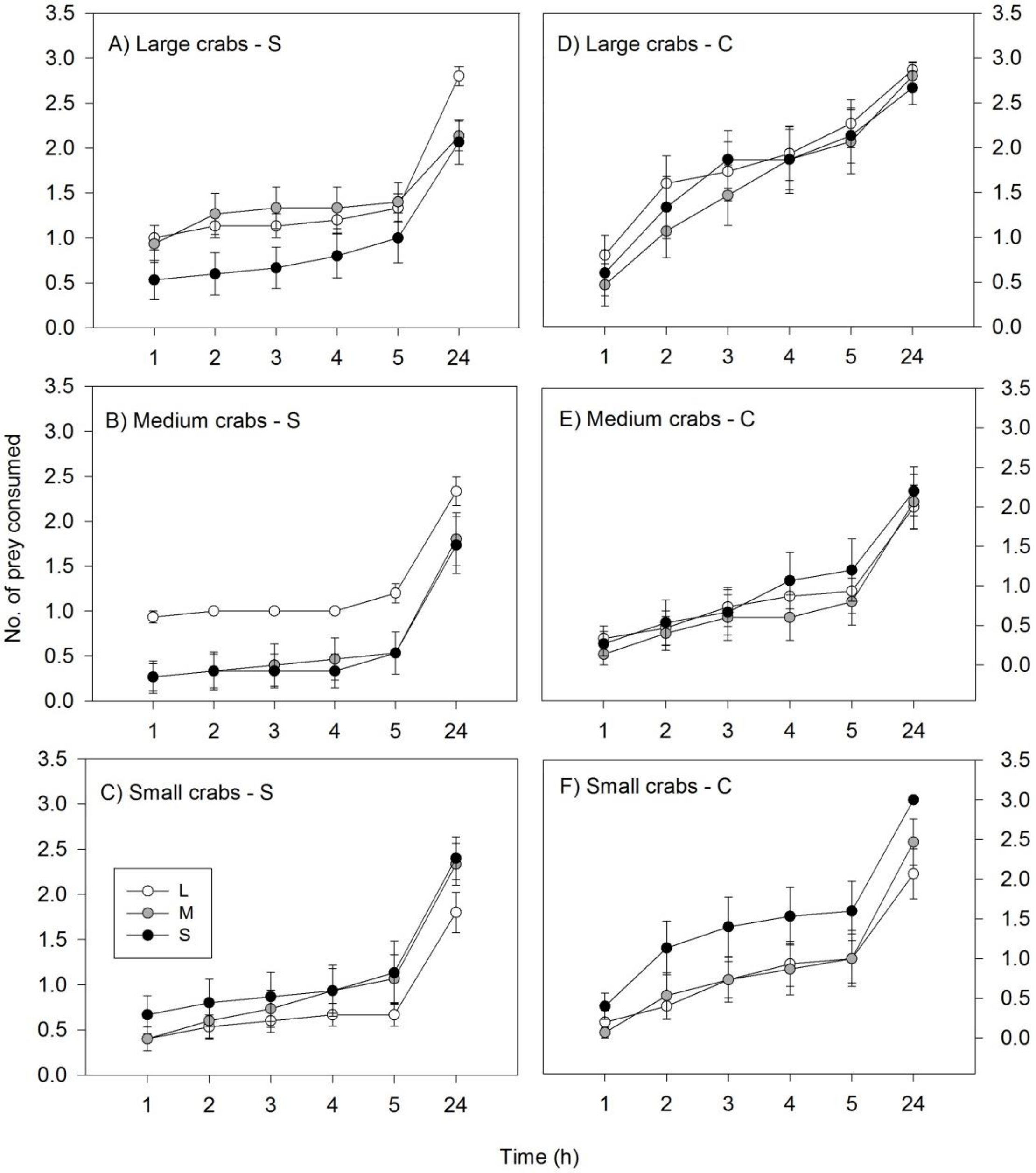
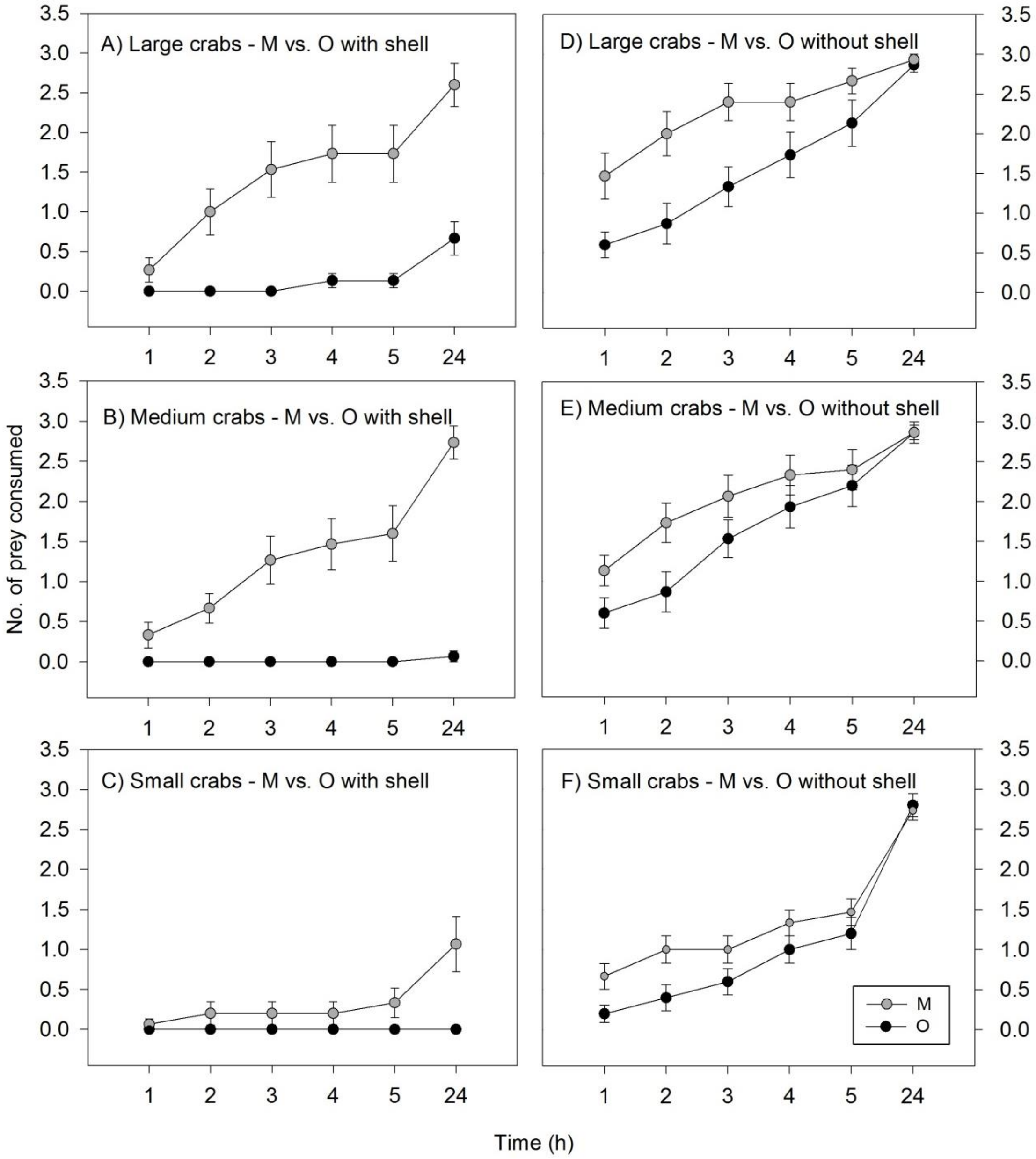

A) Large crabs - S
B) Medium crabs - S
C) Small crabs - S
D) Large crabs - C
E) Medium crabs - C
F) Small crabs - C
L
M
S
No. of prey consumed
Time (h)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
1
2
3
4
5
24

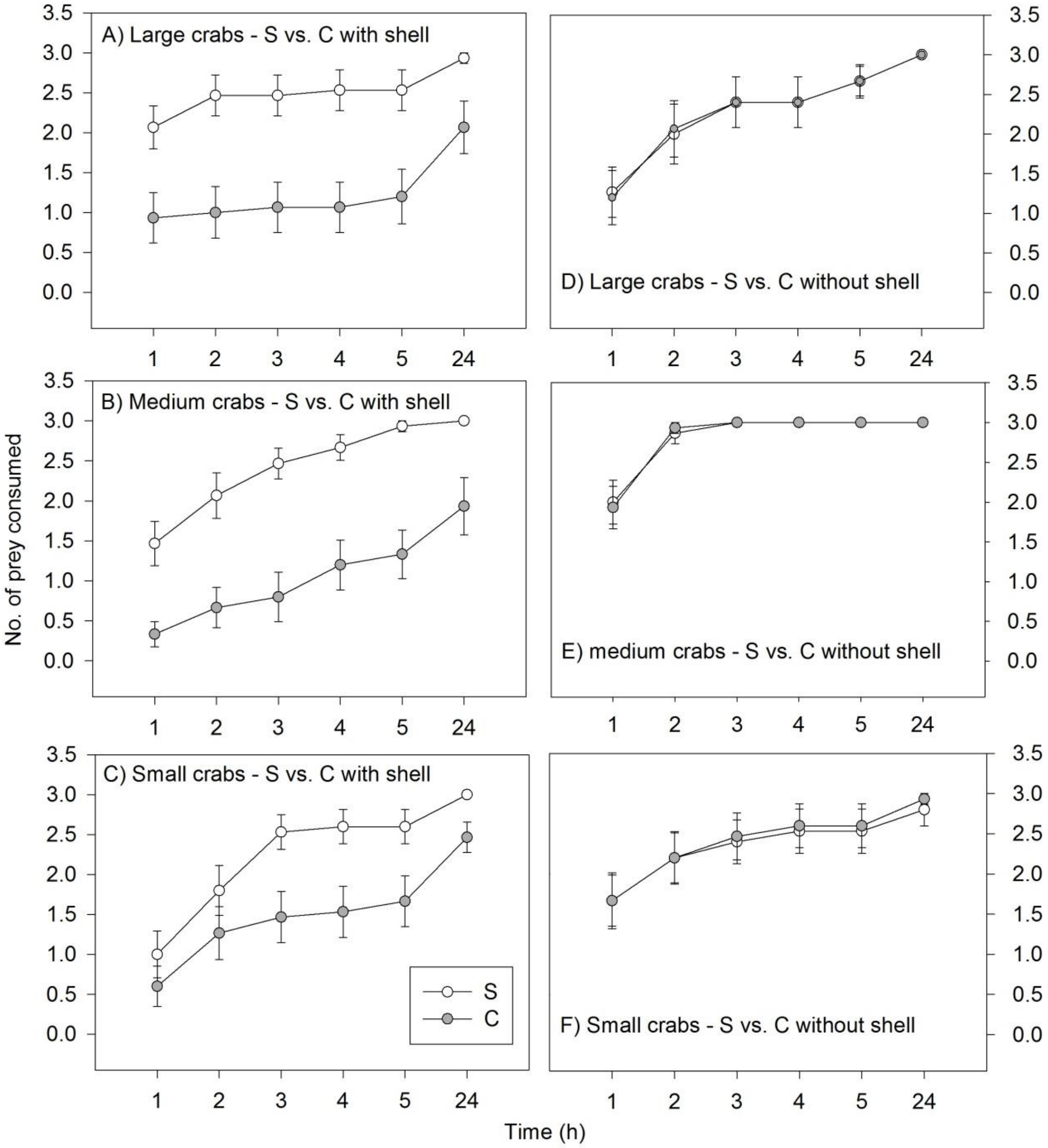
Fig. 3



806

807

808 Fig. 4



A) Large crabs - S vs. C with shell
B) Medium crabs - S vs. C with shell
C) Small crabs - S vs. C with shell
D) Large crabs - S vs. C without shell
E) medium crabs - S vs. C without shell
F) Small crabs - S vs. C without shell
No. of prey consumed
Time (h)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
1
2
3
4
5
24
S
C

Fig. 5

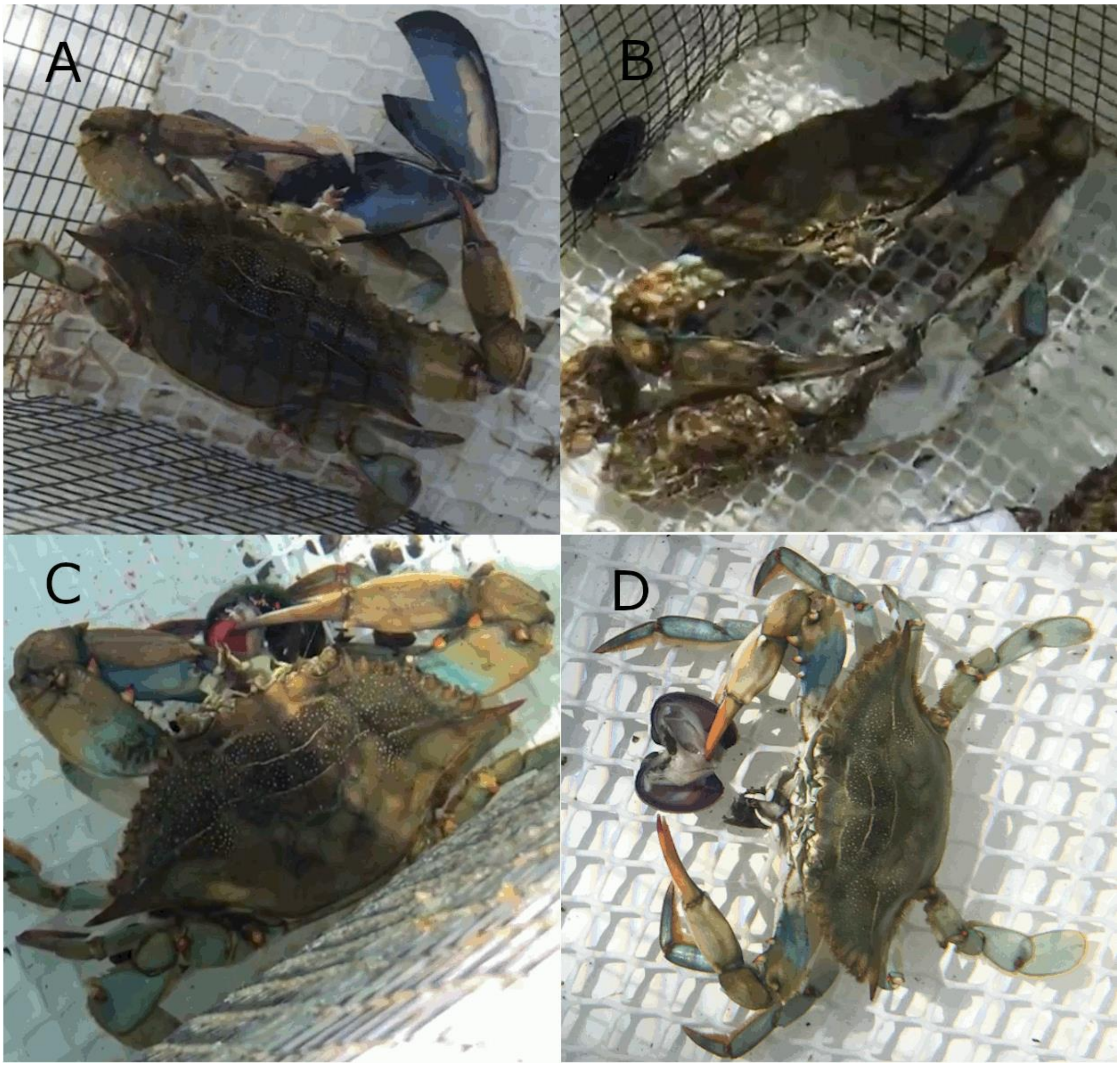


Fig. 6

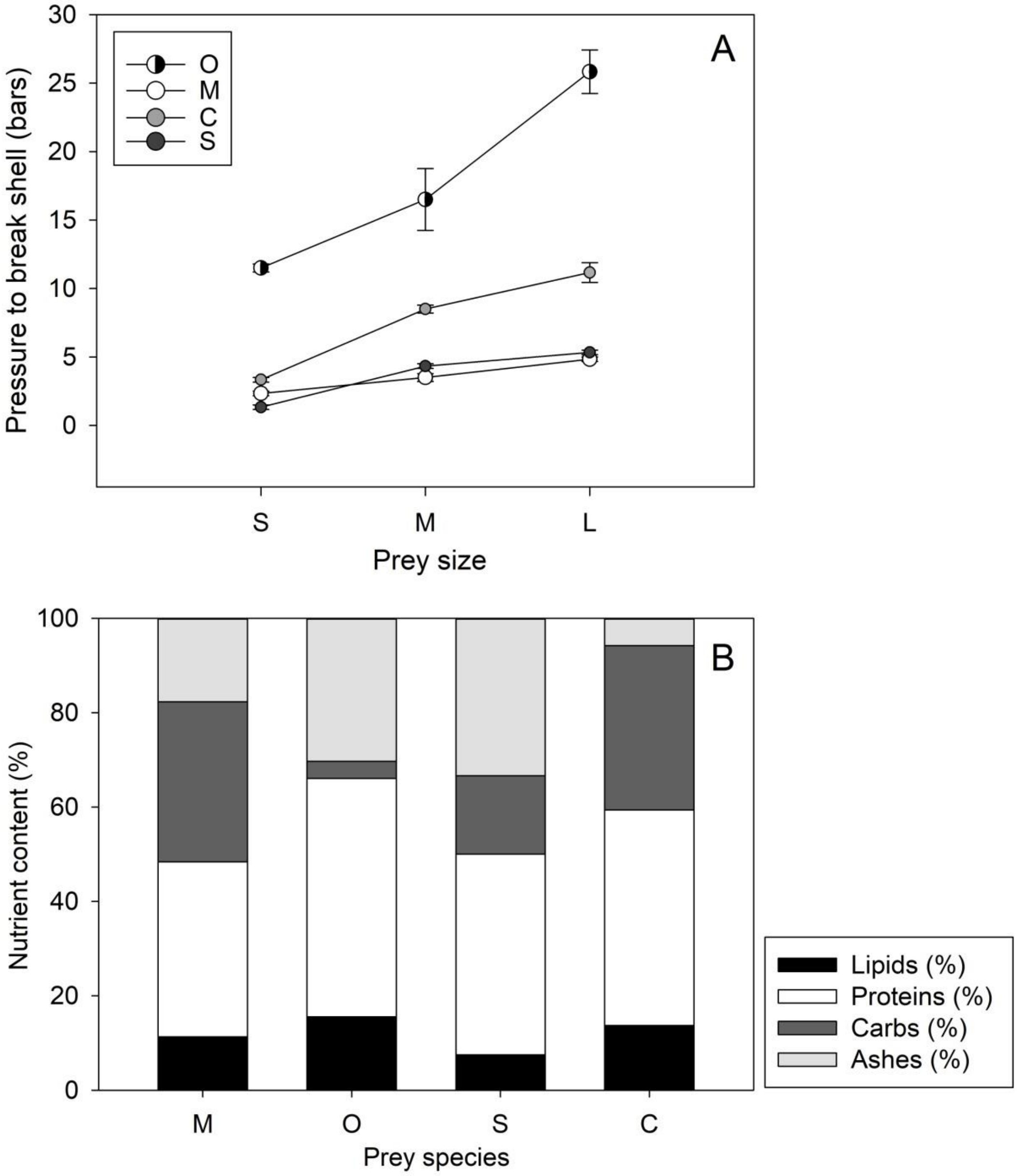

A
O
M
C
S
Pressure to break shell (bars)
30
25
20
15
10
5
0
S
M
L
Prey size
B
100
80
60
40
20
0
Nutrient content (%)
Lipids (%)
Proteins (%)
Carbs (%)
Ashes (%)
M
O
S
C
Prey species

Fig. 7

Table 1 Measures of (a) blue crabs of each size category used during throughout the experiments (N= 120 each) and of (b-e) prey species used in size preference experiments. Individuals were measured to the nearest mm and weighted to the nearest 0.1 g WW (N= 10 for each size and species). In the particular case of the apple snail, the operculum width and the height of the shell are indicated. Errors are SE. For further details and corresponding weight ranges see text.

		Width without spines (mm)	Total width/ operculum width (mm)	Length/ height (mm)	Weight (g WW)
(a) Blue crab	Small	77.6 ± 0.7	91.1 ± 1.1	43.9 ± 0.5	63 ± 1.2
	Medium	104.8 ± 0.7	131.3 ± 1.1	57.5 ± 0.5	157.6 ± 2.1
	Large	128.9 ± 0.7	157.9 ± 0.9	71.2 ± 0.4	307.6 ± 4.2
(b) Mussels	Small		15.5 ± 0.4	27.7 ± 0.8	0.24 ± 0.02
	Medium		19.4 ± 0.2	35.4 ± 0.5	0.73 ± 0.04
	Large		30.4 ± 1.3	61.13 ± 3	3.4 ± 0.4
(c) Oysters	Small		49.7 ± 0.9	69.9 ± 1.8	2.2 ± 0.2
	Medium		51.3 ± 1.9	83.2 ± 2.8	3.2 ± 0.2
	Large		76.9 ± 2.5	121.7 ± 2	10.3 ± 1.2
(d) Apple snail	Small		15.5 ± 0.5	23.2 ± 0.6	0.85 ± 0.06
	Medium		23.3 ± 0.6	33.6 ± 0.9	2.8 ± 0.1
	Large		39.4 ± 1.2	59.4 ± 2.2	18.9 ± 1.8
(e) Asian clam	Small		13.9 ± 0.4	11.9 ± 0.3	0.16 ± 0.01
	Medium		18.3 ± 0.3	17.3 ± 0.3	0.36 ± 0.02
	Large		27 ± 0.5	23.5 ± 0.4	0.90 ± 0.03

Table 2 (a) One-way MANOVA results testing for differences among blue crab sizes (S= small, M= medium, and L= large) used during food preference experiments. Weights of individuals were double square root transformed and total with and length log transformed to meet MANOVA assumptions. (b-e) One-way MANOVA testing for differences among prey sizes of each species. Mussels, oysters, apple snails, and Asian clam variables were arcsinh, square root or double square root transformed to meet MANOVA assumptions. Significant results at $p<$ 0.05 are indicated in **bold.**

MANOVA			
(a) Blue crab	Wilk's λ	$F_{8, 708}$	p
Size= Si	0.0536	293.51	**0.000**
SNK (Width without spines)		L> M> S	
SNK (Total width)		L> M> S	
SNK (Length)		L> M> S	
SNK (Weight)		L> M> S	
(b) Mussel	Wilk's λ	$F_{6, 50}$	p
Size= Si	0.0852	31.52	**0.000**
SNK (Width)		L> M> S	
SNK (Length)		L> M> S	
SNK (Weight)		L> M> S	
(c) Oyster	Wilk's λ	$F_{6, 50}$	p
Size= Si	0.0343	36.64	**0.000**
SNK (Width)		L> M= S	
SNK (Length)		L> M> S	
SNK (Weight)		L> M> S	
(d) Apple snail	Wilk's λ	$F_{6, 50}$	p
Size= Si	0.0243	45.07	**0.000**
SNK (Operculum width)		L> M> S	
SNK (Height)		L> M> S	
SNK (Weight)		L> M> S	
(e) Asian clam	Wilk's λ	$F_{6, 50}$	p
Size= Si		55.92	**0.000**
SNK (Width)		L> M> S	
SNK (Length)		L> M> S	
SNK (Weight)		L> M> S	

Table 3 Friedman's ANOVA X^2 and Kendall's coefficient of concordance (*W*) for ranked consumption rates on marine prey sizes including: a) mussels and b) oysters by each crab size at increasing times from 1 to 24 h after the experiment started (N=15, df= 2). For oysters, only the 24h rates are indicated due to low consumption. Significant differences at *p*< 0.05 are indicated in **bold**. In Wilcoxon matched pairs (WMP) post hoc comparisons indicate significant size pairs (L: Large, M: Medium, and S: Small) at Bonferroni's adjusted *p*< 0.167 for three item comparisons.

Prey species	Crab size	Time	Friedman's χ^2	Kendall's W	*p*	WMP
(a) Mussel	Large	1h	2.24.	0.074	0.3262	
		2h	0.216	0.007	0.8975	
		3h	2.714	0.090	0.2574	
		4h	1.550	0.051	0.4607	
		5h	2.097	0.069	0.3503	
		24h	1.219	0.040	0.5438	
	Medium	1h	2.388	0.079	0.3028	
		2h	5.142	0.171	0.0764	
		3h	12.130	0.404	**0.0023**	M= S>L
		4h	13.377	0.445	**0.0012**	M= S>L
		5h	16.840	0.561	**0.0002**	M> S>L
		24h	19.478	0.649	**0.0000**	M= S>L
	Small	1h	3.500	0.116	0.1737	
		2h	4.727	0.157	0.0940	
		3h	6.972	0.232	**0.0306**	S= M≥L
		4h	9.348	0.311	**0.0093**	S= M≥L
		5h	7.777	0.259	**0.0204**	S= M>L
		24h	7.600	0.253	0.0523	
(b) Oyster	Large	24h	5.600	0.186	0.0681	
	Medium	24h	2.000	0.066	0.3678	
	Small	24h	No consumption			

Table 4 Friedman's ANOVA X^2 and Kendall's coefficient of concordance (*W*) for ranked consumption rates on freshwater prey sizes including: (a) apple snail and (b) Asian clam by each crab size at increasing times from 1 to 24 h after the experiment started (N=15, df= 2). Significant differences at $p < 0.05$ are indicated in **bold**. In Wilcoxon matched pairs (WMP) post hoc comparisons indicate significant size pairs (L: Large, M: Medium, and S: Small) at Bonferroni's adjusted $p < 0.167$ for three item comparisons.

Prey species	Crab size	Time	Friedman's χ^2	Kendall's W	*p*	WMP
(a) Apple snail	Large	1h	5.911	0.197	0.0520	
		2h	7.860	0.262	**0.0196**	
		3h	6.292	0.209	**0.0430**	
		4h	3.534	0.117	0.1707	
		5h	1.148	0.038	0.5630	
		24h	12.842	0.428	**0.0016**	L> M= S
	Medium	1h	14.085	0.469	**0.0008**	L> M= S
		2h	14.085	0.469	**0.0008**	L> M= S
		3h	12.541	0.418	**0.0018**	L> M= S
		4h	11.541	0.384	**0.0031**	L≥ M= S
		5h	11.291	0.376	**0.0035**	L≥ M= S
		24h	1.772	0.059	0.4121	
	Small	1h	0.974	0.032	0.6143	
		2h	0.047	0.001	0.9764	
		3h	0.136	0.004	0.9340	
		4h	0.291	0.009	0.8643	
		5h	0.382	0.012	0.8257	
		24h	4.638	0.154	0.0983	
(b) Asian clam	Large	1h	3.161	0.105	0.2058	
		2h	1.473	0.049	0.4786	
		3h	2.81	0.093	0.2452	
		4h	0.838	0.027	0.6574	
		5h	1.181	0.039	0.5538	
		24h	3.000	0.100	0.2231	
	Medium	1h	2.000	0.066	0.3678	
		2h	0.823	0.027	0.6624	
		3h	0.857	0.028	0.6514	
		4h	4.521	0.15	0.1042	
		5h	4.000	0.133	0.1353	
		24h	0.560	0.018	0.7557	
	Small	1h	6.500	0.216	**0.0378**	
		2h	7.750	0.258	**0.0207**	
		3h	6.642	0.221	**0.0361**	
		4h	7.785	0.259	**0.0239**	
		5h	8.272	0.275	**0.0159**	
		24h	11.272	0.375	**0.0035**	S≥ M= L

Table 5 Wilcoxon matched pairs (WMP) for consumption rates on: (a) marine prey species (mussel: M vs. oyster: O) with and without shell, and (b) freshwater prey species (apple snail: S vs. Asian clam: C) without shell at increasing times from 1 to 24 h after the experiment started (N=15). Significant differences between pairs of items at *p*< 0.05 are indicated in **bold.**

Prey species	Crab size	Time	z	p	WMP	z	p	WMP
				With shell			Without shell	
(a) Mussel vs. Oyster	Large	1h	1.603	0.1088		2.311	**0.0207**	M> O
		2h	2.520	**0.0117**	M> O	2.548	**0.0108**	M> O
		3h	2.803	**0.0050**	M> O	2.667	**0.0076**	M> O
		4h	2.803	**0.0050**	M> O	1.987	**0.046**	M> O
		5h	2.803	**0.0050**	M> O	1.782	0.0747	
		24h	3.179	**0.0014**	M> O	-	-	
	Medium	1h	1.825	0.0678		1.765	0.0775	
		2h	2.520	**0.0117**	M> O	2.52	**0.0117**	M> O
		3h	2.803	**0.0050**	M> O	1.579	0.1141	
		4h	2.803	**0.0050**	M> O	1.352	0.1762	
		5h	2.803	**0.0050**	M> O	0.507	0.612	
		24h	3.295	**0.0009**	M> O	0.000	1.000	
	Small	1h	1.341	0.1797		2.201	**0.0277**	M> O
		2h	1.341	0.1797		2.191	**0.0284**	M> O
		3h	1.341	0.1797		1.774	0.0759	
		4h	1.341	0.1797		2.022	**0.0431**	M> O
		5h	1.603	0.1088		1.467	0.1422	
		24h	2.366	**0.0179**	M> O	0.534	0.5929	
(b) Apple snail vs. Asian clam	Large	1h	2.431	**0.015**	S> C	0.404	0.6858	
		2h	2.934	**0.0033**	S> C	-	-	
		3h	2.934	**0.0033**	S> C	-	-	
		4h	2.934	**0.0033**	S> C	-	-	
		5h	2.803	**0.005**	S> C	-	-	
		24h	2.201	**0.0277**	S> C	-	-	
	Medium	1h	2.51	**0.012**	S> C	-	-	
		2h	2.711	**0.0066**	S> C	-	-	
		3h	2.905	**0.0037**	S> C	-	-	
		4h	2.711	**0.0066**	S> C	-	-	
		5h	3.0594	**0.0022**	S> C	-	-	
		24h	2.2013	**0.0277**	S> C	-	-	
	Small	1h	1.05	0.2936		0.000	1.000	
		2h	1.289	0.1973		0.000	1.000	
		3h	2.52	**0.0117**	S> C	0.534	0.5929	
		4h	2.52	**0.0117**	S> C	-	-	
		5h	2.366	**0.0179**	S> C	-	-	
		24h	2.201	**0.0277**	S> C	-	-	

Table 6 Two-way ANOVA results testing for differences in biomass consumption (gWW) among prey items and blue crab sizes (S= small, M= medium, and L= large). (a) Marine preys (mussels: M vs. oysters: O, all prey sizes included); (b) Freshwater prey (apple snail: S vs. Asian clam: C, all prey sizes included); (c) marine preys (both species included) with shell (S) vs. without shell (WS); and (d) freshwater preys (both species included) with vs. without shell. The homoscedasticity assumption could not be met by transformation and the level of significance was fixed at *p*= 0.01. Statistically significant results at *p*< 0.05 are indicated in **bold.**

ANOVA				
(a) Marine preys	df	MS	*F*	*p*
Prey= P	1	1109.315	158.124	**0.0000**
Size= Si	2	15.818	2.254	0.1113
P x Si	2	2.979	0.424	0.6553
Error	84	7.015		
SNK (P)	M> O			
(b) Freshwater preys	df	MS	*F*	*p*
Prey= P	1	51621.55	631.816	**0.0000**
Size= Si	2	672.52	8.231	**0.0005**
P x Si	2	558.75	6.838	**0.0017**
Error	84	81.70		
SNK (P)	S> C			
SNK (Si)	L> M= S			
(c) Marine preys (S vs. WS)	df	MS	*F*	*p*
Prey= P	1	1875.41	135.058	**0.0000**
Size= Si	2	203.07	14.624	**0.0000**
P x Si	2	49.70	3.580	0.0322
Error	84	13.89		
SNK (P)	WSh > Sh			
SNK (Si)	L= M> S			
(d) Freshwater preys (S vs. WS)	df	MS	*F*	*p*
Prey= P	1	5.964	12.973	**0.0005**
Size= Si	2	0.043	0.093	0.9109
P x Si	2	0.774	1.683	0.1920
Error	84	0.460		
SNK (P)	WSh > Sh			

Table 7 (a) Two-way ANOVA results for differences in the pressure needed to break the shells of the four prey species (M= Mussel, O= Oyster, S= apple snail, and C= Asian clam) at the three study sizes (S= small, M= medium, and L= large). (b) One-way PERMANOVA and pair-wise tests results for differences in the nutritional composition of prey species (i.e., percent proteins, carbohydrates, lipids, and ashes). Statistically significant results at *p*< 0.05 are indicated in **bold.**

(a) ANOVA				
Shell hardness	df	MS	*F*	*p*
Prey= P	3	0.934	366.51	**0.0000**
Size= Si (P)	8	0.132	52.03	**0.0000**
Error	24	0.002		
SNK (P)	O> C> M= S			
SNK (Si)	$O_L > O_M > O_S \geq C_L = C_M > S_L = M_L = S_M = M_M \geq CS > M_S > S_S$			
(b) PERMANOVA				
Nutritional composition	df	MS	Pseudo-*F*	*p* (MC)
Prey= P	3	869.16	23.61	**0.001**
Error	8	36.811		
Total	11			
Pair-wise groups			*t*	*p* (MC)
S-C			0.087	**0.003**
S-O			0.095	**0.035**
S-M			0.109	**0.009**
C-O			0.107	**0.002**
C-M			0.109	**0.009**
O-M			0.096	**0.001**