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Identifying potential predators of the apple snail in the most important invasion area of Europe

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Abstract The only wetland in Europe colonized successfully by the apple snail *Pomacea maculata* Perry, 1810, is the Ebro Delta (Spain). After 10 years, it has become widespread there, despite significant eradication attempts. In spite of its great negative ecological and economic impacts, its incorporation into food webs can result in an abundant potential resource for native and exotic predators. We identified the potential predators, including fishes, amphibians, crustaceans, birds, turtles and mammals in natural conditions using stable isotopic analyses (δ^{13}C and δ^{15}N). Six predator species, three native (two birds and one frog) and three invasive (one crustacean, one turtle and one mammal), were confirmed to consume apple snails. None of the 10 fish species analyzed consumed apple snails, although some have been previously observed consuming this snail under laboratory conditions. This study emphasizes the need to assess the consumption of apple snail, as well as other invasive organisms, by potential predators in natural conditions to confirm the establishment of new trophic relationships and to understand whether these potential predators actually act as biological control agents in nature.

Keywords Biological control · Ebro Delta · Invasive prey · *Pomacea maculata* · Predator–prey relationships · Stable isotopes

Introduction

Freshwater ecosystems are considered more prone to invasion than terrestrial habitats (Tricarico et al., 2016). Several species of the apple snail (genus *Pomacea*), a New World freshwater snail of the family Ampullariidae, have been introduced in aquatic habitats worldwide and are considered highly invasive (Cowie, 2002; Hayes et al., 2012; EFSA PLH Panel on Plant Health, 2013). Two species of this genus of apple snail (*Pomacea canaliculata* (Lamark, 1819) and *P. maculata* Perry, 1810) have

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been widely deliberately or accidentally introduced in the Southeast and East Asia, North America and South America (CABI, 2020a, b). In Europe, *P. maculata* was reported for the first time in the rice paddies and irrigation channels of the Ebro Delta (northeastern Spain) in 2009 (Fig. 1; López et al., 2010). This is the only European wetland successfully colonized by this invasive freshwater mollusk. The invasion presumably came from a fish farm, now closed, that reproduced them for aquariums and was connected to the irrigation channels of the Ebro Delta. Although both species are currently well identified, before 2012 they were not clearly differentiated conchologically and were included in the *Pomacea canaliculata* group (Hayes et al., 2009), until a later review clarified their identity and synonymy (Hayes et al., 2012). Thus, although most published information about their invasiveness, their negative effects in aquatic crops and freshwater ecosystems, and control actions relates to *P. canaliculata*, it is most likely also applicable to *P. maculata* (Martín et al., 2019; CABI, 2020a).

The large economic impact of the apple snail on agriculture is due to its consumption of vast amounts of rice seeds, seedlings and young plants (Cowie, 2002; Joshi & Sebastian, 2006), and the pesticide application costs associated with its control (Horgan et al., 2021). Losses in ecosystem services of the wetlands due to alterations in nutrient cycling and primary productivity (Carlsson et al., 2004a; Gilioli et al., 2017) have led to research efforts to eradicate this invasive species or at least minimize the damage (EFSA Panel on Plant Health, 2014). Methods used to control populations of apple snail include the use of chemical pesticides and/or plant extract molluscicides, changes in cultural management practices, hand eradication and biological control (Cowie, 2002; Horgan, 2017). In the Ebro Delta, the extensive winter flooding of rice paddies with seawater and the application of saponins during the cultivation period are the main tools to control the invasion, although its complete eradication is now unachievable. All of these control methods are aimed at reducing crop damage, especially in rice (*Oryza sativa*) cultivation,

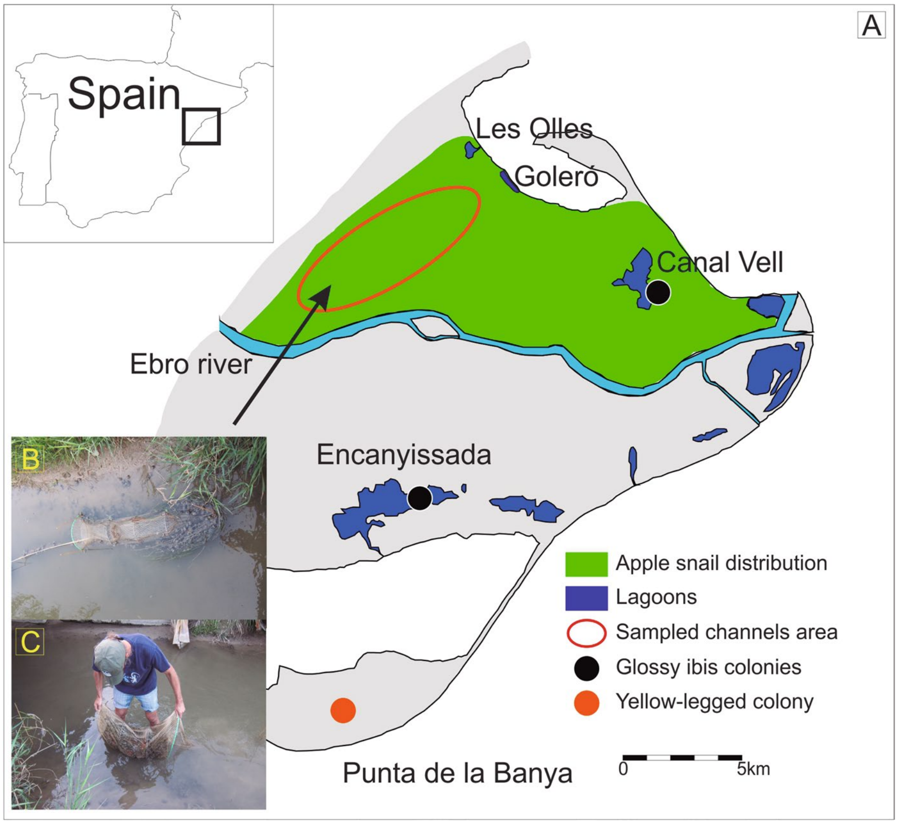


Fig. 1 **A** Distribution of the apple snail and location of the sampling areas of their potential predators in the Ebro Delta, **B** one of the funnel traps used in the irrigation channels for collecting fish, and **C** a massive capture of hundreds of apple snails in this type of fish trap

which is one of the crops most threatened by this invasive species (Horgan, 2017).

Regarding biological control, most of the information about potential predators of the apple snail outside their native distribution range has been studied in laboratory microcosms or in mesocosms conditions, including invertebrates (insects, gastropods, crustaceans and hirudinoids) and vertebrates (fish, reptiles, birds and mammals) (*e.g.* Carlsson et al., 2004b; Yusa, 2006; Yusa et al., 2006; Table S1). Few studies have focused on predation under natural conditions and only for specific predators in each case (*e.g.* Yusa et al., 2000; Cattau et al., 2016; Bertolero & Navarro, 2018; Table S1). Under natural conditions, various factors act on the selection that an organism makes of a potential food resource. Thus, factors ranging from availability and abundance to palatability and recognition as prey may intervene (Goeppner et al., 2020; Cadierno et al., 2017). For that reason, it is not known if all the species cited as potential predators of the apple snail actually use it as a trophic resource under natural conditions, or only consume them under laboratory or mesocosms conditions.

In addition to the recent confirmation of the presence of the apple snail in the diet of the glossy ibis (*Plegadis falcinellus* (Linnaeus, 1766)) breeding in the Ebro Delta (Bertolero & Navarro, 2018) and the predation by the invasive blue crab (*Callinectes sapidus* Rathbun, 1896) in experimental conditions (Prado et al., 2020), other potential predators, either native or invasive species, inhabiting this freshwater ecosystem could be predating on this snail. In fact, field observations of blue crabs preying on adult snails have been common (see video showing a predation attempt at https://www.youtube.com/watch?v=dSkf4NP60xs), as has the presence of vast amounts of snail shells near rat holes and burrows in the vicinity of irrigation channels. For this reason, and as has already occurred with the invasive red swamp crayfish (*Procambarus clarkii* (Girard, 1852)) in similar habitats (Tablado et al., 2010), it is possible that the apple snail has become part of the prey consumed by many species present in this community.

The main objective in the present study was to identify the potential predators of apple snail, including native and invasive species, inhabiting the Ebro Delta (northeastern Spain), the only ecosystem invaded by this species in Europe. For this purpose, the analyses of stable isotopic values of carbon (denoted as δ^{13}C) and nitrogen (denoted as δ^{15}N) in muscle or blood of 18 potential predators, including fish, amphibians, crustaceans, birds, freshwater turtles and mammals, were combined with those of the apple snail. Based on this isotopic approach, it is possible to qualitatively assign the presence of the apple snail as part of the assimilated diet of the different individuals sampled for each of these species. Even though the eradication of apple snail does not seem achievable through biological control, understanding whether in natural conditions it has been successfully incorporated into the food web can help improve management actions considering the control exerted by predators.

Material and methods

Study area and species

The Ebro Delta is considered one of the most important wetlands in the western Mediterranean Sea, and has been declared a Regional Natural Park, Biosphere Reserve and a Ramsar site (a wetland of international importance, designated under the Ramsar Convention; http://www.ramsar.org). The current landscape of the Ebro Delta is dominated by extensive agricultural rice fields (about 65% of the surface area, *ca.* 21,000 ha) and the rest occupied by natural habitats (both freshwater and brackish lagoons, marshes, and dunes). The Ebro Delta is host to a great diversity of birds (343 species) and fishes (101 species, of which 32 species live in freshwater, either permanently or temporarily, with 14 invasive species; López et al., 2012), but the herpetofauna and mammal communities have been greatly depleted (Gosàlbez, 1977; Roig, 2008).

Apple snails and their potential predators (see species and number of individuals collected in Table 1) were captured along rice paddies, irrigation channels, the Ebro River, lagoons (Canal Vell, Les Olles, the green filter of Goleró—an artificial wetland—, and Encanyissada), and vegetated dunes (Punta de la Banya) of the Ebro Delta from 2015 to 2019 (Fig. 1). A total of 18 species of potential predators were sampled (belonging to six classes: 2 crustaceans [CRUS], 10 fishes [FISH], 2 amphibians [AMP], 1 reptile [REP], 2 birds [BIRD] and 1 mammal [MAM]), including native and invasive

Table 1 Sample size (*n*), and mean ± standard deviation of δ^{13}C and δ^{15}N values of the 18 potential predators of apple snail collected in the Ebro Delta. The type of species (native or invasive) is also indicated. The isotopic area overlap between the simulated predator (crustacean, fish, amphibian, reptile, bird and mammal) of apple snail and the species analyzed is also indicated (K95%, K75% and K50%: 95%, 75% and 50% of probability of isotopic area overlap, respectively)

Predators	Type	*n*	δ^{13}C (‰)	δ^{15}N (‰)	K95%	K75%	K50%
Crustaceans							
Red swamp crayfish (*Procambarus clarkii* (Girard, 1852))	Invasive	7	– 29.63 ± 1.39	9.32 ± 1.86	0.00	0.00	0.00
Blue crab (*Callinetes sapidus* Rathbun, 1896)	Invasive	24	– 25.02 ± 2.37	13.13 ± 1.38	5.30	3.70	2.70
Fishes							
Bleak (*Alburnus alburnus* (Linnaeus, 1758))	Invasive	6	– 28.70 ± 1.74	12.76 ± 1.32	0.00	0.00	0.00
Common carp (*Cyprinus carpio* (Linnaeus, 1758))	Invasive	21	– 26.51 ± 2.28	13.57 ± 2.43	0.00	0.00	0.00
Crucian carp (*Carassius auratus/gibelio* (Bloch, 1782))	Invasive	13	– 26.71 ± 2.64	13.90 ± 1.36	0.00	0.00	0.00
Pond loach (*Misgurnus anguillicaudatus* (Cantor, 1842))	Invasive	6	– 27.32 ± 0.70	12.83 ± 0.74	0.00	0.00	0.00
European eel (*Anguilla anguilla* (Linnaeus, 1758))	Native	16	– 26.71 ± 2.64	13.90 ± 1.36	0.00	0.00	0.00
Pumpkinseed (*Lepomis gibbosus* (Linnaeus, 1758))	Invasive	7	– 24.51 ± 0.69	16.85 ± 1.24	0.00	0.00	0.00
Flathead grey mullet (*Mugil cephalus* Linnaeus, 1758)	Native	5	– 33.60 ± 2.81	12.28 ± 1.28	0.00	0.00	0.00
Stone moroko (*Pseudorasbora parva* (Temminck & Schlegel, 1846)),	Invasive	5	– 26.27 ± 3.83	13.04 ± 2.02	0.10	0.00	0.00
Roach (*Rutilus rutilus* (Linnaeus, 1758))	Invasive	5	– 29.39 ± 2.37	11.68 ± 0.61	0.00	0.00	0.00
Wels catfish (*Silurus glanis* Linnaeus, 1758)	Invasive	10	– 26.61 ± 2.25	16.29 ± 2.07	0.00	0.00	0.00
Amphibians							
Bullfrog (*Lithobates catesbeianus* (Shaw, 1802)) (subadults)	Invasive	15	– 27.46 ± 1.01	10.29 ± 0.67	0.00	0.00	0.00
Bullfrog (*Lithobates catesbeianus* (Shaw, 1802)) (tadpoles)	Invasive	15	– 31.21 ± 0.38	14.66 ± 0.48	0.00	0.00	0.00
Perez's frog (*Pelophylax perezi* (López-Seoane, 1885))	Native	3	– 21.52 ± 2.30	10.29 ± 1.89	5.80	6.00	5.00
Reptiles							
Slider turtle (*Trachemys scripta elegans* (Wied, 1838))	Invasive	20	– 27.37 ± 3.20	14.32 ± 2.37	2.50	0.70	0.00
Birds							
Yellow-legged gull (*Larus michahellis* J.F.Naumann, 1840)	Native	28	– 21.67 ± 1.09	10.43 ± 1.04	10.9	8.20	1.60
Glossy ibis (*Plegadis falcinellus* (Linnaeus, 1766))	Native	23	– 24.75 ± 0.71	11.81 ± 0.60	6.60	1.60	0.00
Mammals							
Norwegian rat (*Rattus norvegicus* (Berkenhout, 1769))	Invasive	7	– 24.11 ± 1.43	11.62 ± 2.21	6.30	3.00	1.80

species (number of individuals for each species is indicated in Table 1). Some of the non-native species are catalogued as highly invasive and were captured during specific eradication efforts carried out by the Ebro Delta Natural Park staff.

Muscle tissue from apple snails, crustaceans, fish species, frogs, slider turtles and rats, was sampled and stored frozen at – 20 °C. In the case of the two bird species, 0.1 ml of blood with non-heparinized syringes was sampled from large chicks in their breeding colonies in the Ebro Delta area (Fig. 1). Blood samples were kept refrigerated in the field for 2–3 h and were then stored at – 20 °C.

Stable isotope analysis

All samples were freeze-dried and powdered, and 0.28–0.33 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Laboratory of Stable Isotopes of the Estación Biológica de Doñana CSIC (www.ebd.csic.es/lie/index.html). Samples were combusted at 1,020 °C using a continuous flow isotope ratio mass spectrometry system (Thermo Electron) by means of a Flash HT Plus elemental analyzer interfaced with a Delta V Advantage mass spectrometer, which applies international standards, run every 9 samples; LIE-CV and

LIE-PA, previously normalized with the international standards IAEA-CH-3, IAEACH-6, IAEA-N-1 and IAEA-N-2. Stable isotope ratios were expressed in the standard δ-notation (‰) relative to Vienna Pee Dee Belemnite (δ^{13}C) and atmospheric N_2 (δ^{15}N). Based on laboratory standards, the measurement error (standard deviation) was ± 0.1 and ± 0.2 for δ^{13}C and δ^{15}N, respectively. The C:N ratio of all tissues was always lower than 3.5 ‰, and hence, no correction of the δ^{13}C values was required to account for the presence of lipids in muscle samples (Logan et al., 2008).

Differences in δ^{13}C and δ^{15}N were examined among and within taxonomic predator groups using non-parametric Kruskal–Wallis sum rank and post-hoc Dunn tests (p-adjusted with the Benjamini–Hochberg method). When only two species were included in a taxonomic group, the non-parametric Mann–Whitney *U* test was used or, when the data met the conditions of normality, the parametric *t*-test (equal variance) or Welsh *t*-test (unequal variances) were used. The significance level was set at 0.05. Statistical analyses were conducted using R version 3.5.1 (R Core Team, 2020).

Identification of the potential predators of apple snail using stable isotopic values

Based on the stable isotope values, we estimated the qualitative importance of apple snail for each sampled potential predator. This approach consisted in evaluating the overlap between the isotopic niche of each sampled potential predator with the isotopic niche of a hypothetical crustacean, fish, amphibian, reptile, bird or mammal with stable isotope values corresponding to a diet composed only of apple snails (Fig. 2). Based on this approach (Fig. 2), the presence of some degree of overlap between the isotopic niche of a particular potential predator with the isotopic niche of the hypothetical predator could indicate that

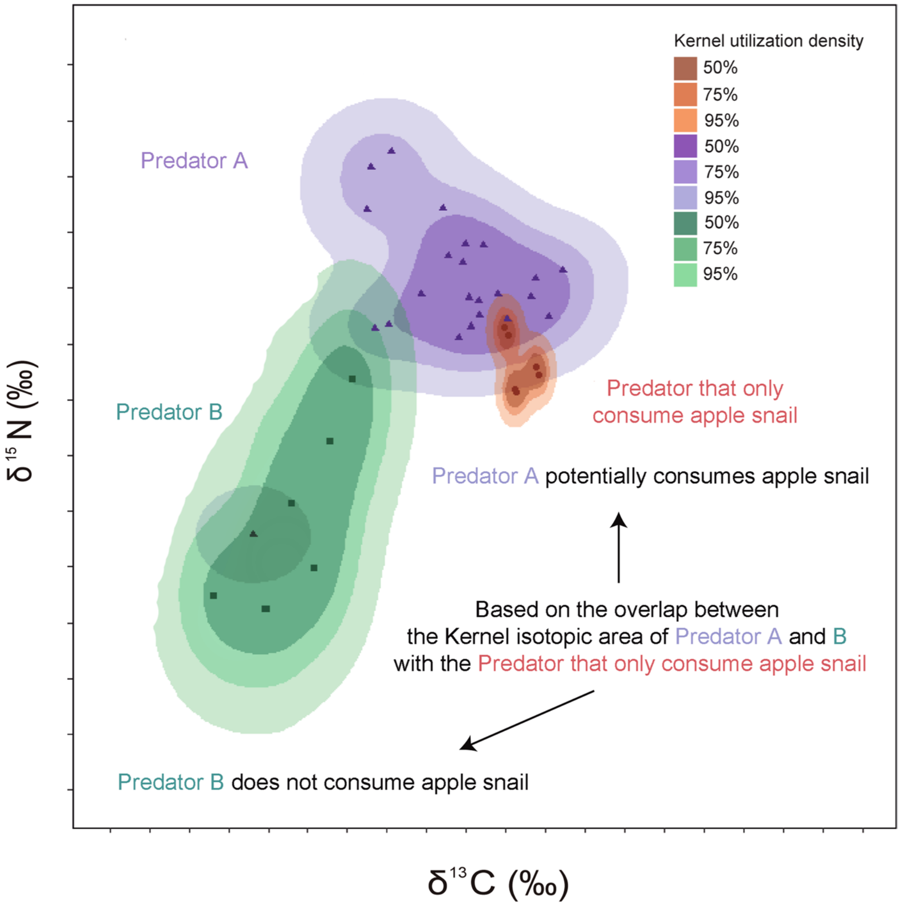


Fig. 2 A conceptual example of assignation of potential depredation of apple snail by two predators (Predator A and B) based on their isotopic niche overlaps with a potential predator that only consume apple snail. Each symbol (circle, triangle and square) corresponds to a single individual predator

this predator could be including apple snail in its diet (see a similar approach in Vigo et al., 2022).

As isotopic niche, we calculated three different kernel utilization density (KUD) estimators that contained 50%, 75% and 95% of the isotopic niche of each species. KUD is estimated across a regular network of equally spaced points, with the extent of the grid larger than that of the observations (Venables & Ripley, 2002, Eckrich et al., 2020). It is important that for some species (see Table 1) the number of sampled individuals was low to obtain accurate KUD estimators. The contour lines used are defined in relation to the Euclidean distance of each observation to the centroid in bivariate space (Robinson, 2021). For these predictions, different diet-to-tissue- discrimination factor values for δ^{15}N (Δ^{15}N) and for δ^{13}C (Δ^{13}C) based on published studies were used for each type of predator (for fishes: $\Delta^{15}\text{N} = 3.56‰$, $\Delta^{13}\text{C} = 1.01‰$; for crustaceans: $\Delta^{15}\text{N} = 3‰$, $\Delta^{13}\text{C} = 1‰$; for amphibians: $\Delta^{15}\text{N} = 3.66‰$, $\Delta^{13}\text{C} = 1.56‰$; for reptiles: $\Delta^{15}\text{N} = 3.4‰$, $\Delta^{13}\text{C} = 1.6‰$; for birds: $\Delta^{15}\text{N} = 2.4‰$, $\Delta^{13}\text{C} = 1.7‰$, for mammals: $\Delta^{15}\text{N} = 2.6‰$, $\Delta^{13}\text{C} = 1.16‰$; Seminoff et al., 2007; Caut et al., 2009; Costalago et al., 2012; Antonio & Richoux, 2014; San Sebastian et al., 2015; Bertolero & Navarro, 2018). The diet-to-tissue discrimination factor is the enrichment in the heavy isotope in predators' tissues relative to the prey consumed due to preferential assimilation of the heavy isotope and preferential excretion of the light isotope (DeNiro & Epstein, 1978, 1981). The qualitative importance of apple snail for each predator was estimated based on the percentage of overlap between the KUD of each species with the KUD of the potential predator that only consume apple snail (Fig. 2). To calculate the KUDs areas and overlaps we used the *rKIN* package (Eckrich et al., 2020) in R version 3.5.1 (R Core Team, 2020). We also checked whether apple snail was isotopically segregated from other potential prey of these predators were present in the study area. For this, we used published data on stable isotope values of sympatric freshwater snails ($n = 5$; Family Lymnaea, *Physa* Draparnaud, 1801, sp.), a freshwater clam ($n = 3$, *Corbicula* Mergele von Mühlfeld, 1811, sp.), water beetles ($n = 5$; *Cybister lateralimarginalis* (De Geer, 1774), *Hydrous piceus* (Linnaeus, 1758)), macrophytes ($n = 3$, *Ceratophyllum demersum* (Linnaeus, 1753)) and algae ($n = 3$) sampled in the study area (authors, unpublished data).

Results

Stable isotopic differences between species

There were significant differences among the six classes of potential predators of apple snail for both δ^{13}C (Fig. 3; Kruskal–Wallis $H = 92.70$, $P < 0.001$; 10 of the 15 pair-wise comparisons were significant: AMP < FISH, CRUS, MAM, BIRD; REP < CRUS, MAM, BIRD; FISH < MAM, BIRD; and CRUS < BIRD) and δ^{15}N (Fig. 3; Kruskal–Wallis $H = 67.94$, $P < 0.001$; 10 of the 15 pair-wise comparisons were significant: MAM, AMP, CRUS < FISH, REP; and BIRD < AMP, CRUS, FISH, REP). Within classes, most species showed significant differences for both δ^{13}C and δ^{15}N.

Among crustaceans, red swamp crayfish had lower values of both δ^{13}C and δ^{15}N than blue crab (Fig. 3; *t*-test δ^{13}C: $t = 4.86$, df = 29, $P < 0.001$; δ^{15}N: $t = 5.92$, df = 29, $P < 0.001$). Among the two species of birds analyzed, glossy ibis had lower δ^{13}C than yellow-legged gull (*Larus michahellis* J.F.Naumann, 1840) (Fig. 3; Welsh *t*-test $t = 12.13$, df = 46.69, $P < 0.001$), but higher δ^{15}N (Welsh $t = -5.93$, df = 44.97, $P < 0.001$). For amphibians, comparing among the stages of the American bullfrog (*Lithobates catesbeianus* (Shaw, 1802)), tadpoles had lower δ^{13}C and higher δ^{15}N than subadults (Fig. 3; Welsh *t*-test δ^{13}C: $t = -13.51$, df = 18.01, $P < 0.001$; *t*-test δ^{15}N: $t = 20.48$, df = 28, $P < 0.001$). On the other hand, among amphibians (only adults or subadults included), bullfrogs had lower δ^{13}C than Perez's frogs (*Pelophylax perezi* (López-Seoane, 1885)) (Mann–Whitney $U = 45$, $P = 0.002$) but similar δ^{15}N ($U = 22.5$, $P = 1$; Fig. 3). Within fishes, δ^{13}C and δ^{15}N values showed significant differences among species (Fig. 3; Kruskal–Wallis δ^{13}C: $H = 34.29$, $P < 0.001$; δ^{15}N: $H = 38.01$, $P < 0.001$; Fig. 3). However, only 9 of the 45 pair-wise comparisons were significant for δ^{13}C and 15 for δ^{15}N ($P < 0.05$; Fig. 3).

Of the 18 potential predators of the apple snail, two fish species [pumpkinseed (*Lepomis gibbosus* (Linnaeus, 1758)) and Wels catfish (*Silurus glanis* Linnaeus, 1758)] occupied the higher isotopic trophic positions, followed by the tadpoles of the American bullfrog and the slider turtle (*Trachemys scripta elegans* (Wied, 1838)) (Table 1; Fig. 3). On the other hand, the lower position of the food web was occupied by red swamp crayfish, followed by the Perez's

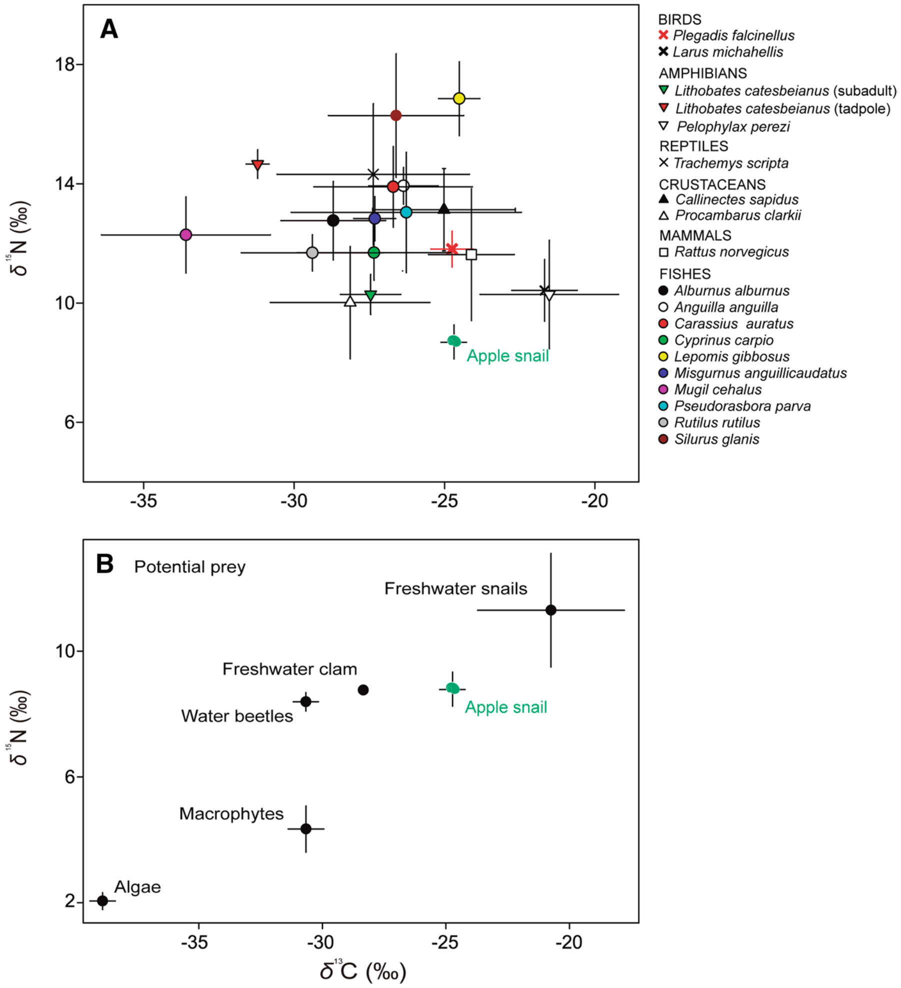
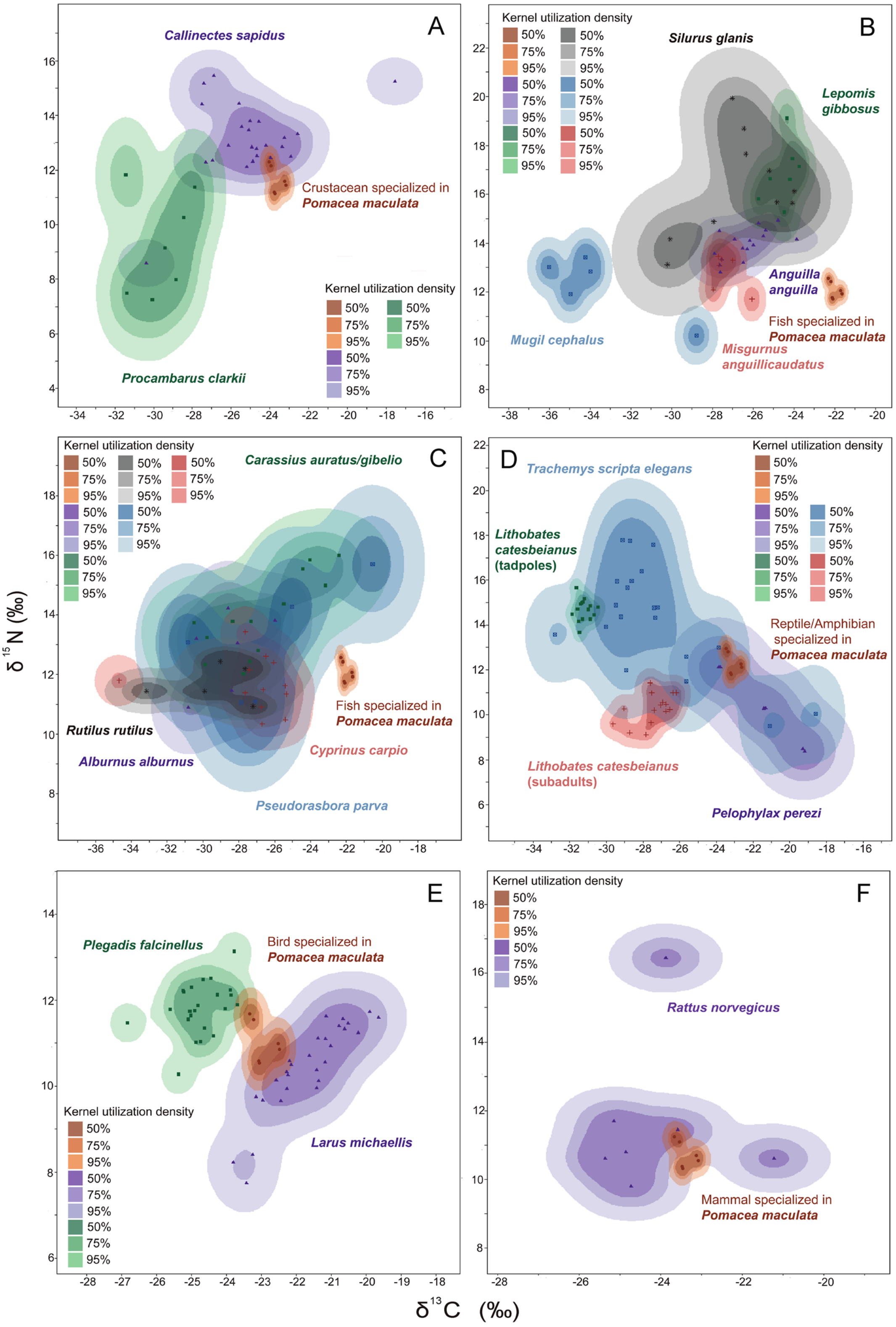


Fig. 3 Mean and standard deviation of δ^{13}C and δ^{15}N values of **A** the apple snail and their potential predators and **B** other potential prey of the predators inhabiting the Ebro Delta

frog, the subadults of the American bullfrog and the yellow-legged gull chicks. The rest of the species occupied intermediate levels in the food web (Table 1; Fig. 3). In addition, we found that the apple snail differed isotopically from other potential prey species present in the food web (Fig. 3).

Expected predators of apple snail based on stable isotopic values

From the expected isotopic values that apple snail predators would have and considering the isotopic fractionation in the predator–prey relationship for each predator group, stable isotopic results showed that the apple snail could be a substantial trophic resource for at least six species (Fig. 4). The glossy ibis and the yellow-legged gull were the main potential predators of apple snail, followed by the blue crab, the Norwegian rat, the Perez's frog and the slider turtle (Fig. 4). The results from isotopic niche overlaps also indicated that none of the fish species included in the study showed stable isotopic values matching the isotopic range for a fish preying exclusively on apple snails (Fig. 4, see Table 1). The main predators that showed higher values of KUDs (KUD95, KUD75, KUD50) overlap with the hypothetical predator of apple snail were the yellow-legged gull (KUD95 = 10.90%, KUD75 = 8.20%, KUD50 = 1.60%) and the glossy ibis (KUD95 = 6.60%, KUD75 = 1.60%), followed by the blue crab (KUD95 = 5.30%, KUD75 = 3.70%, KUD50 = 2.70%), the Norwegian rat (*Rattus norvegicus* (Berkenhout, 1769)) (KUD95 = 6.30%, KUD75 = 3.00%, KUD50 = 1.80%), and the


A
Callinectes sapidus
Crustacean specialized in
Pomacea maculata
Procambarus clarkii
Kernel utilization density
50%
75%
95%
50%
75%
95%
50%
75%
95%
B
Kernel utilization density
Silurus glanis
Lepomis gibbosus
Anguilla anguilla
Fish specialized in
Pomacea maculata
Mugil cephalus
Misgurnus anguillicaudatus
C
Kernel utilization density
Carassius auratus/gibelio
Fish specialized in
Pomacea maculata
Rutilus rutilus
Alburnus alburnus
Cyprinus carpio
Pseudorasbora parva
D
Kernel utilization density
Trachemys scripta elegans
Lithobates catesbeianus (tadpoles)
Reptile/Amphibian specialized in
Pomacea maculata
Lithobates catesbeianus (subadults)
Pelophylax perezi
E
Plegadis falcinellus
Bird specialized in
Pomacea maculata
Larus michaellis
Kernel utilization density
F
Kernel utilization density
Rattus norvegicus
Mammal specialized in
Pomacea maculata
δ15N (‰)
δ13C (‰)

◂**Fig. 4** Isotopic niche and overlap of potential crustacean (**A**), fish (**B**, **C**), amphibian-reptilian (**D**), bird (**E**), and mammal (**F**) predator specialized in apple snail and 18 predator species inhabiting the Ebro Delta. Niche size and overlap estimates were generated for 50%, 75% and 95% contour levels kernel utilization density. The potential predator specialized in apple snail in each panel represents the isotopic niche of expected stable isotopic values of apple snail individuals corrected by specific-group isotopic fractionation factors (see Material and Methods section)

amphibian Perez's frog (KUD95 = 5.80%, KUD75 = 6.00%, KUD50 = 5.00%) (see Table 1). The invasive slider turtle showed a wide isotopic niche (Fig. 4) but a low overlap percentage with the hypothetical apple snail predator (KUD95 = 2.50%, KUD75 = 0.70%, KUD75 = 0%).

Discussion

Once a prey enters a novel habitat, there is potential for a new functional predator–prey relationship to emerge (Thomsen et al., 2014). Therefore, novel prey in the ecosystem need to be identified as a potential resource for predators, as the predators could exert some level of regulation of the invasion. This could be the case of the invasive apple snail in the rice paddies and freshwater wetlands of the Ebro Delta. The apple snail has become an abundant aquatic resource for some opportunistic consumers, such as the glossy ibis (Bertolero & Navarro, 2018). However, ten years since the introduction of the apple snail and its spread across the Ebro Delta, 12 out of 18 of the aquatic predators analyzed in this study have not yet apparently incorporated it into their diets, although some have demonstrated their capacity for predation on apple snails in laboratory and mesocosm conditions (e.g. common carp, the Crucian carp and the red swamp crayfish; Yusa et al., 2006). This discrepancy may be due to the fact that in controlled conditions apple snails are over-exposed to potential predators for several days in very simple environments (e.g. Yeager et al., 2016) and potential predators are not given the option to select preys (apple snails are the only available food). In fact, predatory activity on apple snails under these controlled conditions should only be considered to demonstrate potential for predation and should be confirmed in natural conditions (Yusa et al., 2006).

Although stable isotopes alone cannot completely resolve the consumption of apple snail by its predators due to the limitations of the approach used (the simulation of potential predators based on a predators specialised in apple snail), in combination with published studies and observational events, this work presents the first multi-species study investigating the possible incorporation of apple snail into the diet of several potential predators, including crustaceans, birds, amphibians, fish, one mammal and one freshwater turtle inhabiting the invaded area. Moreover, since the stable isotopic niche of apple snail differed from other sympatric prey, we can assume that a hypothetical predator was reflecting only the stable isotope values of a predator that consumes apple snail exclusively. Based on our results, we found that up to six of these potential predators showed isotopic values that suggest the inclusion of apple snail in their diet. Three of them could be considered as occasional consumers (Perez's frog, slider turtle and Norwegian rat). Feeding studies of Perez's frog have shown that it has a generalist diet, eating gastropods in low proportion (Hodar et al., 1990), similar to other frogs of this genus (Ortega et al., 2016). Thus, due to the great abundance of apple snail in the Ebro Delta, it is plausible that the Perez's frog uses them as food. In the case of Norwegian rats, their capacity to feed on apple snails was previously reported, both in natural and experimental conditions (Yusa et al., 2000, 2006). On the other hand, the slider turtle has been cited as a predator of the apple snail under controlled conditions (Yusa et al., 2006), as well as in a marsh environment in Hawaii (Works & Olson, 2018). In agreement with these studies, the results of this work show that few slider turtles use apple snails as food, which is consistent with their natural history with adults shifting to a more herbivorous diet (Parmenter & Avery, 1990).

In most cases, when birds are exposed to new resources they show dietary conservatism, with the new food undergoing an acceptance process (Marples & Kelly, 1999). In Europe there are no other species of aquatic snails similar to those of the genus *Pomacea* and to date the consumption of gastropods by the two species of birds studied in this study has only been recorded in the Ebro Delta (Bertolero & Navarro, 2018; author's pers. obs.). The two species included in this work have incorporated the apple snail in their diet using different foraging techniques.

Thus, while the glossy ibis swallows whole apple snails and regurgitates the indigestible parts, the yellow-legged gull opens the opercula and gains access to the soft parts (Bertolero & Navarro, 2018). In general, gulls have demonstrated a great learning capacity to eat new food, including invasive mollusks (Cadée, 2001) and decapods (Navarro et al., 2010; Yorio et al., 2020), which can be used as a trophic subsidy. In addition to these two birds, other bird species may also incorporate the apple snail in their diet. For example, in 2018 one empty adult apple snail shell was found in the breeding colony of grey heron (*Ardea cinerea* Linnaeus, 1758) and little egret (*Egretta garzetta* (Linnaeus, 1766)) located in the Ebro Delta, outside the present distribution of the apple snail and where aquatic transport was impossible (Bertolero obs. pers.). For this reason, more studies are needed to confirm the use of apple snails by other aquatic birds.

According to the rice farmers of the Ebro Delta, the recent introduction of the blue crab (Castejón & Guerao, 2013) and its significant spread since 2017 (López & Rodon, 2018) have reduced the number of apple snails (http://neurice.eu/the-blue-crab-an-ally-in-front-of-the-apple-snail-plague/). However, no study has yet quantified the control that the blue crab may exert on the apple snail population. Until now, the only information available is that in laboratory conditions blue crabs consume apple snail adults (Prado et al., 2020). In our case, our results confirm that blue crabs seem to consume apple snails widely under natural conditions. Although the blue crab can predate efficiently on the apple snail, it is also a new invader at the Ebro Delta that may negatively affect both the native fauna (Pla-Ventura et al., 2018; Bertolero, 2021) and the artisanal fisheries (Mancinelli et al., 2017). Thus, the presence of an invasive predator species (blue crab) should not be encouraged as a method to counteract the effects of an invasive herbivore species (apple snail).

Regarding the fish fauna, the 10 species analyzed encompass a wide spectrum of the fish biodiversity present in the Ebro Delta (López et al., 2012). Two abundant species in estuarine environments were also included, namely the European eel (*Anguilla anguilla* (Linnaeus, 1758)) and the Flathead grey mullet (*Mugil cephalus* Linnaeus, 1758). Likewise, most of the species analyzed include benthic fauna in their diets, and specifically gastropods for at least 8 species, based on diet information from Fishbase.org (Palomares & Pauly, 2020). However, the results obtained in the current study indicate that none of these fish species include the apple snail in their diet in the Ebro Delta. This was true even for generalist and highly voracious predators such the Wels catfish or the eel, which include mollusks in their diet (Coop et al., 2009) and the common carp, which consume apple snails in experimental conditions and has been proposed as a useful species for biological control of the apple snails in rice paddies (Wada, 2006; Yusa, 2006; Yusa et al., 2006 and references in Table S1). Thus, based on our results, we suggest that fish species present in the Ebro Delta freshwater ecosystems are unlikely to exert any direct control over the adult apple snail population. Taken together, the results strongly suggest an inherently low predictability of predator–prey relationships for each novel invasion, taking into account solely the species composition and previous knowledge of the diet items of the potential predators.

Finally, the addition of two invasive species in the wetlands and rice paddies of the Ebro Delta, the apple snail (herbivore) at the basal level (this study), and the blue crab (predator) at the upper level (Prado et al., 2020), is expected to cause changes in the food webs. These two non-native species are highly invasive and now widespread in several areas worldwide, making their eradication impossible with current methods. In the case of the blue crab, no attempt has been made to eradicate it, since the government has promoted its fishing as a new commercial species (to allow for this activity, this species is not included in the official list of invasive species of the Spanish government [https://www.miteco.gob.es/es/biodiversidad/temas/conservacion-de-especies/especies-exoticas-invasoras/ce-eei-catalogo.aspx]). Thus, of the several aquatic non-native species introduced in the Ebro Delta in the last century, these latest newcomers, the apple snail and the blue crab, may be restructuring the food webs in a similar way to that of the invasive red swamp crayfish, which has become an important prey for several native species (Tablado et al., 2010). However, it is also possible that unexpected interactions and negative impacts may emerge in the aquatic ecosystem after their introduction (Cucherousset & Olden, 2011) or if one of them is used as a biological control agent (Pearson et al., 2000).

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Author contributions AB and JN conceived the idea and designed methodology; AB, MAL and SR collected the data; JN conducted the isotopic analysis; JN and AB analyzed the data; JN and MV analyzed the isotopic niche overlaps among predators; AB and JN led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability The isotopic values generated from this study are available on request from the corresponding author.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All the scientific work was authorized by the Ebro Delta Natural Park (permits SF/789 and SF/790).

Consent to participate All authors have agreed to participate in this study.

Consent for publication All authors have agreed to publication of this manuscript.

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