

## RESEARCH ARTICLE

# A native bird as a predator for the invasive apple snail, a novel rice field invader in Europe

Albert Bertolero<sup>1</sup>  | Joan Navarro<sup>2</sup> 

<sup>1</sup>Associació Ornitològica Picampall de les Terres de l'Ebre, Amposta, Spain

<sup>2</sup>Institut de Ciències del Mar CSIC, Barcelona, Spain

## Correspondence

Albert Bertolero, c/ La Galera, Associació Ornitològica Picampall de les Terres de l'Ebre, Amposta, 53, 43870 Amposta, Spain.  
Email: albert.bertolero@gmail.com

## Abstract

1. Since 2009 the apple snail *Pomacea maculata* has become a new invader of *Oryza sativa* (rice) fields and wetlands in Europe, only invading the Ebro Delta (north-eastern Spain) thus far. It is considered a highly invasive and damaging species, resulting in large economic losses worldwide.
2. Despite the severe impact of the invasive apple snail on both cultivated and natural wetlands, it has become an abundant potential resource for native avian predators.
3. In this study, stable isotope analysis was used to assess the consumption of the apple snail by the glossy ibis *Plegadis falcinellus* in the Ebro Delta.
4. The results indicate the importance of the apple snail in the diet of this native bird. In particular, isotopic results indicated that apple snails and freshwater coleopterans were the main prey in the diet of glossy ibis chicks, accounting for 26–40% of their diet. Thus, this native bird species could potentially help in the biological control of this invasive snail, but is not expected to eradicate it.
5. Although the spread of this pest in rice fields and wetlands is not desirable, we predict that the apple snail will follow a path similar to other invasive species, such as the red swamp crayfish *Procambarus clarkii*, in establishing itself as part of the wetland food web.

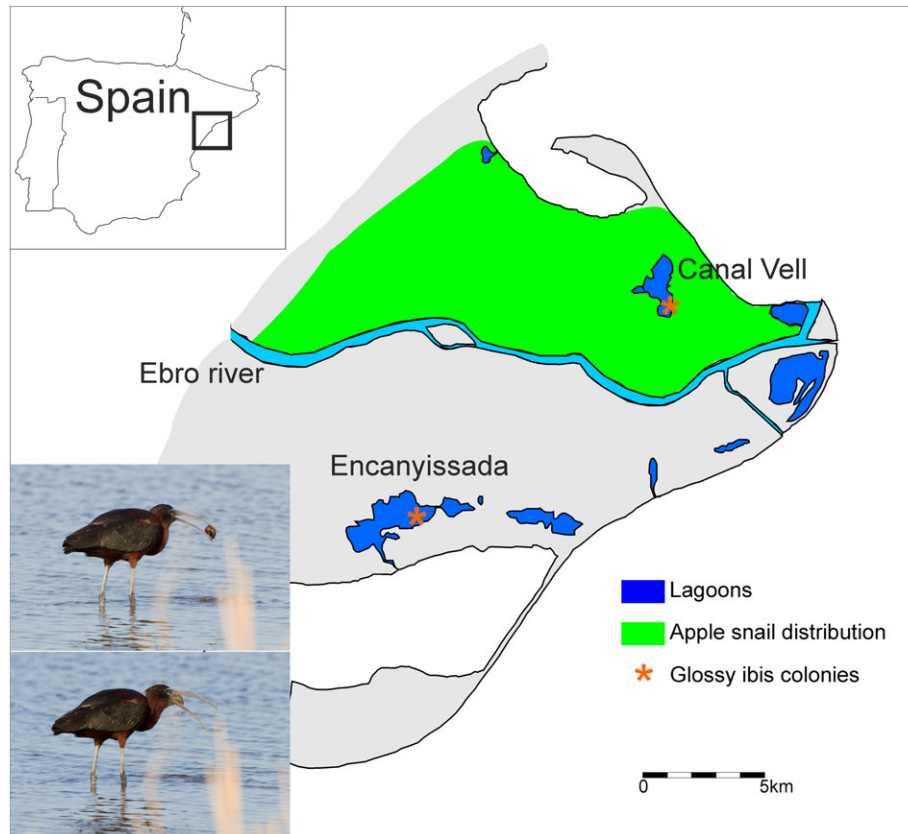
## KEYWORDS

alien species, biological control, Ebro Delta, *Plegadis falcinellus*, *Pomacea maculata*, wetland

## 1 | INTRODUCTION

Aquatic habitats, especially freshwater habitats, are considered more prone to invasion than terrestrial habitats (Tricarico, Junqueira, & Dudgeon, 2016). The invasive apple snail *Pomacea maculata* was detected for the first time in Europe in the *Oryza sativa* (rice) fields and channels of the Ebro Delta (north-eastern Spain) in 2009 (López, Altaba, Andree, & López, 2010). It was described as belonging to the species complex *Pomacea canaliculata* group, and was first reported as *Pomacea insularum* (López et al., 2010). However, a recent revision of this group clarified that the latter is a synonym of *P. maculata*, which is the valid species (Hayes, Cowie, Thiengo, & Strong, 2012). *Pomacea maculata* and *P. canaliculata* are considered highly invasive around the

world (European Food Safety Authority Panel on Plant Health (EFSA PLH), 2013; Hayes et al., 2012), with a large economic impact on agriculture and on natural wetlands (Carlsson, Brönmark, & Hansson, 2004; Cowie, 2002; Joshi & Sebastian, 2006). Adverse impacts in tropical natural wetlands include the reduction or elimination of macrophytes, changes in nutrient flux, and an increase in phytoplankton, which together disrupt the functioning of wetlands, with a shift from clear to turbid waters (Carlsson et al., 2004). Until now, all efforts to eradicate *P. maculata* and *P. canaliculata* have failed, and only some level of control has been achieved in reducing its impact on agriculture or reducing its spread (EFSA PLH, 2013; Horgan, 2017). The apple snail has caused significant damage to rice fields in the wetlands of the northern Ebro Delta (Figure 1). Various actions to eradicate or



**FIGURE 1** Map of the current distribution of the apple snail in the Ebro Delta and the glossy ibis colonies sampled in this study. Insert shows the first observation of a glossy ibis eating an adult apple snail in the Ebro Delta (courtesy of Xavier Curto)

control it have been undertaken, at a cost of €5.3 million in the period 2010–12, and with a budget allocation of €5 million from the Government of Catalonia (Spain) for the period 2014–20 for continuing the work on eradication and control (Generalitat de Catalunya, 2013, 2017).

Several native organisms, including invertebrates (insects, gastropods, crustaceans, and hirudinoids) and vertebrates (fish, reptiles, birds, and mammals), have been reported as predators of eggs, juveniles, or adults of the apple snail species in their areas of introduction (Burlakova et al., 2010; Centre for Agriculture and

Bioscience International (CABI), 2017; Yamanishi, Yoshida, Fujimori, & Yusa, 2012; Yusa, 2006; Yusa, Sugiura, & Wada, 2006a). Despite this great diversity of predators, they include only seven species of birds (Chanyapate, 1997; Horgan et al., 2014; Liang et al., 2013; Teo, 2001; Yusa, 2006; Table 1), and most attention has been paid to domestic ducks used for biological control in rice fields (Liang et al., 2013; Teo, 2001). So far, only one study has estimated the gross daily energetic gain in the endangered population of snail kite *Rostrhamus sociabilis* in Florida feeding on exotic apple snails, compared with feeding on native apple snails (Cattau et al., 2010). Recently, these authors

**TABLE 1** Bird species reported as predators of the apple snail *Pomacea maculata* in their introduced areas

Species	Conditions	References
Mallard, William Siam, Taiwan, Peking, Cherry Valley and Khaki Campbell ( <i>Anas platyrhynchos</i> breeds)	Domestic breed; experimental conditions	Teo, 2001; Yusa, Sugiura, & Wada, 2006a; Yusa, Wada, & Takahashi, 2006b
Shelduck <i>Tadorna tadorna</i>	Domestic breed; experimental conditions	Liang et al., 2013
Muscovy <i>Cairina moschata</i>	Domestic breed; experimental conditions	Teo, 2001
Large-billed crow <i>Corvus macrorhynchos</i>	Wild	Wada, 2006; Yusa, 2006
Asian openbill <i>Anastomus oscitans</i>	Wild	Chanyapate, 1997 (in Yusa, 2006); Sawangproh & Poonswad, 2010
Greater coucal <i>Centropus sinensis</i>	Wild	Chanyapate, 1997 (in Yusa, 2006)
Snail kite <i>Rostrhamus sociabilis</i>	Wild	Cattau, Martin, & Kitchens, 2010; Cattau, Fletcher, Reichert, & Kitchens, 2016; Horgan et al., 2014
Glossy ibis <i>Plegadis falcinellus</i>	Wild	This study

have also shown that the presence of the invasive apple snail improved the demographic parameters of this raptor (Cattau et al., 2016).

The Ebro Delta is considered the second most important Spanish wetland, and was declared a Ramsar site (a wetland of international importance, designated under the Ramsar Convention; <http://www.ramsar.org>) in 1993 for its ecological importance in the Mediterranean area. In addition to extensive agricultural rice fields, this wetland supports a great diversity of birds (more than 350 species), with large breeding colonies of wading birds, among others. The first evidence that birds prey on apple snails in the Ebro Delta appeared in 2015 when a glossy ibis *Plegadis falcinellus* was photographed eating, and swallowing whole, one adult apple snail (Xavier Curto, pers. comm.; Figure 1). Although the diet of the glossy ibis is mainly composed of aquatic coleopterans, odonatan larvae, and crabs during the breeding season (Acosta, Mugica, Mancina, & Ruiz, 1996; Macías, Green, & Sánchez, 2004), in some areas the glossy ibis apparently also includes the invasive red swamp crayfish *Procambarus clarkii* in its diet (Tablado, Tella, Sánchez-Zapata, & Hiraldo, 2010; Toral, Stillman, Santoro, & Figuerola, 2012). Based on these observations, the aim of the present study was to assess the trophic habits of the glossy ibis, and to evaluate and quantify the importance of the apple snail in its diet. For this purpose, stable isotopic values of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in the blood of glossy ibis chicks were analysed during two consecutive years, and Bayesian isotopic mixing models were applied to estimate the potential contribution of each potential prey item in the diet of the glossy ibis.

## 2 | METHODS

### 2.1 | Fieldwork

Glossy ibis is a long-lived medium-sized species (485–580 g) with a widespread distribution, but with scattered breeding populations in southern Europe, Africa, central and southern Asia, Oceania, the Atlantic coast of North America, and the West Indies (del Hoyo, Elliott, & Sargatal, 1992). Their European populations showed a marked regression in the 20<sup>th</sup> century (Burfield & van Bommell, 2004). In particular, the breeding population in Spain was extinct by the early 20<sup>th</sup> century, but in the 1990s new small breeding populations recolonized, and the species was classed as 'Vulnerable' on the Spanish Red List (Madróño, González, & Atienza, 2004). Recently, the Spanish

population in Doñana National Park has increased rapidly, and represents the largest colony in western Europe (Santoro, Green, & Figuerola, 2016). The Ebro Delta Natural Park sustains a small but increasing breeding population (the last census showed ~259 pairs in 2014; Delta de l'Ebre Natural Park, unpublished data). Glossy ibis chicks are fed with regurgitated food carried by adults, and during the breeding season the diet of glossy ibis chicks is similar to that of the adults (Macías et al., 2004). Monitoring chicks by banding them allows biological samples to be taken without capturing the adults (no specific trapping method has been developed for adults of this species). Thus, blood samples of glossy ibis chicks were collected during 2015 from the colony in Canal Vell lagoon ( $n = 13$ ; located in the area invaded by the apple snail) and in 2016 in Encanyissada lagoon ( $n = 9$ ; located outside the invasion area; Figure 1). Sampling chicks from both lagoons allows an assessment of whether glossy ibis breeding outside the area invaded by apple snail can use this trophic resource.

From each chick, 0.1 mL of blood was taken from the tarsus vein with non-heparinized syringes and the samples were kept refrigerated in the field for 2–3 h, and then stored at  $-20^{\circ}\text{C}$ . The potential prey items for glossy ibis were collected during 2015 and 2016, including the apple snail, in rice fields and channels surrounding the Canal Vell lagoon. The potential prey items were selected according to results reported by Acosta et al. (1996) and Macías et al. (2004) (Table 2). Muscle tissue was taken from apple snail, fishes, and frogs, and then stored at  $-20^{\circ}\text{C}$ . Whole insects, red swamp crayfish, and small native gastropods were sampled and stored in the same way.

### 2.2 | Analyses of stable isotopes

Isotope analyses were performed at the Laboratory of Stable Isotopes at the Estación Biológica de Doñana (<http://www.ebd.csic.es/lie/index.html>). All samples of blood and prey were combusted at  $1020^{\circ}\text{C}$  using a continuous flow isotope-ratio mass spectrometry system, by means of a Flash HT Plus elemental analyser coupled to a Delta-V Advantage isotope-ratio mass spectrometer via a CONFLO IV interface (ThermoFisher Scientific, Bremen, Germany). The isotopic composition is reported in the conventional delta ( $\delta$ ) per mil notation (‰), relative to Vienna Pee Dee Belemnite ( $\delta^{13}\text{C}$ ) and atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N}$ ). Replicate assays of standards routinely inserted within the sampling sequence indicated analytical measurement errors of  $\pm 0.1\%$  and  $\pm 0.2\%$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. The standards used were EBD-23 (cow

**TABLE 2** Sample size ( $n$ ) and mean  $\pm$  standard deviation of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the glossy ibis and its potential prey species in the Ebro Delta wetland (north-eastern Iberian Peninsula)

Taxon	Group or species	$n$	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Glossy ibis	Year 2015	13	$11.58 \pm 0.60$	$-24.52 \pm 0.53$
	Year 2016	9	$12.10 \pm 0.51$	$-25.03 \pm 0.86$
Plant	Asian rice <i>Oryza sativa</i>	3	$11.65 \pm 0.13$	$-25.94 \pm 0.11$
Insect	Odonata (larvae)	3	$10.73 \pm 1.41$	$-25.72 \pm 2.41$
	Coleoptera ( <i>Cybister lateromarginalis</i> + <i>Hydrous piceus</i> )	6	$9.49 \pm 1.23$	$-27.84 \pm 3.14$
Crustacean	Red swamp crayfish <i>Procambarus clarkii</i>	3	$11.64 \pm 0.08$	$-24.66 \pm 0.15$
Gastropod	Apple snail <i>Pomacea maculata</i>	3	$8.69 \pm 0.56$	$-24.68 \pm 0.41$
	Other Gastropoda ( <i>Lymnae</i> sp. and <i>Physa acuta</i> )	3	$9.58 \pm 1.13$	$-19.44 \pm 3.57$
Amphibian	Perez's frog <i>Pelophylax perezi</i>	6	$9.46 \pm 2.11$	$-23.95 \pm 3.31$
Fish	Crucian carp <i>Carassius</i> sp.	3	$13.57 \pm 0.30$	$-29.52 \pm 1.09$

horn, internal standard), LIE-BB (whale baleen, internal standard), and LIE-PA (razorbill feathers, internal standard). These laboratory standards were previously calibrated with international standards supplied by the International Atomic Energy Agency (IAEA, Vienna, Austria).

### 2.3 | Isotopic mixing model and statistical analyses

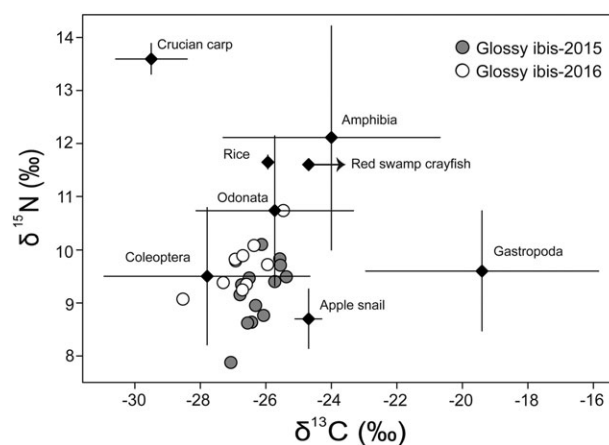
The potential contribution of each potential prey group to the diet of the glossy ibis was estimated using the STABLE ISOTOPE ANALYSIS IN R (SIAR) Bayesian isotopic mixing model (Parnell, Inger, Bearhop, & Jackson, 2010). The SIAR model estimates the potential contribution of each prey item in the diet of the consumer (in this case, the glossy ibis) based on the isotopic values of the consumer and its potential prey. This model runs in the free software R (R Development Core Team, 2016), and allows the inclusion of sources of uncertainty in the data, in particular the variability in the stable isotope ratios of the predator and the potential prey (Parnell et al., 2010). Because the blood isotopically integrates the information during the month before sampling (Bearhop, Waldron, Votier, & Furness, 2002; Pearson, Levey, Greenberg, & Martínez del Río, 2003), the results provide an average of the trophic habits of the chicks throughout their life. Diet tissue discrimination factors, the isotopic enrichment factor of a consumer in relation to the isotopic values of the prey consumed, of 2.4‰ for  $\delta^{15}\text{N}$  and 1.7‰ for  $\delta^{13}\text{C}$ , were used in the Bayesian isotopic mixing model (Caut, Angulo, & Courchamp, 2009). As there are no species-specific enrichment factors published for the glossy ibis, the values used were from the review by Caut et al. (2009). In addition, Welsh's tests were used to compare the differences in both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values between years and colonies.

## 3 | RESULTS

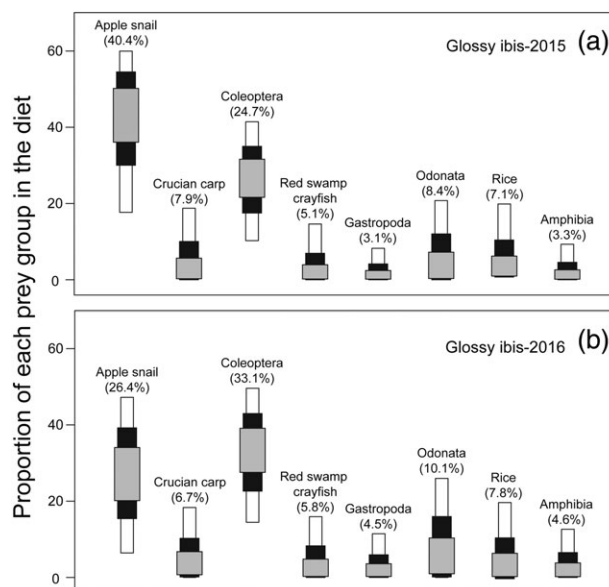
Significant differences were found in the values of  $\delta^{15}\text{N}$  ( $F_{1,19} = 4.73$ ,  $P = 0.042$ ), but not in the values of  $\delta^{13}\text{C}$  ( $F_{1,19} = 2.47$ ,  $P = 0.141$ ), between the two colonies and years (Table 2). The values of blood isotopes in the glossy ibis were between the values of some of their potential prey groups (Figure 2). Although small differences between years and breeding sites were found, the diet of the glossy ibis, based on the SIAR models, was composed mainly of apple snail and coleopterans, followed by larvae of odonatan, carp, rice, crayfish, amphibians, and gastropods (Figure 3).

## 4 | DISCUSSION

This study is the first report of the regular incorporation of apple snail in the diet of the glossy ibis, at least during the breeding season. The glossy ibis has resident populations in the Ebro Delta and may prey on apple snails outside of the breeding season, but this should be confirmed by sampling birds during that period. It is possible that in winter, however, when rice fields are dry and apple snails are buried in mud (Yusa, Wada, & Takahashi, 2006b), the birds can only prey on apple snails in the channels that retain water. Other studies have reported observations of wild bird species feeding on invasive apple snails (Sawangproh & Poonswad, 2010; Wada, 2006; Yusa, 2006),



**FIGURE 2** Mean and standard deviation stable isotope values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (‰) in the blood of glossy ibis chicks (circles), and of their potential prey groups (not drawn when standard deviation was less than the size of the mean symbol), in the Ebro Delta Natural Park (north-eastern Spain)



**FIGURE 3** Results of the SIAR model (with 95, 75, and 50% credibility intervals) showing the estimated prey contributions of the diet of glossy ibis chicks during the breeding seasons of 2015 (a) and 2016 (b) in Ebro Delta Natural Park. Numbers in parenthesis indicate the 50% percentile

and the positive perception of snail kite predation by farmers in Ecuador (Horgan et al., 2014). The snail kite is an endangered species in the USA, where a previous study (Cattau et al., 2010) found that juveniles feeding on invasive apple snails showed energetic deficiencies. However, a more recent work (Cattau et al., 2016) found that the populations of snail kite that feed on this new prey improved their demographic parameters, particularly juvenile survival, compared with snail kite feeding on native snails. Although natural local predators of the apple snail can act as a biological control agent, we do not expect them to eradicate this pest, because predator-prey relationships normally only regulate prey numbers (Newton, 1998). On the other hand, in Europe, no other similar snails are present,

and thus for local predators they are a novel food resource with which they must become familiar (i.e. learning to break the shell on the ground, as rats (*Rattus norvegicus*) do; to open the opercula and gain access to the soft parts, as yellow-legged gulls (*Larus michahellis*) do; or to swallow it whole and regurgitate the undigestible parts, as glossy ibis do; Figure 1).

The apple snail reached high densities in the rice fields and channels of the Ebro Delta a few years after its initial detection in 2009 (estimated at 31.5 g m<sup>-2</sup> wet weight (Gilioli et al., 2017) or assessed at 9–33 individuals m<sup>-2</sup>, with a maximum of 200 individuals m<sup>-2</sup> (M. A. López, pers. comm., May 2017)), causing some agricultural damage and large economic losses. Despite this severe adverse impact on agriculture and natural wetlands, it has also become an abundant resource in the food web for potential predators. Several studies have demonstrated that when an alien species is established and becomes abundant in an area, it can be used as a trophic subsidy (Barbar, Hiraldo, & Lambertucci, 2016; Navarro et al., 2010; Rodríguez, 2006; Speziale & Lambertucci, 2013; Tablado et al., 2010). The invasion of apple snails in the Ebro Delta follows this pattern, and the results of this study show that at least one bird species, the glossy ibis, has regularly incorporated it into its diet quite quickly after colonization (after a maximum of 6–7 years). The possibility that other predators in the Ebro Delta currently consume the apple snail (e.g. other bird species, rats, or fishes) cannot be excluded, but new research is necessary to identify these species and the degree to which the apple snails are consumed. In a similar case, the invasive red swamp crayfish, introduced to Spain in 1973, is today fully integrated in the food web as an important prey item for several predator species (Tablado et al., 2010), including birds in the Ebro Delta (e.g. Navarro et al., 2010). Thus, we predict that the apple snail will follow the same path as the red swamp crayfish in the food web, even though the spread of this pest in rice fields and wetlands is undesirable.

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## ORCID

Albert Bertolero  <http://orcid.org/0000-0002-6834-2600>

Joan Navarro  <http://orcid.org/0000-0002-5756-9543>

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