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Stopping Winter Flooding of Rice Fields to Control Invasive Snails Has no Effect on Waterbird Abundance at the Landscape Scale

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The invasive apple snail (*Pomacea maculata*) appeared in 2010 in the Ebro Delta Natural Park, an important area for rice production and waterbird conservation in the eastern Mediterranean. To control crop damage, farmers stopped flooding their rice fields in winter, an agri-environmental scheme (AES) applied for more than 20 years in some European and American regions to favor flora and fauna from wetlands, including wintering waterbirds. Thus, apple snail control is controversial because of its potential side effects on international waterbird conservation efforts. Despite the fact that 10 years have passed since the first flooding limitations, and the alarms raised by the managers of the Natural Park, the side effects of apple snail management on waterbird conservation have not been evaluated. Here we fill this gap by analyzing a 35-year time series to assess whether abundance trends of 27 waterbird species, from five functional groups, decreased in the Ebro Delta after stopping winter flooding. We considered the effects of confounding local factors by also assessing trend changes in l'Albufera, a similar nearby not invaded wetland where flooding has not been interrupted. In addition, as a control of the positive effect of winter flooding, we also assessed whether abundance trends increased in both wetlands after applying this AES winter flooding. Our results showed complex and decoupled trend changes across species and geographical areas, without statistical evidences, in general or for any particular functional group, on the positive effect of winter flooding in both wetlands neither on the negative effect of its cessation in Ebro Delta. These results suggest the safety of this apple snail control in terms of waterbird abundance at a landscape scale. In addition, these results question, at least in two important wintering areas in Europe, the attractor role associated with the flooding agri-environmental scheme applied for decades.

Keywords: agri-environmental schemes, Ebro Delta, invasive species management, *Pomacea maculate*, side effects, wetlands, biological invasion, decision making

INTRODUCTION

It is widely recognized that invasive species cause negative impacts on the environment, the economy and human health (Mack et al., 2000; Pimentel et al., 2000, 2005; Doherty et al., 2016; Diagne et al., 2021), and that, due to globalization, biological invasions are growing at a dizzying rate (Seebens et al., 2017, 2021). Therefore, invasive species management plays a crucial role in global, national and local policy agendas. To better manage invasive species, policymakers and managers require to fully understand not only the effectiveness of management actions against invasive species, but also their side effects on the invaded communities. The best example of this dual focus is the use of pesticides. For a long time, pesticides have been the most widely used practice to control and eradicate invasive and non-desirable native species, albeit with detrimental effects not only on the environment and biodiversity but also on human health (Pimentel, 1971; Geiger et al., 2010; Beketov et al., 2013). These impacts prompted the regulation and eventual restriction of their use [e.g., European Union, 1991; European Union, 1992; OTA (US Congress Office of Technology Assessment), 1995]. In recent decades, more environmentally friendly alternatives to pesticides have emerged, although they are not without side effects. It is therefore essential to enhance our knowledge of the side effects of any management against invasive species, especially in fragile areas such as wetlands.

In the last century, humans have caused the degradation of up to 80–90% natural Mediterranean wetlands (Finlayson and Davidson, 1999; Perennou et al., 2012; Davidson, 2014; Lefebvre et al., 2019), mostly because of the development and intensification of agriculture (CEC (Commission of European Communities), 1995; Heimlich, 1998; Duncan et al., 1999). The reduction in the extension and quality of wetlands affect the diversity and abundance of waterbirds at flyways scale (Czech and Parsons, 2002; Bellio et al., 2009; Donnelly et al., 2020; Fan et al., 2021). To mitigate this impact, the European Commission has invested enormous amounts of money over the last three decades through the Common Agricultural Policy (CAP) encouraging farmers to adopt agri-environmental schemes (AES) to reconcile the impacts of agriculture on biodiversity in Europe (EEC Regulation 2078/92; Ansell et al., 2016). Since the turn of the century, one important AES associated with the protection of flora and fauna in wetlands is the flooding of rice fields in winter (RD 708/2002; Martínez-Eixarch et al., 2017). Rice field flooding has multiple positive impact for birds (Czech and Parsons, 2002; Elphick and Oring, 2003; Fasola et al., 2010; Ibáñez et al., 2010; Pernollet et al., 2015; Koshida and Katayama, 2018; Sesser et al., 2018). But specifically this AES, among other benefits, provides stopover sites during pre- and post-breeding migrations, as well as foraging and resting habitats during winter (hereafter winter flooding; RD 708/2002; Fasola and Ruiz, 1996; Sánchez-Guzmán et al., 2007; Lourenço and Piersma, 2008; Santiago-Quesada et al., 2014; Marco-Méndez et al., 2015; Gencat, 2018). Winter flooding has been mostly applied in Spain, because of its strategic geographical location for migratory birds (Longoni, 2010; Marco-Méndez et al., 2015; Pernollet et al., 2015).

In the Ebro Delta Natural Park (NE Spain; **Figure 1**), one of the most important bird areas in southwest Europe included in Natura 2000 network and an important rice producing areas in the country, the application of winter flooding started in 1999 but it was interrupted in some fields from 2010, and in all fields from 2014 (Martínez-Eixarch et al., 2017; Curcó and Brugnoli, 2019), to control the invasive apple snail (*Pomacea maculata*). However, this control has raised concerns about the consequences for waterbirds[1,2,3]. Based on these concerns, we hypothesize that stopping flooding to control apple snail and leaving the rice fields dry after harvest might negatively affect the capacity of the Ebro Delta to host wintering birds. However, this hypothesis has not been tested empirically.

Here we used a 35 year long time series to test whether the abundance trend of 27 waterbird species, from five functional groups, declined in the Ebro Delta after stopping winter flooding to manage the invasive apple snail, thereby reducing the suitable area for waterbirds. To ensure that our inferences are robust and avoid spurious relationships due to local trend variations unrelated to winter flooding, we also studied the changes in abundance trends of the same 27 species in l'Albufera Natural Park, another nearby Spanish wetland also included in Natura 2000 network (ca. 180 km south in the same flyway; **Figure 1**), and also a key site for waterbird conservation. In l'Albufera, winter flooding started on a similar period (2001) but was not interrupted because it was not invaded by the apple snail. To provide insights on the potential side effect of the control of the apple snail, we tested two specific and complementary hypotheses. Our first hypothesis is that stopping the winter flooding to control the apple snail has had a negative side effect on waterbird abundance in the Ebro Delta. If this is the case, then we expect to detect a decline in the trend of waterbird abundance in the Ebro Delta after the cessation of winter flooding, that is not mirrored in l'Albufera. Our second and complementary hypothesis is that the onset of winter flooding favored the presence of more waterbirds. Then, we expect an increase in the trend of waterbird abundance in both wetlands after the application of winter flooding (i.e., a positive control). Additionally, we also contemplated that regional and global factors can affect species abundances and alter our expectations, especially in species with different breeding and wintering ranges. So, we also examined whether the existing trend changes supported the negative effect of stopping winter flooding under the hypothetical cases that abundance of waterbird species would have increased or decreased in both wetlands during our study period due to regional or global factors.

To evaluate these hypotheses is challenging because population dynamics can vary over time in a complex and different way for each species. Thus, we searched for trend changes in the time series of the 27 seven species and two wetlands. We latter evaluated whether the number of species

[1]https://elpais.com/ccaa/2016/08/14/catalunya/1471196493_389899.html

[2]https://www.elperiodico.com/es/barcelona/20190205/delta-ebre-aves-hibernantes-caracol-manzana-7286374

[3]https://www.lavanguardia.com/local/tarragona/20200219/473661835262/proponer-volver-inundar-arrozales-delta-ebro-frenar-descenso-aves-invernantes-tarragona.html

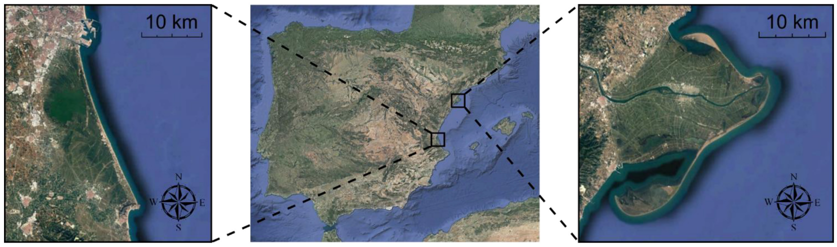

FIGURE 1 | Satellite image of Spain (center), l'Albufera (left), and Ebro Delta (right). Source: Google Earth.

with trend changes supporting our hypotheses was higher than that expected if population abundance of those species varied in random way. We explored the existence of a general pattern for the 27 species as well as for the species of each of the five functional groups.

MATERIALS AND METHODS

Study Areas

The Ebro Delta is a 32,000 ha wetland on the Mediterranean coast of NE Spain (40°43′N 00°43′E) (**Figure 1**). The Ebro Delta is divided in two hemideltas (hereafter southern and northern hemideltas). The geological and hydrological dynamics of the Ebro delta has been altered multiple times by human action (see details in Mañosa et al., 2001; Cardoch and Day, 2002; Ibañez and Prat, 2003; Genua-Olmedo et al., 2016). Currently the flow is totally controlled by dams and water canalizations. Two main channels, one on each hemidelta, provide water from the river to the rice cultivation by a network of irrigation ditches (Mañosa et al., 2001). The Ebro Delta was awarded with the category of Natural Park in 1983. Its surface was extended in 1986 (7,736 ha) and was designated as a Ramsar site in 1993. A total of 12,000 ha including the Natural Park and other wetlands and rice fields are included in the Natura 2000 Network (Ibáñez et al., 2010). These awards evidence the high international value of this area which supports up to 30,000 pairs of nesting waterbirds, and 180,000 wintering individuals annually (accessed in January 2021)[4].

L'Albufera is a wetland of 21,120 ha in the east of Spain (39°17′N, 00°20′E) with a coastal lagoon that receives water from streams, rivers and ditches. The lagoon is surrounded by sandy dunes and marshlands mainly devoted to rice crops and orchards (**Figure 1**). Rice crops are flooded in winter because of the application of this AES. The whole wetland, including rice fields, marshlands, the lagoon and the dunes were awarded the category of Natural Park in 1986 (20,956 ha); Special Protection Area (SPA) and Site of Community Importance (SCI) under Natura 2000 network in 1990; and as a Ramsar site in 1991. See Soria (2006) and Martín et al. (2013) for further details on l'Albufera. This wetland hosts more than 10,000 waterbird breeding pairs, some of them endemic or threatened, and an average of 80,000 individuals of waterbirds (accessed in January 2021)[5].

[4]https://rsis.ramsar.org/es/ris/593

[5]https://rsis.ramsar.org/es/ris/454

Rice cultivation at the large scale started in eighteenth century in l'Albufera and in the second half of the nineteenth century in the Ebro Delta. Since, rice crops have hugely modified the natural landscape in both wetlands (see Mañosa et al., 2001; Cardoch and Day, 2002; Soria, 2006; Torregrosa et al., 2021 for details). For instance, in the Ebro Delta, natural habitats covered ca. 28,000 ha (88%) at the beginning of 20th, but only ca. 8,000 ha nowadays. Rice fields are currently the most extended land use in Ebro Delta (ca, 21,000 ha; 66% of the wetland; Martínez-Vilalta, 1996; Mañosa et al., 2001; Genua-Olmedo et al., 2016; Pla et al., 2019) and in l'Albufera (ca 15,000 ha; 70% of the wetland; Martín et al., 2013; Torregrosa et al., 2021); making the management of these rice fields very relevant for wetland biodiversity.

Waterbird Time Series

We analyzed a winter time series of censuses of 27 waterbird species from 1985 to 2020 in the Ebro Delta and l'Albufera Natura 2000 sites, from the International Waterbird Censuses (IWC) carried out every year in mid-January coordinated by Wetlands International. In both wetlands, sequential partial censuses of permanent sectors were annually performed, covering all the wetland (both natural and artificial habitats). Since, sequence distribution of census reduces the likelihood of double counting or false zeros, we used as our dependent variable the summed absolute counts of individuals for each species of waterbirds. See **Supplementary Material** for data on summed absolute counts (**Supplementary Data Sheet 1**) and data manipulation due to sampling absences **Supplementary Appendix A**.

To evaluate the effects of winter flooding, we classified waterbirds in five functional groups: (1) surface birds: *Anas acuta, Anas crecca, Anas platyrhynchos, Mareca penelope, Mareca strepera, Spatula clypeata, Tadorna tadorna;* (2) diving birds: *Aythya ferina, Aythya fuligula, Netta rufina*, and *Fulica atra*; (3) short-billed/legged waders: *Calidris alpina, Calidris minuta, Charadrius alexandrinus, Charadrius hiaticula, Vanellus vanellus;* (4) long-billed/legged waders: *Calidris pugnax, Limosa limosa, Recurvirostra avosetta, Tringa erythropus, Tringa nebularia, Tringa ochropus;* (5) large wading species: *Ardea alba, Ardea cinerea, Bubulcus ibis, Egretta garzetta,* and *Phoenicopterus roseus*.

Apple Snail

Apple snails (*Pomacea* spp.) are freshwater species native to South and Central America (Kwong et al., 2010; Hayes et al., 2015) presumably introduced in the Ebro River in 2009 as an accidental release from an exotic pet animal importer (López et al., 2010; Joshi and Parera, 2017). The apple snail spread rapidly throughout the Ebro Delta (i.e., rice fields, channels, and river) despite the efforts to control it. One year after its release, the apple snail had invaded 576 ha of rice fields in the northern hemi delta. Two and five years later, the extension of the invaded rice fields, respectively, increased, respectively, by about three and five times. In 2018, the apple snail was invading most of the northern hemidelta (about 9,000 ha), but significant damage in the southern hemidelta was protected by management (see details in Gencat, 2018).

Pomacea spp. predominantly feeds on macrophytes, preferring species with a low dry matter content (Carlsson et al., 2004; Carlsson and Lacoursiere, 2005; Burlakova et al., 2009). However, apple snails also feed on irrigated crops such as rice causing huge economic losses (Naylor, 1996; Cowie, 2002). In rice fields, the apple snail consumes the seed and the rice seedling (Gencat, 2018).

In the Ebro delta, there have been several measures to control the expansion and eradicate the apple snail: physical barriers in the rice fields and in the channels that irrigate them, disinfection of machinery, flooding of the fields and channels with saline water, and the use of pesticides, the most extended management (Gencat, 2018). Here we focus on the interruption of winter flooding because of its potential side effects on waterbird abundance.

This management strategy has caused annual mortalities of the apple snail up to 65–99% (Gencat, 2018). Despite control efforts, due to apple snails' high reproductive capacity and mobility across the irrigation network, it has expanded to the Ebro River (Hayes et al., 2015; Joshi and Parera, 2017).

Winter Flooding

Before the definition of AES winter flooding by the European Commission, winter flooding was already promoted at the small scale in the Mediterranean region for hunting purposes (Fasola and Ruiz, 1996; Elphick et al., 2010). But AES funding commits to extending the flooding of the rice fields for at least four more months (RD 708/2002). In the Ebro Delta, winter flooding as an AES implementation started in 1999 and was partly limited from 2010 by asking a special requirement (Martínez-Eixarch et al., 2017; Gencat, 2018). However, from 2014, AES winter flooding was no longer encouraged because of the new Rural Development Program (2014–2020) of Catalonia to control apple snail (Martínez-Eixarch et al., 2017; Curcó and Brugnoli, 2019). In contrast, in l'Albufera, AES implementation started in 2001 and has not been interrupted since then.

As our time series are from 1985 to 2020, in the Ebro Delta we can identify three periods: (i) before the implementation of AES winter flooding, 1985–1998; (ii) during the implementation of AES winter flooding before apple snail invasion, 1999–2009, and (iii) after stopping the AES winter flooding to control the apple snail, 2010–2020. In contrast, in l'Albufera we identify two periods: (i) before the implementation of AES winter flooding, 1985–2001; and (ii) during the implementation of AES winter flooding, 2001–2020. Thus, the duration of the implementation of the AES winter flooding is different in the Ebro Delta than in l'Albufera, mainly because of the management of the apple snail in the Ebro Delta.

Analyses of Trend Changes

To test the influence of winter flooding of rice fields on waterbird species abundance, we need to know (i) whether abundance trends of waterbirds varied over time; if they did, (ii) whether these trend changes occurred before or after altering flood management, to infer causality, and (iii) if the observed increase or decrease of waterbird abundance trends is consistent with our predictions.

To obtain this information for each of the 27 species in the two wetlands, we performed piecewise regressions. These analyses consider the potential non-linearity between the dependent and independent variables through multiple and consecutive lineal regressions; therefore, this statistical tool can be considered as an intermediate approach between lineal and additive models. In particular, piecewise regressions statistically evaluate whether a time series is best fitted with a single linear regression or with multiple linear regressions, penalizing the addition of new linear regressions (see Naumova et al., 2001; Zeileis et al., 2002; Muggeo, 2003; Toms and Lesperance, 2003; for more details). If the relationship between the dependent and independent variable is best fitted with more than one linear regression, in our study case, the analyses provide the estimated year of the trend change, as well as the estimated abundance trend of waterbirds before and after the changes.

To perform piecewise regressions, we used the R package segmented (Muggeo, 2003, 2008, 2017) because it is able to identify multiple changes in abundance trends across years. Changes in abundance trends are observed at the interception between two consecutive different linear regressions. To run models with the segmented package, we first modeled the abundance of the species (log scale) with the years through the function "lm" of the R package stats (R Core Team, 2020). We then executed the function "segmented" of the R package segmented with the output of the previous model (Muggeo, 2003, 2008, 2017; see R help for programming details). We also calculated the confidence intervals for the changes in trend with the function "confint" of the R package stats (Muggeo, 2017; R Core Team, 2020).

To make our inferences independent from potential methodological subjectivities, we performed a sensitivity analysis. In particular, we also searched for changes in abundance trends with the function breakpoints of the R package "strucchange" (Zeileis et al., 2002, 2003; see **Supplementary Appendix B** for details).

Tested Hypotheses

To test our hypothesis, we specifically assessed whether there were statistically significant changes in waterbird abundance trends after (i) the onset of winter flooding, our positive control test on the influence of winter flooding, and (ii) the cessation of AES winter flooding to control the apple snail. We then evaluated whether the linear regression estimated after the trend change matched our prediction (i.e., had a steeper or lower slope; see below). Finally, we tested whether the number of cases that matched our predictions was higher than expected if the changes in the abundance trend were by chance. We assumed that, by simple chance, a species had a 50% chance of changing its abundance trend in each wetland, with 25% increasing and 25% decreasing.

Regarding the particular hypotheses, if the onset of AES winter flooding had a positive effect, we expect the abundance trend of species to increase in both wetlands. As changes in the habitat suitability may have delayed effects on communities and populations (Menéndez et al., 2006; Kuussaari et al., 2009), we were conservative and studied the abundance trends in the whole AES flooding period. We finally assessed whether the number of species whose trends matched our predictions in both wetlands was greater than 3; a value resulting from rounding the multiplication of the 25% probability of decreasing in the Ebro Delta, the 50% probability of remaining constant in l'Albufera and the 27 species of waterfowl; $0.25 \times 0.50 \times 27 = 3.37$).

On the other hand, if stopping winter flooding to control the apple snail in the Ebro Delta had a negative side effect on abundance of one waterbird species, we expect the abundance trend of that species to decrease in the Ebro Delta, but to remain constant in l'Albufera. To consider potential delayed effects, we also evaluated the abundances trends in the whole "No AES flooding" period. Finally, we assessed whether the number of species whose trends matched our predictions in both wetlands was greater than two; a value resulting from rounding the multiplication of the 25% probability of decreasing in the Ebro Delta, the 25% probability of decreasing in l'Albufera, and the 27 species of waterbirds; $0.25 \times 0.25 \times 27 = 1.68$). We tested it by using Fisher's exact test.

For evaluating the potential negative effect of stopping winter flooding, we also considered in our expectations the possible influence of regional or global factors such as climate changes (Sutherland, 1998; Knudsen et al., 2011; Lehikoinen et al., 2013) and other human activities such as persecution and pesticides pressure (Fasola et al., 1996; del Moral et al., 2003; Isenmann, 2004), habitat transformation and deterioration (Gauthier et al., 2005; Sanderson et al., 2006) or supplementary feeding (Plummer et al., 2015; Greig et al., 2017). Due to the lack of specific data for our studied periods (e.g., Waterbird Population Estimates; Delany and Scott, 2006), we could not control for these effects by evaluating the match between local and regional or global trends (Rendón et al., 2008; Toral and Figuerola, 2010). However, we may assume that any factor altering regional or global abundance trends would similarly influence local trends in both the Ebro Delta and l'Albufera due to their proximity. In particular, if regional or global factor caused increases in population trends, and the cessation of winter flooding affect waterbird abundance, the abundance trends of waterbirds would increase in l'Albufera, while in the Ebro Delta they would decrease, remain constant, or even increase, but less than in l'Albufera. Alternatively, if any factor caused decreases in population trends, and the cessation of winter flooding affect waterbird abundance, the abundance trends would decrease in l'Albufera and the Ebro Delta, but more in the latter one due to the expected negative effect of limiting winter flooding. So, we also explored whether the number of species with trend changes supporting the negative effect of winter flooding was higher than that expected by chance, but under a scenario where regional or global population abundance would have increased or decreased during the "No AES flooding" period.

To consider group-specific effects of winter flooding, we also performed one Fisher's exact test for each of the five functional groups. Then, we performed "$1 + 5$" Fisher's tests per hypothesis. We used Fisher's exact test due to expected values were less than five. We conducted Fisher's exact test through the r function "fisher.test" from the R package base (R Core Team, 2020).

RESULTS

During the AES flooding period (Ebro Delta: 1999–2009; l'Albufera 2001–2009), no species increased their abundance trend in both the Ebro Delta and l'Albufera (i.e., our prediction about the positive effect of winter flooding). Intuitively, the number of species matching our prediction was not higher than expected by chance when considering all species together or when considering particular functional groups (*p*-values were always 1). In eight species, the abundance trend increased in just one of the two areas but remained constant in the other one (**Figure 2** and **Supplementary Figure 1**; i.e., *Mareca penelope, Spatula clypeata, Netta rufina, Vanellus vanellus, Recurvirostra avosetta, Ardea cinerea, Bubulcus ibis,* and *Egretta garzetta*). On the other hand, and contrary to our expected effect of winter flooding, the abundance trend of 10 species declined in one or both wetlands (**Figure 2** and **Supplementary Figure 1**; *Anas acuta, Charadrius hiaticula, Limosa limosa, Recurvirostra avosetta, Ardea alba, Ardea cinerea, Bubulcus ibis, Egretta garzeta,* and *Phoenicopterus roseus*).

During the no AES flooding period, 2010–2020, i.e., after the invasion of the apple snail and the cessation of winter flooding in the Ebro Delta, only in four out of 27 species the abundance trends decreased in the Ebro Delta, while they did not change in l'Albufera (i.e., our prediction; **Figures 2**, **3**; *Anas crecca, Ardea Alba, Fulica atra,* and *Limosa limosa*). These four species did not belong to a specific functional group (i.e., 2 surface species, 1 long billed/legged wader, and 1 large wading species). Importantly, the number of species with changes supporting the potential negative effect of stopping winter flooding was lower than expected by chance when considering all species together or when considering individual functional groups (*p*-values in Fisher's exact test: analyses with all the 27 species = 0.33; surface birds = 0.23; diving birds = 1; short-billed/legged waders = 1; long-billed/legged waders = 0.50; and large wading species = 0.50). On the other hand, and contrary to our expectation, the abundance trend of nine species declined only in l'Albufera or in both wetlands. See summary information on the occurrence and direction of trend changes for the 27 species in the two studied areas in **Figure 2**. For full details on trend changes, regression coefficients, and residual plots see **Supplementary Figures 1–4** and **Supplementary Tables 1, 2**.

As for our complementary analyses, when theoretically considering that regional or global factors might have decreased

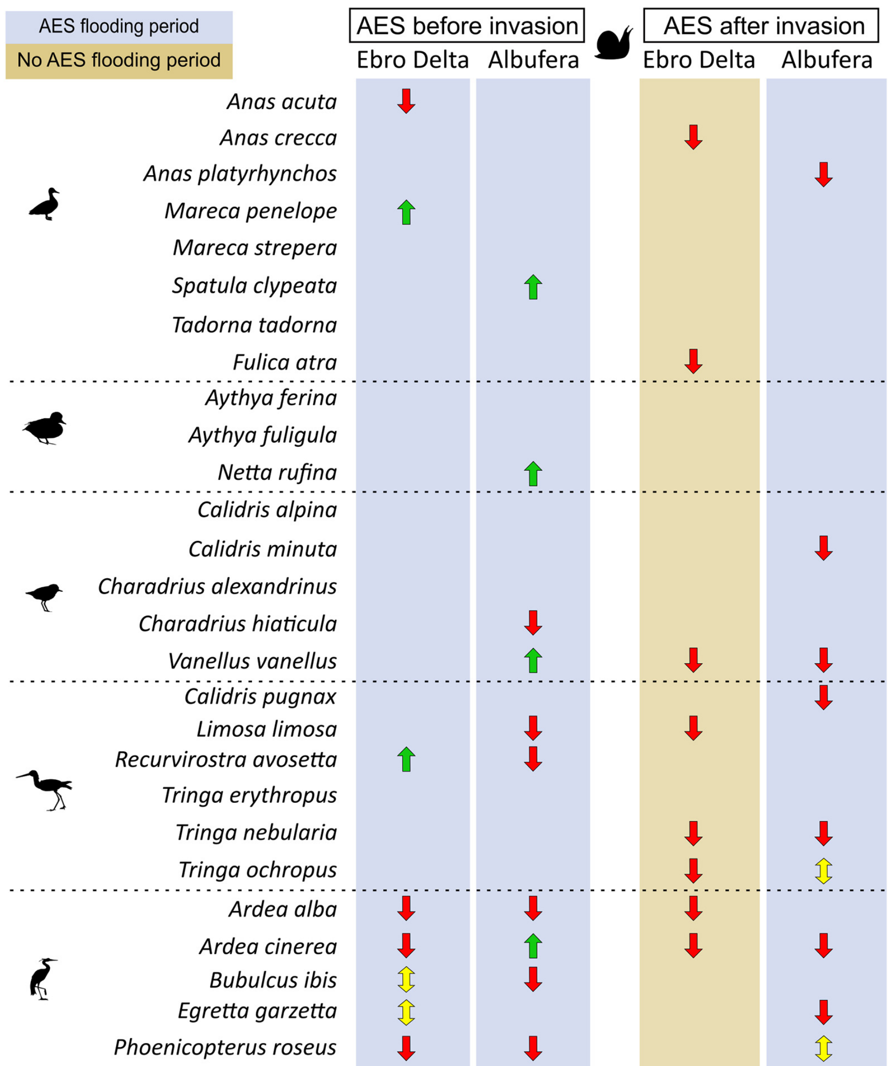

FIGURE 2 | Trend changes of waterbird abundance in the Ebro Delta and l'Albufera in two periods defined by the invasion of the apple snail in the Ebro Delta (left block: AES before invasion; right block: AES after invasion). The two blue rectangles in the block "AES before invasion" indicate that rice fields were flooded in winter in the Ebro Delta and l'Albufera due to the AES. While the brown and blue rectangles in the block "AES after invasion" indicate that rice fields were flooded in winter in l'Albufera, but not in the Ebro Delta due to apple snail control. Information on the block AES before invasion aims to test our hypothesis on the positive effect of winter flooding on trend in waterbird abundance, while information on the block AES after invasion aims to test our hypothesis on the negative side effect of stopping winter flooding (see hypothesis in main text). The green and red arrows indicate the occurrence of a, respectively, statistically significant increase or decrease in the abundance trend in that period. The yellow arrows indicate that there were multiple and antagonistic trend changes, or those changes were too complex to draw any conclusion. This figure does not inform on existing trend changes before the onset of the AES winter flooding. For full details on all trend changes, their timing, and the abundance trends before and after trend changes (see **Supplementary Figures 1–4** and **Supplementary Tables 1, 2**).

waterbird populations during our populations study, no trend change supported the negative effect of stopping winter flooding (**Figure 2**). When considering that regional or global factors might have increased waterbird populations, only *Ardea cinerea* and *Tringa nebularia* showed trend changes matching our prediction (**Figures 2**, **4**). But in the case of *Tringa nebularia*, it can be considered a statistical artifact due to the absence of the species in l'Albufera in the first decade of the time series (**Figure 4**). In any case, the lack of statistical evidence on the negative effect of stopping winter flooding holds even when also considering these two latter species (both *p*-values of Fisher's exact test for long billed/legged and large wader birds = 0.22).

There were also trend changes unrelated with the study period of our predictions. In the basic flooding period, before the implementation of AES winter flooding, 14 species varied their abundance trends (Ebro Delta: *Anas crecca, Ardea alba, Ardea cinerea, Calidris alpina, Calidris minuta, Fulica atra, Recurvirostra avosetta, Tringa ochropus, Vanellus vanellus*; l'Albufera: *Anas crecca, Ardea alba, Ardea cinerea, Calidris pugnax, Charadrius hiaticula, Egretta garzetta, Fulica atra, Mareca strepera, Phoenicopterus roseus, Recurvirostra*

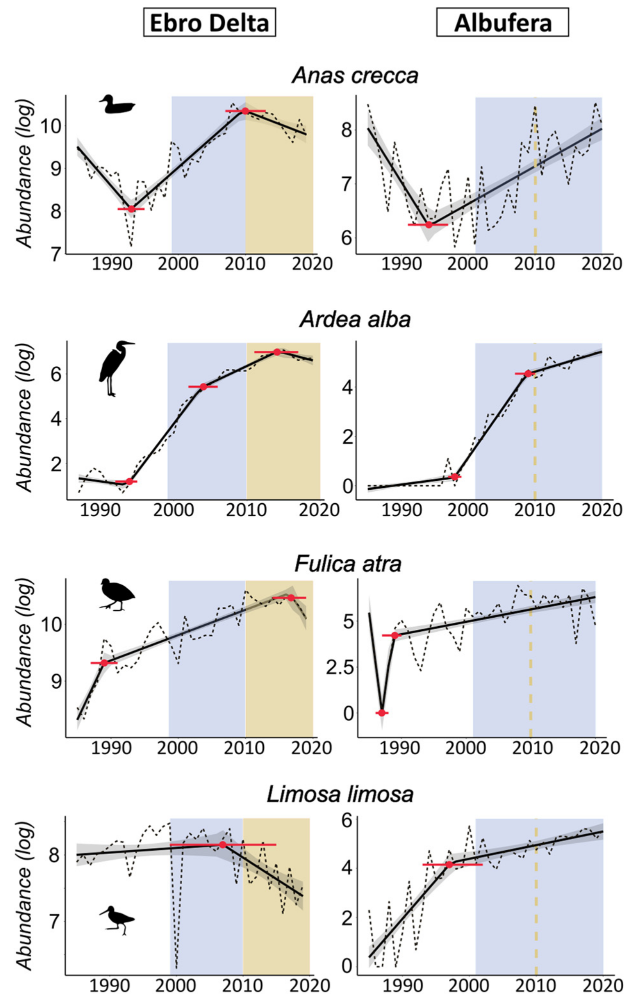

FIGURE 3 | Estimated trends in waterbird abundance and years of change. The dotted lines show the annual censuses of four species in the Ebro Delta and l'Albufera (left and right plots, respectively). The solid black line and gray shading, respectively, indicate the estimated abundance trends in each period and their uncertainty. The red dot indicates the estimated year in which the abundance trend changed (i.e., significant break point identified), while the red line represents its associated uncertainty. The blue and brown backgrounds, respectively, indicate the AES flooding period and no AES flooding period. As the cessation of the flooding was only conducted in the Ebro Delta to control the invasion by apple snail, in the plots of the l'Albufera (right side) there is only one blue background, but with a vertical dashed brown line indicating the year when the no AES flooding period started in the Ebro Delta. See **Supplementary Figure 1** for details of all species.

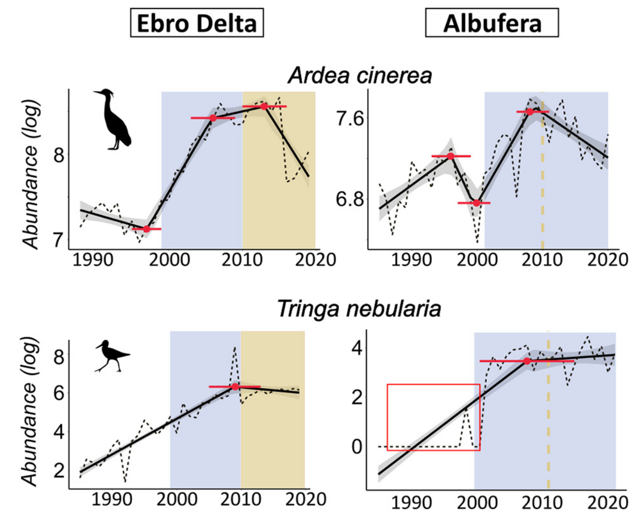

FIGURE 4 | Estimated trends in *Ardea cinerea* and *Tringa nebulosa* abundance and years of change. For details on plot elements see the legend of **Figure 3**. For details on model outputs see **Supplementary Figure 3** and **Supplementary Table 1**.

avosetta, Tringa ochropus; **Supplementary Figure 1**). The sensitivity analyses showed qualitatively similar results (**Supplementary Figure 2**).

DISCUSSION

Due to public concern from different stakeholders about the potential negative side effect of controlling the apple snail by stopping winter flooding of rice fields in the Ebro Delta, one of the most important wetlands in the western Mediterranean for migratory birds, we empirically analyzed whether the abundance trend of 27 waterbird species decreased after its cessation. In order not to misattribute stochastic local variations as evidence of the negative effect of limiting winter flooding, we also assessed whether the abundance trends of the same species were constant in l'Albufera, an expectation for a nearby wetland where winter flooding was not stopped because of the absence of the apple snail. Our study provides timely news and supports that such a negative side effect on waterbird abundance trends at a landscape scale is not evident for waterbirds in general or for any particular functional group.

Alternative Explanations

There might be several non-exclusive explanations for a generalized lack of effect across species. First, we might not have detected the expected signals in the period of partial flooding of AES because there was a migration of birds from the Ebro Delta to l'Albufera. But if this were the case, we would expect to see a negative trend change in the Ebro Delta and a positive one in l'Albufera, something that does not occur in any case. Secondly, demographic and environmental factors beyond winter flooding might have affected abundance trends at regional or global scale. Certainly, variations in migration timing may affect bird counts and introduce noise in the time series values (Ferrer, 1982; Rendón et al., 2008). However, if such variations would mask trend changes, we should have no detected so many different trend changes. Additionally, our analyses also rejected that some regional or global factors masked the signal by increasing or decreasing global population abundances during our study period.

Overall, the absence of evidences supporting the positive impact of winter flooding and of the negative impact of its cessation support that flooding alone is not sufficient to make fields suitable for waterbirds and attract them (Elphick et al., 2010). Moreover, the presence of nine trend changes suggesting that winter flooding has a negative effect on abundance trends, and the presence of 14 trend changes prior to any alteration in the flood management, strongly suggest that the detected trend changes are not attributed to winter flooding of rice fields but to external and unknown factors (Almaraz and Amat, 2004; Rendón et al., 2008; Schummer et al., 2010; Toral and Figuerola, 2010; Márquez-Ferrando et al., 2014).

The abundance of waterbird species varied in a complex way across species, years, and nearby geographical areas. This complexity and decoupling challenges the effectiveness of conservation managements designed for multiple species, such as winter flooding. Understanding population fluctuations and their causes is fundamental to take appropriate conservation actions, especially when already threatened species show downward trends. Future studies are therefore urgently needed to elucidate the mechanisms underlying inter- and intra- species variation.

Differences and Similarities With Previous Studies

The observed landscape-scape role of flooded rice fields located in natural wetlands during winter seems to contrast with that of flooded rice fields when they are the main source of water, such as those located in areas away from coastal marshes that are naturally flooded by autumn and winter rains (Sánchez-Guzmán et al., 2007), or those located in low water areas that are artificially flooded in summer due to the hydrological cycle of rice cultivation (e.g., Fasola et al., 1996; Parejo and Sánchez-Guzmán, 1999; Tourenq et al., 2000; Maeda, 2001; Ramo et al., 2013). Thus, the role of flooded rice fields to attract higher abundances of waterbirds may depend on the surrounding habitat (Elphick, 2008; King et al., 2010), and potentially be more qualitative than quantitative.

The absence of a clear signal of the positive effect of winter flooding, and our suggestion of a qualitative rather than quantitative role, seems to deviate from widely disseminated discourse in the literature on the positive effect of rice field flooding (e.g., Elphick and Oring, 1998, 2003; Ackerman et al., 2006; Elphick, 2008; King et al., 2010; Tajiri and Ohkawara, 2013; Koshida and Katayama, 2018). Possibly, this discrepancy is because those studies compared waterbird abundance in flooded and non-flooded rice fields (field–scale management) or studied the effects of landscape-scale rice fields on specific snapshots, whereas we studied the effects of altered rice field flood management on the time series of waterbird abundance at a landscape scale.

However, we did find discrepancies with a study focused on waterbird abundance at the landscape scale, and coincidentally focused on our two studied wetlands (Pernollet et al., 2015). Since we used similar, if not the same, datasets, the differences between our conclusions might rely on the statistical approximation used. Pernollet et al. (2015) observed that the estimated trend of the total abundance of six dabbling ducks was higher in the period after the starting of the AES. However, by estimating the trends in two predefined periods such as before and after the onset of AES, it is not possible to detect whether the trend change occurred before or after the occurrence of the hypothetical causal factor. For instance, in *Anas crecca* and *Mareca penelope* abundance trend in the period 1985–2000 was lower than in the period 2000–2010, but the trend started to change before the AES initial application (**Figure 3** and **Supplementary Figure 1**). On the other hand, abundance trends calculated using the abundance of all species together (e.g., Pernollet et al., 2015) may be only reflective of trends in the most abundant or variable species (depending on whether abundances were scaled or not, respectively; i.e., masking effects). Thus, considering these analytical arguments, our conclusion is that the effect of winter flooding on abundance is neither obvious nor general or group-specific in these two wetlands.

MANAGEMENT IMPLICATIONS AND CONCLUDING REMARKS

Our results cast doubts on the role as an attractor of waterbirds of flooding rice fields after harvest, a management that is becoming common in multiple parts of the world (e.g., Europe, United States and Japan; Yamamoto et al., 2003; Lourenço and Piersma, 2008; Elphick et al., 2010). Certainly, our results are limited to two wetlands, and should therefore be taken with caution. But due to there are limited information on the effect of flooded rice fields at the landscape scale (King et al., 2010; but see Chan et al., 2007; Elphick, 2008; Santiago-Quesada et al., 2014), and available information is mostly limited to time series of few species (Rendón et al., 2008; Toral and Figuerola, 2010; Márquez-Ferrando et al., 2014; Herbert et al., 2018), our study raises the question of whether policy-makers, managers, and scientists really know the role at large spatial scale of agro-environmental measures that have been applied for decades. Thus, our study is of interest to different stakeholders aiming to improve the conservation status of waterbirds by applying this or similar AES (Sesser et al., 2018). To understand the role of flooded rice fields as an attractor of wintering waterbirds, future studies can compare the abundances at a landscape scale of areas with new and abandoned rice fields cultivated with flooding techniques and with and without any other source of water (e.g., time series in before-and-after changes in the availability of flooded rice fields). The major challenge is to perform well-controlled (natural) experiments, especially at large scale (Carpenter, 1998; Elphick et al., 2010; King et al., 2010).

Our results provide important and timing insights about the controversial control of the apple snail in the Ebro Delta (unpubls. comments)(see text footnote 1–3). However, flooding of rice fields can influence waterbirds beyond their abundance. For instance, flooded rice fields are important for bird roosting, resting, nesting, and foraging (Manley et al., 2004; Lourenço and Piersma, 2008; Fujioka et al., 2010; Pierluissi, 2010; Strum et al., 2013; Santiago-Quesada et al., 2014).

We therefore claim to be cautious in using only abundance as an indicator of the conservation status of waterbirds in aquatic ecosystems (Green and Figuerola, 2003). On the other hand, the management of invasive species can be complicated due to their legacy effects (Hobbs and Humphries, 1995; Zarnetske et al., 2010). For example, the elimination of the apple snail could have a negative impact on *Plegadis falcinellus*, a bird species that feeds on it (Bertolero and Navarro, 2018; Curcó and Brugnoli, 2019). Additionally, literature strongly support that alterations in flood managements of rice fields can affect many other taxa not considered here (Machado and Maltchik, 2010; Katayama et al., 2015; Koshida and Katayama, 2018; Baba et al., 2019). Therefore, we emphasize that our study is important, but one of the pieces of a complex puzzle. And, therefore, its information should not be taken in isolation to argue for the future application of this and similar conservation measures. To make the right decisions, policy-makers and managers need a holistic perspective on all the direct and indirect effects of managements against invasive species.

Overall, our results highlight the importance of evaluating the side effects of managing species and evaluating the effectiveness of ongoing or previously applied conservation measures (e.g., Ibáñez et al., 2010; Martínez-Eixarch et al., 2017). Our results are highly relevant for conservation practitioners since funds to conserve biodiversity are limited (Echols et al., 2019) and future water availability can be scarce in many regions (Ferguson et al., 2018; Kristvik et al., 2019). Because the number of threatened habitats and species as well as biological invasions is set to increase dramatically in the coming decades (Seebens et al., 2017, 2021), it is urgent to accelerate research to meet future, but close, ecological and conservation challenges.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

RB-M conceived the presented idea, performed the analyses, wrote the first version of the text, proposed the analytical approach, and all authors discussed it. RB-M and PV obtained the data. All authors discussed the results and contributed to the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.688325/full#supplementary-material

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