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Waterbird impacts on widgeongrass *Ruppia maritima* in a Mediterranean wetland: comparing bird groups and seasonal effects

Hector Rodríguez-Pérez and Andy J. Green

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We studied the effect of waterbirds on the submerged macrophyte *Ruppia maritima* in eleven fish ponds within Doñana Natural Park (SW Spain). Separate exclosure designs allowed us to exclude flamingos or all waterbirds from 3×3 m plots within the ponds and compare them with control plots. Four experiments were conducted for three month periods at different points of the annual cycle with varying bird densities. Flamingos and wildfowl (ducks and coot) had significant negative additive effects on the presence of aboveground (leafs and shoots) or belowground (roots) parts of *Ruppia* at all times of the year. For plots where *Ruppia* was present, aboveground biomass was significantly higher in all-bird exclosures than in controls or flamingo exclosures. Presence and biomass of this annual plant varied significantly between seasons as did the density of seeds in sediments. Seasonal changes in seedbank densities were consistent with consumption by birds. There were no significant treatment × season interactions for *Ruppia* presence, aboveground biomass or seeds. This is the first exclosure study to compare the effects of waterbirds on submerged macrophytes at different times throughout the annual cycle, and the first to compare simultaneously the effects of different bird groups. Our findings refute previous suggestions that major effects of waterbirds are limited to temperate regions and to periods of early growth or when major concentrations of migratory wildfowl are formed in autumn. Flamingos are important in structuring shallow wetlands in the Mediterranean, and possibly many other regions.

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Submerged vascular plants have a vital role in non-marine aquatic ecosystems, influencing nutrient dynamics and water chemistry, modulating the structure and dynamics of pelagic and benthic food webs, and increasing the physical habitat diversity (reviewed by Jeppesen et al. 1998). Changes to the status of submerged macrophytes owing to herbivory or other factors can have major consequences for the whole ecosystem (Scheffer et al. 1993, Van Donk and Otte 1996, Perrow et al. 1997).

Most studies of herbivory in aquatic systems have been made on insects, even though other groups such as crayfish, fish and waterbirds cause bigger reductions in macrophyte biomass (Lodge et al. 1998). Although several studies have shown that waterbirds can have an important effect on the standing crop of submerged macrophytes, they have been restricted to wildfowl (ducks, swans and coots) and largely concentrated in temperate areas of North America, Europe and New Zealand (Lodge et al. 1998, Marklund et al. 2002). It has been suggested that major effects of waterbirds on macrophytes may only be observed in temperate waters and during periods of early growth or in autumn when macrophyte productivity is low and wildfowl form migratory concentrations (Perrow et al. 1997, Mitchell and Perrow 1998). There is a lack of information from

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non-temperate (e.g. Mediterranean) regions to test this hypothesis. There is also a lack of studies comparing effects of wildfowl in different parts of the annual cycle, and addressing the impacts of other waterbirds such as flamingos.

Greater flamingos (*Phoenicopterus ruber*) have undergone a dramatic increase in population size in the Mediterranean region since 1970 and are now one of the most abundant waterbirds in terms of biomass (Johnson 1997, Wetlands International 2002). Without direct evidence, previous workers (Gallet 1950, Duarte et al. 1990, Grillas et al. 1993) have suggested that flamingos trample and uproot beds of widgeongrass *Ruppia* and other macrophytes while feeding in Mediterranean wetlands. Whilst this has not previously been tested experimentally, exclosure experiments elsewhere have shown that flamingos can have a marked effect on benthic organisms (Hurlbert and Chang 1983, Glassom and Branch 1997a, 1997b). Greater flamingos are also reported to cause significant damage in ricefields (Tourenq et al. 2001), and we suggest that greater flamingos are ecological engineers (Fig. 1). Here we present an exclosure study comparing the effects of greater flamingos and wildfowl on *Ruppia maritima* in Doñana, southwest Spain, during distinct three month periods of the annual cycle.

The strength of effects on macrophytes recorded by exclosure experiments depends partly on the timing and duration of the experiment and how it relates to the macrophyte life cycle (Mitchell and Wass 1996). It also depends on waterbird phenology. As far as we know, ours is the first exclosure study to compare the effects of waterbirds on submerged macrophytes at different times throughout the annual cycle. It is also the first exclosure study to address simultaneously the impacts of different groups of waterbirds using the same area, or to assess the influence of changes in waterbird density throughout the annual cycle.

Fig. 1. Aerial photograph of craters made by greater flamingos feeding in the sediments of ponds in Veta la Palma. The flamingos were flushed immediately before the photo was taken. Author Héctor Garrido/Equipo de Seguimiento de Procesos Naturales.

Study area

We carried out exclosure experiments in Veta la Palma (36°57′N, 6 °14′W), a private fish-farm divided in 52 regular ponds within Doñana Natural Park. The ponds were constructed in 1992–1993 on top of what was natural marshland in the Guadalquivir estuary. All the ponds are shallow (average 30 cm, maximum depth 50 cm) and flat-bottomed with a total combined surface area of 2997 ha. The fish species cultured are European seabass (*Dicentrarchus labrax*), flathead mullet (*Mugil cephalus*), gilthead seabream (*Sparus auratus*) and eels (*Anguilla anguilla*) as well as Atlantic ditch shrimp (*Palaemonetes varians*). Each pond is dried out under rotation approximately every two years to extract fish. Ponds are interconnected via canals and permanent flow of water taken from the Guadalquivir estuary maintains dissolved oxygen levels (see Frisch et al. 2005 for more details).

The dominant submerged macrophyte is *R. maritima*, forming extensive beds during spring and early summer. Small patches of *Potamogeton pectinatus* occur in some ponds in years of lower salinity. Most of the shoreline is bare mud and regular dredging to prevent siltation of peripheral canals used to extract fish from the ponds has restricted development of vegetation, which is dominated by *Arthrocnemum macrostachyum* and *Suaeda* spp. with some small patches of *Phragmites australis* and *Scirpus maritimus* in few ponds. Mean monthly air temperature during the study varied from 11.8°C in February to 23.5°C in July. Mean annual precipitation is 562 mm year^{-1} with a range of 158–1062 mm year^{-1} (Castroviejo 1993). Salinity during our study varied from 7 g l^{-1} during winter months of high rainfall to 15 g l^{-1} at the end of September, after the dry summer months typical of the Mediterranean region. pH ranged between 9.3–10.4.

Material and methods

Aerial surveys of waterbirds of the whole Veta la Palma estate (2997 ha) were carried out monthly during the study as part of regular surveys of Doñana National Park and its surroundings. All birds are counted, including those on the water and those in flight (most of which are flushed by the plane). Eleven ponds (range 26–114 ha, a total of 697 ha) were selected for the exclosure experiments so as to include ponds that had been flooded for different lengths of time but were not scheduled to be dried out during the experiments. All exclosures and controls were 3 × 3 m in size, and we used two exclosure treatments. One treatment used a 2 cm mesh nylon net tied to a square PVC frame suspended from four iron poles (2 m long, 10 mm diameter) pushed into the pond bottom in each corner. Wires were also

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attached from the top of the poles to the centre of the net to prevent it from touching the water surface. At the sides of the square frame, 20 cm of net hung vertically down to prevent birds from passing underneath. This design allowed fish to enter but decreased utilisation by ducks, coot and other birds. The netting did not affect water movement. There was no algal growth and shading effects were close to zero.

The second treatment excluded only flamingos by taking advantage of their much greater height. Four iron poles were placed in each corner of the square as before, and a wire tied round them at a height of 70–75 cm from the bottom (i.e. 30–65 cm above the water surface). This height was selected after trials confirming that other birds pass comfortably underneath the wire and freely enter the exclosures. The controls consisted merely of the four iron poles. Eurasian coots *Fulica atra* and ducks were frequently observed feeding inside controls and flamingo exclosures, whereas birds were never observed inside all-bird exclosures. No evidence of waterbird grazing, such as clipped stems, was observed inside the all-bird exclosures.

Each experimental block contained one exclosure of each kind plus a control, separated by 10 m. Treatments were randomly located within each block. There were two experimental blocks in each pond, separated by 25 m. Both blocks were placed in the western part of each pond, aligning all exclosures and controls at an equal distance from the pond edge (approximately 20 m for the first experiment), so as to expose them equally to the dominant southwesterly wind with an equal and relatively low fetch.

Seeds, aboveground and belowground parts were sampled three months after installing the exclosures. When establishing the exclosures, we only sampled the seedbank. This was owing to their small size and the need to limit disturbance to allow us to study effects on the invertebrate community simultaneously (H. Rodríguez-Perez and A. J. Green, unpubl.). The experiment was repeated four times between July 2001 and September 2002, moving the exclosures and controls 10 m in towards the centre of the pond at the beginning of each new experiment. There was no change in depth towards the centre of the ponds. Owing to the lack of a significant fringe of emergent vegetation, there was no windbreak along the pond edge and no gradient in waterbird density from the edge to the centre of ponds that could have influenced waterbird effects as we moved controls and exclosures between experimental periods (Weisner et al. 1997). There was slight overlap between experimental periods caused by the time required to sample and move exclosures and controls in eleven ponds. This usually took two weeks, but bad weather caused some delays. The first experimental period ran from 13 July to 30 October 2001 (Oct 01 from hereon), coinciding roughly with the post-breeding period for

waterbirds. The second period, from 22 November 2001 to 27 February 2002 (Feb 02 from hereon) corresponded with the wintering period. The third period from 7 March to 6 June 2002 (Jun 02 from hereon) covered the pre-breeding and early breeding periods. The fourth period, from 12 June to 10 September 2002 (Sep 02 from hereon) covered the breeding and early post-breeding period. Each individual exclosure or control was set for 82–92 days in Oct 01, 95–105 days in Feb 02, 88–96 days in Jun 02 and 83–91 days in Sep 02. In a given pond and period, all exclosures and controls were established or sampled on the same day.

One vegetation sample was taken from the northeast corner of each exclosure and control so as to avoid stepping inside and disturbing spots where separate invertebrate samples were taken. The water depth in the sampling points ranged from 10 to 39 cm (mean $\pm$ se $= 27 \pm 8$ cm). A PVC pipe section of 40 cm diameter was inserted into the mud and all the water extracted. Then all *Ruppia* stems and leaves (hereafter aboveground parts) were carefully cut at the base with finger nails and removed. Three cores of mud were taken from within the pipe area to study roots (hereafter belowground parts). Each core sample was of 5.5 cm diameter and 10 cm depth. Aboveground parts were rinsed in the lab, dried at 80°C for 48 h and then weighed. The number of seeds attached to inflorescences in the aboveground parts were also counted. Such seeds were only recorded in the Jun 02 samples. Mud samples were rinsed with tap water in a 0.5 mm sieve to extract belowground parts which were then dried and weighed.

Seed bank effects were also studied, counting the seeds in separate core samples. Three cores of 5.5 cm diameter and 5 cm depth were taken in each exclosure. These samples were taken both when installing the exclosures and three months later. Seed bank data were available for an earlier experimental period, from 3 April 2001 to 11 July 2001 (Jul 01 from hereon) coinciding with the waterbird breeding period. In contrast, there were no data for the Sep 02 period.

The total number of ponds used for analyses varied from 9 to 11 between experimental periods owing to unforeseen drainage of some ponds. Those ponds which only had data for some of the periods were included in our analyses, as the methods used were robust. However, we repeated our analyses using only the nine ponds used throughout the study and found no important differences (results not shown).

Statistical methods

We used generalized mixed linear models (GLMs, McCullagh and Nelder 1989) to analyze the effects of treatment (all-birds exclosure, flamingo exclosure or control) and experimental period on *Ruppia*. Pond and

block nested within ponds were included as random factors, using the GLIMMIX macro (SAS Institute 1996). Treatment and sampling period were included as fixed factors of three and four levels respectively. Post-hoc analyses of least-squared means were carried out to identify significant differences among levels of fixed factors. Tests on the effects of each predictor were performed using F-statistics (Crawley 1993).

Data on presence/absence of aboveground or belowground parts in our samples were analysed via logistic regression with a binomial error and a logit link function. To analyse the effects of treatment and sampling period on samples where *Ruppia* was present, we also conducted GLMs of the non-zero biomass data (log transformed to overcome heteroscedasticity) using an identity link and normal error distribution. Owing to the high proportion of zeros, there was no suitable transformation that enabled analysis of all the data (i.e. including zeros). With aboveground biomass as the dependent variable, belowground biomass (log transformed) was included as a covariable to test the hypothesis that treatment and period influenced the relative amounts of above and below ground parts.

The change in density of *Ruppia* seeds in sediments (log transformed) between the beginning and end of each experimental period was also analyzed in a GLM with an identity link and normal error distribution. For samples with non-zero aboveground part biomass, the number of seeds found on plants in June 2002 was analyzed in a GLM with a poisson error distribution and log link function. The biomass of aboveground or belowground parts was included as a covariable together with treatment to test the hypothesis that waterbirds influenced the relative abundance of seeds for a given plant biomass.

We initially considered pond age (the time for which each pond had been flooded at the beginning of the first experimental period) as an additional predictor (results not shown). Pond age was considered as a fixed factor of four levels corresponding to four age categories (ponds filled for <6 months, 7–12 months, 13–18 months and >19 months). The partial effect of pond age was never significant (P >0.1 in all cases).

Results

Waterbird counts

Peak numbers of waterbirds in the study site occurred during the post-breeding period, reaching more than 50 000 in November 2001 (Fig. 5). Numbers were also high in winter, and were lowest in March and April (Fig. 5). Numbers of the largely herbivorous coots and ducks followed a similar pattern, peaking in October–November during the post-breeding period. Numbers of flamingos reached a peak in July–August at the height

of their breeding season (Fig. 5). In terms of biomass, flamingos were easily the most important group of birds in the area (Fig. 6). The mean density of ducks and coot in Veta la Palma was 2.6 ha^{-1}. The mean density of flamingos was 3.9 ha^{-1} (densities were calculated based on the total pond area of 2997 ha). The most abundant wildfowl species recorded (in order of decreasing abundance) were *Fulica atra*, *Anas platyrhynchos*, *A. clypeata*, *A. Penelope*, *A. acuta*, *A. strepera*, *Netta rufina* and *Atythya ferina*, although *A. penelope*, *A. clypeata* and *A. acuta* were only present in winter. The *Anas* dabbling ducks accounted for 96% of the total number of ducks counted.

Presence/absence of *Ruppia*

The proportion of samples in which *R. maritima* aboveground parts were recorded varied consistently between treatments, with lowest presence in controls, highest in all-birds exclosures and intermediate values in flamingo exclosures (Fig. 2). Both treatment and period had highly significant effects on presence of aboveground parts in a logistic regression (Table 1). Post-hoc tests revealed significant differences between all treatments (flamingos vs all-birds, $t_{213} = -3.40$, p =0.0008; controls vs all-birds, $t_{213} = -5.36$, p <0.0001; controls vs flamingos, $t_{213} = -2.55$, p =0.01). Among sampling periods, Jun 02 had a significantly higher presence than the others (Oct 01 vs Jun 02, $t_{213} = -3.43$, p = 0.0007; Feb 02 vs Jun 02, $t_{213} = -3.59$, p =0.0004; Jun 02 vs Sep 02, $t_{213} = 4.12$, p <0.0001), whereas there was no significant difference among the other three periods. The interaction between treatment and period was not significant ($F_{6,207} = 0.19$, p =0.97).

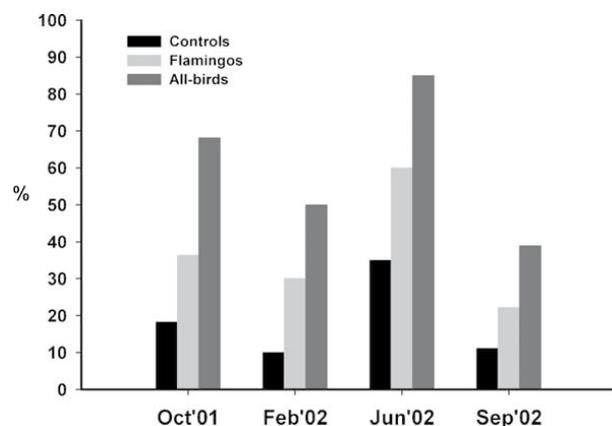Similar results were observed for the presence/absence of belowground parts, with highly significant effects of

Fig. 2. The percentage presence of *Ruppia maritima* aboveground parts in the three treatments (all-bird exclosures, flamingo exclosures and controls) at the end of each experimental period. Dates refer to the end of the three month experiments.

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Table 1. Summary of generalized linear models testing the partial effects of treatment (factor of three levels) and period (factor of four levels) on the presence/absence for aboveground and belowground parts of *R. maritima*. Pond and block were included as random factors using GLIMMIX, with binomial error and a logit link. See methods for more details. All-bird exclosure treatment and period Sep 02 were aliased. Significant results are written in bold face.

	Aboveground						Belowground					
	Estimate	SE	df_N	df_D	F	p	Estimate	SE	df_N	df_D	F	p
Intercept	−0.27	0.58					−1.5	0.53				
Treatment			2	213	14.9	**<0.0001**			2	213	12.96	**<0.0001**
Control	−2.62	0.49					−2.13	0.43				
Flamingo	−1.41	0.41					−1.13	0.37				
Period			3	213	7.19	**0.0001**			3	213	7.70	**<0.0001**
Oct 01	0.57	0.53					2.28	0.54				
Feb 02	0.45	0.54					0.97	0.56				
Jun 02	2.25	0.55					2.05	0.54				

treatment and sampling periods (Table 1). Post-hoc tests revealed significant differences between all three treatments, with flamingo exclosures showing intermediate root presence (control vs flamingo, $t_{213} = -2.32$, p = 0.021; control vs all-birds, $t_{213} = -4.98$, p <0.0001; flamingo vs all-bird, $t_{213} = -3.07$, p = 0.002). There were also significant differences between most periods, except for Oct 01 vs Jun 02 and Feb 02 vs Sep 02 (Oct 01 vs Feb 02, $t_{213} = 2.97$, p = 0.003; Oct 01 vs Sep 02, $t_{213} = 4.22$, p <0.0001; Feb 02 vs Jun 02, $t_{213} = -2.42$, p = 0.016; Jun 02 vs Sep 02, $t_{213} = 3.77$, p = 0.0002). The interaction between treatment and period was not significant ($F_{6,207} = 0.47$, p = 0.83).

Biomass of *Ruppia*

Only non-zero values were included in analyses of biomass. For aboveground parts, biomass varied seasonally with highest values recorded in all-birds exclosures (Fig. 3). Treatment and sampling period both had highly significant effects (Table 2). Post-hoc tests revealed significant differences between all-birds exclosures and the other two treatments (control vs all-birds, $t_{67} = -2.77$, p = 0.007; flamingo vs all-birds, $t_{67} = -5.33$, p <0.0001; control vs flamingo, $t_{67} = 1.15$, p = 0.25). Among sampling periods, Jun 02 had a significantly higher biomass than the others (Oct 01 vs Jun 02,
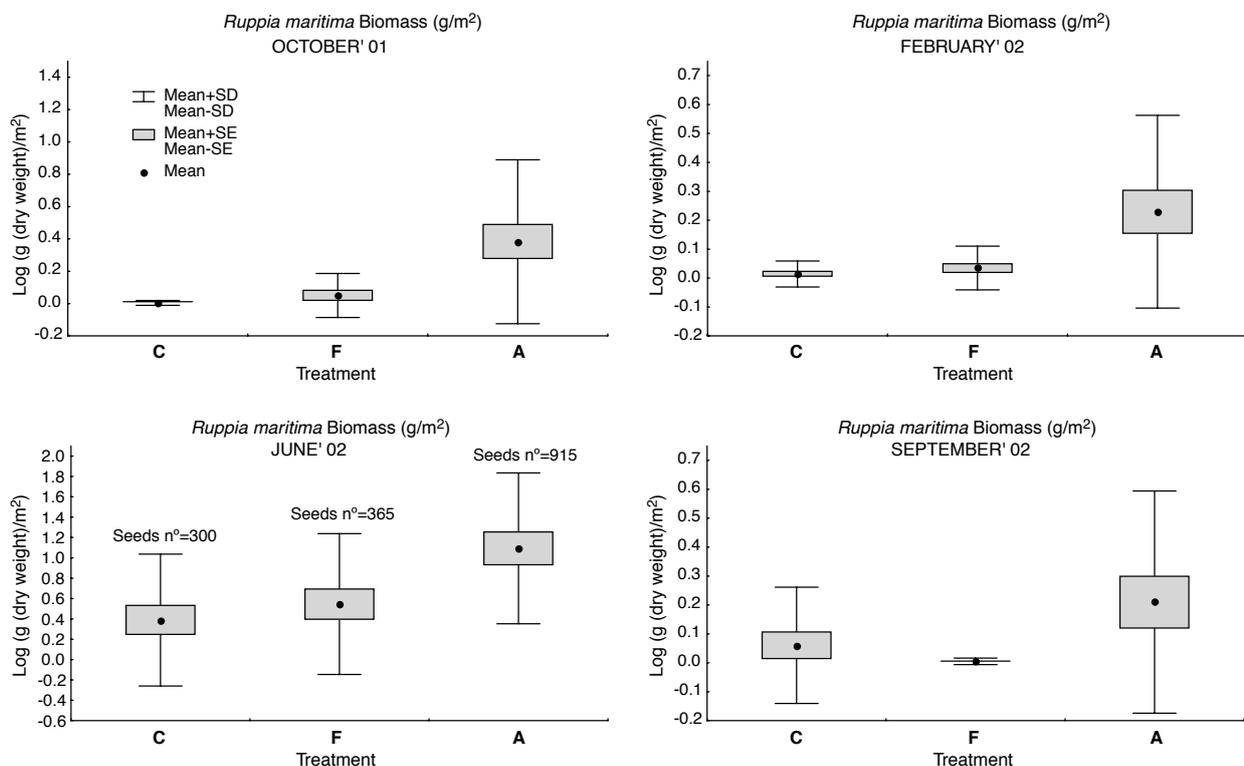

Fig. 3. Aboveground *R. maritima* biomass (g m^{-2}, log$_{10}$ transformed) at the end of each experimental period, including all data (i.e. not excluding zeros as for Table 2). C: control, F: flamingo exclosure and A: all-bird exclosure. The numbers of seeds recorded in June 2002 refer to the total numbers of seeds counted in inflorescences. Note the change of scale on the y axis.

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Table 2. Summary of generalized linear models testing the partial effects of treatment (factor of three levels) and period (factor of four levels) on the biomass (g m^{-2}, log$_{10}$ transformed, non-zero values only) of aboveground and belowground parts of *R. maritima*. Pond and block were included as random factors using GLIMMIX, with normal error and a identity link. See methods for more details. All-bird exclosure treatment and period Sep 02 were aliased. Significant results are written in bold face.

	Aboveground						Belowground					
	Estimate	SE	df$_N$	df$_D$	F	p	Estimate	SE	df$_N$	df$_D$	F	p
Intercept	0.08	0.24					−0.92	0.36				
Treatment			2	67	14.96	**<0.0001**			2	50	4.68	**0.013**
Control	−0.64	0.23					0.16	0.56				
Flamingo	−0.92	0.17					−1.05	0.71				
Period			3	67	18.86	**<0.0001**			3	50	10.57	**<0.0001**
Oct 01	0.08	0.25					0.55	0.39				
Feb 02	0.17	0.27					0.001	0.41				
Jun 02	1.33	0.25					0.64	0.38				

$t_{67} = -6.33$, $p < 0.0001$; Feb 02 vs Jun 02, $t_{67} = -5.27$, $p < 0.0001$; Jun 02 vs Sep 02, $t_{67} = 5.35$, $p < 0.0001$). The interaction between treatment and period was not significant ($F_{6,61} = 1.70$, $p = 0.13$).

Similar results were recorded for root biomass with both treatment and period having significant effects (Table 2). Post-hoc tests showed that the only significant difference between treatments was that between the two classes of exclosures (flamingo vs all-birds, $t_{50} = -2.94$, $p = 0.005$; control vs flamingo, $t_{50} = 0.98$, $p = 0.33$; control vs all-birds, $t_{50} = -1.56$, $p = 0.13$). Among sampling periods, Jun 02 again had a significantly higher biomass than the others (Oct 01 vs Jun 02, $t_{50} = -4.62$, $p < 0.0001$; Feb 02 vs Jun 02, $t_{50} = -3.66$, $p = 0.0006$; Jun 02 vs Sep 02, $t_{50} = 3.06$, $p = 0.0036$). The interaction between period and treatment was marginally significant ($F_{6,50} = 2.33$, $p = 0.047$). For periods Oct 01 and Feb 02, root biomass was lowest in controls and was much higher in all-birds exclosures than in the other treatments. In contrast, in Jun 02 and Sep 02 root biomass was highest in controls and lowest in flamingo exclosures.

When including belowground biomass as a covariable, the effects of period ($F_{3, 84} = 13.68$, $p < 0.0001$) and treatment ($F_{2,84} = 4.26$, $p = 0.017$) on aboveground biomass remained significant. The partial effect of belowground biomass was highly significant ($F_{1,84} = 52.71$, $p < 0.0001$). Whilst controlling for belowground biomass, aboveground biomass remained significantly higher in Jun 02 than in other periods, and higher in all-bird exclosures (post-hoc tests for treatments: flamingo vs all-birds, $t_{84} = -0.1208$, $p = 0.007$; control vs flamingo, $t_{84} = 0.0213$, $p = 0.7119$; control vs all-birds, $t_{84} = -0.099$, $p = 0.08$).

Ruppia seeds

There were no differences among treatments in the change in seed densities in sediments from the beginning to the end of each period, but there were highly significant differences between periods (Table 3,

Fig. 4). Post-hoc analysis showed that the change in seed numbers for Jul 01 was more positive than for other periods (Jul 01 vs Oct 01, $t_{551} = 7.03$, $p < 0.0001$; Jul 01 vs Feb 02, $t_{551} = 5.11$, $p < 0.0001$; Jul 01 vs Jun 02 $t_{551} = 3.95$, $p < 0.0001$). The change in seed numbers for Jun 02 was also more positive than for Oct 01 ($t_{551} = -3.07$, $p = 0.002$) and for Feb 02 ($t_{551} = -1.97$, $p = 0.049$). The interaction between period and treatment was not significant ($F_{6,543} = 0.51$, $p = 0.8$).

For Jun 02, we found no evidence of a treatment effect on the number of seeds recorded on plants. Treatment did not have a significant effect on seeds when considered on its own ($F_{2,16} = 2.34$, $p = 0.12$) nor while including aboveground ($F_{2,15} = 0.10$, $p = 0.90$) or belowground biomass as covariables ($F_{2,13} = 2.88$, $p = 0.092$). Both aboveground ($F_{1,15} = 22.82$, $p = 0.0002$) and belowground ($F_{1,13} = 5.98$, $p = 0.029$) biomass had a significant effect on seed numbers. Both aboveground and belowground biomass were highly correlated with the number of seeds on plants (n = 15, aboveground, r = 0.80, p < 0.01, seeds per sample = −50.753 + 3.218 aboveground dry biomass (g per sample); belowground,

Table 3. Summary of generalized linear models testing the partial effects of treatment (factor of three levels) and period (factor of four levels) on the change in density of *R. maritima* seeds in sediments from the beginning to the end of each period (n m^{-2}, log$_{10}$ transformed). Pond and block were included as random factors using GLIMMIX, with normal error and a identity link. See methods for more details. All-bird exclosure treatment and period Jun 02 were aliased. Significant results are written in bold face.

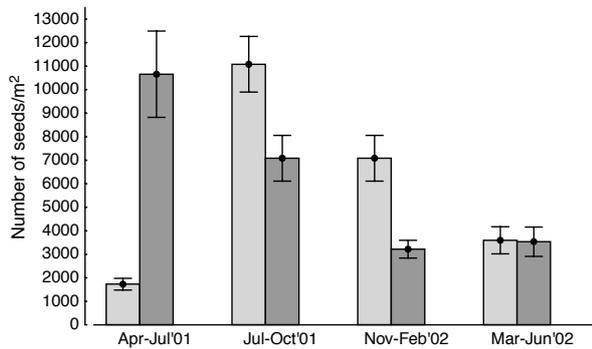|	Seed bank					
	Estimate	SE	df$_N$	df$_D$	F	P
Intercept	2.16	0.014				
Treatment			2	549	0.07	0.94
Control	−0.004	0.01				
Flamingo	−0.0007	0.01				
Period			3	549	17.35	**<0.0001**
Jul 01	0.06	0.01				
Oct 01	−0.049	0.02				
Feb 02	−0.017	0.02				

Fig. 4. Changes in *R. maritima* seed densities (mean+se) recorded in the top 5 cm of sediments from the beginning to the end of each period. Light bars show seed densities at the beginning of each period and dark bars at the end. All treatments were pooled together.

$r = 0.89$, $p < 0.01$, seeds per sample $= -19.546 + 92.210$ belowground dry biomass (g per sample).

Fig. 6. Mean biomass of flamingos and wildfowl during experimental periods in Veta la Palma. Calculations were based on the average aerial counts for each species for each period and body mass as cited by del Hoyo et al. (1992). Dates refer to the end of the three month experiments.

Discussion

Contrary to previous suggestions (Perrow et al. 1997, Mitchell and Perrow 1998), our study shows that major effects of waterbirds on submerged macrophytes are neither restricted to temperate waters nor to periods of early growth or in autumn when macrophyte productivity is low and wildfowl form migratory concentrations. Previous studies in Europe have concentrated on coot and dabbling ducks, for which the strongest impacts have been recorded during autumn and winter congregations that coincide with the end of the plant growing season (Lodge et al. 1998), or during the initial phase of reestablishment of macrophytes following biomanipulation (Lauridsen et al. 1993, 2003). Waterbirds have previously been shown to inhibit growth of *R. maritima*
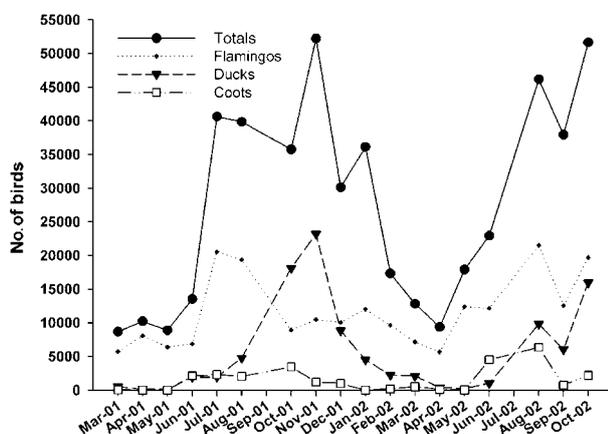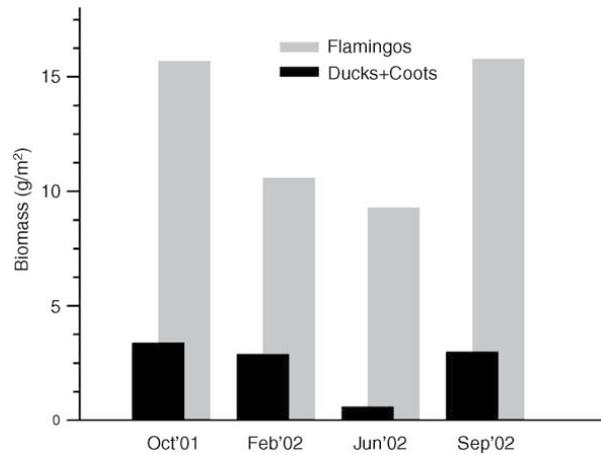

Fig. 5. Aerial counts of waterbirds in Veta la Palma from March 2001 to October 2002. Totals represent all birds counted in the area, including shorebirds, gulls, herons, etc.

planted experimentally in our study area (Figuerola and Green 2004).

Despite major seasonal variation in the abundance of *R. maritima* and of waterbirds (especially wildfowl), we found no evidence of seasonal changes in the relative effect of excluding flamingos or all waterbirds on *Ruppia* presence or aboveground part biomass. In the absence of information on plant growth rates and feedback effects, we can not translate our results into accurate measures of the proportion of primary productivity or standing crop that was consumed by birds (Mitchell and Wass 1996). Nevertheless they suggest there is no simple relationship between herbivory effects and herbivore density in our study system. Likewise, Marklund et al. (2002) found no significant correlation between waterfowl density and their effects on submerged vegetation in a meta-analysis of previous studies.

Since the biomass of submerged vegetation was relatively low in our study area and particularly low in autumn and winter, the strong effects of waterbirds at all times provides no evidence for thresholds below which there is no herbivory because birds move on to search elsewhere. Verhoeven (1980) reported a threshold of $8.8 \, \text{g DW m}^{-2}$ (equivalent to 0.94 on the y axis of Fig. 3) for coots feeding on *Ruppia*, a value much higher than the biomass we recorded in three of our four study periods (Fig. 3). Similar values were cited by Mitchell and Perrow (1998) for swans feeding on *Potamogeton* tubers or filamentous algae. The position of thresholds is likely to depend on the abundance of food in alternative patches, and the general low abundance of submerged vegetation in our study system may be one reason why any thresholds were much lower (Marklund et al. 2002). The ability of omnivorous ducks and coot to switch to feeding on invertebrates and seeds is likely to enable

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them to persist in Doñana despite the lack of submerged vegetation. During winter, the mild temperatures and lower energy demands for birds at Mediterranean wetlands may lead them to stay on depleted feeding grounds and persist partly on fat reserves rather than risking a migration to some other site.

Based on overall means for the whole study (including zero data), we found that *R. maritima* aboveground part biomass in controls was only 22% of that when all birds were excluded and only 71% of that when flamingos were excluded. This figure for all birds is much higher than that recorded in most previous studies of avian herbivory, even though our densities of waterbirds were much lower than most (Lodge et al. 1998, Marklund et al. 2002). Such strong effects of birds may partly be due to the combination of wildfowl feeding on aboveground parts and flamingos uprooting plants owing to physical disturbance caused by their feeding behaviour (Fig. 1). Other stressors in our study area such as high salinity (Murphy et al. 2003) or fish (Cardona et al. 2001) may also diminish the capacity of *R. maritima* to respond to damage caused by waterbirds. Although none of fish species present are strict herbivores, their movements and defecation are likely to favour phytoplankton at the expense of macrophytes.

Abundance of *R. maritima* varies considerably between years in our study site, and was higher in 2001 than in 2002 (Fig. 2–4). These were years of good and average rainfall respectively, when 30 000 ha of natural, temporary marshland close to our study site was also flooded from November to June. In drier years, the densities of birds in Veta la Palma are much higher because the temporary marshes are dry while the ponds remain flooded, although with a higher salinity. In such dry years, the effects of birds on *R. maritima* appear to be even stronger (J. Figuerola and A. J. Green, unpubl.).

Many submerged macrophytes are only weakly anchored in the sediments by their belowground parts and are particularly vulnerable to disturbance (Combroux et al. 2001, Capers 2003). Ours is the first study to demonstrate a deleterious effect of greater flamingos on submerged vegetation, but our results are compatible with observations by previous authors (Gallet 1950, Hurlbert and Chang 1983, Montes and Bernués 1991) and the capacity of flamingos for modifying their environment (Fig. 1) owing to their treading action during feeding (Johnson 1997). The true impact of flamingos may have been underestimated by our data since wildfowl are likely to have been attracted to feed in flamingo exclosures where *R. maritima* was relatively more available. Conservation measures have led to a roughly tenfold increase in the numbers of greater flamingos in the Mediterranean region since the 1960s (Wetlands International 2002). This has coincided with a major loss of wetland habitat over the same period

(Finlayson et al. 1992), and the resulting increase in density is likely to have increased the role of flamingos in structuring aquatic ecosystems in the Mediterranean region.

As well as by grazing and physical disturbance, wildfowl and particularly flamingos are likely to have a further harmful effect on macrophyte growth owing to the nutrients added via faeces and the turbidity caused while feeding (Wass and Mitchell 1998). When feeding, flamingos disturb the sediment, mobilise transfer of nutrients from sediments into the water column and increase the turbidity for some time after they have left (Comin et al. 1994, Glassom and Branch 1997b). This increase in turbidity by sediment resuspension reduces the available light for macrophytes, favouring a shift to a turbid water state dominated by phytoplankton (Søndergaard et al. 1992, Scheffer et al. 1993). Our exclosures were too small to study these effects, since feeding activity in adjacent areas also raised turbidity within exclosures.

Ruppia maritima can be either an annual or a perennial (Verhoeven 1979, Valdés et al. 1987), but is annual in our study area with no underground storage organs. This makes it easier to understand the effects of grazing and other waterbird activities on future biomass than for the many macrophytes with storage organs. Although we could not demonstrate a direct effect of avian exclusion on seed production, the strong effect on aboveground and root biomass coupled with the correlation between biomass and seed production makes it clear that more seeds are produced in the absence of birds. Thus waterbird impacts in one year are likely to influence *Ruppia* growth the following year. However, it is not clear how many of the extra seeds produced in the absence of birds would be lost before entering the seed bank, e.g. via consumption by other organisms. Whilst controlling for belowground biomass, the aboveground biomass remained higher in all-birds exclosures. This is likely to be because ducks and coot graze on green parts when they are available and have relatively less impact on root biomass.

We observed a weakly significant interaction between period and treatment for positive belowground biomass values. This suggests paradoxically that exposure to waterbirds reduced presence of roots in Jun 02 and Sep 02 but increased biomass for those samples where roots were present. This result may have been a type I error.

We were surprised not to detect any influence of birds on seed densities in sediments, since at our study site *R. maritima* seeds are an important component of the diet of ducks and coots (Figuerola et al. 2002) and probably also flamingos (Johnson 1997). A flamingo found dead at Veta la Palma in January 2004 contained 246 *R. maritima* seeds in its gizzard, together with benthic ostracods. It is possible we were sampling areas

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that were deeper than those preferred by ducks for feeding on seeds (Guillemain and Fritz 2002). However, it seems more likely that the reduced loss of seeds from sediments within exclosures was not detected because of the extreme variation in seed densities in our samples, or because of continuous horizontal movements of seeds in sediments associated with wave action, fish activity or bird activity. While consuming seeds, flamingos mix up sediments with their treading action, and are likely to bring seeds into the top 5 cm layer we sampled from deeper down, thus making it harder for us to detect seed loss. During autumn and winter periods, we found a major decrease in seed densities during our experiments which is attributable to loss from predation (Fig. 4).

The effects of waterbirds on submerged vegetation we have recorded have major consequences for the functioning of aquatic ecosystems. More studies of waterbird effects in non-temperate wetlands and more studies comparing different periods of the annual cycle are required before we can understand when, where and why avian effects on submerged vegetation are important. Flamingos and other non-herbivorous waterbirds should be included in such studies. Flamingos are likely to be very important in structuring coastal wetlands and shallow lakes in large parts of the world, and management measures designed to increase their populations (Martos and Johnson 1996) may have major implications for ecosystem functioning.

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