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# Response of specialized birds to reed-bed aging in a Mediterranean wetland: Significant changes in bird biomass after two decades

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**Abstract** Traditional fish farming carried out in wetland is declining in many countries of Mediterranean Europe. This decline can lead to a lack of management of the reeds that tend to age progressively. In this work we compared, through a wide temporal range (2001-2019), the densities of four habitat-specialized birds (warblers), strictly linked to *Phragmites australis* reed-beds in a coastal wetland on the Tyrrhenian central Italy. In this wetland, following the abandonment of fish farming, the average density of reeds significantly decreased, and both the average reed diameter and habitat heterogeneity showed a significant increase. Comparing 2001 to 2019, we observed an increase in the total density of breeding warblers. The two species of *Acrocephalus* (*scirpaceus* and *arundinaceus*), and *Cettia cetti* showed a marked increase in density, while *Cisticola juncidis* showed a clear decrease. More particularly, a significant increase in *Cettia cetti* ($p < 0.001$) and a decrease in *Cisticola juncidis* ($p < 0.05$) emerged when comparing bird biomasses. Species diversity and evenness were more high in 2019 than in 2001. Our data suggest that: (i) these species could be considered indicators of long-term reed-bed changes and (ii) their biomass may be used as a more effective metric when compared to abundance.

**Keywords** *Cettia cetti*; *Cisticola juncidis*; *Acrocephalus* sp.; warblers; *Phragmites* reed-beds; heterogeneity; indicators

## Introduction

In wetlands where fish farming is carried out, *Phragmites australis* reed-beds are actively managed through mowing, fire and flooding (Dawson and Kern-Hansen 1979; Poizat and Corivelli 1997; Francová et al. 2019). Nevertheless, there are evidences that these activities can impact animal communities (Vadász et al. 2008; Broyer and Calenge 2010; Zacchei et al. 2011; Trnka et al. 2014), in particular wetland birds that are ecologically linked to water-related habitats (Poulin et al. 2004, 2005; Schmidt et al. 2005; Mortelliti et al. 2012).

During the last decades, traditional freshwater fish farming carried out in wetland is declining in many countries of Mediterranean Europe (Causarano and Battisti 2009). This can lead to a lack of management of the reeds that tend to age progressively. With the progressive advancement of the reed aging dynamics, there is (i) an increase in the average diameter and a reduction in the density of the reeds, (ii) a reduction of the heliophilous species along the habitat edges (rush-beds) and, (iii) due to progressive landfill, an increase in the density of shrubs (and plant biomass) inside reed stands, making this habitat more heterogeneous (Ostendorp 1993).

Among water-related species linked to *Phragmites* reed-beds, warblers represent a specialized ecological guild (sensu Verner 1984) of passerine birds (Báldi and Kisbenedek 1999; Leisler and Schulze-Hagen 2011). This guild include many species adapted to different stages of reed-bed succession (e.g. Martìnez-Vitalta et al. 2002; Baldi 2006). In particular two warblers of genus *Acrocephalus* (Reed warblers, *A. scirpaceus* and Great Reed warbler, *A. arundinaceus*) are abundant in dense and monospecific stands where *Phragmites* reed growth conditions are excellent (Catchpole 1973, 1974; Martìnez-Vitalta et al. 2002; Poulin et al. 2002); Cetti's warbler (*Cettia cetti*) is instead a more generalist edge species, linked to ecotonal conditions and/or to reed-beds and hygrophilous vegetation with higher heterogeneity (e.g. reeds aged with shrubs; Araújo et al. 2016). Finally, Fan-tailed warbler (*Cisticola juncidis*) is a small-sized species inhabiting rushbeds, and uncultivated or ephemeral areas of first colonization of the reeds, where rush-beds can be dominant (Campedelli et al. 2009).

In this work we compared, through a wide temporal range (2001-2019), the densities of these four warblers with respect to different habitat characteristics of *Phragmites australis* reed-beds in a small remnant Mediterranean wetland. During this time span, reed-beds have not been managed since fish farming activity has been abandoned in 2004 (Battisti, pers. obs.). Consequently, reed-beds have undergone a progressive expansion in size area, and aging,

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therefore increasing the reed mean diameter and decreased their reed density. Moreover, with the aging of the reeds a large number of shrubs progressively led to a higher local environmental heterogeneity. In this regard, our hypothesis was that these changes could differently affect abundance and biomass of local populations of the target warblers, in particular: (i) increased habitat heterogeneity should induce an increase in the abundance and biomass of the Cetti's warbler (an edge species), (ii) an increase in reed-bed size area should increase the habitat availability for *Acrocephalus* spp. (and consequently absolute abundance and normalized density), and (iii) a decrease in surrounding rush-beds should decrease these metrics in Fan-tailed warbler. Our aim was also to test the role of these species as indicators of different states of reed-bed aging, selecting population metrics that are more sensitive and effective in detecting this environmental change.

## Materials and methods

### *Study area*

The study area is included in the "Palude di Torre Flavia" natural Monument (central Italy; 41°58'N; 12°03'E), a small protected wetland (40 ha) on the Tyrrhenian coast (Special Protected Area according to the Directive 2009/147/EC of the European Parliament and of the Council of 30 November on the conservation of wild birds), relict of a larger wetland drained and transformed by land reclamation during the last century (Battisti 2006; Battisti et al. 2006) (Fig. 1). At the landscape scale, this area shows characteristics of a remnant fragment of wetland inside an agricultural and urbanized matrix. At the local scale, it shows a semi-natural heterogeneity with *Phragmites australis* reed-beds, channels used for fish farming from 1938 (mainly, managing stocks of *Anguilla anguilla* and three species of mullets, *Mugil cephalus, Liza saliens, Liza ramada*). From 2004, activity of fish management (e.g. flooding, reedbed mowing and fire; Battisti et al. 2009) was definitively stopped. Near the reed-bed stands there are flooded meadows with *Carex hirta, Juncus acutus* and Cyperaceae corresponding to *Juncetalia maritimi* habitat type according the "Habitat" Directive 92/43/EC. The water in the wetland is mainly of meteoric and sea storm origin and flow from surrounding areas is scarce (Battisti 2006; Battisti et al. 2008). Climate is xeric-meso-Mediterranean (Tomaselli et al. 1973; Blasi and Michetti 2005).

### *Sampling techniques*

Reed-bed vegetation structure

To quantify the reed bed structure, a number of plots of 0.25-$m^2$ were randomly selected (24 in 2001, 25 in 2018). In each plots the diameter at breast height (h: 1.40 cm) and the number of reeds were measured, obtaining respectively the average values of reed diameter and density (number of reeds/0.25 $m^2$). We used a professional gauge with an approximation of 0.1 mm.

Reed-bed cover and heterogeneity have been quantified using multi-spectral and multi-temporal datasets considering a time span of about 20 years. In particular, two specific scenes acquired in April 2001 and April 2019 were analyzed. The 2001 data-set was obtained from the TM

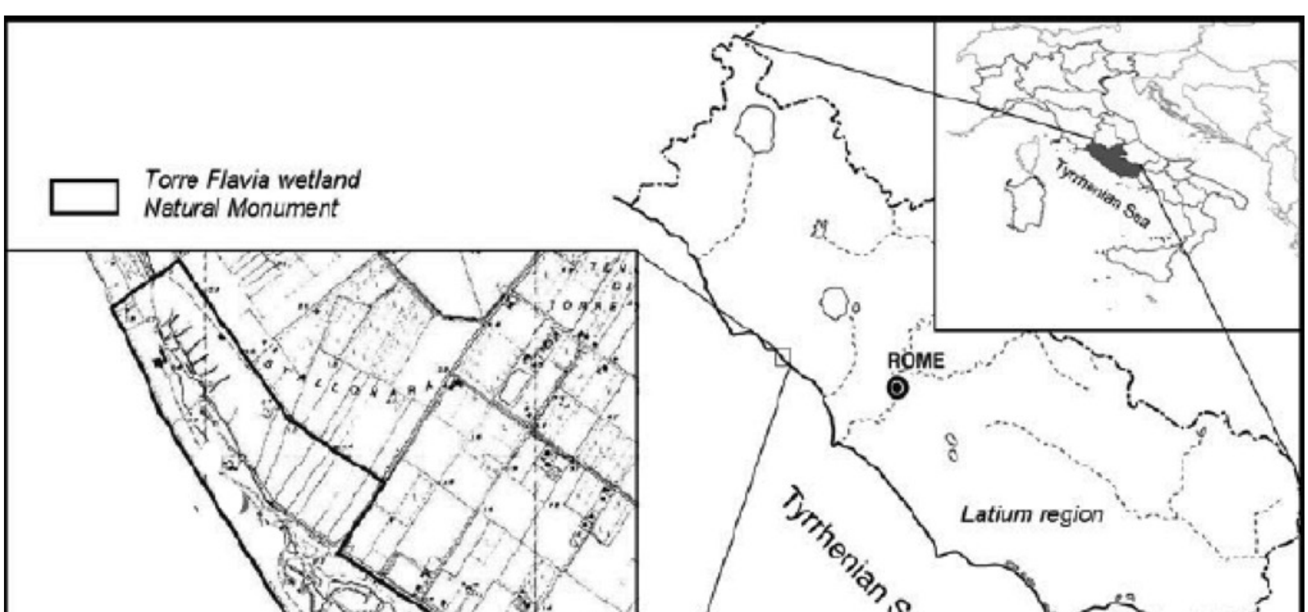


Figure 1. The study area (Torre Flavia wetland, central Italy).

(Thematic Mapper) sensor on board of LANDSAT5 satellite, while the 2019 dataset was obtained from the Operational Land Imager (OLI) sensor on board of the LANDSAT8 satellite. The geometric resolution for both images were 30 m/pixel.

The normalized differential vegetation index (NDVI) was then processed in order to identify areas characterized by different degrees of vegetation coverage in the study area. The NDVI index is closely related to the health status of vegetation, more particularly with its leaf area, size area and biomass, and indirectly to total habitat heterogeneity (for an application in reed-bed habitats, see Bresciani et al. 2009). Therefore, low index values indicate areas with poor coverage and plant heterogeneity while, on the contrary, high index values are found in those areas where there is a high vegetation cover, biomass and heterogeneity, and a high presence of biomass.

Reed-bed-related bird species

As targets, we selected four highly specialized wetland-related species linked to different age stages of *Phragmites australis* reed-beds: i.e. Fan-tailed warblers (*Cisticola juncidis*), linked to rush-beds and early stages of *Phragmites* reed-beds; Reed warbler (*Acrocephalus scirpaceus*) and Great Reed warbler (*A. arundinaceus*): having a high habitat suitability for dense and homogeneous reed-beds and Cetti's warbler (*Cettia cetti*), an edge species, linked to high heterogeneous reed-beds with presence of shrubs.

To obtain data about their density, both in 2001 and in 2019 spring period, we carried out a mapping method (Bibby et al. 2000) in the same 11-ha wide area characterized by an extended reed-bed surrounded from rush-beds (*Bolboschoenus maritimus, Juncus* spp.) and uncultivated lands (details in Causarano and Battisti 2009; Malavasi et al. 2009).

During each breeding seasons (March-July), a number of periodic field visits were carried out with a comparable sampling effort (26 hours of sampling; both in 2001 and 2019). In each visit, the observer collected data following a non-linear transect (2,200 m-long in both of periods) in early morning (07.00-10.00 h a.m.) covering the whole study area (Bibby et al. 2000). Contacts (i.e. records of each individual bird) were noted on a local map (scale 1: 2,000 from 1: 10,000 Technical Regional Map; Regione Lazio 1990). Species-specific maps were created and species-specific territories were obtained following the clustering procedure described in Bibby et al. (2000). One point was given to territories (i.e. clusters of individual species-specific contacts) completely inside the study area and 0.5 point to edge territories (i.e. clusters partially included in the 11-ha study area; Bibby et al. 2000). We considered a "territory" as a range area inside which a species pair was considered to breed (i.e. one territory = one breeding pair; Bibby et al. 2000). Birds flying very higher than 25 m were not considered. Since the selected target species are sensitive to water stress (i.e. abrupt changes in water level in reed-beds; Battisti et al. 2006; Zacchei et al. 2011), we performed these samplings during spring period, when water depth (range: 40-80 cm) was comparable between years.

### *Data analysis*

We analysed the data at the species level for Cetti's warbler and Fan-tailed warblers. Data from the two *Acrocephalus* species have been considered both at the species level and in the aggregate (for statistical analyses), due the low local density of Great Reed warbler.

The following parameters were calculated: (i) n: number of species-specific territories; (ii) breeding pair density (D), as a measure of normalized abundance, expressed as number of territories (i.e. breeding pairs)/10 ha and calculated for each species and all species ($D_{tot}$); (iii) relative frequency for each species (frD) as the ratio: specific density / $D_{tot}$; (iv) consuming biomass (Cb; in g/10 ha; calculated as: $Cb = Scb^{0.7}$ (Salt 1957), where Scb, or standing crop biomass, is the total body mass of all censused individuals, in g/10 ha). This value is directly proportional to energy removed by individuals from environment (Salt 1957). We used consuming biomass more than standing crop biomass, because the former explained better the specific variations of metabolic rhythm mainly related to individual size. To calculate the biomass values, mean body mass values were obtained from the data archives of the local bird ringing station (Sorace et al. 2015); (v) relative frequency in Cb for each species (frCb) expressed as the ratio: specific Cb/total Cb.

Finally, at the guild level we calculated the Shannon diversity index (H'; Shannon and Weaver 1963, as $H' = -\Sigma f_i \ln f_i$, where $f_i$ is the frequency of i-th species), and the evenness index ($J = H'/H'_{max}$; Lloyd and Ghelardi, 1964; where H' is the Shannon diversity index and $H'_{max} = \ln S$; Pielou 1966; review in Magurran 2004). We obtained values of Shannon diversity and evenness indices both using abundance and biomass frequencies.

Density values and related averaged and median values (and interquartile range, IQR) were compared using non-parametric Mann-Whitney U test between 2001-2019 breeding seasons. To compare relative frequencies among years we used the $\chi^2$ test (Fowler and Cohen 1992; Dytham 2011). Significance levels were set at $p < 0.01$ level. To perform the analyses, we used the statistical package SPSS 13.0 for Windows.

## Results

### *Reed-bed structure (size, structure and heterogeneity)*

From 2001 to 2018 the average density of reeds per plot decreased significantly (2001: 53.21±16.97; median value: 56; IQR: 26, n = 1277; 2018: 22.92±14.34; median value: 20; IQR: 22; n = 573; Z = –5.002, $p < 0.001$; U Mann-Whitney test), while the average diameter showed a significant increase (2001: 0.44 cm±0.24; median value: 0.37; IQR: 0.28, n = 1277; 2018: 2.35±2.13; median value: 2.0; IQR: 2.0, n = 564; Z = –23.647, $p < 0.001$; U Mann-Whitney test).

In 2001 the average value of the NDVI index was 0.06, while in 2019 this value was significantly higher (NDVI = 0.36), indicating a net increase in reed-bed size area, plant biomass and habitat heterogeneity.

***Reed-bed-related bird species***

The total number of territories of the four target species increased from 20 (2001) to 32 (2019). Comparing 2001 to 2019 breeding season, abundance of Cetti's warbler and *Acrocephalus* spp. showed a net increase in abundance, while Fan-tailed warbler showed a decrease (Table 1). We could not detect significant differences in density frequencies between 2001 and 2019 (Cetti's warblers: $\chi^2 = 2.708$, $p = 0.100$; Fan-tailed warbler: $\chi^2 = 1.782$, $p = 0.182$; *Acrocephalus* spp.: $\chi^2 = 0.039$, $p = 0.843$). Considering the biomass frequencies, we could not detect significant changes in the *Acrocephalus* spp. ($\chi^2 = 0.605$, $p = 0.437$); however a significant increase in Cetti's warbler biomass ($\chi^2 = 13.32$, $p < 0.001$) and a significant decrease in Fan-tailed warbler ($\chi^2 = 6.041$, $p = 0.014$) emerged.

We observed an increase of Shannon H' diversity and evenness when comparing 2001 to 2019. This increase is more pronounced when these indices have been calculated using frequencies in consuming biomass.

## Discussion

Following the abandonment of the fish farming activity from 2001 to 2019, the reed-bed in the Torre Flavia wetland increased in structure (i.e. higher mean diameter of reeds and lower density), size area (with a corresponding reduction in rush-beds) and habitat heterogeneity. Consequently, we observed: (i) an increase (in abundance, biomass and frequency) both in *Acrocephalus* species, strictly linked to dense and homogeneous reed-beds, and in Cetti's warbler, an edge species linked to heterogeneous habitats, and (ii) a decrease of Fan-tailed warbler, strictly linked to rush-beds and uncultivated lands. However, differences appear more pronounced when considering biomass frequencies. In this regard, among the metrics utilized, frequencies calculated on biomass appear more sensitive to detect habitat changes when compared to frequencies calculated on abundance.

Since this sensitivity is correlated with reed-bed changes in size, structure and habitat heterogeneity, we suggest that, at least in the Mediterranean region, these warblers could represent good indicator species, *sensu* Heink and Kowarik (2010), i.e. a component used to measure and evaluate environmental phenomena, conditions or changes. Indeed, these species meets many of the typical criteria requested for a biological indicator (Noss 1990; Rüdisser et al. 2012): they (i) have a stable zoological systematic and taxonomy; (ii) are relatively common, widespread, and medium-sized species, easy to detect using simple sampling techniques (e.g. mapping method) with a minimum field and economic effort; (iii) are sensitive to specific phenomena (in our case, reed-beds changes in size, structure and heterogeneity); (iv) are universally applicable and spatially comparable since they are largely diffused, at least in the Mediterranean basin (Hagemeijer and Blair 1997). Finally, interpretation and communication of indicator results may be easily carried out through simple, quantitative, and reproducible uni-variate metrics as, for example, the normalized density and biomass, analogously to what reported, as for example for forests birds (e.g., Lorenzetti and Battisti 2007; Roberge and Angelstam 2009) and synanthropic ones (Battisti and Zullo 2019).

It is strategic to have available indicators showing the response of biodiversity components to environmental change. In this regard, following a DPSIR approach (Carr et al. 2007), local biomass could be a sensitive metric for these indicators at the species/population level, useful to assess (i) the reed-bed state and impacts (due to threats acting on reed-beds: mowing, fire, etc.); and (ii) management response (conservation outcomes): in this last case these species could be used in monitoring to test the success of conservation projects testing before and after the effectiveness of management actions carried out in wetland reserves (see Haila 1985; see Before-After-Control-Impact Approach in Underwood 1992). Finally, these species could provide information on other rarer and co-occurring water-related species, inhabiting the same reed-beds, but that are less detectable (e.g. reed-bed ardeids of conservation concern as Little bittern *Ixobrychus minutus*).

However, this work has some limitations. First, the pattern is only local and therefore field data can be influenced by not considering large-scale factors. For example,

Table 1. Comparison between 2001 and 2019 of number of territories (n), normalized abundance (density, D) and relative frequency (frD), standing crop biomass (Scb), consuming biomass (Cb) and relative frequency (frCb) for the four selected target birds belonging to the reed-bed related guild. Shannon diversity and evenness values both for abundance ($H'_{ABB}$ and $J_{ABB}$) and biomass ($H'_{BIOM}$ and $J_{BIOM}$) have been reported.

| | 2001 | | | | | | 2019 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | n | D | frD | Scb | Cb | frCb | n | D | frD | Scb | Cb | FrCb |
| *Cettia cetti* | 1.5 | 1.36 | 0.074 | 32.64 | 11.47 | 0.116 | 10.5 | 9.54 | 0.328 | 228.96 | 44.86 | 0.322 |
| *Cisticola juncidis* | 7 | 6.36 | 0.35 | 122.11 | 28.89 | 0.292 | 4.5 | 4.09 | 0.141 | 78.53 | 21.21 | 0.153 |
| *Acrocephalus scirpaceus* | 10.5 | 9.55 | 0.52 | 208.19 | 41.97 | 0.424 | 16 | 14.55 | 0.5 | 317.19 | 56.35 | 0.405 |
| *Acrocephalus arundinaceus* | 1 | 0.91 | 0.05 | 55.15 | 16.56 | 0.167 | 1 | 0.91 | 0.031 | 55.15 | 16.56 | 0.119 |
| *Acrocephalus* sp. plur. | 11.5 | 10.46 | 0.57 | 263.34 | 58.53 | 0.591 | 17 | 15.46 | 0.531 | 372.34 | 72.91 | 0.524 |
| Tot | 20 | 18,18 | 1 | 418.09 | 98.89 | 1 | 32 | 29.09 | 1 | 679.83 | 138.98 | 1 |
| $H'_{ABB}$ | 1.05 | | | | | | 1.10 | | | | | |
| $J_{ABB}$ | 0.75 | | | | | | 0.79 | | | | | |
| $H'_{BIOM}$ | 0.99 | | | | | | 1.27 | | | | | |
| $J_{BIOM}$ | 0.72 | | | | | | 0.92 | | | | | |

a generalized increase in Cetti's warbler densities on a European continental scale is known (Robinson 2007), and this fact could affect our conclusions. Secondly, our coastal wetland is small and isolated. Therefore, effects due to habitat fragmentation on sensitive warblers (see for *Acrocephalus* spp.: Celada and Bogliani 1993; Paracuellos 2006; Benassi et al. 2009; Mortelliti et al. 2012) could affect our data. Thirdly, we carried out only a coarse-grained comparison between two years at the extreme of a relatively large time period. In this regard, it could be more useful to obtain data allowing to examine a more fine-grained year-by-year trends.

However, despite these limitations, to the best of our knowledge this is the first paper evidencing a strict linkage between reed-bed aging (following a fish farming abandonment) and a set of different species belonging a wetland specialized guild, where biomass emerged as an interesting sensitive metric. Therefore, we stimulate further studies to corroborate our first evidences about the role of these guild of reed-bed related species as indicators and as key attribute for its species biomass.

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