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AVIAN RESPONSES TO REED BED MANAGEMENT: A STUDY IN THE TORRE FLAVIA WETLAND (ROME, ITALY)

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Riassunto

Le zone umide, in particolar modo paludi, lagune e torbiere, offrono numerosi servizi ecologici alla popolazione umana ed ospitano una notevole biodiversità in ambito di specie animali e vegetali. In particolare, tra i vertebrati, un elevato numero di specie è rappresentato dagli uccelli, che frequentano ed utilizzano gli habitat di palude, che possono offrire risorse trofiche, protezione e siti idonei alla nidificazione. Tra gli uccelli maggiormente legati alle zone umide, sono presenti anche numerose specie migratrici, tra cui la cannaiola comune (*Acrocephalus scirpaceus*), il cannareccione (A*. arundinaceus*) ed il forapaglie castagnolo (*A. melanopogon*) che svernano in Africa (le prime due) o nell'area mediterranea (la terza) e, si riproducono nelle zone umide italiane e centro-nordeuropee caratterizzate da estesi canneti. La presenza di determinate specie di uccelli all'interno di un ecosistema palustre è strettamente legata alle caratteristiche dell'habitat, in particolar modo la struttura del canneto, che ne costituisce una componente tipica. Una corretta conservazione e manutenzione del canneto è di fondamentale importanza per la salvaguardia sia delle zone umide, che delle numerose specie legate all'habitat del canneto, molte delle quali minacciate a livello europeo e globale. All'interno del monumento naturale "Palude di Torre Flavia", la manutenzione e gestione del canneto è avvenuta a fine agosto 2022 tramite attività di sfalcio che hanno interessato circa il 25% dell'intero canneto. Durante i mesi primaverili, la ricrescita delle canne ha interessato le zone precedentemente sfalciate, portando alla formazione di un nuovo canneto, con caratteristiche strutturali differenti rispetto al canneto maturo. Se il taglio del canneto ha un effetto positivo sul mantenimento di questo specifico habitat nel lungo periodo, esso determina modificazioni dell'intero habitat che potrebbero influenzare la comunità di uccelli presente all'interno della riserva naturale, in particolar modo le specie legate al canneto maturo. Questa ricerca ha quindi come principali obiettivi: (i) la caratterizzazione della struttura degli habitat di canneto, (ii) il censimento delle specie di uccelli all'interno dell'intera riserva naturale e (iii) lo studio di eventuali risposte comportamentali da parte della comunità ornitica alle attività di gestione del canneto. I risultati ottenuti indicano la presenza di differenze sia nella struttura dell'intero canneto, comparata rispetto agli anni precedenti, che nelle caratteristiche strutturali tra il canneto maturo ed il canneto sfalciato. A livello di specie ornitiche, i risultati indicano come (i) il

canneto nuovo venga selezionato negativamente dalla maggior parte delle specie e, (iii) siano presenti differenze significative nel numero di record di quattro specie di uccelli all'interno delle due tipologie di canneto. Tuttavia, analisi più approfondite su specie particolarmente legate al canneto maturo, evidenziano come il numero di territori non differisce significativamente rispetto agli anni precedenti. Di conseguenza, le risposte alle attività di sfalcio del canneto devono essere interpretate multi-fattorialmente, tenendo conto dell'ecologia di ciascuna specie e delle possibili fluttuazioni nel numero di individui all'interno delle popolazioni.

Abstract

Wetland ecosystems host a high number of bird species, especially due to the reed bed habitats, that provide trophic resources and breeding sites. A correct management and conservation of the reed bed is essential to preserve both the bird wetland communities and wetland ecosystems. In the "Palude di Torre Flavia" natural monument, mowing activity were carried during September 2022 on a large reed bed area. Bird responses to the cutting activities are expected especially for the reed-bed related birds. To census the bird community was used a mapping method (Bibby et al., 2000) with a total of nineteen field visits (38 hours), and the collected records digitalized in QGIS 3.16.0 maps using a 2 meters buffers and centroids. The study area was stratified in five different sub habitats with QGIS 3.16.0 tools and, using the abundance of the species within each sub habitat, habitat preference analyses (i.e., habitat use and selection) were performed, focusing on reed beds. Also, the number of territories, guild, ecological parameters and both the Shannon-Wiener and evenness indices, were estimated for four reed bed species, and compared with previous sampling years results. Phenological analyses were made on bird censused species. Focusing on reed beds, data about the structure of both the entire and mowed reed bed were obtained quantifying several ecological parameters, such as stem density and diameter, within 25 randomly chosen plots. Comparison of the structural characteristics of the reed beds were made between different sampling years, and between mowed and mature reed bed.

1. Introduction

1.1 Wetlands ecosystems

Wetlands are important ecosystems providing several ecological functions (e.g., flood water control, nutrient retention, food web support; Baker, 2009) and services (e.g., Turner et al., 2000; Varin et al., 2019).

Despite the difficult of defining precisely what is and what constitutes a wetland, the Ramsar Convention (1971) definition is widely accepted worldwide by governments and NGOs (Turner et al., 2000) and consists in wetlands defined as 'areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt including areas of marine water, the depth of which at low tide does not exceed 6 m' (Ramsar Convention, 1971).

This definition acknowledges the habitat complexity that characterizes the wetland ecosystems, with both aquatic and land components (Turner et al., 2000). This habitat heterogeneity provides ecological niches for both animal and vegetal communities (e.g., Cronk & Fenessy, 2001; Kačergytė et al., 2021; Soultan et al., 2022), and services for human society (Fig. 1.1), such as fertility, nutrients for agriculture and water supply (Turner et al., 2000; Varin et al., 2019).

Despite the awareness about the importance of the wetland ecological services (Fig 1.1), globally losses have continued, especially because of the prevailing consideration that the services are considered to be of little or no value (or even negative), resulting in the ongoing destruction, modification, fragmentation, or loss of wetlands (Turner et al., 2000). In fact, wetlands are one of world's most important and threatened ecosystems (Verones et al., 2013; Hu et al., 2017), extensively reduced by human activities, especially in Europe, North America, and Asia (e.g., Mitsch & Gosselink, 2000; Verhoeven, 2014; Fluet-Chouinard et al., 2023). In Europe, 80% wetlands are estimated to be already lost (Verhoeven, 2014).

The loss and degradation of wetland ecosystems are caused mostly by changing in the land use, for agriculture and water drainage (Pfadenhauer & Grootjans, 1999; Turner, 2000), sea erosion (Paprotny et al., 2021), industrialization and drought as a response to climate change (Middleton & Kleinebecker 2012; Hu et al., 2017).

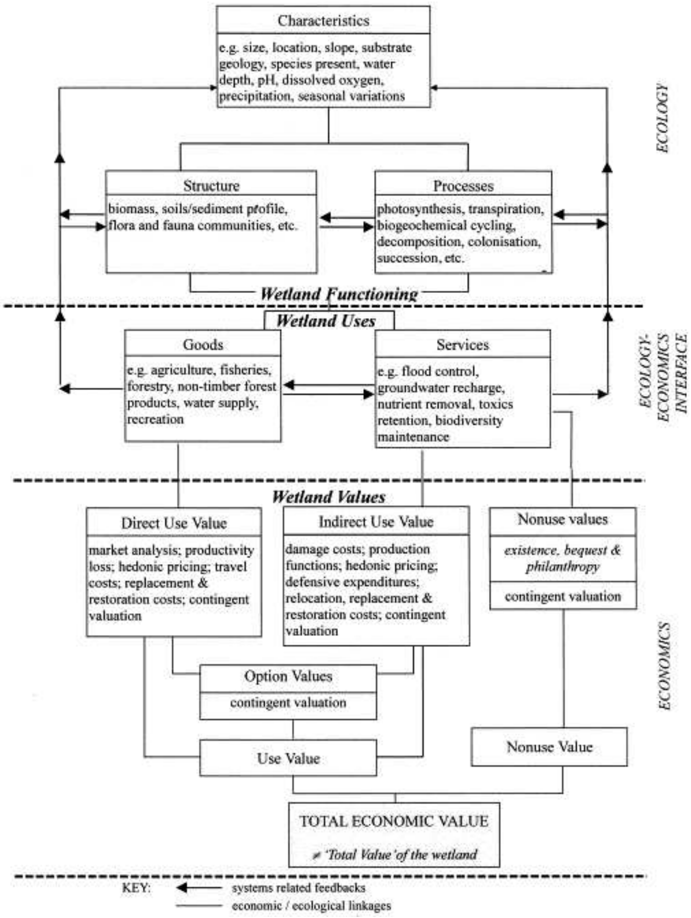


Fig. 1.1: Connections between wetland functions, uses, and values. (From Turner et al., 2000)

1.2 *Phragmites australis* reed bed

In Europe, wetland areas are widely dominated by monospecific stands of Common reed *Phragmites australis* (Schmidt et al., 2005; Mérő et al., 2017) and the presence of extensive stands can be observed especially in lakes, rivers, stagnant water, and artificial bodies (Bibby & Lunn, 1982). The phenology of the species can be divided in different phases: (i) the germination phase, in which new stems emerge from the rhizomes (or stolon) or by seed germination (Schmidt et al., 2005), (ii) the vegetative phase, in spring and early summer, in which both the new and old green shoots (or culms), grow, and achieve their highest relative growth rate (RGT) in April-May (Engloner, 2009), (iii) the reproductive phase with the seed dispersal

(Duncan et al., 2017) and (iv) the dormant phase of the plant during winter, or possible die and persist as dry matter (Fig. 1.2). The expansion of the stands is mainly described as a consequence of the asexual reproduction, by rhizomes or stolon (Engloner, 2009).

Contradictory results were obtained by numerous studies on *P. australis*, especially in the plant morphology changes during the vegetative phase (Engloner, 2009). Despite the different results, the overall structure of the *P. australis* reed bed change over the years, becoming less dense and with a higher number of dry stems. Also, the diameter stems are considered to became thicker over the years. (Ostendorp, 1993; Battisti et al., 2021).

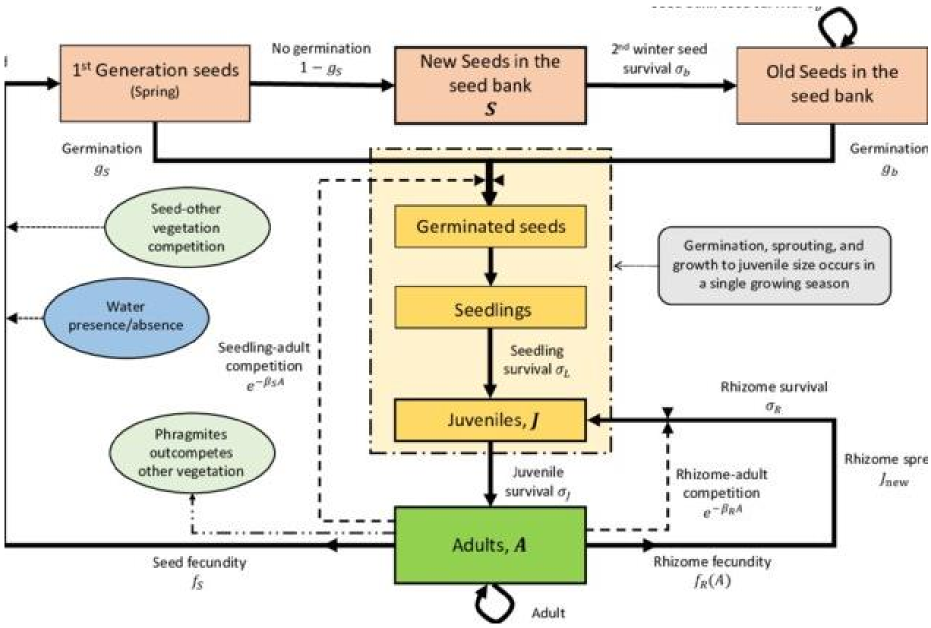


Fig. 1.2: Schematization of *P. australis* life cycle. (From Duncan et al., 2017)

1.3 Reed bed related birds

Focusing on animal species, *P. australis* reed beds host a high number of habitat specialist species, including insects, spiders, and birds, especially passerines (Ostendorp, 1993; Tscharntke, 1999; Schmidt, 2005) mainly because of the vegetation structure and food supply (e.g., Martínez-Vilalta et al., 2002; Poulin et al., 2002). Bird species are principally ecologically related with vegetation cover and water bodies (Paracuellos, 2006), especially for food (Schmidt et al., 2005) and for nesting and feeding sites during migratory or breeding periods (Bibby & Lunn, 1982)

The communities of the European water-related birds and related *to P. australis* are composed mainly by warbler species (Leisler & Schulze-Hagen, 2011), adapted to different stages of reed-bed succession (Martìnez-Vitalta et al. 2002; Battisti et al., 2021). In particular, two warblers of genus *Acrocephalus*, *A. scirpaceus* (Eurasian reed warbler) and *A. arundinaceus* (Great Reed warbler) are abundant in common reed beds (Martìnez-Vitalta et al. 2002; Poulin et al. 2002). Less common Acrocephalidae warblers are the sedge warbler, *A. schoenobaenus* and the Moustached warbler, *A. melanopogon*. Several studies about the breeding habitat selection by Acrocephalus warblers are found in the scientific literature (e.g., Poulin et al., 2002; Poulin & Lefebvre, 2002). Other bird species commonly found in wetland areas are *Cettia cetti* (Cetti's warbler), a more generalist edge reed bed species linked to ecotonal areas (Battisti et al., 2021), and *Cisticola juncidis* (Zitting cisticola) preferring rush bed habitats (Godinho & Rabaça, 2010). Among wetland related species, Anseriformes, Gruiformes and Pelecaniformes are also well represented in the bird community (e.g., Lemoine et al., 2007), using differently wetland habitats (Krapu et al., 2010), depending on their behavior or ecological characteristics (Paracuellos, 2006).

The future of reed beds and their birds is a crucial point for wetland conservations. Soultan et al. (2022) found that about 45 non-passerines wetland birds will reduce about 20% of their distribution range in 50 years. In 2021, many wetland bird species were classified as of concerning conservation status in Europe; for example, in the last IUCN Red List for Italian breeding birds, *A. melanopogon* and *A. schoenobaenus* have been classified as Vulnerable (VR) and Critically Endangered (CR), respectively (IUCN 2021).

To understand how to protect the wetland-bird species, it is necessary to study the relative importance of different ecological factors at a local and regional scales (Soultan et al., 2022). Studies of the wetland ecosystems have shown that several environmental factors are crucial for the wetland bird communities, such as the size of the wetland (Benassi et al., 2009), connectivity among wetlands (Moreno-Mateos et al., 2009), and structure of reed bed (Battisti et al., 2021). As example, *Ixobrychus minutus*, *A. scirpaceus* and *A. arundinaceus* are known as area-sensitive species (Baldi & Kisbenedek 1999), dependent on *P. australis* reed bed, and sensitive to the habitat loss and fragmentation (Paracuellos, 2006). Unfortunately, despite a good knowledge of most bird species ecology, comprehensive studies on the preference of the habitats by wetland's species are still scarce, especially within European reed bed stands (Šťastný & Riegert, 2021).

Famiglia	Specie	ssp	Nome comune	Categoria pop. Italiana 2012	Categoria pop. Italiana 2021
Acrocephalidae	*Hippolais polyglotta*		Canapino comune	LC	LC •
	Acrocephalus melanopogon		Forapaglie castagnolo	VU	EN
	Acrocephalus schoenobaenus		Forapaglie comune	CR	CR
	Acrocephalus palustris		Cannaiola verdognola	LC	NT
	Acrocephalus scirpaceus		Cannaiola comune	LC	LC
	Acrocephalus arundinaceus		Cannareccione	NT	NT

Fig. 1.3: Red list classification of the population of Acrocephalidae breeding species in Italy, in 2012 and 2021. (Modified from: Lista Rossa Uccelli Nidificanti in Italia 2021, https://www.iucn.it/liste-rosse-italiane.php)

1.4 Conservation and management of the reed bed

Habitat and ecosystem management can provide benefits to species, abundance, and population size (Ausden, 2007), and to entire communities (Mérő et al., 2017; Lehikoinen et al., 2017; Maphisa et al., 2017).

In wetlands, habitat management usually includes *P. australis* reed beds maintenances (Lougheed et al., 2008) which is mostly done by harvesting, grazing, burning, mowing, or flooding (Valkama et al., 2008). The effects of the habitat management depend on several factors, such as type, temporal frequency, spatial extent, and local intensity of the management actions (McCabe & Gotelli, 2000). The reed management activities can have an impact on both the structure of the reed bed, such as stronger regrowth of the reeds (Graveland, 1998) and on animal and plants reed bed related species, especially for their survival and conservation (Mérő et al., 2017).

The mowing activity is usually carried on in winter (Poulin & Lefebvre, 2002), to facilitate mechanical harvest for conservation or commercial purposes, such as thatching, building material and crops industry (Valkama et al., 2008). The direct effects of the mowing activity on animal species are studied for both vertebrates and invertebrates (e.g., Valkama et al., 2008). For invertebrates, the reed cutting might reduce the abundance of arthropod species, causing damage on all the trophic webs (Schmidt et al., 2005). Focusing on birds, the mowing activity prevents nestbuilding by breeding passerines (Poulin & Lefebvre, 2002; Poulin et al., 2002) due to the remotion of the dry reeds. In particular, Great reed warblers build nests in unburned parts or little changed areas of the reed bed (Mero et al. 2017). Other studies focused on how the mowing activity changed the trophic resources for reed bed birds (e.g., Schimdt, 2005), predation rate and number of individuals (Valkama et al., 2008).

Despite the wide scientific literature on the impact of mowing activities on wetlands birds, few information is available on how the management of the reed bed influences both the population parameters of reed-nesting songbird and more generally non threatened species (Mérő et al., 2017).

1.5 Research aims and hypotheses

The focuses of this research are at both reed bed and bird community level, in particular on the reed bed sub habitat and the possible effects of reed bed management on the bird community. The main research aims are: (i) the census of the bird community, (ii) the historical analyses of territories and ecological guild parameters of four reed bed related bird species, the characterization of (iii) the entire reed bed and (iv) mowed reed bed, (v) bird ecological analyses (use and habitat selection) with focus the on mature and mowed reed bed, and (vi) bird phenological analyses.

We expect that, due to the ageing of the reeds, the structure of entire reed bed has changed during the sampling years. Also, due the management activities, the new mowed reed bed should present different structural characteristics than the mature reed bed, especially in stem density and number of dry stems. At bird community level, the mowing activities (i.e., mowed reed bed) can possibly affect, either positively or negatively, the wetland bird community. In particular, mature reed bed related species (e.g., *A. scirpaceus*) will probably avoid mowed zones. Other less specialized reed bed birds, such as ecotonal or not-strictly wetland species, can be affected positively or unaffected by higher heterogeneity habitat within the study area.

2. Materials and methods

2.1 Study area

The study area is a twelve hectares zone included in the "Palude di Torre Flavia” natural monument, a protected wetland on the Tyrrhenian coast in central Italy (41°58'N; 12°03'E), situated between the municipalities of Cerveteri and Ladispoli (Rome, central Italy). In accordance with the Italian directive 79/409/EEC “Birds”, the protected area has been classified as a Special Protection Area (SPA) (SPA IT 6030020). Additionally, a Site of Community Importance (SIC), "Secche di Torre Flavia" (SCI IT 6000009; Dir. 92/43/EEC "Habitat"), is situated in the pelagic area, designated for the protection of *Posidonia oceanica*. The protected area, covering an area of 48 hectares, at a landscape level is a relic of a larger wetland, drained and transformed during the last century (Battisti, 2006), while at local level the natural monument hosts different habitats and key-species, including *Phragmites australis* reed-beds, rush habitats, flooded areas and channels previously used since 1938, and until 2004, for fish farming (*Anguilla anguilla*, *Mugil cephalus*, *Liza saliens*, and *Liza ramada*; Battisti et al., 2021). The water within the area is primarily of meteoric, sea storm origin, and from the inflow of surrounding areas (Battisti, 2006). The climate in this area is xeric-meso-Mediterranean (Blasi & Michetti, 2005).

The “Palude di Torre Flavia” natural monument is included in the network of Long-Term Ecological Research (LTER) managed by the Ministry of Agricultural, Food, and Forestry Policies. Activities such as birdwatching, environmental education, scientific bird ringing, and conservation projects, especially for *Charadrius alexadrinus*, and some dune plant species, are carried out periodically.

The *P. australis* reed bed within the study area represents a natural habitat that host several wetland bird species but is also strongly related to the human presence, as it reduces the visibility of the area, become invasive, especially on human pathways and obstruct water channels. Therefore, a good maintenance of the reed bed areas is necessary for the wealth of the entire wetland ecosystem, and for a sustainable and secure fruition of the reserve by humans. To allow the aging and growing of the *P. australis* reeds, no mowing, cutting, or burning reed bed activities were carried out since

2004. In late August 2022, due to the extremely invasive reed bed stands, approximately 15,000 m^2 of mature reed bed were mowed artificially, significantly changing the structure of the entire wetland. In the following spring, stems emerged within the previously mowed zones, characterizing the areas with new reed bed stands that exhibit a different structure than the mature reed beds.

Fig. 2.1: Photo of the "Torre Flavia" wetland. *P. australis* reed bed stands can be seen at the edge of the water channel.

2.2 Bird census and surveys methods

Depending on the aim of the research project, several methods are used to sample and census bird community (Bibby, 2003). First methods were described in late 1950s studies: moon watching (Tunmore, 1956), to study nocturnal migration of birds, a mapping method (Enemar et al., 1959), or comparison between different standard census methods (e.g., Taylor, 1965). Some of the most well-known bird survey methods are still based on the use of line transect and point transect, or count-points, (e.g., Mounir et al., 2022; Xu et al., 2022), which allow to standardize the study area, repeating the sampling multiple times to quantify and subsequently compare the results obtained at different times, which can be within the same day or, on a broader scale, different years (Gregory et al., 2004).

One of the differences between census methods and survey methods is that the firsts do not require correction for detectability; the search effort, number of visits and the recording units should be considered in the planning activity to possibly census every single observation during field sampling (Gregory et al., 2004).

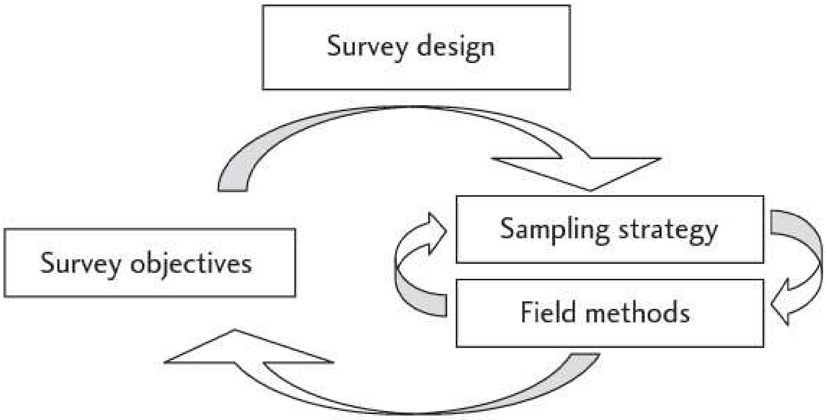


Fig. 2.2: Feedback loops between survey design, sampling strategy, field method and survey objectives. (From: Gregory et al., 2004).

One of the most used methods for bird censusing is the mapping method (Gregory et al., 2004), and is widely used to census the bird territory in a certain area (e.g., Lohmus, 2023) and to study bird ecology (e.g., Lopez et al., 2023). This method consists of periodic visits within the study area, annotating all the bird contacts on a paper map, handier during field works (Bibby et al., 2000). The field surveys should be made along pre-established paths, covering the entire area of interest, to obtain the maximum number of species contacts (Bibby et al., 2000; Gregory et al., 2004).

Other parameters need to be considered while planning the field visits. The sampling months, the day-time surveys and the type of bird contacts sampled (Bibby et al., 2000), depend mainly on the ecology of the bird sampled, while the walking speed, the number of visits, the number of sampling hours per survey, to standardize the work field (Gregory et al., 2004).

The mapping method is widely used to censusing birds; however, several limitations of this method are the observer's skill in recognizing bird species, the precise noting bird contacts on map, the detectability of bird species, and weather conditions (Bibby et al., 2000).

The results of mapping, when standardized to a single study area, could be compared if all variables (route length, travel time, bird species surveyed, the same type of contacts surveyed) were the same during each sampling year (Bibby et al., 2000; Gregory et al., 2004).

2.3 Field sampling

To census the bird community was used the previously described mapping method (Bibby et al., 2000) and to possibly compare the results, the mapping parameter were the same used in Battisti et al. (2021).

A total of 19 visits were carried out, each of two hours, between the end of March and the beginning of June. The choice of the sampling months was decided due to census the bird during the breeding period (March-June), when the birds are easier to contact, especially with territory songs.

Each visit covered the twelve hectares zone within the "Palude di Torre Flavia" natural monument. During every visit, in the first hours of the morning (between 06.00 a.m and 09.00 a.m., depending on the dawn time), the researcher would follow a 2,2 km non-linear transect, especially designed to cover all the study area and to possibly sample the highest number of birds record within all the different habitats.

All the visits were carried out in the morning hours just before dawn, because of the most active period of the birds during the entire day (e.g., Bibby et al., 2000, Jain, 2005).

Also, five established contact-points (41°57'41.58" N, 12°2'47''.30 E; 41°57'32.39" N, 12° 2'53.77" E; 41°57'39.46" N, 12°2'53.71" E; 41°57'37.97" N, 12°2'59.29" E; 41°57'33.70" N, 12° 2'58.43" E) were used to better sample the bird contact in both the dense reed bed, *Rubus ulmifolius* bushes and flooded areas. In each contact-points, the time spent was 10 min.

To avoid bias in the record sampling, the starting and ending points of each visit were randomly chosen with QGIS 3.16.0 tools (QGIS.org; qgis: randompointsinsidepolygons), along the non-linear transect. Furthermore, severe weather days, such as rainy or windy days, were avoided, due to lack of bird contacts and difficulties of obtaining data on field (Bibby et al., 2000).

Fig. 2.3: Satellite photo of the study area defined by the white line. The area is situated within the natural monument of "Palude di Torre Flavia", outlined by the orange line. Yellow dots are the contact points designed to cover all the study area. Source: Google Earth Pro (April 2022).

2.3.1 Bird data and analyses

To study the bird community was decided to collect direct contacts of bird species, classified as "sight", if the individual was seen during the visits, "song", when the researcher would hear the reproductive song of a species, and "call", when the bird would emit any non-reproductive vocal sign. Also, combination of two types of contacts (i.e., "sight-song", "sight-call", "song-call") were collected during the visits.

The choice of different type of records was decided due to the different ecology and behaviors of the bird species within the study area. As example, territorial, small, and elusive land species, such as *A. scirpaceus* and *C. cetti* would be mostly contacted with songs, while medium size, aquatic birds, as *F. atra*, with calls and sight records.

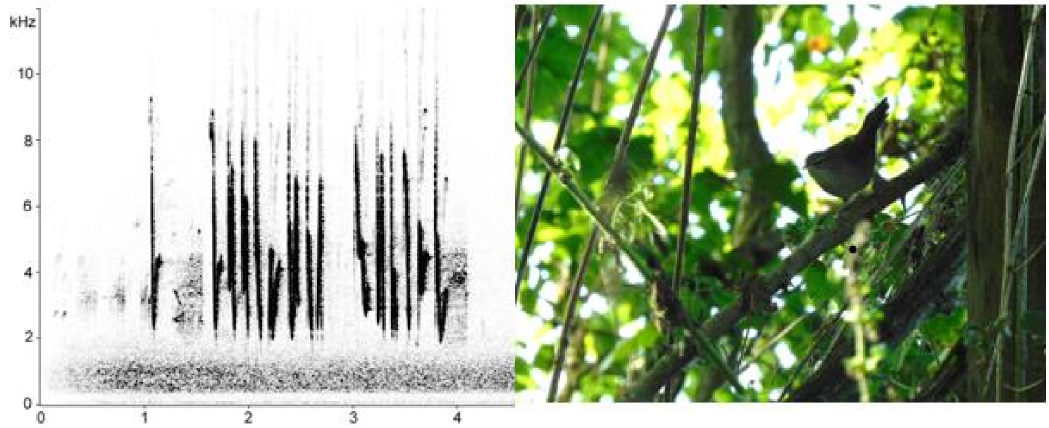


Fig. 2.4: Sonogram of *C. cetti* territorial song (left) and photo of the species (right). (Modified from: www.fssbirding.org).

To estimate the number of territories of four reed bed related species (*A. arundinaceus*, *A. scirpaceus*, *C. cetti* and *C. juncidis*), during every survey were noted on paper map bird intra-specific interactions, such as contemporary territorial bird songs (i.e., two song records collected at the same time and from different positions; See 2.5).

Following the same protocol described in Battisti et al. (2021), to obtain a fine-scaled geo-localization of the birds, records of birds flying higher than 25 meters were not collected during the field surveys. Also, all the contacts were recorded with the helping of GPS software (Google Earth Pro).

Due to the sensibility of the bird species to the water stress, the water level was considered during the experimental design and sampling were made when the average water depth (40-80 cm) was comparable during years (Battisti et al., 2021).

During every work visit, all the bird records were on the most recent available map (a satellite photo 1:1800, Google Earth April 2022); no March-June 2023 digital and paper maps (Google Earth, LANDSAT/Copernicus) were available during the entire sampling months.

Since no abrupt changes within the study area were registered in the previous years, the assumption of stability was used to consider equally in sizes and extension each sub-habitat, between April 2022 and 2023 sampling months, especially reed bed areas (i.e., reed bed stands, islands and reed bed edges).

Fig. 2.5: Comparison between satellite photos of the study area. June 2015 (up-left), August 2017 (up-right), July 2019 (down-left) and April 2023 (down-right). No changes within the area can be observed between the years, except for reed density and life-cycle phases of *P. australis* (i.e., different colors) within reed bed areas. (From Google Earth Pro).

After the work field, all the bird contacts were digitalized using QGIS Software 3.16.0. To better geolocate all data, as .raw files were used the same work-field map (Google Earth Pro April 2022), with the plugin "Quick Map Services" and (ii) two aerial photos of the study area, obtained by drones during June 2018 and 2023, appropriately georeferenced with ten GPS control points.

With the "New Shapefile" QGIS 3.16.0 tool, a total of 19 shapefiles were created, each one containing the bird records sampled during the respective field survey. Every shapefile contained a specific set of data; for each bird record was annotated the species ("id"), the contact type ("seen", "song" or "call" or a combination of both, "seen-song", "song-call" and "seen-call", and the respective set of GPS coordinates.

Since the species contacts collected during the work field have a certain degree of spatial uncertainty, during the digitalization of single bird record, was applied a 2 meters buffer and then calculated the centroid with QGIS 3.16.0 tools, respectively "Buffer" (ID: native:buffer) and "Centroids" (ID:

native:centroids). Only centroid GPS coordinates of each bird records were used for the all the bird and habitat analyses of this study.

Using all the digitalized data, were measured the total number of records per species, and the number of each record types per species. The total number of records per species was calculated by summing all the types of single species contacts. Combinations of two different record types (i.e., "sight-song", "sight-call" and "song-call") were counted as single bird record.

Also, using QGIS 3.16.0 tool "New map" and all the available bird digitalized records, for each bird censused species were created species-specific maps.

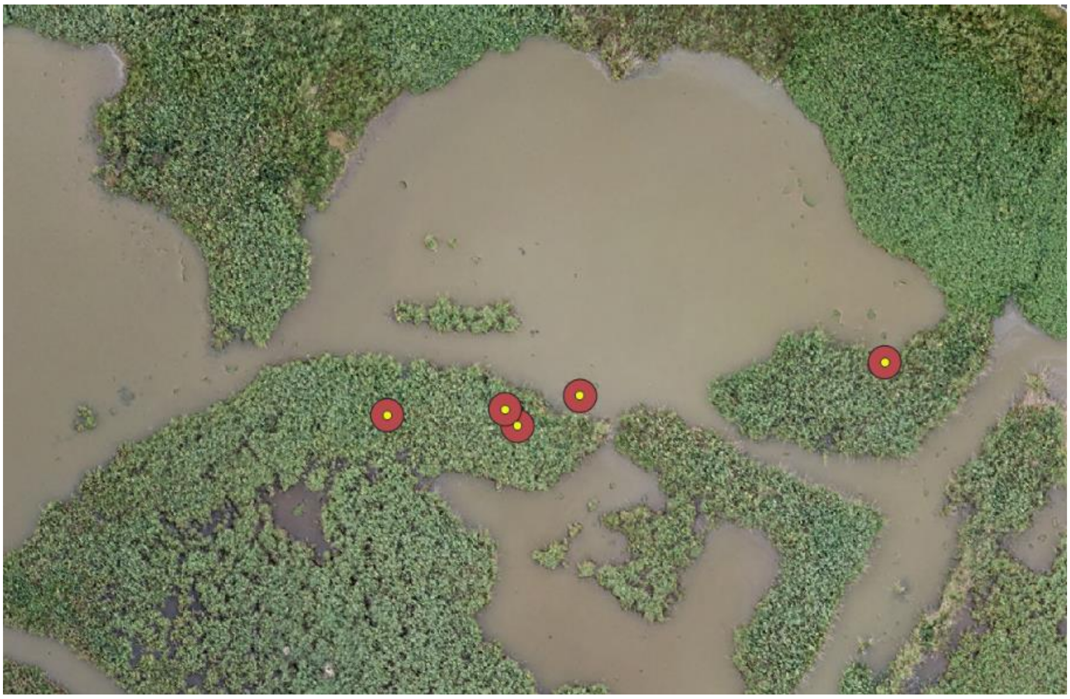


Fig. 2.6: Example of the digitalization of five bird records with the relatives 2-meters buffers (red areas) and centroids (yellow points). Only centroids coordinates were used for bird-habitat analyses.

2.4 Sub habitat characterization

To study and characterize the heterogeneity of wetland habitats, the sampling area was subdivided in five distinct sub habitats: "Mature reed bed" (MR), "Mowed reed bed" (MO), "Rush area" (RA), "Water area" (WA) and "Ecotone area" (EA).

“Mature reed bed” (MR) refers to reed bed areas that have not been subjected to cutting activities since 2004, while “Mowed reed bed” (MO) refers to the reed bed grown within previously mowed areas. “Rush area” (RA) are natural zones characterized by the dominant presence of *Juncus effusus*, “Water area” (WA) refers to basins and water channels that are periodically flooded during the spring season and “Ecotonal area” (EA) are areas characterized by the presence of various vegetational communities, with a predominance of *Quercus ilex* and *Rubus ulmifolius*.

The habitat characterization of the study area was made using QGIS 3.16.0 software and tools. As .raw files were used the same map and drone photos utilized for the digitalization of bird records (See 2.3.1).

To have a better habitat characterization were also used a vegetation map of the natural reserve (Guidi, 2008) and the 1:1500 map of the mowing activity carried out by “Città Metropolitana di Roma”.

Fig. 2.7: Particular of the different sub-habitats within the “Torre Flavia” wetland. In the photo can be noted the mature reed bed (dark green), *R. ulmifolius* bushes (light green), rush areas (brownish areas) and water areas. (A fine resolution drone 2018 photo).

Within QGIS layers, with the “New Shapefile” tool, a total of five shapefiles were created, each one containing the respective sub habitat areas, and adjusted with “Repair shapefile” tool.

To check the accuracy and better geo-localize the edges of each sub habitat, five field surveys were carried out during spring 2023, covering all the area utilizing GPS software (Google Earth Pro). All the shapefiles were then exported in Google Earth Pro and each sub habitat size was calculated as the sum of the respective areas.

Differences in the edges and extensions of the sub habitats were mitigated by (i) the large size of every sub habitat and (ii) no anthropogenic or natural habitat abrupt changes are registered within the study area during 2022-2023.

2.5 Bird Habitat preference

To study the bird habitat preference, the bird data were first skimmed: only species with more than 10 records were used for habitat analyses.

After the skimming process, all the species were then divided into two distinct groups: aquatic and land species. Due to ecological and physiological constraints, the land bird habitat analyses were made without considering WA.

The abundance of species was first obtained by counting the number of records (centroids) within each respective sub habitat, and then used to measure (i) the total number of records per sub habitat, (ii) the number of species recorded for each sub habitat and (iii) the number of records of each species per sub habitat. Also, using the abundance of each species, bird habitat-use and habitat selection were performed focusing on the MR and MO sub habitat.

Habitat-use analyses were made to observe differences in the use of single sub habitats by bird species: chi square analyses were performed with both single species records counted within each habitat and the total number of records.

Habitat selection analyses were performed to observe if a species is significatively selecting a particular sub habitat of the study area, performing chi square analyses, using both the total frequency of species records within each sub habitat, and the frequency of the respective sub habitat.

Habitat use and selection chi square analysis were made with Past 4.3.0. (Hammer et al., 2001)

To focus on the differences in the number of records between MR and MO, were selected species with more than 5 records within at least one reed bed habitats. The records were then normalized for the sub habitats size, and χ^2 analyses performed with PAST 4.3.0.

Fig: 2.8: Example of three *C. juncidis* records. All the records have been classified as RH contacts (RH = grey layer).

2.5.1 Feinsinger niche breadth index

To better study the sub habitat specialization of the species, was calculated the Feinsinger niche breadth index, also called Proportional Similarity Index, PS, or Czekanowski's index (Feinsinger et al., 1981). The index is commonly used (e.g., Elafri et al., 2017) to measure the differences between the frequency distribution of resources used by a population and the frequency distribution of resources available. The niche breadth index values range from 1.0, when a population is extreme generalist and uses resources in proportion to their availability, to near-zero values, when a population is extreme specialized exclusively on the rarest resource (Feinsinger et al., 1981).

Starting from the frequency of every bird species within each sub habitat and the frequency of the respective sub habitat, the niche breadth index (PS) was calculated as following:

$$PS = 1-0,5 \sum |pi-qi$$

In the formula, pi is the frequency of bird contact in each sub habitat (i.e., used resources) while the qi is the frequency of the sub habitat (i.e., the available resources). All the habitat frequencies were used to calculate the PS of the aquatic bird species, while WA frequency was not used for the land species.

2.6 Bird guild analyses

2.6.1 Cluster and territories

The number of territories of the four reed-bed related species were estimated using the clustering methodology described in Bibby et al. (2000), and the protocol used in Battisti et al. (2021). Those species are related to different age stages of *P. australis*. *C. juncidis* is linked to rush beds and early stages of the stems, *A. scirpaceus* and *A. arundinaceus* to the mature reed bed, while *C. cetti* on heterogeneous reed beds and shrubs (Battisti et al., 2021).

To compare the results with previous 2001 and 2019 studies, of the total number of visits 13 days were chosen randomly (26 hours of sampling). All the four species song contacts and the intra-specific (i.e., contemporary songs), sampled during each work field, were extrapolated for each visit. By using the bird data, species-specific maps were created and estimated both the number of clusters for each species and the number of effective territories.

For Bibby et al. (2000), to estimate a cluster by using bird records, two contacts for eight or less visits should be recorded, while three contact of a species for nine or more day of work field. Furthermore, two contacts of a bird should be registered at least ten days apart, due to avoid temporary migrants present in the study area only for few days. Cluster estimated by this method are the territory of the individuals of the bird species within the study area, and "territory" is the area in which a couple of bird is in reproductive phase (Bibby et al., 2000).

Following the previously described criteria, single clusters for each species were estimated using considering a minimum of three records and at least one contemporary songs.

To focus on clusters located within the study area, the final number of territories was obtained by giving 1 point to a territory totally inside in the study area, while 0,5 point to a territory within edge zones.

To observe possible trends, the number of territories of each species was compared with 2001 and 2019 results with χ^2 test, using Past 4.3.0.

2.6.2 Ecological parameters

Starting from the number of territories of the four bird species, were calculated the following parameters: (i) breeding pair density as a normalized measure of the abundance of the species, expressed as number of territories per 10 hectare, measured for each species (D) and for all the species (Dtot); (ii) the relative frequency for each species (frD) measured as the ratio normalized density of single species and the total density (D/Dtot); (iii) the consuming biomass for each species (g/10 ha), measured as Cb = Scb^0.7 (Salt, 1957) where Scb, or standing crop biomass, is the total body mass of all the individuals censed. This parameter is directly proportional to the energy removed from the bird individuals from the environment and directly linked to the individual size and the metabolism of the species (Salt, 1957).

To calculate the biomass values was used the mean body mass values of the species, obtained by data collected during the bird ringing activity at "Palude di Torre Flavia" natural monument (Sorace et al. 2015); and (v) the relative frequency of Cb of each species, calculated by the ratio: Cb/Cb tot.

Biomasses results were compared with previous studies made in 2001 and 2019 in Battisti et al. (2021) with χ^2 test, in Past 4.3.0.

Fig. 2.9: Photo of nets used for bird ringing activity within the "Palude di Torre Flavia" natural monument.

2.6.3 Shannon-Weaver index and evenness

To measure the diversity within the bird guild, composed by the four-reed bed related species, both the ecological Shannon-Weaver index (H'; Shannon and Weaver 1963) and the guild evenness (J; Lloyd e Ghelardi, 1964, Pielou, 1966; revision in Magurran 2004) were calculated.

The Shannon index is used to measure the diversity at different levels, from genes to entire ecosystems (Konopiński, 2020). At species level, the index considers both species richness (the number of species) and the number of individuals within each species.

The higher the index value, the greater the diversity within the community, due to greater richness of species and a more equal distribution of individuals. Instead, low index values indicate the presence of dominant species in terms of the number of individuals or a low total number of species. The Shannon-Wiener index was calculated as follow:

Shannon-Wiener index: $H' = -\Sigma fi \ln fi$

In the formula, fi is the frequency of the i-species. As fi, was used the standardized frequencies calculated on the breeding pair density (D).

The evenness index is typically used to the distribution of the individuals within a species community. The value of the evenness index can range from 0 to 1, depending on the distribution of individuals. Values around 0 and 1 indicate, respectively, an equal or unequal distribution of individuals within the community species (Jost, 2010). The evenness index is calculated as follows:

$$\text{Evenness index: } J = H' / H'max$$

In the formula, H' is the Shannon-Weiner index and H'max = ln S, where S is the number of species in the guild. In this study S = 4.

The Shannon-Weiner and evenness index were calculated with both standardized frequency of abundance and species biomass and compared with previous sampling 2001 and 2019 years (Battisti et al., 2021). Differences among the indices would indicate an increase or decrease in the diversity within the bird guild.

2.7 Phenological analysis

The phenological analyses were conducted using only bird song contact; total contacts would not provide information on which record changed over time and both sight contacts and calls are not specifically linked to seasonality. Species with less than 10 song contacts were excluded for the analysis.

To study the phenology of the species, the sampling days were divided in comparable groups, called decades. However, due to the different number of sampling days in each month (especially because of the severe weather in May 2023), the distribution of the days within each decade is not equally.

To obtain comparable groups, the days were divides in four decades: (i) the beginning of the mapping period and the end of March (24th, 28th, 29th, and 30th), (ii) first half of April (4th, 6th, 12th, 14th), (iii) late April days (18th, 19th, 20th, 27th, 28th), and (iv) all the days of May (5th, 9th, 10th, 15th, 24th). The last mapping day, June 1st, was not included in the last decades and not used for phenological analyses.

To normalize the number of days within the decades, was calculated the means of the song records of each decade and statistical analyzed using χ^2 test when the sum of the respective song contact means were > 5, while

Fisher exact test for sum of the means <5. All the statistical analyses were performed with Past 4.3.0.

2.8 Quantification of the reed bed structure

2.8.1 Reed bed within the study area

To characterize the structure of the entire reed bed was followed the same methodology used in Battisti et al. (2021): a total of 25 plots measuring 0.5 x 0.5 meters were randomly selected using QGIS 3.16.0 tool (ID: qgis:randompointsinsidepolygons), within the entire reed bed of the study area.

Random plots are a simplification and approximation of the area and should allow for the characterization of the entire reed bed habitat, avoiding excessive sampling effort.

In the initial reed bed study conducted in 2001, the selection the two main plot parameters, the number and extension, was primarily based on the type of the habitat and on the sampling effort: the reed bed is a fundamentally homogeneous habitat, and it was preferred to use numerous small plots rather than few larger ones. During the work field, to avoid the damaging of the reed bed and subsequently the nesting site for bird species, each plot was measured with a 0,5 x 0,5 meters square, made with plant residuals.

Within each plot, using professional calibers, field meter, and GPS devices (Google Earth Pro) a total of 11 reed bed structural parameters were quantified (Tab. 2).

To quantify the biotic characteristics of the *P. australis* reed bed, reeds were first divided in two groups: taller than 140 cm and shorter than 140 cm. The choice of the 140 cm threshold value was decided in the previous reed bed study (Battisti et al., 2021) both to the ease in the height and diameter reed measurements and a primarily distinction of the mature reeds, higher than 140 cm. Differences in the number of stems higher than 140 cm should be seen in the structure of the 2023 reed bed, compared to the previously sampling years due to the mowing activities.

Tab. 2. Reed bed ecological structural parameters.

	Reed bed structural parameters
Stems higher than 140 cm	N° stems
	Stem density
	N° green/dry stems
	Mean stem diameter at half height (cm)
	Mean stem diameter at 140 cm (cm)
Stem lower than 140 cm	N° stems
	Stem density
	N° dry/green stems
	Stem height (cm)
	Mean stem diameter at half height (cm)
	Vegetation cover
	Plot submerged

The total number of stems per plot was obtained by counting each stem individually, and to avoid double-counting, a distinctive mark was applied to each reed using a non-invasive marker. Subsequently, was observed whether each stem was in a vegetative state ("g", green) or not ("d", dry). For each stem higher 140 cm was calculated the diameter at 140 cm and at half-height, while for stems lower than 140 cm, the diameter at half-height and the total stem height, measured to the last reed internode.

Mean densities were calculated from the respective total number of stem groups and the mean stem diameters measured using the diameter value of each reed in both groups. Statistical analyses ANOVA and Kruskal-Wallis were performed with PAST 4.3.0. to observe differences in the stem diameters within each group.

For the two qualitative analyses, specifically related to the reed bed plots, "Vegetation Cover" and "Submerged plots" refer respectively to the presence of other macrophytes than *P. australis* ("1" if presence or "0" if absence) and the presence of water covering part or all the plot ("1" if presence or "0" if absence).

The values of the parameters of the entire reed bed that were compared with previous studies (Battisti et al., 2021) for 2001 and 2018 sampling years are: (i) the mean stem density and (ii) the mean stem diameter at 140 cm of reeds higher 140 cm. Those parameters reflect the total structure and aging of the reed bed; a thicker diameter of the stems and a lower density of the reeds are expected during the ageing of the reeds (Ostendorp, 1993). Comparisons were made using similar number of plots per sampling years, (24 for 2001 and 25 for 2018) and same sampling period. To observe differences between the sampling years, ANOVA and Kruskal-Wallis statistical tests were performed using PAST 4.3.0.

2.8.2 Mowed reed bed

To quantify the mowed *P. australis* reed bed in the study area were used 25 plots, measuring 0,5 x 0,5 meters, randomly chosen using QGIS 3.16.0 tools (ID: qgis:randompointsinsidepolygons), within the mowed reed-beds area. Within each plot, appropriately geolocated (Google Earth Pro), a total of seven variables were measured using: square 0,5 m x 0,5 m (See 2.6.1), professional calibers and field meters.

Within each plot, since the number of measurable stems was considerable and would have required significant sampling effort, to collect data was used the stratified randomization method (Aoyama, 1954), used in previous reed bed studies (e.g., Schmidt et al., 2005). Ten stems were randomly selected for the measure of three parameters: (i) the number of green/dry stems, (i) the half-height diameter of the stem, and (iii) the height of single stems (to the last internode).

Additionally, for each plot, 3 qualitative variables were quantified: (i) the presence of other macrophytes than *Phragmites australis* ("cop-veg"), which was scored as 0 or 1 respectively on presence or absence of vegetation, (ii) if the plot was submerged or not ("plot-water"), scored as 0 (water absence) or 1 (water presence), and if within the plot were measured > 40 stems (1) or not (0). The threshold value was given due to the mean stem number measured within the majority of the plots.

For the qualitative variables were calculated the percentage of plots with different vegetation cover, submerged and with more than 40 stems.

The mean half height diameter and mean stem height were calculated using all the stem values. To observe differences within each plot, statistical analyses ANOVA and Kruskal-Wallis were performed on both the parameters made with Past 4.3.0.

Fig. 2.10: Photo of the mowed reed bed within the study area. Can be noted the high density of the green stems and the total homogeneity of the sub-habitat.

2.8.3 Mature and mowed reed bed comparison

The comparison between the mature and the mowed reed bed within the study area was important to observe differences in the structure the two habitats. Due to the previously described quantification methods (See 2.8 and 2.9), to compare the mature reed bed and the mowed reed bed were used 10 plots for each habitat. From the 25 plots used to quantify the structure of the entire reed bed of the study area, 10 random plots were selected from the total number of mature reed bed plots, using the before-made sub habitat characterization (MR). For the mowed reed bed, 10 plots were chosen randomly from the 25 plots used to quantify the same sub habitat.

Due to the number of stems (10) measured within each plot representing the mowed reed bed, ten stems were randomly selected from the total stems of each plot representing the mature reed bed. Comparisons were between 100 stems of both the mature and the mowed reed bed, 10 for each plot of the two groups.

To study the differences of the two habitats, were compared the following parameters: (i) number of total green stems, (ii) number of total dry stems (iii) Mean diameter of the stems at half-height, (iv) number of plots with n stems > 40, (v) number of plots with vegetation cover different than *P. australis* and (vi) number of submerged plots.

For the qualitative variables were compared the percentages of both plots and stems, while to measure differences in the mean stem diameter, ANOVA analysis was performed using PAST 4.03.

3. Results

3.1 Bird mapping

During the 19 visits carried out in the study area, a total of 21 bird species were censused (Tab. 3.1): *A. arundinaceus* (Great reed warbler), *A. melanopogon* (Moustached warbler), *A. scirpaceus* (Eurasian reed warbler), *Anas platyrhynchos* (Mallard), *Carduelis carduelis* (European goldfinch), *C. cetti* (Cetti's warbler), *Chloris chloris* (European greenfinch), *C. juncidis* (Zitting cisticola), *Cygnus olor* (Mute swan), *Fringilla coelebs* (Eurasian chaffinch), *Fulica atra* (Eurasian coot), *Galerida cristata* (Crested lark), *Gallinula chloropus* (Common moorhen), *Motacilla flava* (Western yellow wagtail), *Parus major* (Great tit), *Rallus aquaticus* (Water rail), *Saxicola rubicola* (European stonechat), *Serinus serinus* (European serin), *Curruca melanocephala* (Sardinian warbler), *Tachybaptus ruficollis* (Little grebe) and *Turdus merula* (Common blackbird).

The species are classified in 4 Orders (Anseriformes, Gruiformes, Passeriformes, and Podicipediformes) and 13 Families (Acrocephalidae, Alaudidae, Anatidae, Cettidae, Cisticolidae, Fringillidae, Motacillidae, Muscicapidae, Paridae, Podicipedidae, Rallidae, Sylviidae and Turdidae).

Tab. 3.1. Censused bird species and the respective total number of contacts.

Order	**Family**	**Species**	**N° rec.**
Anseriformes	Anatidae	*Anas platyrhynchos*	139
Anseriformes	Anatidae	*Cygnus olor*	2
Gruiformes	Rallidae	*Fulica atra*	360
		Gallinula chloropus	48
		Rallus acquaticus	1
	Alaudidae	*Galerida cristata*	2
	Acrocephalidae	*Acrocephalus arundinaceus*	5
		A. melanopogon	13

Passeriformes		*A. scirpaceus*	120
	Cettidae	*Cettia cetti*	574
	Cisticolidae	*Cisticola juncidis*	135
	Fringillidae	*Carduelis carduelis*	3
		Chloris chloris	28
		Fringilla coelebs	2
	Fringillidae	*Serinus serinus*	24
	Motacillidae	*Motacilla flava*	1
	Muscicapidae	*Saxicola rubicola*	1
	Paridae	*Parus major*	23
	Sylviidae	*Curruca melanocephala*	52
	Turdidae	*Turdus merula*	23
Podicipediformes	Podicipedidae	*Tachybaptus ruficollis*	81

For the 21 species, a total of 1639 bird contacts were sampled, divided in 943 song records (57,5%), 661 visual records (40,4%) and 35 calls (2,1%) (Fig. 3.1). The most contacted species are *C. cetti* (574) *F. atra* (360), *A. platyrhynchos* (139), *C. juncidis,* (135)*, A. scirpaceus* (120) and *T. ruficollis* (81) representing the 86,1% of total records (1409).

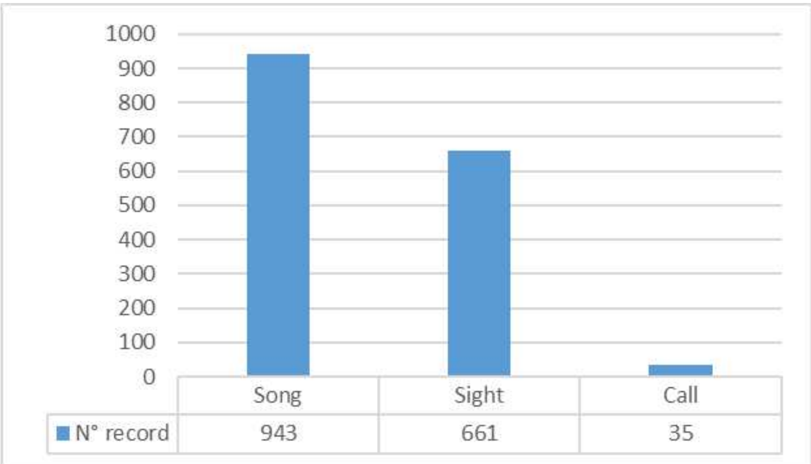


Fig. 3.1: Total number of records divided in the three types.

The higher number of sight records were collected for all the aquatic bird species (Tab 3.2), with a predominance of *F. atra* records (340), followed by *A. playrynchos* (139), *G. chloropus* (45) and *T. ruficollis* (32). For the song records, the species most recorded are prevalently land species, with a high number of *C. cetti* records (559), followed by *C. junicidis* (121), *A. scirpaceus* (105) and T. ruficollis (49). For the last record type, a higher number of calls were recorded only for *F. atra* (20) and *P. major* (6). The bird records, divided in types and for each sampling days, are shown in Supplementary Data Tab. 1, 2, and 3, respectively for sight, songs, and call records.

Tab. 3.2. The number of records per species, divided by each type.

Species	**Sight record**	**Song record**	**Call record**	**TOT**
A. arundinaceus	4	1	0	5
A. melanopogon	1	12	0	13
A. scirpaceus	15	105	0	120
A. platyrhynchos	139	0	0	139
C. carduelis	0	3	0	3
C. cetti	15	559	0	574
C. chloris	4	22	2	28
C. juncidis	14	121	0	135
C. melanocephala	11	37	4	52
C. olor	2	0	0	2
F. coelebs	2	0	0	2
F. atra	340	0	20	360
G. cristata	0	2	0	2
G. chloropus	45	0	3	48
M. flava	1	0	0	1
P. major	10	9	6	25
R. acquaticus	1	0	0	1
S. rubicola	1	0	0	1
S. serinus	2	22	0	24
T. ruficollis	32	49	0	81
T. merula	23	0	0	23

3.2 Sub habitats

The extension of the entire study area, calculated with GIS 3.16.0 analyses, is 120,574 m^2, (approximately 12 hectares), while the five sub habitats have respectively an extension of 43,177 m^2 (35,8%) for mature reed bed (MR), 38,397 m^2 (31,7%) for water areas (i.e., water channels and pounds; WA), 16,286 m^2 (13,51%) for rush areas (RA), 14,696 m^2 (12,5%) of mowed reed bed (MO), and finally, 8,018 m^2 (6,6%) of ecotonal areas (EA) (Table 3.3).

The sub habitat frequencies calculated without WA are 0.52, 0.18, 0.20 and 0.1 respectively for MR, MO, RA, and EA sub habitats.

Tab. 3.3. Sub habitat sizes and frequencies.

Sub Habitat	MR	MO	WA	RA	EA	Tot
Sub Habitat size (m^2)	43177	14696	38397	16286	8018	120574
Area proportion	0.358	0.125	0.317	0.133	0.067	1
Area proportion without WA	0.524	0.183	-	0.195	0.098	1

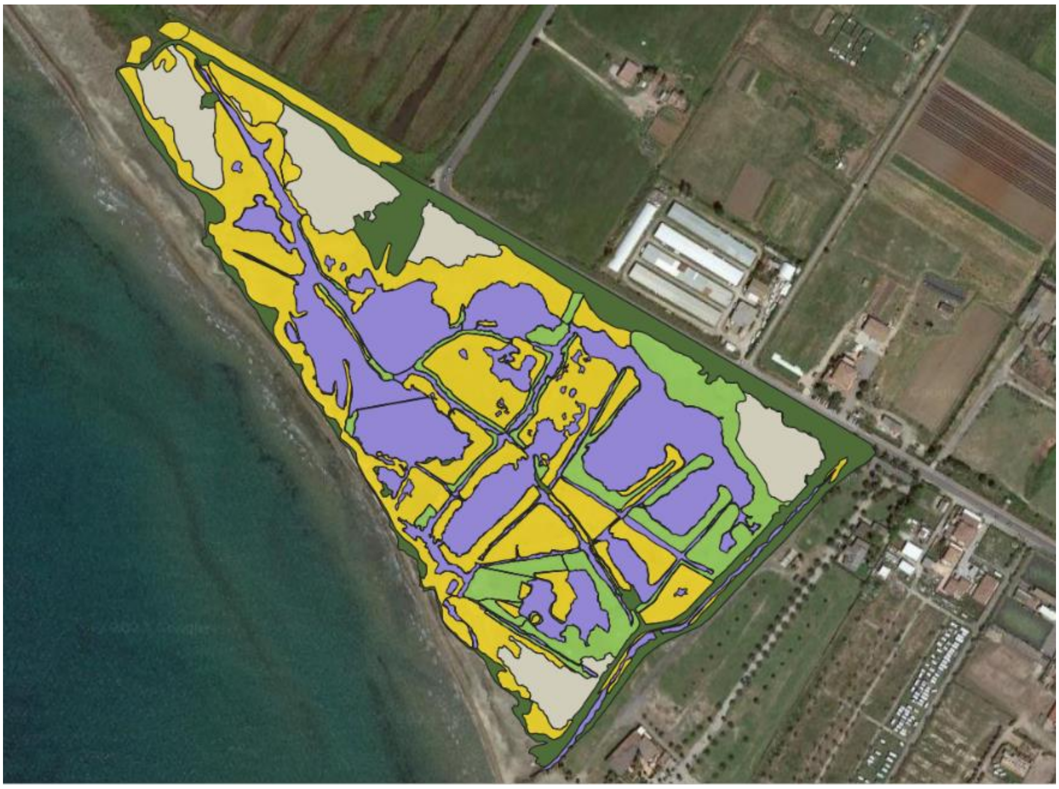
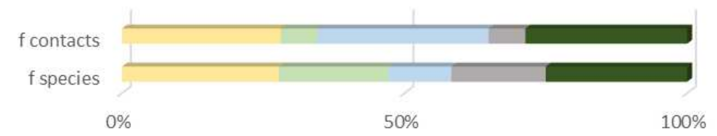


Fig. 3.2: The results of the sub habitat characterization of the twelve hectares study area. Yellow, light green, dark green, blue, and grey respectively represent the mature reed bed (MR), the mowed reed bed (MO), rush areas (RA), water areas (WA) and ecotonal areas (EA).

3.3 Bird habitat analyses

Of the total number of species censused, habitat preferences analyses were conducted for 13 bird species, 8 classified as land species, *A. melanopogon, A. scirpaceus, C. cetti*, *C chloris, C. juncidis*, *P. major*, *S. serinus*, *C. melanocephala* and *T. merula*, and 4 aquatic species *A. platyrhynchos*, *F. atra*, *G. chloropus* and *T. ruficollis*.

The greatest number of species were contacted in MR (10), followed by EA (9), MO (7), RA (6) and WA (4). Considering the total number of bird contacts (1622), 491 were in WA, 464 in EH and 455 in MR. The two habitats with the lowest number of records were RA and MO, with the same number of records, 106 (Fig 3.4).



	f species	f contacts
MR	0,278	0,281
MO	0,194	0,065
WA	0,111	0,303
RA	0,167	0,065
EA	0,25	0,286

Fig. 3.4: Frequencies of the bird contacts and species within each sub habitat.

For land species, a total of 994 records were collected and the habitats with the highest number of total observations were in EA (463) and MR (392), followed by RA (105) and MO (32). For aquatic species total observations were 628, distributed mainly in WA (491), MO (74), MR (61) and in RH (1) and EA (1) with few contacts (Fig 3.5). Zitting cisticola and Cetti's warbler are the land species with records within the greatest number of sub habitats (4; Tab 3.4), while for aquatic species, the Mallard and the Little Grebe were those distributed across the largest number of sub habitats (4).

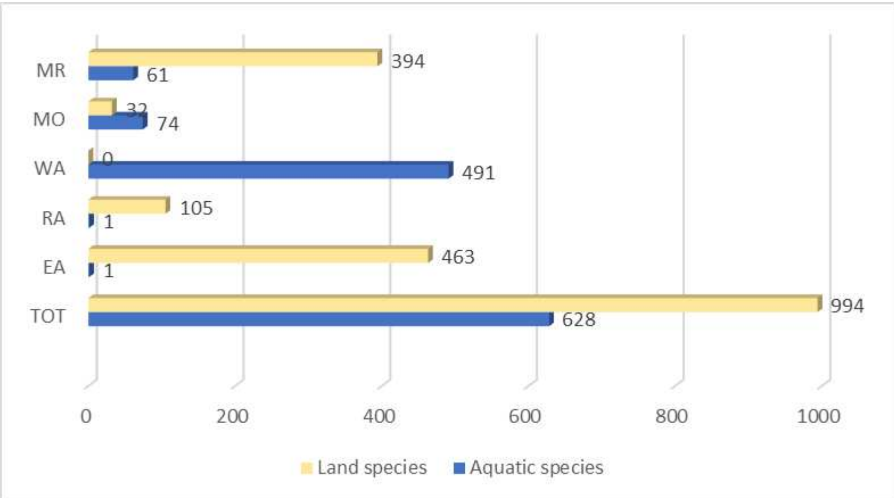


Fig. 3.5: Number of total contacts for both land and aquatic species within each sub habitat.

Table 3.4. Type and number of habitats in which were contacted land and aquatic species.

Land species	**Habitat type (without WA)**	**TOT Hab.**
A. melanopogon	MR	1
A. scirpaceus	MR, MO	3
C. cetti	MR, MO, RA, EA	4
C. chloris	EA	1
C. juncidis	MR, MOR RA, EA	4
P. major	RA, EA	2
S. serinus	EA	1
C. melanocephala	MR, MO, EA	3
T. merula	MR, EA	2
Aquatic species	**Habitat type (with WA)**	**TOT Hab.**
A. platyrhynchos	MR, MO, WA, RA	4
F. atra	MR, MO, WA	3
G. chloropus	MR, MO, WA, EA	4
T. ruficollis	MR, MO, WA	3

Focusing on each land and aquatic species (Tab. 3.5), the Moustached warbler contacts were distributed exclusively in MR (13); for Eurasian reed warbler the highest number of records were collected within the same habitat (MR; 109). The highest number of contacts for the Greenfinch (28), European serin (24), Great tit (24), and the Common blackbird (20) were collected within EA. Cetti's warbler records were sampled mainly between EA (314) and MR (217), while for the Zitting cisticola, mostly in RA (78). For all the aquatic species, most of the records were sampled within habitat WA: 300, 105, 54 and 32, respectively for the Common Coots, Mallard, Little Grebe, and the Common Moorhen.

Tab. 3.5. Total records of species per habitat.

Species	**MR**	**MO**	**WH**	**RA**	**EA**	**Tot**
A. melanopogon	13	0	0	0	0	13
A. scirpaceus	109	10	0	0	1	120
A. platyrhynchos	14	19	105	1	0	139
C. cetti	217	19	0	24	314	574
C. chloris	0	0	0	0	28	28
C. juncidis	45	3	0	78	9	135
F. atra	22	38	300	0	0	360
G. chloropus	7	8	32	0	1	48
P. major	0	0	0	1	24	25

S. serinus	0	0	0	0	24	24
C. melanocephala	7	0	0	2	43	52
T. merula	3	0	0	0	20	23
T. ruficollis	18	9	54	0	0	81
Total	455	106	491	106	464	1622

Results of the land (Supplementary data Tab. 4) and aquatic (Supplementary Data Tab. 5) bird habitat use analyses indicate that all the species are differentially using the 5 different sub habitats within the study area: the p-values are significant ($p < 0.001$) for all the bird land and aquatic species under study. Therefore, not all the habitats are used equally by the species: reflecting how each species is ecologically associated with specific habitats and how the distribution of records is not equal for every sub habitat.

The MR and MO habitat selection analyses for land bird species are shown in Tab 3.6, while the analyses for all the sub habitats can be seen in Supplementary Data Tab. 3. P-values are not significative only for RH contacts of *P. major, T. merula* and *S. serinus*, indicating the non-selectivity of the sub habitat by the three species.

For aquatic species, the results of the two reed bed selection analyses iare shown in Tab. 3.7, while all the other habitat analyses in Supplementary Data Tab. 4. P-values are not significative for all the aquatic species in MO, and *G. chloropus* in EA.

Tab 3.6. Land bird selectivity for mature reed bed (MR) and mowed reed bed (MO).

	MR			**MO**		
Land species	Fr	χ^2	p	fr	χ^2	P
A. melanopogon	1	11.741	< 0.001	0	2.613	0.093
A. scirpaceus	0.908	70.468	< 0.001	0.083	7.446	< 0.05
C. cetti	0.378	49.634	< 0.001	0,033	82.804	< 0.001
C. chloris	0	30.987	< 0.001	0	6.097	< 0.05
C. juncidis	0.333	19.941	< 0.001	0.022	22.536	< 0.001
P. major	0	27.668	< 0.001	0	5.444	< 0.05
S. serinus	0	26.562	< 0.001	0	5.226	< 0.05
S. melanocephala	0.135	31.827	< 0.001	0	11.323	< 0.001
T. merula	0.130	14.386	< 0.001	0	5.009	< 0.05

Tab. 3.7. Aquatic bird selectivity for MR and MOR.

	MR			**MO**		
Aquatic species	Fr	χ^2	P	fr	χ^2	P
A. platyrhynchos	0.101	40.026	< 0.001	0.137	0.284	0.594
F. atra	0.061	137.87	< 0.001	0.106	0.894	0.344
G. chloropus	0.146	9.406	< 0.05	0.167	0.899	0.343
T. ruficollis	0.222	6.502	0.010776	0.111	0.088	0.767

For the comparison between normalized bird records respectively in MR and MO, analyses were made on 9 bird species. Significant differences in the frequency between the two reed bed habitats were observed for 4 species: *A. melanopogon*, *A. scirpaceus, C. cetti* and *C. junicidis* (Tab 3.8).

Tab. 3.8. Statistical analyses of bird records in MR and MO.

	MR	**MO**		
Species	N° record	N° record	χ^2	P
A. platyrhynchos	14	19	0,871	0,351
A. melanopogon	13	0	3,693	0,055
A. scirpaceus	109	10	19,633	< 0,001
C. cetti	217	19	40,821	< 0,001
C. juncidis	45	3	8,954	< 0,05
F. atra	22	38	1,248	0,264
G. chloropus	7	8	0,358	0,550
C. melanocephala	7	0	1,264	0,261
T. ruficollis	18	9	1,515	0,218

3.3.1 Feinsinger indices

The Feinsinger niche breadth index (PS) was calculated for 13 bird species (Tab. 3.9). Lowest values of the index for land species were obtained for *C. chloris* (0.10), *S. serinus* (0.10), *T. merula* (0.23) and *C. melanocephala* (0.27). Species that show intermediate values of PS are *C. juncidis* (0.46), *P. major* (0.52), *A. melanopogon* (0.53), *C. cetti* (0.55). Among land species, the higher value of PS was calculated for *A. scirpaceus* (0.72). Among aquatic species, *F. atra* is the most selective species, with a PS of 0.46, followed by the intermediate values of *A. platythrynchos* (0.50), *G. chloropus* (0.52), and *T. ruficollis* (0.62).

Tab. 3.9. Feinsinger niche bredth index (PS) calculated for each bird species.

Species	PS
A. melanopogon	0.53
A. scirpaceus	0.72
A. platyrhynchos	0.50
C. cetti	0.55
C. chloris	0.10
C. juncidis	0.46
F. atra	0.46
G. chloropus	0.52
P. major	0.52
S. serinus	0.10
C. melanocephala	0.27
T. merula	0.23
T. ruficollis	0.62

3.4 Guild analyses

3.4.1 Cluster and territories

During the mapping of the study area, a total of 856 song records of the four reed-bed related species were collected. Of the total number of records, 605 (70,7%) were observations of *C. cetti*, 126 (14,7%) of *A. scirpaceus,* 120 (14%) of *C. juncidis*, and 5 (0,6%) of *A. arundinaceus* (Tab 3.10).

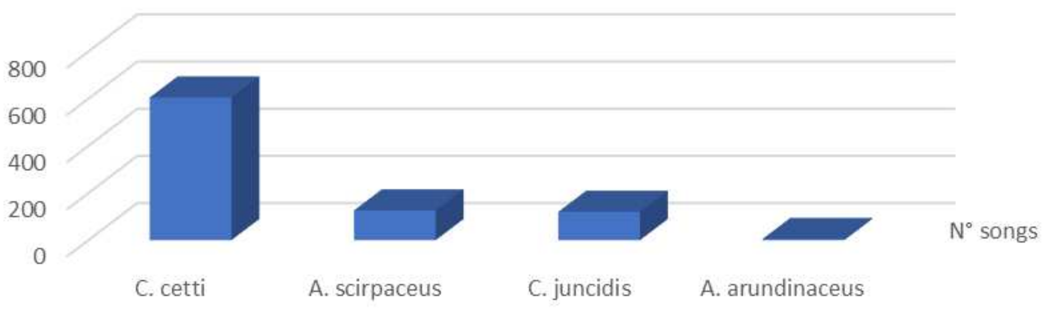


Fig. 3.6: Total song contacts of the four reed-bed related species.

Each species shows a particular trend of the number of song contacts during the breeding season (See 3.5). However, for the Great reed warbler, due to the limited data availability, it was not possible to confirm an increase in the number of records between different days.

Of the total number of song contacts, 527 records were used to estimate the number of territories (Tab. 3.10) and 129 of them were classified as simultaneous songs.

For the Eurasian Reed Warbler, based on 65 records, including 45 simultaneous ones, 15 clusters were estimated, 14 located entirely within the study area. Cetti's Warbler clusters, using 387 contacts and 62 simultaneous songs, were estimated to be 10, with only 1 at the edge of the study area. The Zitting cisticola, with a total of 80 records, including 22 simultaneous ones, had 3 estimated clusters, with 2 of them within the study zone. Finally, the Great Reed Warbler, based on only 3 records and no calls or inter/intraspecific interactions, was present in only one territory. Therefore, the total number of clusters is 31, calculated based on 129 simultaneous calls out of 535 records, resulting on a total score of 27.5 territories.

Tab. 3.10. Bird song records, clusters, and territories.

Species	**N° songs**	**N° contemporary song**	**N° clusters**	**N° territories**
C. juncidis	80	22	3	2.5
A. scirpaceus	65	45	15	14.5
A. arundinaceus	3	0	1	1
C cetti	387	62	10	9.5
Total	527	129	31	27.5

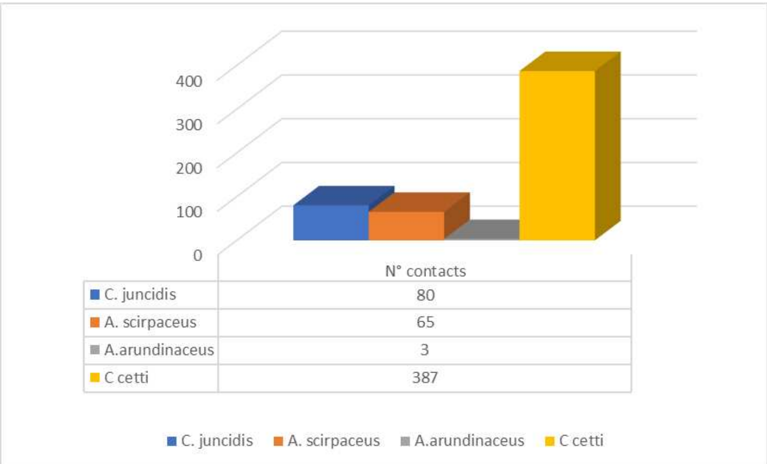


Fig 3.7: Number of song contacts for each guild species.

3.4.2 Ecological analyses

The normalized territories (i.e., territories for 10 ha), and the consuming biomass for each species is shown in Tab. 3.11. The estimated normalized territories C*. juncidis* are 2.28, for *A. scirpaceus* 13.18, 0.91 for *A. arundinaceus* and 8.64 for *C. cetti,* for a total of 25 bird species territories. The consuming biomass is 14.06, 52.59, 16.55, and 41.84 g/10 ha for C*. juncidis, A. scirpaceus, A. arundinaceus and C. cetti* respectively.

At the guild-level, The Shannon index and evenness calculated on species territory frequencies and consuming biomass are respectively 1.04 and 0.75 for territories, while 1.24 and 0.9 for biomasses (Tab 3.12).

Tab. 3.11. Ecological analyses of the bird gild.

Species	**N**	**D**	**frD**	**Scb**	**Cb**	**frCb**
C. juncidis	2.5	2.28	0.09	43.64	14.06	0.11
A. scirpaceus	145	13.18	0.53	237.36	52.59	0.42
A. arundinaceus	1	0.91	0.04	55.09	16.55	0.13
C. cetti	9.5	8.64	0.35	207.27	41.84	0.33
Total	27.5	25	1	593.37	125.04	1

Tab 3.12. Shannon index (H') and evenness (J) calculated for the bird guild.

H' Abb	1.04
J Abb	0.75
H' Biom	1.24
J Biom	0.90

The total number of territories has changed from 20 in 2001, 32 in 2019 (+60%) and 27.5 in 2023 (-14,06%; Tab. 3.13). For two species, the Eurasian reed warbler and Cetti's warbler, the higher number of territories were in 2019. For *A. scirpaceus*, 10.5 territories were estimated within the study area in 2001,16.5 in 2019 and 14.5 in 2023, while for *C. cetti,* 1.5 in 2001, 10.5 in 2019 and 9.5 in 2023 (Fig. 3.4). The Zitting cisticola showed a negative trend in the number of territories, 7 in 2001, 4.5 in 2019 and 2.5 in 2023, and

Finally, the single territory of the Great reed warbler has not changed during the three years of study. Comparing the number of standardized territories of each species between the three study years, no significative differences were observed in the number of territories (Supplementary Data Tab. 5). No significative differences were also observed for the 2019-2023 comparison (Tab. 3.14).

Tab 3.13. Comparisons of the bird guild parameters in the different years.

	Species	N°	D	frD	Scb	Cb	frCb
	C. juncidis	2.5	2.28	0.09	43.64	14.06	0.11
2023	*A. scirpaceus*	14.5	13.18	0.53	287.36	52.59	0.42
	A. arundinaceus	1	0.91	0.05	55.15	16.56	0.13
	C. cetti	9.5	8.64	0.35	207.27	41.84	0.33
	Total	27.5	25	1	593.42	125.05	1
	C. juncidis	4.5	4.09	0.14	78.53	21.21	0.15
2019	*A. scirpaceus*	16	14.55	0.5	317.19	56.35	0.41
	A. arundinaceus	1	0.91	0.03	55.15	16.56	0.12
	C. cetti	10.5	9.54	0.33	228.96	44.86	0.32
	Total	32	29.09	1	679.83	138.98	1
	C. juncidis	7	6.36	0.35	122.11	28.89	0.29
2001	*A. scirpaceus*	10.5	9.55	0.52	208.19	41.97	0.42
	A. arundinaceus	1	0.91	0.05	55.15	16.56	0.17
	C. cetti	1,5	1.36	0.074	32.64	11.47	0.12
	Total	20	18.18	1	418.09	98.89	1

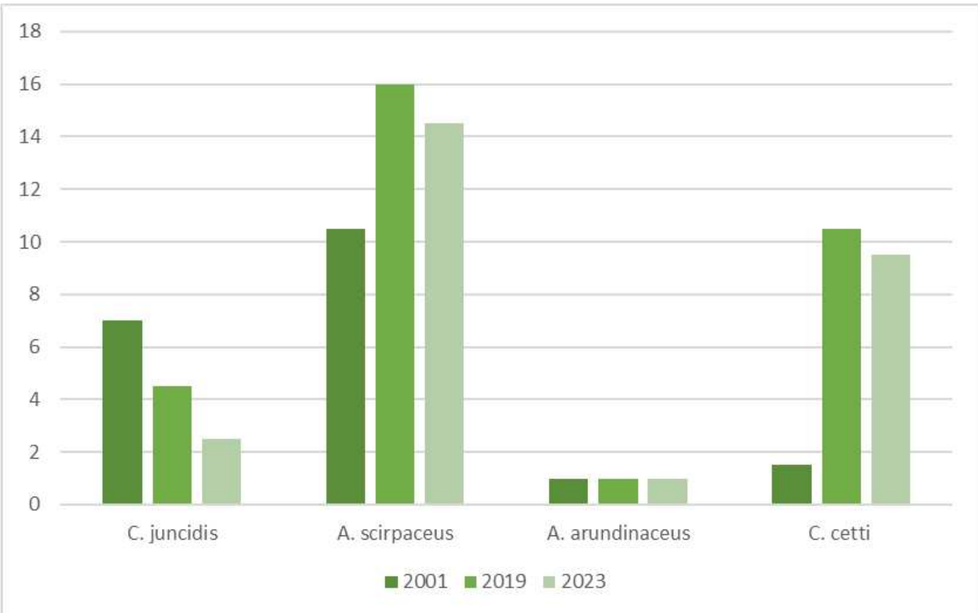


Fig. 3.8: Comparison between the number of territories of each species, in the three sampling years (2001, 2019, 2023).

The total consuming biomass (Cb) of the gild species, changed during the three years: 98.89 in 2001, 138.98 in 2019 and 125.05 in 2023 (Supplementary Data Tab. 6). Considering the consuming biomass of all the years, statistical differences were found for two of the four gild species: Eurasian reed warbler and Cetti's warbler. Nonetheless, focusing on the comparison between 2019 and 2023, no statistical differences are observed in the consuming biomass for the species (Tab. 3.14.).

Tab. 3.14. Comparisons between D and Cb of 2019 and 2023.

Species	**D** χ^2	**P**	**Cb** χ^2	**P**
C. juncidis	5.484	0.064	12.13	0.351
A. scirpaceus	0.095	0.95	0.081	0.824
C. cetti	4.786	0.091	16.47	0.893

The comparison of the Shannon-Wiener index and evenness between 2001, 2019 and 2023 indicates that the values of all the indices were higher in 2019 (Tab. 3.15.). In particular, the Shannon-Wiener index calculated on the normalized breeding abundance of species (H' abb) increased from 2001 (1.05) to 2019 (1.1) of 4.75% and then a decrease of 5.75% in 2023 (1.04), the lowest value of the three years.

The evenness of the bird species abundance (J abb) also shows the same general trends: during the 2019 (0,79) an increase of 5,33% of the 2001 value (0.75) and in 2023 the same percentage decrease in 2023 (0.75). For both the indices calculated on the Consuming biomass, H' biom and J biom, have respectively an increase of 28.3% (1.27) and 27.8% (0.92) referring to 2001 and a decrease of 2.36% and 3.26% in 2023.

Tab. 3.15. Shannon and evenness indices for the years 2001, 2019 and 2023.

Years	**H' abb**	**J abb**	**H' Biom**	**J biom**
2001	1.05	0.75	0.99	0.72
2019	1.1	0.79	1.27	0.92
2023	1.04	0.75	1.24	0.89

3.5 Phenological analysis

Results of song phenological analyses were conducted on 9 species (Tab. 3.16): *A. melanopogon, A. scirpaceus*, *C. cetti*, *C, chloris*, *C. juncidis*, *P. major*, *S. serinus*, *C. melanocephala* and *T. ruficollis.* Differences in mean song records between the decades were statistically significant for 3 of 9 species studied: *A. scirpaceus*, *A. melanopogon*, and *C. cetti* (Tab. 3.17). For *A. melanopogon*, the mean song contacts decreased from 2.25±2.63 and 0.75±0.96 respectively for the first and second decades, to 0 contacts in the third and fourth decades. Also, *C. cetti* had a similar negative trend, with 48.8±14.97 mean song contacts and 31.3±5.62 in the first two decades and 29.6±3.2 and 18.4±8.2 in the last two. In contast, *A. scirpaceus* showed an opposite trend, with an increase in the number of contacts in the last decades, from 0 and 1.75±1.71 (first and second decades) to 3,6±3.65 and 14,6±1.14 in the third and fourth.

Tab. 3.16. Mean bird song records per decades.

Species	**1 decade**	**2 decades**	**3 decades**	**4 decades**
A. melanopogon	2.25±2.63	0.75±0.96	0	0
A. scirpaceus	0	1.75±1.71	3.6±3.65	14.6±1.14
C. cetti	48.8±14.97	31.3±5.62	29.6±3.2	18.4±8.2
C. chloris	2.25±2.22	1.25±0.5	1.2±1.1	0.2±0.45
C. juncidis	6.5±3.7	7±4.83	6.4±3.21	6.2±2.28
P. major	0	0.75±1.5	1.2±1.64	0.4±0.55
C. melanocephala	2.5±3.32	1.5±1	2.8±2.39	1.2±1.79
S. serinus	2.75±1.5	0.75±0.96	0.4±0.55	1±0.71
T. ruficollis	4±1.83	2.75±0.96	2.4±1.82	2±2.35

Tab 3.17. Statistical analyses for the mean song contacts of the bird species.

Species	**χ^2**	**χ^2 p**	**Fisher p**
A. melanopogon	-	-	0.0444
A. scirpaceus	36.40	< 0.001	-
C. cetti	19.46	< 0.001	-
C. chloris	-	-	0.85
C.juncidis	0.09	0.99	-
P. major	-	-	1
C. melanocephala	0.8	0.837	2.5

S. serinus	-	-	0.54
T. ruficollis	1.31	0.7273	-

3.6 Structure and ageing of reed bed

The results of the quantification of the reed bed environmental parameters through plots are shown in Table 3.18.

Of a total of 368 stem higher than 140 cm were measured, 238 (64.7%) were dry, and 130 (25.3%) green. The mean stem diameter measured at 140 cm height is 0.59 ± 0.24 cm, while the mean diameter measured at the half height is 0.65 ± 0.34 cm. For the stems higher than 140 cm, the density per the total number of plots was also calculated, resulting in 14.72 ± 19.37. The high standard deviation indicates the heterogeneity of the new reed bed, with both mature and mowed reed bed areas.

For stems lower than 140 cm, the total number within each is 278, 44 stems (15,8%) being dry and 234 (84,2%) green. The average diameter measured at half height is 0.15 ± 0.42 cm, and the density is 11.12 ± 14.76. In this group as well, the high standard deviation indicates the heterogeneity of the reed bed. The mean height of the stems < 140 cm is 90.63 ± 33.47 cm.

Out of 25 plots, within 9 plots (36%) were observed other macrophytes than *P. australis*, indicating that common reed is the dominant species in this sub habitat. Additionally, 9 out of 25 plots (36%) are submerged, with a mean water depth of 11.59 ± 8.59 cm.

As shown in Tab 3.19. and 3.20., the differences between the stem diameters and heights of the two reed bed types were statistically significant.

Table 3.18. Quantification of biotic and abiotic parameters of the entire reed bed.

	Parameters	**Values**
Stems higher than 140 cm	N° stems	368
	Stem density	14.72±19.37
	N° green stems	130
	N° dry stems	238
	Mean stem diameter at half height (cm)	0.65±0.34

	Mean stem diameter at 140 cm (cm)	0.59±0.24
Stems lower than 140 cm	N° stems	278
	Stem density	11.12±14.76
	N° green stems	234
	N° dry stems	44
	Stem height (cm)	90.63±33.47
	Mean stem diameter at half height (cm)	0.15±0.42
	Vegetation cover	9/25
	Plot submerged	9/25

Table 3.19. Statistical analysis on stems > 140 cm.

Stems higher 140 cm	**Values**	**p ANOVA**	**p K-Wallis**
Stem diameter at 140 cm (cm)	0.59±0,24	p < 0,001	p < 0,001
Stem diameter at half height (cm)	0.65±0.34	p < 0,001	p < 0,001

Table 3.20. Statistical analysis for parameters of stems < 140 cm.

Stems lower 140 cm	**Values**	**ANOVA**	**K-Wallis**
Stem height (cm)	90.63±33.47	p < 0,001	p < 0,001
Stem diameter at half height (cm)	0.15±0.42	p < 0,001	p < 0,001

The number of total stems > 140 cm, measured and compared during all the sampling years, was considerably decreased, with 1277 measured in 2001, 573 in 2018 and 279 in 2023 (Tab 3.21). Same negative trend for the mean stem density, with 53.21±16.97 in 2001, 22.92±14.34 in 2018 and 14.72±19.36. The higher mean stem diameter value was calculated for the 2018, with 0.60±0.4 cm, followed by 0.42±0.14 cm in 2001, and 0.42±0.14 cm in 2023. Statistical analyses made on both the biological parameters, were significant for all the sampling years, and in particular in 2018 and 2023 comparison (Tab 3.22).

Tab. 3.21. Biotic reed bed parameters compared between 2001, 2018 and 2023.

	2001	**2018**	**2023**
Stem number	1277	573	279
Mean stem density	53.21±16.97	22.92±14.34	14.72±19.36

Mean stem diameter (cm)	0.44±0.24	0.60±0,4	0.42±0.14

Tab. 3.22. Statistical analyses on mean stem density and mean stem diameter.

	ANOVA p	**Kruskal- Wallis p**
Mean stem density	< 0,001	< 0,001
Mean stem diameter (cm)	< 0,001	< 0,001

3.7 Mowed reed bed structure

The results of quantification of the 8 environmental variables measured for each of the 25 plots of the mowed reed bed are shown in Tab. 3.23. Of the total number of stems measured, all stems (250) were in the vegetative phase (green), with a mean half-height diameter of 0.47±0.13 cm, and the mean height of 103.01±24.76 cm. Differences between the stem half-height diameters and heights were significant (Tab. 3.24). All the plots had more than 40 stems, indicating a high density of total reed stems in the young reed bed. Two plots (8% of the total) had different vegetation than *P. australis*, while 12 plots (48%) were flooded, with a mean water depth of 4.28±6.11 cm.

Tab. 3.23. Ecological parameters of the mowed reed bed.

Mowed reed bed variables	**Values**
N° of green stems	250
N° of dry stems	0
Plot with more than 40 stems	25
Stem diameter (cm)	0.47±0.13
Stem height (cm)	103.01±24.76
Veg plot	2
Sub plot	12
Water depth (cm)	4.28±6,11

Tab. 3.24. Stem heights and half-height diameters analyses.

Parameter	**Values**	**ANOVA p-value**	**Kruskal-Wallis p**
Stem diameter (cm)	0.47±0.13	p < 0,001	p < 0,001
Stem height (cm)	103.01±24.76	p < 0,001	p < 0,001

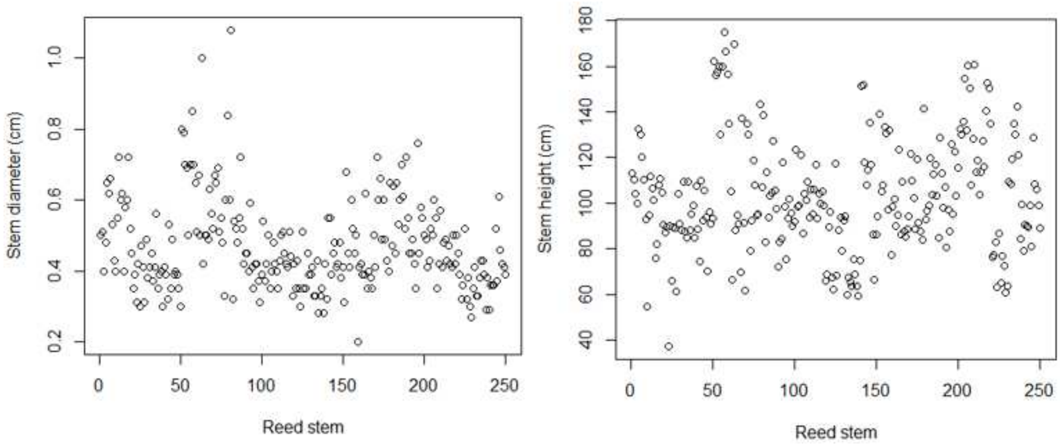


Fig. 3.9. Distribution of stem diameters (left) and height values (right). (R Studio *https://www.R-project.org/.)*

The comparison between the two reed beds has revealed important ecological differences (Tab. 3.25). The stem of the mature reed bed has mean diameter of 0.63 ± 0.03 cm, while the stem of the mowed reed bed has an average diameter of 0.48 ± 0.01 cm. A significant difference was found in the stem diameters of the two groups.

The number of green stems is equal to the total number of reeds measured within the mowed reed bed (100), while approximately two-thirds (31 out of 100) of the reeds measured within the mature reed bed are dry.

For the qualitative variables, few plots with vegetation different from *P. australis* are presents in both the reed beds, 1 in the mowed reed bed and 2 in the mature reed bed. The number of submerged plots is similar for both groups, with 8 in the mowed reed bed and 6 in the mature reed bed. However, a significant difference can be observed in the number of plots with a reed density greater than 40. In the mature reed bed, only 3 out of 10 plots have a reed density of fewer than 40 stems, while in the mowed reed bed, all the plots have a stem density greater than 40.

Tab 3.25. Comparison between MR and MO variables.

Variable	**MR**	**MO**	**p**
Diameter half height (cm)	0.63±0.03	0.48±0.01	< 0,001
Number of green stems	31	100	
Number of dry stems	69	0	
Vegetational cover	1	2	
Submerged plots	8	6	
Plot < 40 stems	3	10	

4 Discussion

4.1 Reed bed structure and comparisons

The quantification of the reed bed allowed to study the structure of the two reed bed types and to compare the results with previous sampling years.

To study the entire reed bed, the subdivision of the stems in the two groups was necessary to define the differences within the internal structure. The two groups exhibit very distinct characteristics, especially in the biotic parameters. Stems higher than 140 cm presented higher values in the mean half height diameter, mean density, and total number of dry stems. For both the stem groups, the high standard deviation in the mean densities suggests a marked variability within the number of stems within each plot. The methodology used may be not suitable for measuring the density of the plots, especially using threshold values. Comparing the mean density and mean stem diameter of mature stems (higher than 140 cm), between the three sampling years has allowed to study the changing of the reed bed structure over years. The mean stem density has decreased significantly, possibly due to the aging of the reed bed (Ostendorp, 1993) and reed mowing activity, that have decreased the number of mature stems per plot. The mean stem diameter showed an overall positive trend in the increasing value between the sampling year. Without any other significant natural and anthropic changes of the reed bed recorded since 2004, differences in both the parameters could be mainly caused by both the reed bed ageing and mowing activities, in particular the decreasing of density.

The quantification of the mowed reed bed structure was fundamental to study the abiotic and biotic characteristics of the sub habitat. From the results obtained, MO is characterized by a high number of green stems. Also, both the mean stems diameter (0.47±0.13 cm) and height (103.01±24.76 cm) shows a significant growth of the stems. The statistical analyses demonstrate that significant differences are measured for both the parameters when compared between all plots, suggesting a heterogeneity within each plot due to the different growing conditions. However, despite the high variability, the mean diameter and mean height values are of fundamental importance for characterizing the mowed reed bed.

The comparison between the mowed and mature reed bed evidenced the differences within the structure characteristics that are most observable in the field, such as density, reed diameter and the vegetative state of the reeds. From the results, the mature reed bed has a larger mean diameter, a significant number of dry reeds and a less density in the number of stems per plot, compared to the mowed reed bed. Other parameters, such as vegetational cover, number of flooded plots and water depth, cannot be used to clearly distinguish between the two types of reed beds.

4.2 Bird community

4.2.1 Bird-habitat analyses

The high number of the total bird contacts was obtained mainly due to the choice of carrying out the censing mapping during both the breeding period and the habitat heterogeneity of the study area. The habitat characterization of the study area indicates a marked sub habitat heterogeneity, with a predominance in extension of both dense environments (such as *P. australis* mature reed bed areas and ecotonal zones) and water open habitats (WA). In particular, MR areas are highly fragmented in reed islands and extensive stands, while ecotonal areas (EA) are especially dense zones due to the predominant mediterranean vegetation, such as *R. Ulmifolius* shrubs and *Q. ilex* woods, in particular along human paths and natural reserve edges. WA is characterized by numerous water channels, and artificially irrigated ponds, crucial for maintaining the marsh and wetland ecosystem; RA is fragmented in five differently zones, located at the edges of the study area and between reed beds and ecotonal zones. The last sub habitat (MO) is characterized by mowed reed bed islands located in southeast and central areas, where reeds were overgrowing and causing damage to the water channels and human path.

Of the most song contacted species, *C. cetti*, *C. juncidis*, *A. scirpaceus* and *T. ruficollis*, exhibit the typical characteristics of elusive songbirds related to *P. australis* reed bed, shrubs, and bushes (Eurasian reed warbler and Cetti's Warbler), or water habitats (Little grebe). Their records were collected mostly within dense (MR and EA) sub habitats. *A. arundinaceus,* a typical mature reed bed species, was encountered few times during the mapping visits, even if the species is considered to breed within the study area (Battisti et al., 2020). The low number of observations is probably caused due to the inadequate census method for the species, or a more dilution effect of the song contacts caused by the small number of individuals. Species with less than 10 contacts, *C. melanocephala*, *C, chloris*, *S, serinus*, and *P. major,* are strictly ecotonal species and their songs indicate possibly suitable nesting sites. Few records were collected also for temporary migratory species, such as *M. flava*, *S. rubicola*, and non-songbird species like *C. olor*, *A. platyrhynchos, F. atra*, and *G. chloropus*. Several records were sampled for *A. melanopogon*, a species that does not breed within the study area, but the suitability of the habitat and the record of the species could potentially lead to future nesting within the reserve.

The elevated number of sight records was collected within open water areas (WA). Most of the censused species are medium-size aquatic birds (*F. atra, A. platyrhynchos, G. chloropus,* and *T. ruficollis*), were easy to contact especially in open areas while transit and foraging. Species with less than 10 sight contacts are the elusive songbirds described before (*C. cetti*, *A. scirpaceus*, *C. junicidis*), strictly ecotonal species (*T. merula* and *P. major*) and primarily singing species such as *C. chloris* and *S. serinus*, whose visual records were mainly sampled when the individuals were hidden in the tree crown.

Bird calls were sampled especially for both not-singing birds, such as the aquatic birds, *F. atra* and *G. chloropus*, and to collect more records within dense mature reed bed habitat. The bird calls were sampled in a high number only for *F. atra*.

The use and selection habitat analyses confirmed the known ecology of each surveyed bird species, with a primary distinction among reed birds, aquatic species, and birds with a preference for ecotonal zones. For all the species, the habitat uses analyses results were significative, indicating that species were using differently the sub habitats within the reserve. The selection habitat analyses results indicates that almost all the sub habitats are significantly selected, positively or negatively, by the species, exception for rush areas (RA), ecotonal areas (EA) and the mowed reed bed (MO), respectively for *P. major, T. merula* and *S. serinus* (RA), *G. chloropus* (EA), and the four aquatic species (MO).

Focusing on the comparison between the MR and the MO, significative differences in the number of records between the two sub habitats, were found for 4 of the 9 studied species. As expected with the initial hypothesis, significant differences were observed for reed bed related species, *A. melanopogon*, *A. scirpaceus, C. cetti* and *C. juncidis,* while for the aquatic species and *C. melanocephala*, no significant different were found. For other species was not possible to make specific comparisons between the two sub habitats, due to the limited number of records within reed beds.

4.2.2 Bird Territories

Within the study area, the total number of territories surveyed slightly decreased from 2019. Focusing on the distribution of territories, the species with the most clusters and estimated territories is *A. scirpaceus*. The high number of territories, within 43,000 m^2 of mature reedbed, indicates that the reserve provides a heavily utilized habitat for the species. The species' nesting in the area was estimated in Battisti et al. (2021), while reproductive pairs can also be found in Lazio (Lardelli et al., 2022). Despite the mowing activities, the number of normalized territories of the species has decreased not significatively between 2019 and 2023. For the other species, the significant number of *C. cetti* territories can be attributed to the presence of ecotonal zones and open environments. Despite the management activities increased the habitat heterogeneity in the study area, the comparison of standardized territories between the 2019 and 2023 has not shown significant differences. For *C. juncidis,* the negative trend in the number of territories in the sampling years could be possibly explain by a locally decrease in the number of individuals and in a progressive changing of the reed bed structure over the years. For the last species, *A. arundinaceus*, was estimated the presence of a single territory during the 2023 mapping. The limited number of records and territories of this species is possibly caused by the fragmentation of the reed bed within the study area.

4.2.3 Consuming biomass, Shannon-Weiner index ed evenness

The Consuming biomass (Cb), the Shannon-Weiner index and evenness were compared to study possible changes not only in the number of territories, but also in both the ecological parameters and diversity of the species guild, especially in the distributions of the individuals within each species. The comparison between 2019 and 2023 indicates that the Consuming biomass have not changed significantly between the two years because of the similar number of territories (i.e., individuals censused).

For the Shannon-Weiner and evenness indices calculated with Cb and the number of territories, highest values were obtained for the 2019, with an increase from 2001 and a slightly decrease in the 2023. The increases in 2019 reflects the greater number of territories of the bird species, while the decrease between 2019 and 2023 suggests fluctuations in the populations of the bird studied, especially concerning the Eurasian Reed warbler.

In general, both the Shannon-Weiner and evenness indices, calculated with the species territories, are more stable across the different years than the biomasses indices, more sensitive to variation of the guild composition. The same results were observed in Battisti et al. (2021).

4.2.4 Phenological analysis

The results of the phenological analyses indicates that the song record means differs between the decades for three bird species: *A. scirpaceus*, *A. melanopogon*, and *C. cetti*. The first species, the Eurasian reed warbler, is the only species that had a positive song contacts trend in all the decades. The species is migratory and starts to emit territorial vocalizations once arriving at its nesting site (Catchpole, 1973).

A. melanopogon exhibits a particular song contact trend. The higher mean values are in the first two decades and are attributed to the songs singed while passing through the natural reserve to the north breeding sites. Monitoring this species demonstrates the passage of this migratory species through reed habitats of the study area, during its migration to the North Europe.

Finally, the decrease in the number of song contacts of *C. cetti* is primarily due to the ending of the breeding period, started in late March.

5. Conclusion

The structure of the entire reed bed within the study area is continually changing, due to both the reed aging and the management activities. In our results, as expected by the ageing of the reed bed and possibly influenced by the mowing activities, the entire reed bed structure was characterized by less mature stem density and smaller stem diameters, compared with the pre-mowed years 2001 and 2018. Also, comparisons between the mature reed bed and mowed reed bed showed differences in several structure parameters. As expected, the mowed reed bed has a higher stem density, no dry stems, and a lower mean stem diameter.

The response of the bird community to the mowing activities was a specific negative use and selection of MO by land species, and a not significant selection of MO, by aquatic species. Focusing on MR and MO contacts, significative differences were found in 4 of the 9 species. As expected with the initial hypothesis, differences were significative for the most reed bed related species: *A. melanopogon*, *A. scirpaceus, C. cetti* and *C. junicidis.* Low number of records within the mowed reed bed possibly indicates that the species avoid MO during the major bird activity hours.

At guild level, the comparisons of the ecological parameters with previous years indicates an overall higher value of the parameters in the last pre-mowing year (2019), but no significative reduction of reed bed related species territories was found. Most plausible hypothesis are: (i) bird species reduced the territories and (ii) the territories did not cover the entire pre-existing mature reed bed. Future studies should focus on studying the distances and sizes of territories of the Common reed warbler territories.

The limitations of this study are the small local scale sampling and the use of bird contacts. Large-scale factors, such as population fluctuations, can influence pattern and field data, while birds contacts are not directly associated with the number of individuals of all the species within a sampling area; the high number of *C. juncidis* records was probably collected for a small number of individuals.

Despite the limitations, our study suggests that a correct management of the reed bed within the "Torre Flavia" wetland is essential for the goodness of the entire ecosystem and should take in consideration both the bird community responses to the mowing activities, prevalently negative, and the conservation of the mature reed bed habitat within the natural reserve.

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6. Supplementary data

Tab. 1. Song records for each species collected during the sampling days (March, April, May, and June).

	March				April									May					June
°th	21	28	29	30	4	6	12	14	18	19	20	27	28	5	9	10	15	24	1
A	2	0	6	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	1	2	4	2	0	1	8	7	13	15	14	15	16	7
C	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0
F	63	60	39	32	29	34	36	25	32	26	27	33	28	31	18	20	14	9	3
G	5	1	3	0	2	1	1	1	2	2	0	2	0	0	1	0	0	0	1
H	12	4	5	5	3	6	5	14	8	3	6	11	4	3	7	5	9	7	4
I	3	0	7	0	0	2	2	2	2	2	2	1	7	0	0	4	0	2	1
J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	2	0	0	0	3	0	1	1	0	1	0	0	0	0	0
Q	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S	2	2	5	2	1	0	2	0	1	0	0	1	0	2	1	1	1	0	1
T	3	2	6	5	2	4	3	2	4	0	4	1	3	5	4	0	1	0	0
U	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Species legend: A = *A. melanopogon*, B = *A. scirpaceus*, C= *A. arundinaceus*, D = *A. platyrhynchos*, E = *C. carduelis*, F = *C. cetti*, G = *C. chloris*, H = *C. juncidis*, I = *C. melanocephala*, J = *C. olor*, K = *F. coelebs*, L = *F. atra*, M = *G. cristata*, N = *G. chloropus*, O = *M. flava*, P = *P. major*, Q = *R. acquaticus*, R = *S. rubicola*, S = *S. serinus*, T = *T. ruficollis*, U = *T. merula.*

Tab. 2. Sight records for each species collected during the sampling days (March, April, May, and June).

	March				April									May					June
°th	21	28	29	30	4	6	12	14	18	19	20	27	28	5	9	10	15	24	1
A	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	1	0	0	1	1	1	0	0	1	4	2	3	1
C	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	0	0
D	5	7	7	16	9	10	16	19	5	9	6	8	2	4	3	1	7	3	2
E	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0
F	3	1	0	0	0	4	1	2	0	1	1	1	0	1	0	0	0	0	0
G	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1
H	4	0	0	1	0	1	1	0	0	0	0	0	0	1	2	1	2	0	1
I	0	2	1	0	0	1	0	2	0	0	1	0	1	3	0	0	0	0	0
J	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	14	23	23	18	15	28	29	21	19	24	20	19	20	15	10	16	16	8	2
M	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
N	3	1	4	1	3	5	4	5	2	9	3	0	2	0	1	0	0	2	0
O	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	2	0	0	3	1	0	0	1	1	1	0	0	0	0	1
Q	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
T	5	0	0	1	2	3	0	1	3	1	2	4	3	1	0	3	1	0	2
U	1	2	5	2	1	0	2	0	1	4	0	0	1	0	0	0	0	4	0

Species legend: A = *A. melanopogon*, B = *A. scirpaceus*, C= *A. arundinaceus*, D = *A. platyrhynchos*, E = *C. carduelis*, F = *C. cetti*, G = *C. chloris*, H = *C. juncidis*, I = *C. melanocephala*, J = *C. olor*, K = *F. coelebs*, L = *F. atra*, M = *G. cristata*, N = *G. chloropus*, O = *M. flava*, P = *P. major*, Q = *R. acquaticus*, R = *S. rubicola*, S = *S. serinus*, T = *T. ruficollis*, U = *T. merula.*

Tab. 3. Call records for each species collected during the sampling days (March, April, May, and June).

	March				April									May					June
°th	21	28	29	30	4	6	12	14	18	19	20	27	28	5	9	10	15	24	1
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0
J	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L	5	1	3	2	3	0	2	2	0	0	1	0	0	0	0	1	0	0	5
M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2
O	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	0	0	2	0	0	0	0	1	1	2	0	0	0	0	0
Q	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
U	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Species legend: A = *A. melanopogon*, B = *A. scirpaceus*, C= *A. arundinaceus*, D = *A. platyrhynchos*, E = *C. carduelis*, F = *C. cetti*, G = *C. chloris*, H = *C. juncidis*, I = *C. melanocephala*, J = *C. olor*, K = *F. coelebs*, L = *F. atra*, M = *G. cristata*, N = *G. chloropus*, O = *M. flava*, P = *P. major*, Q = *R. acquaticus*, R = *S. rubicola*, S = *S. serinus*, T = *T. ruficollis*, U = *T. merula.*

Tab. 4. Usage habitat analyses for the land species.

	MR	**MO**	**RA**	**EA**		
Land species	**fr**	**fr**	**fr**	**fr**	χ^2	**P**
A. melanopogon	1	0	0	0	52	< 0,001
A. scirpaceus	0,908	0,083	0	0,01	415,65	< 0,001
C. cetti	0,378	0,033	0,042	0,547	597,01	< 0,001
C. chloris	0	0	0	1	112	< 0,001
C. juncidis	0,333	0,022	0,578	0,067	143,91	< 0,001
P. major	0	0	0,04	0,96	89,76	< 0,001

S. serinus	0	0	0	1	96	< 0,001
C. melanocephala	0,135	0	0,038	0,827	125,02	< 0,001
T. merula	0,130	0	0	0,87	64,174	< 0,001

Tab. 5. Usage habitat analyses for the aquatic species.

	MR	MO	WA	RA	EA		
Aquatic species	**fr**	**Fr**	**fr**	**Fr**	**fr**	χ^2	**p- value**
A. platyrhynchos	0,101	0,137	0,755	0,01	0	347,07	< 0,001
F. atra	0,061	0,106	0,833	0	0	1146	< 0,001
G. chloropus	0,146	0,167	0,667	0	0,021	88,177	< 0,001
T. ruficollis	0,222	0,111	0,667	0	0	155	< 0,001

Tab. 6 Song record of the bird guild species per day of sampling.

Sampling days	*C. juncidis*	*A. scirpaceus*	*A. arundinaceus*	*C. cetti*	TOT
24-mar	11	0	0	68	79
28-mar	4	0	0	67	71
29-mar	5	0	0	41	46
30-mar	5	0	0	35	40
04-apr	3	0	0	31	34
06-apr	8	1	0	42	51
12-apr	7	3	0	39	49
14-apr	16	4	0	30	50
18-apr	7	2	0	32	41
19-apr	3	1	0	30	35
20-apr	6	2	0	32	40
27-apr	11	7	0	32	50
28-apr	3	8	2	26	39
05-mag	4	14	0	31	49
09-mag	7	17	1	21	46
10-mag	6	18	1	22	47
15-mag	9	16	0	13	38
24-mag	7	20	0	9	36
01-giu	4	7	0	4	15
Tot	126	120	5	605	856

Tab. 7. Comparison of standardized breeding density (D) between the three sampling years.

Species	2001	2019	2023	χ^2	P
C. juncidis	6.36	4.09	2.28	5.48	0.06
A. scirpaceus	11.82	25	22.72	0.10	0.95

C. cetti	9.55	14.55	13.18	4.79	0.09

Tab. 8. Comparisons of the Consuming biomass (Cb) between the three sampling years.

Species	**2001**	**2019**	**2023**	**χ^2**	**P**
C. juncidis	28.89	21.21	14.06	12.13	0.002
A. scirpaceus	70	117.77	110.99	0.08	0.96
C. cetti	41.97	56.35	52.59	16.47	0.00002

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