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URBAN WASTE-WATER TREATMENT PLANTS AS HOTSPOTS FOR BIRDS: AN ENVIRONMENTAL ASSESSMENT HIGHLIGHTS THE ROLE OF A SINGLE DOMINANT GULL

F. CERFOLLI[1], C BATTISTI[2]*

[1] Department of Ecological and Biological Sciences (DEB), Tuscia University, Viale dell'Università snc, Viterbo, 01100, Italy
[2] "Torre Flavia" LTER (Long Term Ecological Research) Station, Città Metropolitana di Roma Capitale, Servizio Aree protette, Parchi Regionali, Viale G. Ribotta 41, Rome, 00144, Italy
**Corresponding author: c.battisti@cittametropolitanaroma.it*
ORCID: https://orcid.org/0000-0002-2621-3659

ABSTRACT. - Waste-water treatment plants (WWTPs) are still little explored in their ecological role. This work reports data obtained from standardized sampling for the two largest Italian WWTPs, to obtain seasonal patterns (late winter, spring, and autumn) of univariate diversity metrics in bird communities. The ecological conditions, linked above all to the high availability of water, with the presence of mud and trophic resources, and heterogeneous features (buildings, trees, and hedges), allow the presence of water-related and synanthropic bird species, using the site as a seasonal stopover and wintering sites. The large availability of biomass of invertebrates, linked to the sludge from the water-waste treatment plants, can provide an important trophic resource during spring migration. The richness, diversity and evenness did not differ significantly between the two plants analysed, both having comparable size and heterogeneity. The highest Simpson dominance values were recorded in autumn with species frequency concentrated in a few abundant species. Detrended Correspondence Analysis (DCA) shows a close association between the autumn period and the dominant Black-headed Gull (*Chroicocephalus ridibundus*), in both treatment plants, with possible implication on the spreading on zoonosis.

Key words: Water-waste treatment plants, Water-related birds, Detrended Correspondence Analysis, Chroicocephalus ridibundus, zoonosis

INTRODUCTION

In urban areas, Mediterranean water reservoirs can perform many ecosystem services (Panuccio *et al.* 2017, Di Santo *et al*. 2021). Among these, water-waste treatment plants (hereafter, WWTPs) represent a peculiar typology (Fraquelli & Giandrone 2003). The presence of infrastructures serving the various phases of water treatment and purification (reservoirs, cisterns, canalizations), with the consequent high heterogeneity of the environmental mosaic and the location along watercourses, attracts a large number of vertebrates (i.e., birds and amphibians). This has been, in recent times, studied for water treatment systems based on constructed wetlands and phytoremediation plants (Frederick & McGehee 1994, Andersen *et al*. 2003, Hsu *et al*. 2011, Orlowski 2013), but not yet explored for treatment systems using engineering technologies and infrastructures (Harebottle *et al.* 2008), for which there is evidence of a relative environmental impact (Brandi *et al.* 2000, Venkatesh & Brattebø 2011, Alves *et al*. 2012, Johnson 2019). This work illustrates the data obtained from standardized periodic samplings on water-related birds carried out within two large WWTPs located along the Tiber North and South of Rome (central Italy), evidencing seasonal patterns. The monitoring activities, conducted within the WWTPs, have made it possible to put forward some hypotheses for the sustainable management of biodiversity in these sites. To our knowledge, this is the first assessment on bird diversity of large urban waste-water treatment plants in the Mediterranean area.

METHODS

The Rome North WWTP, the largest in Italy, is located on the right bank along the Tiber River, north of Rome, at km 9,200 of the Flaminia road (41°57'47"N; 12°29'45" E) and collects sewage from the Northern urbanized areas of Rome (Bufalotta, Nomentana, Nuovo Salario, Serpentara, Cassia and Flaminia) through three sewer pipes. The treatment plant is divided into two sections built in subsequent times and includes a complex structure consisting of two separate but interconnected systems for the treatment of sewage and sludge (water flow: about 3 mc/sec; 95 x 10^3 mc/year overall, corresponding to 780,000 inhabitants; Fig. 1).

The Rome South WWTP is located on the orographic left of the Tiber river, south-west of Rome, at km 10,700 of the Ostiense road (41°48'47.9"N; 12°25'31.6" E) and contains, also in this case, two treatment lines (biological oxidation and biofiltration, sludge line with mechanical dehydration; water flow:> 10 mc/sec; 320 x 10^3 mc/year overall, corresponding to > 700,000 inhabitants). Both of the water plants show a 40 ha in size, each one (further details on the Tiber drainage system in Del Monte *et al*. 2016; for details on the surrounding Campagna Romana landscape: Grapow & Fanelli 1993; Fig. 1). For the characterization of bird communities, we used the quantitative point count method with fixed radium (50 m; Bibby *et al*. 2000, Sutherland 2006). In three subperiods (late winter: March; spring: May-June; autumn: October and November), we carried out periodic and replicated standardized sampling in two different years (2018: Rome North; 2019: Rome South), randomly locating 14-point counts in each site. The sampling points were kept at a distance of at least 100 meters from each other, assuming this as the minimum distance to avoid pseudo-replication of the data (i.e., the multiple counting of the same record; Battisti *et al*. 2014). A sampling session lasting two minutes was carried out at each station, at least one hour after sunrise, mainly between 07.00 and 11.00 a.m., repeated monthly. During each session, the number of individuals of each species observed in flight, on the ground or indirectly contacted through vocalizations, songs or other signs of presence was recorded. We avoided days with rain precipitations and strong winds (possible underestimation of the field data; Bibby *et al*. 2000). At species level, we obtained the number (n) of individuals and the relative frequency (fr_i, as n/N, where N is the total number of individuals; species with p_i> 0.05 were considered dominant). At community level, we obtained the: (i) number of species (S as non-normalized richness); (ii) the Margalef index (normalized richness; Dm; calculated as Dm = S-1/lnN; representing a value of normalized species richness,

where S is the total species richness and N the total number of surveys; (iii) the Shannon-Wiener diversity index ($H' = - \Sigma fr_i \times \ln fr_i$, where fr_i is the relative frequency of any species; Keylock 2005); (iv) the evenness index (e = H '/ lnS; Jost, 2010), (v) the Simpson's dominance concentration index ($d = \Sigma fr_i^2$; for a review of the univariate metrics, Magurran 2004, Magurran & McGill 2011; see also Ricotta 2005). To focus on the role of WWTPs for waterbirds, data have been analysed also at this guild level. The guild of strictly waterbirds was locally represented by six species: Mallard (*Anas plathyrynchos*), Grey Heron (*Ardea cinerea*), Common Sandpiper (*Actitis hypoleucos*), Green Sandpiper (*Tringa ochropus*), Black-headed Gull (*Chroicocephalus ridibundus*), Yellow-legged Gull (*Larus michahellis*). For this guild we calculated the relative frequency of occurrence (as $Fr_g = \Sigma fr_w$, where fr_w is the relative frequency of any waterbird species) in each month of sampling.

To test the significance of the differences between the median values, we used a non-parametric Mann-Whitney U test. The χ^2 tests was performed to compare relative frequencies (Dytham 2011). From the waterbird species/month matrix, we performed a Detrended Correspondence Analysis (DCA) to test for the linkage among months and species in each treatment plants (Hill & Gauch 1980). Systematic order and nomenclature follow Baccetti *et al.* (2021).

Fig. 1. - Map of the two study areas (Rome North and Rome South WWTPs)

RESULTS

We obtained 4950 records (1670 in Rome North, 3280 in Rome South WWTPs) belonging to 41 species (Table I). Black-headed Gull (*Chroicocephalus ridibundus*) appeared the most frequent species, with highest dominance in autumn ($fr_i > 0.1$, in both the WWTPs; Table I). At community level, the number of both absolute and normalized species appeared higher in late winter

(March) than in autumn (October-November). Both the Shannon-Wiener diversity and the evenness indices showed a similar trend. The highest dominance concentration values were recorded in autumn (Table I).

Mean species richness (both absolute and normalized) not differ between WWTPs (absolute species richness: U = 12; normalized: U = 9, both p > 0.05; Mann-Whitney U test; Fig. 2). Analogously, the paired comparison among mean values of Shannon-Wiener diversity index, evenness and Simpson dominance index did not show a significant difference between plants (p > 0.05; Mann-Whitney U test; Fig. 3). At guild level, we observed a significant difference in frequency among months in both the WWTPs (Rome North: χ^2 = 690.29; Rome South: χ^2 = 205.31, both p < 0.001, d.f. = 4). Trend in relative frequency of the waterbirds shows the highest values in autumn (October-November), although with a difference between WWTPs (Fig. 4). The Detrended Correspondence Analysis (DCA) shows a clear link between a single waterbird species (the Black-headed Gull, *Chroicocephalus ridibundus*) and the autumn period (autumn-winter; Figs. 5, 6).

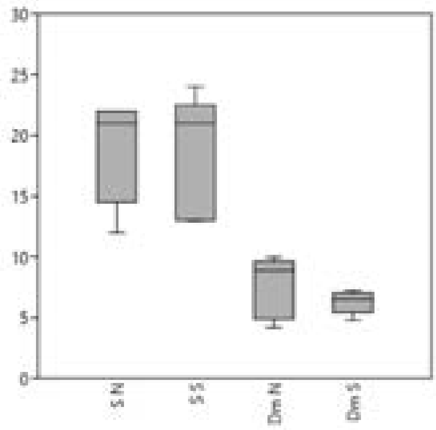

Fig. 2. - Mean values of species richness (S) and normalized species richness (D_m) both for the WWTPs of Rome North (N) and Rome South (S)

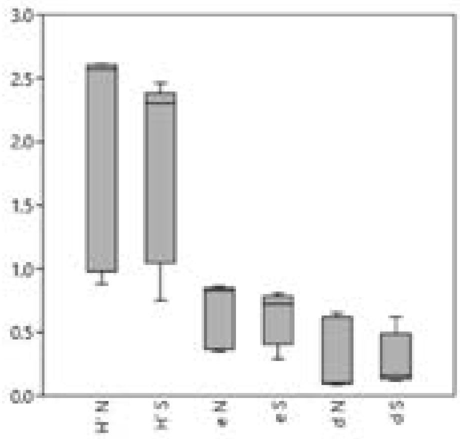

Fig. 3. - Mean values of Shannon-Wiener diversity index (H'), evenness (e), and Simpson dominance index (d) both for the water treatment plants of Rome North (N) and Rome South (S).

	Rome North					Rome South				
	late winter	spring		autumn		late winter	spring		autumn	
Species	**Mar**	**May**	**Jun**	**Oct**	**Nov**	**Mar**	**May**	**Jun**	**Oct**	**Nov**
Phasianus colchicus				0.002						
Anas platyrhynchos W	0.178	0.099	0.013	0.048	0.020	0.044	0.077	0.025	0.013	0.006
Columba livia f. domestica	0.030	0.115	0.017	0.024	0.024	0.022	0.067	0.049	0.007	
Apus apus			0.164				0.025			
Bubulcus ibis										0.005
Ardea cinerea W									0.002	
Actitis hypoleucos W			0.009		0.001					
Tringa ochropus W						0.004				
Chroicocephalus ridibundus W	0.040	0.027	0.060	0.802	0.760	0.338		0.153	0.437	0.192
Larus michahellis W		0.005	0.056		0.032	0.12	0.225	0.074	0.018	0.005
Milvus migrans							0.004			
Merops apiaster							0.007			
Picus viridis		0.011				0.004		0.006		0.001
Falco tinnunculus	0.010					0.004		0.018	0.002	0.001
Falco peregrinus						0.004				
Psittacula krameri	0.040	0.016	0.030	0.014	0.011	0.067	0.011		0.031	0.001
Corvus monedula							0.232	0.025	0.013	0.009
Corvus corone cornix	0.178	0.154	0.095	0.005	0.020	0.129	0.126	0.221	0.024	0.01
Cyanistes caeruleus	0.010	0.011	0.004			0.004	0.004			
Parus major	0.020	0.011	0.013			0.004	0.004			
Cisticola juncidis		0.011	0.009			0.027	0.007	0.018		
Hippolais polyglotta		0.005								
Delichon urbicum			0.112				0.021	0.037		
Hirundo rustica	0.010	0.143	0.194		0.001	0.004	0.021	0.018	0.009	
Phylloscopus collybita	0.059				0.005	0.004				0.004
Cettia cetti	0.010		0.004		0.001	0.013	0.011	0.006		
Aegithalos caudatus						0.004				
Sylvia atricapilla	0.040	0.060	0.026			0.004		0.013		
Sylvia melanocephala	0.010									
Certhia brachydactyla	0.010	0.005						0.006		
Troglodytes troglodytes	0.040	0.005	0.009	0.010	0.001					
Sturnus vulgaris	0.079	0.077	0.056	0.005	0.073	0.049	0.035	0.141	0.412	0.764
Turdus merula	0.079	0.060	0.026	0.027	0.001	0.018	0.025	0.012		
Erithacus rubecula				0.002	0.013				0.013	0.001
Passer italiae	0.099	0.055	0.052	0.039	0.019	0.084	0.074	0.104		
Passer montanus						0.013		0.012		
Anthus pratensis					0.001					
Motacilla alba		0.016	0.017		0.013	0.013	0.021	0.055	0.018	0.003
Chloris chloris	0.020	0.082	0.030	0.014			0.004	0.006		
Carduelis carduelis	0.020	0.005								
Serinus serinus	0.020	0.022	0.004			0.018	0.004			
N	**101**	**182**	**232**	**412**	**743**	**225**	**285**	**163**	**451**	**2156**

Table I. - Relative frequencies (fr_i) of the bird species recorded in the two water treatment plants during the study period (late winter: March; spring: May-June; autumn: October-November). W: waterbird species.

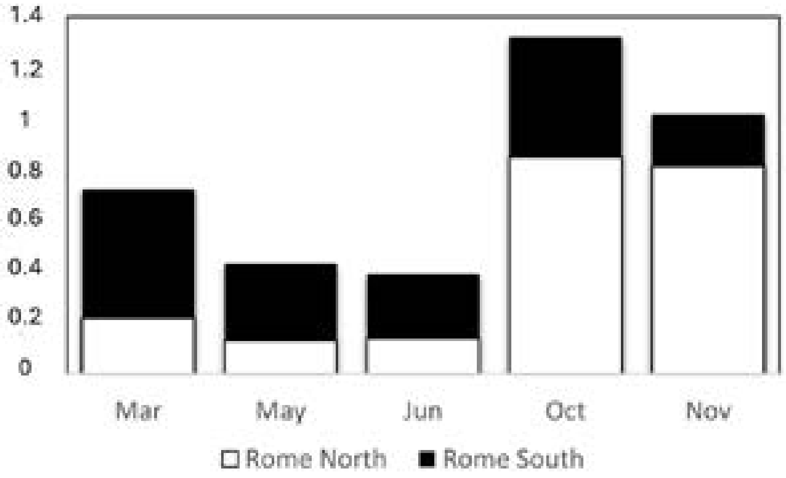

Fig. 4. - Trend of relative frequency of the guild of strictly waterbird species in the two water treatment plants (Rome North: white; Rome South: black).

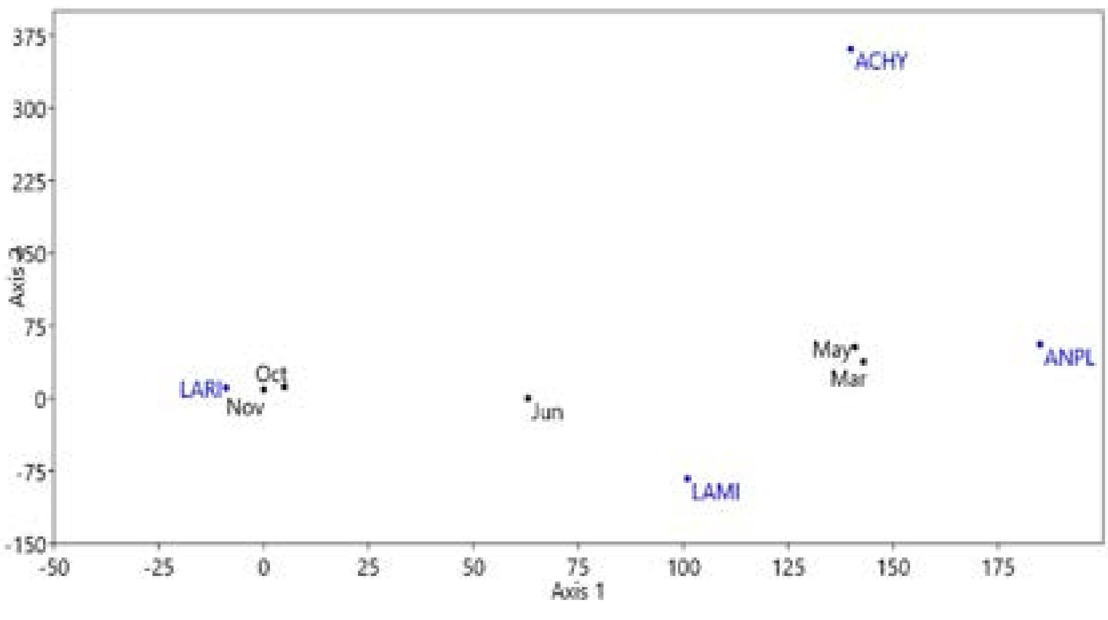

Fig. 5. - Detrended Correspondence Analysis (DCA; Axis 1 and axis 2) on the waterbird species/months in the Rome North water treatment plant. LAMI: *Larus michahellis*; LARI: *Chroicocephalus ridibundus*; ACHY: *Actitis hypoleucos*; ANPL: *Anas platyrhynchos*.

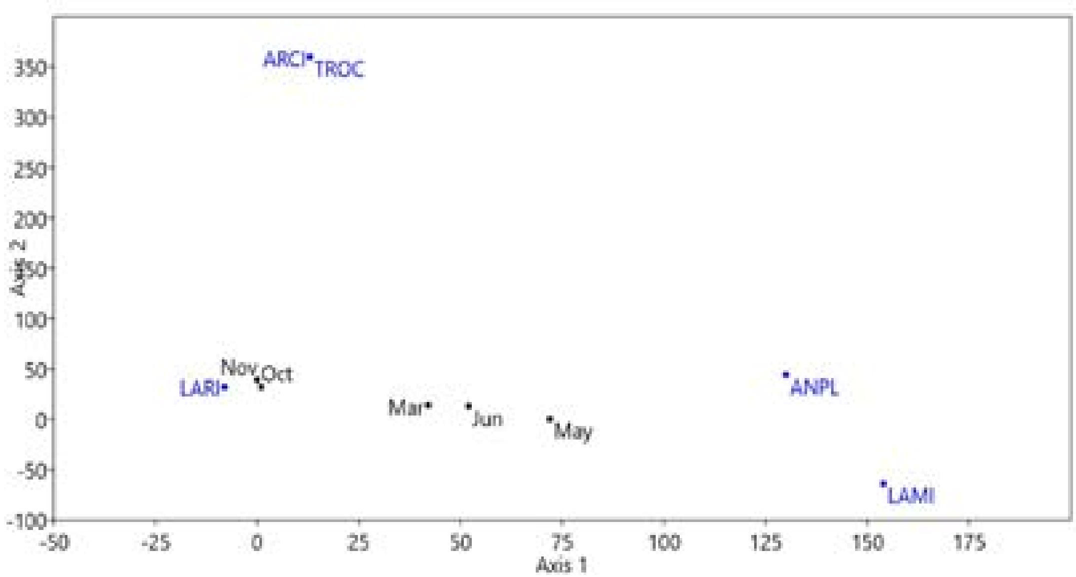

Fig. 6. - Detrended Correspondence Analysis (DCA; Axis 1 and axis 2) on the waterbird species/months in the Rome South water treatment plant LAMI: *Larus michahellis*; LARI: *Chroicocephalus ridibundus*; ACHY: *Actitis hypoleucos*; TROC: *Tringa ochropus*; ANPL: *Anas platyrhynchos*; ARCI: *Ardea cinerea*.

DISCUSSION

In the two urban water-waste treatment systems, bird species use the site as a seasonal stop-over, especially in late winter and spring (also favoured by the location of the WWTPs, in both cases near a large freshwater river: the Tiber, the longest watercourse of central Italy). More particularly, an increase in relative frequency of the water-related guild was observed in autumn (October and November), although local factors may explain differences in pattern between the two WWTPs. In the spring period the availability of a large amount of invertebrate biomass (mainly, Diptera, Chironomids; pers obs), linked to the sludge from the purification tanks, can provide an important trophic resource in the breeding period and during spring migration (e.g. such as *Apus apus* and swallows, see Arena *et al*. 2011).

Other species, strictly synanthropic and not strictly linked to riverine habitats (e.g., *Streptopelia decaocto*, *Columba livia* f. domestica, *Sturnus vulgaris*, corvids, sparrows, ecotonal finches) or recently introduced in the surroundings (*Psittacula krameri*; e.g., Dodaro & Battisti 2014; Battisti & Fraticelli 2023), use trees and green areas for nesting even at high densities. From the collected data it emerges that the richness in species, the diversity and the evenness did not differ significantly in the two WWTPs, since these have an area of comparable habitat pattern and size area. The highest

Simpson dominance values were recorded in the autumn when fewer species were detected, each with a relatively large number of individuals; thus, the frequency of the species (or 'dominance') has been concentrated in a few very abundant species: this is a well-known pattern for birds occurring in natural and artificial Mediterranean wetlands (Redolfi De Zan *et al*. 2011, Zacchei *et al*. 2011).

Among the water-related species, the Black-headed Gull was found to be the autumn dominant in both the WWTPs, as highlighted by the Detrended Correspondence Analysis (DCA). In this regard, water treatment plants may play an important role for this species during this period. Analogously to other water-related birds, this species hosts several pathogens (e.g., belonging to genus *Salmonella*, *Campylobacter, Escherichia*; Literák *et al*. 1992, Sixl *et al*. 1997, Liao *et al*. 2019): in this regard, the high concentrations observed may deserve to attention since water treatment plants could act as hubs for possible epidemic zoonosis expanding in surrounding wet areas.

The peculiar ecological conditions, linked above all to the high availability of water, with the presence of mud and trophic resources connected to them, combined with the buildings, open areas, trees and hedges, allow the presence of water-related and synanthropic species. In this regard, even small environmental restoration actions (e.g., phytoremediation tanks with *Phragmites australis* reeds or other autochthonous aquatic macrophytes) could increase the richness and diversity of vertebrate animal communities, reducing the synanthropic component in favour of more specialized communities of water-related birds, including species of conservation concern (see Harebottle *et al*. 2008, Murray & Hamilton 2010, Murray *et al*. 2014).

To our knowledge, this is the first assessment in large urban waste treatment plants for Mediterranean area. In this regard, this type of environmental assessment, in addition to increasing the ecological knowledge of sites still little investigated in this respect, can stimulate environmental projects (*sensu* Battisti *et al*. 2020) carried out in water treatment plants and aimed at increasing bird diversity. These interventions, once communicated, will be able to re-frame these plants also with a view to environmental sustainability (e.g., Kärrman 2001, Chen *et al*. 2020).

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