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Assessment of Odonate assemblages in the Agro Pontino (Latina, Central Italy) using two biotic indices (Insecta: Odonata)

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Abstract
Odonate assemblages were surveyed in seven sites within the context of the EU LIFE project GREENCHANGE that aims to preserve biodiversity and to enhance the ecological value of the agro-ecosystems of Agro Pontino (Latina, Central Italy) through interventions of ecological restoration. In total 16 species were recorded during the *ante-operam* surveys in 2019, which represent 28% of the Odonate fauna of the Lazio Region. None of them has a significant conservation interest, however *Coenagrion castellani*, an endemic Italian species, was observed in a small rivulet near the Pantanello site during an additional field survey. The application of OHI (Odonate Habitat Index) and ORI (Odonate River Index) allowed a detailed characterization of the dragonfly assemblages that are mainly dominated by eurytopic and euryecious species typical of standing waters. The assemblages of Pantanello and Fosso Epitaffio are notable exceptions thanks to the presence of sensitive lentic and lotic species. The main pressures affecting Odonate assemblages identified in the study area are the alterations of aquatic and riparian vegetation, the hydromorphological degradation of running waters, limited water quality and the presence of the invasive crayfish *Procambarus clarkii*.

Key words: dragonflies, damselflies, Odonate River Index, Odonate Habitat Index, biodiversity loss, ecological restoration, ecological assessment.

Introduction

In Italy, a large number of ecosystems have a worrying conservation status. These are largely hygrophilous habitats and ecosystems typical of coastal and plain areas (Comitato Capitale Naturale 2021), most affected by transformations of the territory aimed at finding spaces for human activities. As in the European Union (EU), agriculture, urbanization, modification of the water regime are among the main factors threatening the conservation of biodiversity, also for habitats and species of community interest (EEA 2020; Ercole et al. 2021). In particular, the intensification of agriculture has led to the elimination of natural elements once widespread in and on the edges of agricultural areas, such as hedges, tree patches, ponds, and wetlands, strongly reducing structural diversity and the availability of resources of agro-ecosystems (Firbank 2005; Henle et al. 2008).

This strong ecological impoverishment has determined the decrease in the populations of many farmland species of birds, pollinators, and other taxa typical of landscape mosaics and traditional low intensity farming areas (Baldi et al. 2013; Berg et al. 2015; Sánchez-Bayo & Wyckhuys 2019; Wagner D.L. 2020; Calvi et al. 2018; Brambilla 2019; Rete Rurale Nazionale & Lipu 2021; Battisti et al. 2022).

In recent years, awareness has increased regarding the role that biodiversity and ecosystems play for the economic and social well-being of the population, guaranteeing the provision of numerous Ecosystem Services (MEA 2005; Haines-Young & Potschin 2010; Mace et al. 2018; Jurjonas et al. 2023; Emerson 2024) and this has led - also thanks to the impetus given by international policies and regulations such as the UN Decade on Ecosystem Restoration and the Nature Restoration Law in the EU - to the launch of numerous ecosystem restoration projects. Many of these concerned the ecological restoration of degraded agroecosystems, in consideration of the importance that agricultural areas have as habitats for many species, for their role as buffer areas and for their ability to guarantee, in some contexts, the maintenance of adequate levels of ecological connectivity.

Ecological restoration projects, understood according to the definition given by SER (2004) as "intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity and sustainability", re-

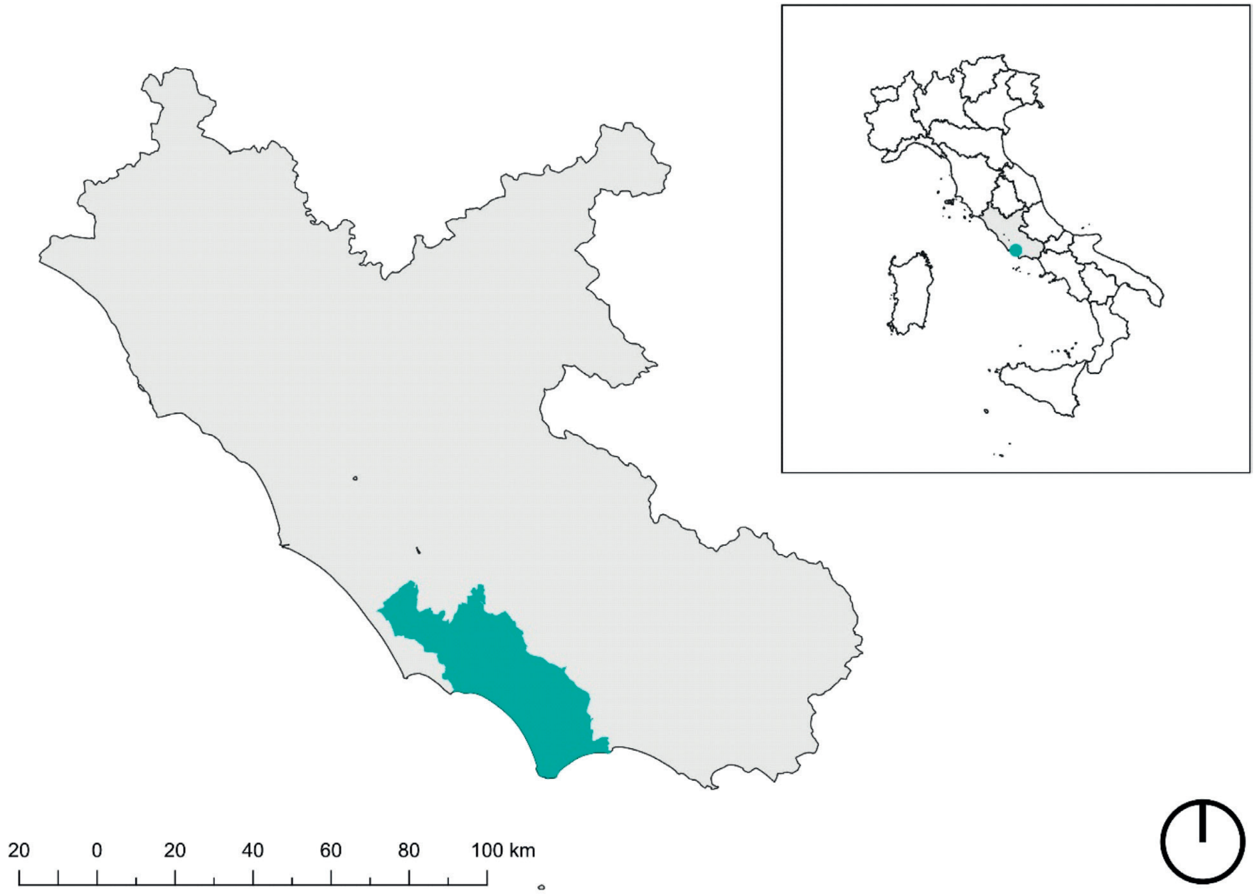

Fig. 1 – Geographical location of the study area (Lazio, central Italy). On the right: geographical location within Italy

quire adequate monitoring systems to evaluate the effectiveness in the recovery of degraded ecosystems and the more general benefits on biodiversity. For this reason, it is important to choose a well-articulated system of indicators, which measures different components and can provide an adequate assessment of the complexity of ecosystems. For example, the choice of indicator species must be very accurate, evaluating very carefully how much the variation in the dynamics of a single population is truly representative of the changes in the ecosystem as a whole (Burger 2006; Siddig et al. 2016).

The EU project GREENCHANGE (LIFE17 NAT/IT/000619) aims to preserve biodiversity and to enhance the ecological value of the agro-ecosystems of the Agro Pontino (Central Italy) and Maltese rural areas. Specific conservation actions carried out in the Agro Pontino consist in the re-naturalization of sections of rectified canals (i.e. re-meandering and replacement of the alien and infesting vegetation with autochthonous species), development of linear structures (i.e. hedges) for strengthening connections between ecosystems and the creation of hygrophilous and hydrophilic habitats such as wetlands, temporary ponds, hygrophilous and mesophilous woods.

A complete set of bioindicators was selected to evaluate the success of the interventions of restoration, both in the aquatic and terrestrial habitats: vegetation, dragonflies, amphibians, reptiles, birds and bats. Among them, dragonflies occupy an important role in the assessment of aquatic ecosystems due to their amphibious life cycle and because of their well-known ecological requirements. They provide information about the ecological integrity (i.e. vegetation structure and hydrological regime and connectivity) and habitat heterogeneity of both the aquatic breeding sites and the surrounding terrestrial areas (Corbet 2004; Simaika & Samways 2012; Rocha-Ortega et al. 2021). Moreover, the results of Odonate surveys can be summarized in biotic indices. There are valid examples developed in different geographical contexts: e.g. central and Mediterranean Europe (Dragonfly Association Index, Chovanec et al. 2015; Odonate River Index, Golfieri et al. 2016; Odonata Community Index – Corsica, Berquier et al. 2016) and Africa (Dragonfly Biotic Index, Simaika & Samways 2009; African Dragonfly Biotic Index, Vorster et al. 2020).

In this paper, we present the results of the dragonfly surveys carried out in 2019, i.e. before the above-mentioned interventions of ecological restoration. Our work implements the limited literature about Odonate distribution in the Latina Province and it represents the first application of the Odonate River Index (Golfieri et al. 2016) to assess the ecological conditions of a river system in central Italy.

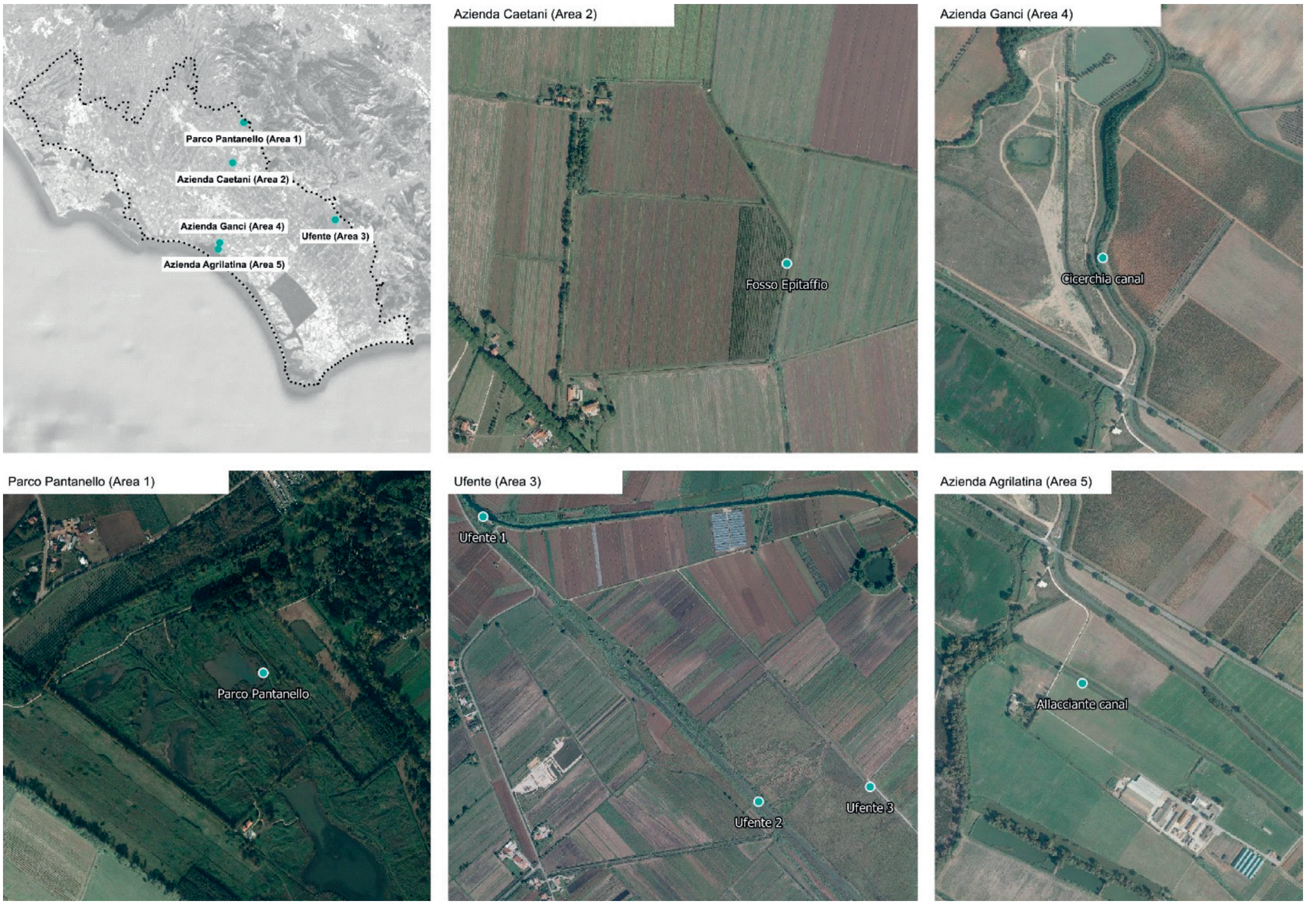

Fig. 2 – Geographical distribution of the sampling sites in the Agro Pontino area.

Study area

The Agro Pontino is the largest alluvial plain in Central Italy (Latina Province, Lazio Region), with an extension of about 1180 km^2 (Fig. 1). The current layout of this large area is the result of the so-called "great integral reclamation" of the 1920s, which completely transformed the original landscape, draining the vast system of ponds and marshes and making the land available for agriculture and urban settlements. The hydrographic system has been completely upset and today is characterized by a large network of drainage canals, overall about 200 km long, and by some natural watercourses, which have undergone numerous interventions over the years with the consequent alteration of the natural hydromorphological dynamics. The Agro Pontino is currently undergoing extensive transformation due to intensive agriculture, industrialization, and urban sprawl (Magaudda et al. 2020). The natural areas are now reduced to small isolated fragments within a heavily anthropized matrix.

Odonate surveys were carried out in seven sites (Fig. 2), both lotic and lentic, located in the alluvial plain with an altitude that ranges between 0 and 25 m. Three sites (Ufente 1, Ufente 2 and Ufente 3) were located along the Ufente River, the largest natural river in the Agro Pontino, and its surrounding canals.

The Ufente 1 site is a heavily hydromorphological modified reach of Ufente river, surrounded by intensive agricultural areas. The vegetation is impoverished and made up of strips of reeds with *Arundo donax* and other nitrophilous species such as *Urtica dioica, Galium aparine* and *Silene alba*. The Ufente 2 and Ufente 3 sites are on two canals, with extremely low-flow rates, located on the opposite edges of a large natural area, characterized by three dominant types of vegetation: a paucispecific community with *Arundo donax*, a reed bed with *Phragmites australis* and extensive meso-hygrophilous grasslands, with species as *Juncus articulatus*, *Juncus inflexus, Carex otrubae, Carex distans, Holoschoenus vulgaris, Bolboschoenus maritimus*, which can be classified as habitat of Community interest *6420 - Mediterranean tall humid herb grasslands of the Molinio-Holoschoenion*. The area in which these two sites are located is included in the SPA IT6030043 *Monti Lepini* and borders on the SCI IT6030043 *Laghi Gricilli*.

The site in the Pantanello Park is a large (i.e. about 3.4 hectares) permanent pond fed by the springs of the Ninfa River, characterized by the following prevalent vegetation types: reed beds with *Phragmites australis*, stable meso-hygrophilous grasslands, extensive and dense *Rubus ulmifolius* bushes. This area borders the SCI IT6040002 *Ninfa*.

Table 1 – Environmental variables measured at the study site (EV: emerging vegetation; SV: submerged vegetation) and geographical coordinates.

Site	Shading (%)	EV (%)	SV (%)	pH	Electrical conductivity (µS/cm)	Latitude (N)	Longitude (E)
Ufente 1	15	15	70	7.56	1032	41.27.34	13.06.10
Ufente 2	5	15	0	7.66	947	41.26.59	13.06.56
Ufente 3	15	10	0	7.82	1034	41.27.14	13.06.55
Pantanello Park	5	15	5	8.09	355	41.34.34	12.57.06
Fosso Epitaffio	5	20	0	8.00	484	41.31.36	12.55.57
Cicerchia canal	5	5	0	7.67	1266	41.24.53	12.54.15
Allacciante canal	0	5	0	7.59	880	41.24.37	12.54.20

The Fosso Epitaffio, Cicerchia canal and Allacciante canal are all located within farms, with vegetation dominated by nitrophilous species such as *Arundo donax, Rubus ulmifolius, Urtica dioica, Galium aparine, Raphanus raphanistrum, Rumex crispus*. In the Cicerchia canal, there is also a population of *Robinia pseudoacacia*. The most purely hygrophilous vegetation is represented by isolated specimens of *Salix alba, Phragmites australis, Iris pseudacorus*, *Carex* sp.pl. Allacciante and Cicerchia canal are drainage canals with low flow, located near the brackish Fogliano Lake (included in the Circeo National Park), while the Fosso Epitaffio is a ditch, with more pronounced flow velocity.

Materials and methods

The study sites were characterized considering the following environmental variables: presence of aquatic vegetation, shading and water chemical parameters (i.e. pH

Table 2 – Odonata species in the study sites (B: breeding species; O: observed species).

	Ufente 1	Ufente 2	Ufente 3	Pantanello Park	Fosso Epitaffio	Cicerchia canal	Allacciante canal
Zygoptera							
Calopteryx splendens	**O**				**B**		
Sympecma fusca				**B**			
Coenagrion pulchellum	**B**						
Erythromma viridulum	**B**			**B**			
Ischnura elegans	**B**	**O**	**O**	**B**	**B**	**B**	**B**
Platycnemis pennipes					**B**		
Anisoptera							
Aeshna mixta		**O**	**O**	**B**		**B**	**O**
Anax imperator				**B**		**O**	
Onychogomphus forcipatus					**B**		
Crocothemis erythraea	**O**	**O**	**O**	**B**		**B**	**B**
Trithemis annulata	**B**	**B**				**B**	
Libellula fulva					**B**		
Orthetrum cancellatum		**B**	**O**	**B**		**B**	**O**
Orthetrum coerulescens				**O**		**B**	
Sympetrum fonscolombii	**B**			**B**			
Sympetrum striolatum				**B**			

and electrical conductivity). The presence of aquatic vegetation was visually assessed and expressed through the percent cover of two vegetation categories (i.e. submerged and emergent vegetation) in a 3m wide swath along the banks. Percent shading of the study site by riparian vegetation (100–0% sky in the canopy over water) was visually assessed, while pH and electrical conductivity were measured with a XS (PC5 model) water analyzer.

Odonate surveys were conducted on sunny, calm days between 3 May and 30 Sep 2019. Each study reach was surveyed four times between May and September (i.e. about every six weeks), excluding the sites Ufente 1 and Ufente 2 that were surveyed only three times, due to the limited accessibility. A transect of 100 m was surveyed along the banks in both lentic and lotic sites, with the exception of the sites Cicerchia canal and Ufente 1 because of the presence of inaccessible riparian vegetation. We dedicated 30 minutes to the observation of adult dragonflies and 30 minutes for exuviae collection every time a study site was investigated.

Adult dragonflies were observed with binoculars and when possible they were photographed, to allow a subsequent control of the identifications carried out in the field. Some adult individuals were caught with an insect net, for identification, and then released. The number of individuals observed and their behaviour were also recorded in the field for each species. Identification of adult dragonflies was carried out following Dijkstra & Lewington (2006). Exuviae were searched along the banks, on emergent and riparian vegetation, up to 2 m from the water edge. The exuviae were preserved dry, then hydrated for one day and finally transferred in 70% ethanol. Identification was conducted in the laboratory with a binocular microscope following Carchini (2016), Gerken & Sternberg (1999) and Doucet (2011).

The classification of a species as breeding in a study site was based on the following criteria: (i) presence of teneral individuals, (ii) direct observation of tandem and copulating pairs and ovipositing females, (iii) direct observation of territorial behaviour, (iv) adults in abundance class 3 or 5; (v) presence of exuviae. The abundance classes were defined following Golfieri et al. (2016).

For each study site we calculated the following metrics: (i) total number of breeding species, (ii) number of sensitive breeding species, (iii) total number of breeding families, (iv) value of the Odonate Habitat Index (OHI, Chovanec & Waringer 2001).

The OHI describes the hydrological regime and the lentic-lotic character of a site. OHI values range from 1, indicating a lotic Odonate assemblage, to 5, indicating an assemblage typical of a lentic and temporary site. The calculation of OHI is based on data about breeding species and requires the species-specific Habitat value (HV) and the Indication weight (IW), and the estimated abundance (A) of the species, expressed in classes with increasing values from 1 to 5. IW ranges from 1, for eurytopic species, to 5, for stenotopic species. Sensitive species have an IW value greater than or equal to 3. HV values range from 1, that indicates species typical of lotic sites, to 5, for species that breed in lentic and temporary sites. To calculate OHI we applied the IW and HV values revised for the geographical context of northern Italy by Golfieri et al. (2016) and we also assessed such values for the Mediterranean species *Trithemis annulata*, on the basis of the authors' expert judgement.

Table 3 – Odonate metrics of the study sites.

Site	Species	Sensitive species	Families	OHI
Ufente 1	5	2	2	3.05
Ufente 2	2	0	1	2.93
Ufente 3	0	0	0	//
Pantanello Park	9	2	4	2.94
Fosso Epitaffio	5	3	5	1.41
Cicerchia canal	6	1	3	3.06
Allacciante canal	2	0	2	2.82

For the three sites located along the Ufente River and its surrounding canals it was also calculated the Odonate River Index (ORI, Golfieri et al. 2016). This biotic index was developed to evaluate the ecological condition of river corridors in northern Italy and it requires the survey of a set of sampling sites that represent the diversity of aquatic habitats of the river corridor along a gradient of connectivity from the main channel to disconnected ponds in the floodplain. The calculation of ORI is based on five metrics, obtained pooling together data of all the considered sites: (i) number of breeding species, (ii) number of sensitive breeding species, (iii) number of breeding families, (iv) OHI mean, (v) OHI range. ORI values range from 0 to 1, representing, respectively, totally altered and undisturbed conditions of the river corridor and they are associated to one of the five classes quality classes considered for the ecological assessment of the water bodies according to the EU Water Framework Directive 2000/60 (Golfieri et al. 2016).

Nonparametric Spearman's r correlation coefficient was used to evaluate the relationships between the environmental variables and the OHI metrics calculated at each study site. Statistical analyses were carried out using PAST software (version 4.13; Hammer et al. 2001).

Results

The values of the environmental variables measured in the study sites are presented in Table 1. Aquatic vegetation and shading by riparian vegetation are limited, except at Ufente 1, characterized by a significant presence of submerged vegetation. As regards water chemical parameters, pH is slightly basic (i.e. between 7.56 and 8.09), while electrical conductivity varied between 355 e 1266 μS/cm. The lowest values of electrical conductivity were registered at Pantanello and Fosso Epitaffio, directly fed by spring water.

A total of 16 dragonflies species, 6 Zygoptera and 10 Anisoptera, were found considering both sampling methodologies. Dragonflies were observed at all the study sites but no breeding species were recorded at Ufente 3 site (Table 2 and Table 3). *Ischnura elegans* was the most diffuse zygopteran species, being observed at all study sites, while *Sympecma fusca*, *Coenagrion pulchellum* e *Platycnemis pennipes* were recorded at only one site (Table 2). As for Anisoptera, *Crocothemis erythraea* was the most diffuse species (i.e. in six sites), while *Onychogomphus forcipatus*, *Libellula fulva* and *Sympetrum striolatum* were observed at only one site (Table 2). The observed species are not listed in the Annexes of the EU Habitats Directive 92/43, as well in categories of risk of the Red List of the Italian Dragonflies (Riservato et al. 2014a), but *C. pulchellum* is evaluated as Near Threatened.

Nevertheless, it is worth underlining that during a field survey near Pantanello Park, we observed a male exemplar of *Coenagrion castellani* in small rivulet (Rio Cieco) at the border of the area that will be restored. *C. castellani* is an endemic Italian species (Dijkstra et al. 2023) with a significant conservation interest, being classified as "Vulnerable" in the IUCN Red List of Threatened Species (De Knijf et al. 2023). Pantanello and Cicerchia canal host the most diverse Odonate breeding assemblages with respectively 9 and 6 species (Table 3). Pantanello Park is a protected area with well-preserved agro-ecosystems and it has been the object of significant interventions of re-naturalization like the conversion of traditional agricultural fields into woods and open pastures with shrubs. Moreover, the investigated lentic site is fed by high-quality spring waters (e.g. low electrical conductivity), and its riparian vegetation is managed with conservation-based criteria. The highest number of sensitive breeding species and of breeding families was recorded instead at Fosso Epitaffio, due to the presence of stenotopic species such as *Onychogomphus forcipatus*, *Libellula fulva* e *Calopteryx splendens*. These species, as well as *Platycnemis pennipes,* that are typical of lotic waters, were found only in this site, characterized by specific water conditions (i.e. low electrical conductivity and more pronounced flow velocity).

On the other hand, Ufente 2 and the Allacciante canal host extremely poor and simplified assemblages, with only two breeding species. These sites are located in more impacted agricultural contexts, with significant pressures related to the management of aquatic and riparian vegetation, and to chemical water quality, underlined by higher values of electrical conductivity.

The OHI was around 3 for most of the study sites and this value describes Odonate assemblages typical of permanent lentic water bodies, dominated by eurytopic and euryecious species (e.g. *Ischnura elegans*, *Crocothemis erythraea*, *Orthetrum cancellatum*) thanks to their tolerance to the alteration of habitats, as well as of the chemical water quality. However, more sensitive lentic species like *Coenagrion pulchellum*, *Erythromma viridulum* and *Sympecma fusca* were observed at Pantanello Park and at the Ufente 1 site. Only the Fosso Epitaffio has a lower value (i.e. 1.41) that indicates a rheophilic Odonate assemblage. Due to the lack of breeding species, it was not possible to calculate the OHI for the Ufente 3 site.

The ORI was calculated for the three sites located along the Ufente River and its surrounding canals and its value resulted to be 0, corresponding to the lowest class of ecological status (i.e. bad).

No significant statistical relationships were found between the environmental variables and the OHI metrics calculated at each of the study sites.

Discussion and conclusions

The number of Odonate species observed represents about 28% of the species actually known for the Lazio Region (58 species; Riservato et al. 2014b; La Porta et al. 2023). In the literature, no specific paper focuses on the Odonate assemblages breeding in the canals that drain the Agro Pontino. Nevertheless, some studies referring to the Lazio region or to the entire province of Latina report the presence in this area of *Coenagrion castellani* and *Lindenia tetraphylla* (Calvario et al. 2008; Giunti et al. 2009). Recently, Novaga et al. (2020) have reported some data from the literature relating to Odonates of conservation interest observed in the wetlands of the plain areas close to the Lepini Mountains, highlighting the presence of *Coenagrion castellani* and *Cordulegaster trinacriae.* Investigations concentrated specifically in areas close to that of our study were carried out by Angelici (1982) and Nardi (1993), who reported the species observed in the adjacent Lepini Mountains but they present some data about the area of Ninfa that we investigated at the Pantanello site.

The Odonate richness, i.e. 17 species considering also the opportunistic observation of *C. castellani*, and the composition of the assemblages is comparable to the above-mentioned studies. Angelici (1982) and Nardi (1993) list 13 and 19 Odonate species respectively, and they indicate the presence of *C. castellani* at Ninfa. Our observation confirms the presence of the species in this area and what indicated by Domeneghetti et al. (2017) regarding the most recent reports of *C. castellani* in Lazio region, all of which occurred in lowland or low-hill areas and mostly within protected areas.

Most of the study sites, with the exception of Pantanello and the Fosso Epitaffio, host simplifed assemblages characterized by eurytopic and euryecious species, due to the alterations of aquatic and riparian vegetation, of the hydromorphological conditions and poor water quality. The presence of the invasive crayfish *Procambarus clarkii*, that was observed in all the study sites during the herpetological field surveys, also with abundant populations (CIRF 2020), is another element that has a strong negative influence on dragonflies at larval stages (Siesa et al. 2014).

The results of this study confirm that Odonate assemblages are good describers of the ecological conditions of their aquatic breeding sites and the surrounding terrestrial areas (Remsburg & Turner 2009; Martín & Maynou 2016; Cunningham-Minnick et al. 2019; Rocha-Ortega et al. 2021; Worthen et al. 2021); in addition, dragonfly-based indices are practical and efficient tools to summarize this information (Berquier et al. 2016; Golfieri et al. 2018; Vorster et al. 2020; Petrovičová et al. 2021).

The ORI application to the three sites located along the Ufente River and its surrounding canals highlights a heavily impacted ecological condition which is summarized as being of "bad" quality class. This evaluation derives from the low numbers of breeding species, sensitive species and families, as well as from the lack of diversification between the Odonate assemblages. However, it is worth remembering that this was the first application of the ORI outside of the original geographical context and river typology considered for its development, i.e. the corridors of large Alpine rivers. Moreover, in this study the ORI was applied to assess the condition of a system made up of a riverine site and two draining canals, which is not the typical configuration of channels and ponds that can be found in a large natural river corridor. Nevertheless, the application of the ORI allowed to underline the main anthropic alteration of the sites located along the Ufente River and its surrounding canals. The interventions of restoration planned in the study sites are expected to improve the richness and composition of the Odonate assemblages as observed in previous studies carried out in Italy and in other countries (Funk et al. 2009; Samways & Sharratt 2010; Subrero et al. 2013; Termaat et al. 2015).

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