APPLICATION OF THE HABITAT FISH BIOLOGICAL INDEX (HFBI) FOR THE ASSESSMENT OF THE ECOLOGICAL STATUS OF PO DELTA LAGOONS (ITALY)

APPLICAZIONE DELL' HABITAT FISH BIOLOGICAL INDEX (HFBI) PER LA VALUTAZIONE DELLO STATO ECOLOGICO DEGLI AMBIENTI LAGUNARI DEL DELTA DEL PO

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Abstract

Fish fauna is one of the biological quality elements useful for assessing the ecological status of European transitional water bodies, for the purposes of applying the Water Framework Directive. For the assessment of the ecological status of Italian transitional aquatic ecosystems, the multimetric index "Habitat Fish Bio-Indicator" (HFBI), based on fish fauna, was developed and validated at national level. This paper reports the results of a study on the shallow-water fish fauna of 5 lagoon environments of the Po Delta (Northern Adriatic, Italy) which aimed to evaluate the ecological status of these water bodies by calculating the HFBI. The results made it possible to describe the taxonomic composition and the functional and trophic structures of the fish community characteristic of these delta lagoons. The shallow water fish assemblage was characterized by the presence of juvenile stages of marine migrating species (i.e., grey mullets of the genus *Chelon*, the sea bream *Sparus aurata* and the sea bass *Dicentrarchus labrax*) and by species of estuarine residents (i.e., small gobies *Knipowitschia panizzae*, *Pomatoschistus marmoratus*, and *P. canestrinii*, the silverside *Atherina boyeri* and the killfish *Aphanius fasciatus*). The guilds of hyperbentivores/zooplanktivores, microbentivores and detritivores were the most represented in the fish assemblage of the of investigated lagoons. The HFBI index, applied on an annual basis, made it possible to highlight marked differences in the ecological status of the Po Delta lagoons. Only Caleri lagoon resulted in a good state, Marinetta and Canarin lagoons in a moderate state, while Barbamarco and Scardovari lagoons resulted in a poor ecological state. Eutrophication, resulting from the large inputs of nutrients with freshwater from the Po River branches, and the modification of lagoon morphologies, a consequence of anthropogenic activities, are the main pressure factors that determine the ecological status of these transitional water bodies.

Riassunto

I pesci sono uno degli elementi di qualità biologica utile per la valutazione dello stato ecologico dei corpi idrici di transizione europei, ai fini dell'applicazione della Direttiva Quadro sulle

Acque. Per la valutazione dello stato ecologico degli ecosistemi acquatici di transizione italiani è stato messo a punto e validato a livello nazionale l'indice multimetrico HFBI, basato sulla comunità ittica. In questo lavoro sono riportati i risultati di uno studio sulla fauna ittica di basso fondale di 5 ambienti lagunari del Delta del Po (Adriatico Settentrionale, Italia), che ha avuto lo scopo di valutare lo stato ecologico di questi corpi idrici mediante il calcolo dell'HFBI. I risultati hanno permesso di descrivere la composizione tassonomica e la struttura funzionale e trofica del popolamento ittico caratteristico di queste lagune deltizie. Il popolamento ittico di basso fondale delle lagune indagate è risultato caratterizzato dalla presenza degli stadi giovanili di specie di migratori marini (ad es., i cefali del genere *Chelon*, l'orata *Sparus aurata* e la spigola *Dicentrarchus labrax*) e da specie di residenti estuarini (ad es., le specie di piccoli gobidi *Knipowitschia panizzae*, *Pomatoschistus marmoratus* e *P. canestrinii*, il latterino *Atherina boyeri* e il nono *Aphanius fasciatus*). Le guilds degli iperbentivori/zooplanctivori, dei microbentivori e dei detritivori sono risultate le più rappresentate nel popolamento ittico degli ambienti lagunari del delta del Po. L'indice HFBI, applicato su base annuale, ha permesso di evidenziare marcate differenze di stato ecologico delle lagune indagate. Una sola delle lagune indagate (Caleri) è risultata in uno stato buono, due lagune (Marinetta e Canarin) in stato moderato, mentre le lagune di Barbamarco e Scardovari sono risultate in uno stato ecologico scarso. L'eutrofizzazione, conseguente agli ingenti apporti di nutrienti con le acque fluviali dei rami del Po, e la modifica delle morfologie lagunari, conseguenza delle attività antropiche, risultano essere i principali fattori di pressione che determinano lo stato ecologico di questi corpi idrici di transizione.

Introduction

Fish fauna represents an important biological component of transitional coastal environments due to the role played by fish in the functioning of lagoon and estuarine ecosystems, in guaranteeing ecological connectivity with coastal marine ecosystems, and in terms of fishery yields (McLusky & Elliott, 2004; Perez-Ruzafa *et al.*, 2011). Furthermore, fish have been used successfully to assess the environmental quality of a variety of aquatic ecosystems, including transitional coastal waters. (e.g., Soto-Galera *et al.*, 1998; Whitfield & Elliott, 2002). Indeed, several attributes of fish communities, both structural and functional, have been shown to be sensitive to a variety of anthropogenic pressures affecting transitional coastal ecosystems (Pasquaud *et al.*, 2013; Zucchetta *et al.*, 2016, 2020).

In the context of the European Directive 2000/60/EC (Water Framework Directive, WFD), 4 biological quality elements (EQBs) are mandatory to assess the ecological status of transitional water bodies and fish fauna is one of them. As a consequence, the implementation of WFD has led to the development of numerous fish-based biological indices for the ecological assessment of different types of transitional water bodies across Europe (Franco *et al.*, 2009; Peres-Domingues *et al.*, 2012; Pasquad *et al.*, 2013; Lepage *et al.*, 2016; Zucchetta *et al.*, 2021).

For the assessment of the ecological status of Italian transitional ecosystems based on the EQB fish fauna, the multimetric index "Habitat Fish Bio-Indicator" (HFBI) was developed (Catalano *et al.*, 2017; Franzoi *et al.*, 2019). Starting from the approach of Franco *et al.* (2009), the index was built selecting the metrics (measures that summarize the characteristics, both structural and functional, of the fish community) most correlated with the anthropogenic pressures affecting fish fauna. This process has allowed to obtain an index sensitive to ecological status variations caused by human activities in the lagoon ecosystems (Zucchetta *et al.*, 2020).

Lagoons (or *sacche* in Italian) of the Po Delta are transitional water bodies characterized by extensive shallow waters, a high hydro-morphological dynamism and marked changes in environmental characteristics, both abiotic and biotic, on a seasonal basis (Maicu *et al.*, 2018).

Furthermore, they are subject to a variety of anthropogenic pressures that have changed their natural ecological characteristics. Although there is a lot of knowledge on Po Delta coastal lagoons (e.g., Carrada *et al.*, 1987; Verza & Cattozzo, 2015; Sfriso *et al.*, 2016, 2021; Maicu *et al.*, 2018), even with regard to the fish fauna that populates these ecosystems (Giannini *et al.*, 1979; Gandolfi *et al.*, 1985; Rossi, 1986; Maccagnani *et al.*, 1985; Franzoi *et al.*, 1985, 1989a, 1989b, 1993; Lanzoni *et al.*, 2010, 2021; Milardi *et al.*, 2018, 2019), there are no comparative works that assess the ecological status of fish fauna of different delta lagoons.
A study of the fish assemblage of 5 lagoons of the Po Delta was recently conducted. In this paper, the data collected throughout this study were used to assess their ecological status by applying the HFBI index. The results obtained were compared and discussed with current knowledge on the environmental quality of the investigated ecosystems.

Study area

The study was carried out in five lagoons of the Po Delta, from north to south: Caleri, Marinetta, Barbamarco, Canarin and Scardovari (Figure 1). These lagoons have very shallow water bodies, with different morphological characteristics and characterized by a various degree of connection with the sea and with the river branches (Maicu *et al.*, 2018). The main production activity within these lagoons is the breeding of bivalve mollusks, especially Philippine clams (*Ruditapes philippinarum*) (Verza & Cattozzo, 2015).

Caleri

It covers an area of about 9.8 km^2 and has an average depth of 1.4 m (Maicu *et al.*, 2018). It communicates with the sea through a mouth about 100 m wide, while it is connected to the south with the Marinetta lagoon (Figure 1). Fresh water supplies are scarce, and mostly come from the irrigation water used in the area surrounding the lagoon.

Marinetta

It has an area of about 3.5 km^2 and an average depth of about 1.8 meters. It extends from the island of Albarella to the last stretch of the Po di Levante, and it is connected with the sea to the north-east through a 400 m wide inlet (Maicu *et al.*, 2018). To the south it is connected with the Vallona Lagoon (Figure 1).

Barbamarco

It has an extension of about 6.8 km^2 and an average depth of 1 m. It communicates with the sea through two inlets, 55 (north mouth) and 70 m wide (south mouth) (Maicu *et al.*, 2018). It receives fresh water from the river branches of the Po both in the northern and southern parts (Figure 1).

Canarin

It has an area of about 6.4 km^2 and an average depth of 0.9 m; currently, the lagoon is connected to the sea through a single inlet, approximately 200 m wide (Maicu *et al.*, 2018). It is connected to the north with the Basson Lagoon. It receives fresh fluvial and irrigation waters in the north, west and south (Verza & Cattozzo, 2015) (Figure 1).

Scardovari

It covers an area of approximately 28.1 km^2 and the average depth is approximately 1.6 m. The northern part, more confined, has a greater depth than the southern part. The latter communicates with the sea with two inlets (Figure 1), 225 m wide in the North-East and 845 m in the South-West. Fresh water enters in the lagoon through a small channel connecting it with the Po di Gnocca, and through two pumping stations located on the western shore (Maicu *et al.*, 2018).

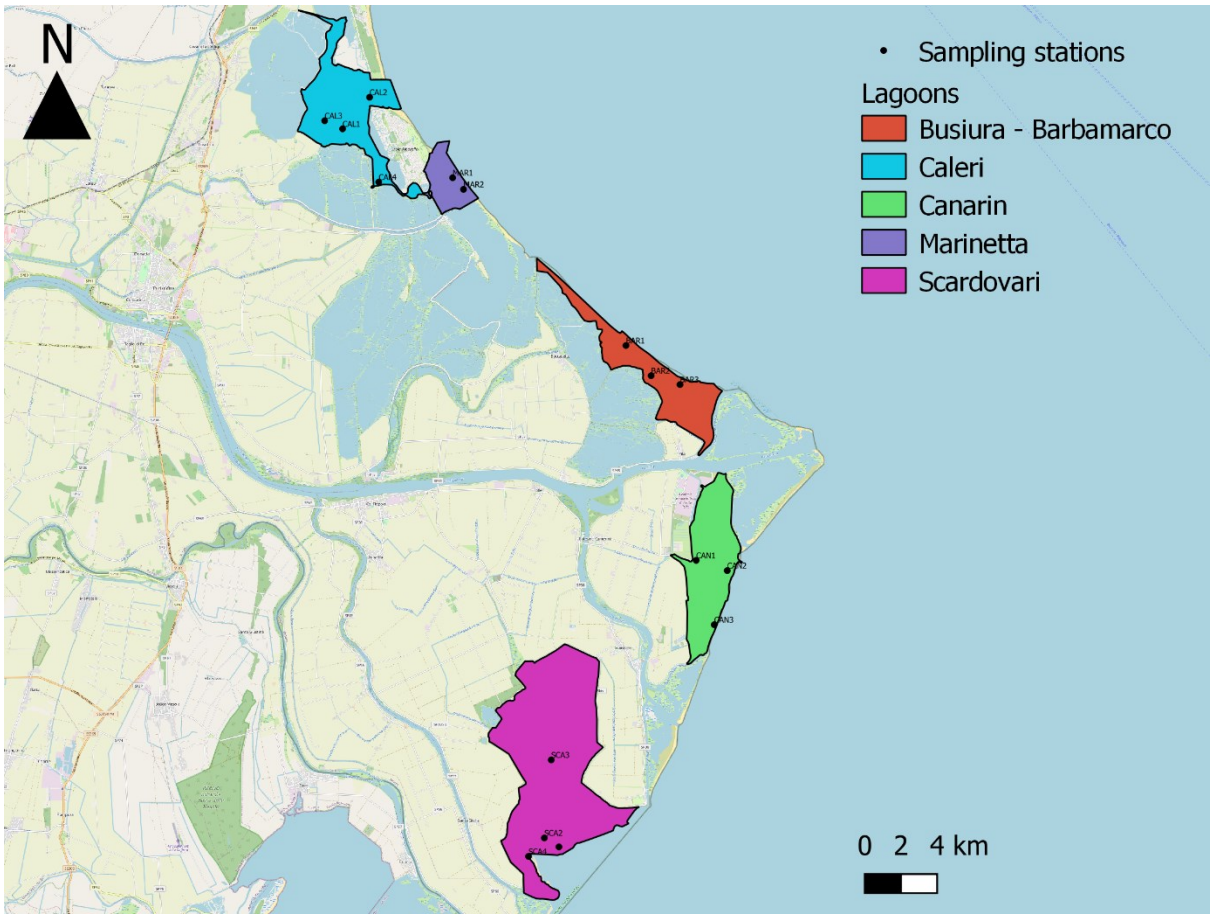

Figure 1 – Map of the Po Delta: the investigated lagoons are indicated with different colors. The location of the sampling stations is reported in each lagoon.
Figura 1 – Mappa del Delta del Po: le lagune studiate sono indicate con differenti colori. In ogni laguna è riportata la localizzazione delle stazioni di campionamento.

Material and methods

The sampling of the fish fauna was conducted in shallow water stations (water depth <1.5 m) representative of the different types of habitats and bottom characterizing the investigated lagoons. The number of stations per lagoon took into account their different extension: 2 stations in Marinetta, the smallest lagoon; 3 stations in Barbamarco and Canarin, being of intermediate size; 4 stations in Caleri and Scardovari, the two largest lagoons (Table 1; Figure 1). In Scardovari, sampling was conducted only in the southern part, given the absence of areas with water depths of less than 1.5 m in the northern part. The sampling methods were those provided for by the ISPRA protocol for the application of the Habitat Fish Biological Index (Catalano *et al.*, 2017). Two samplings were carried out per lagoon, one in spring (May 2021) and one in autumn (October 2021). The ichthyofauna samples were collected using a tightly meshed beach seine (internode distance 2 mm in the central bag and 4 mm in the wings), 10 m long and 2 m high in the center. Two replicates per sampling date were carried out for each station. During each sampling operation, the surface of the seabed explored by the instrument (towing length x opening distance of the net) was calculated, in order to standardize the capture data in terms of sampled surface units. The sampling effort (area explored per replicate) was kept as constant as possible. All sampled specimens were identified at the species level, counted and weighed (±0.01 g or ±0.001 g), and total abundance and biomass were calculated and standardized over 100 m^2. The species found in the samples were then classified into Estuarine

Use Functional Guilds (EUFG) and Feeding Mode Functional Guilds (FMFG) (Franco *et al.*, 2008, 2009; Catalano *et al.*, 2017; Zucchetta *et al.*, 2021).
Non parametric Kruskal-Wallis test was carried out to verify differences among lagoons, and they were considered significant for $p<0.05$ (R-Studio in R version 4.3.1). The level of similarity of fish assemblages among sites and seasons was tested running a non-metric Multidimensional scaling analysis (nMDS) (PRIMER 7 Version 7.0.23). For the nMDS analysis the data were Square root transformed and the Bray-Curtis similarity matrix was used.
The HFBI consists of six metrics based on fish community diversity and structure (Catalano *et al.*, 2017): total biomass/total abundance ratio (average individual weight); biomass density of bentivorous species (micro- and macro-bentivorous); Margalef index calculated on the biomass of the dominant species (species that make up 90% of the total biomass); Margalef index calculated on the biomass of migratory species (estuarine-dependents and estuarine-opportunists); Margalef index calculated on the biomass of bentivorous species; Margalef index calculated on the biomass of hyperbenvorous/zooplanktivorous/piscivorous species.
These metrics are considered to be the most sensitive to the main categories of anthropogenic pressures affecting Italian transitional ecosystems (Franco *et al.*, 2009, 2010; Zucchetta *et al.*, 2020):

- morphological and coastline changes: these include changes in the morphology and hydrodynamism of lagoon water bodies, and the habitats that constitute them;
- use of resources and of the seascape: includes the pressures deriving from activities connected with the direct and indirect exploitation of biological resources and lagoon spaces;
- effects on environmental quality: they include the quality of environmental matrices present in the lagoon ecosystem.

As prescribed in the HFBI application protocol (Catalano *et al.*, 2017), the six metrics included in the index, expressed as ecological quality ratios, were calculated for each sampling station and date.
In addition to being sensitive to anthropogenic pressures, the HFBI is built considering different reference conditions, depending on the sampling period (spring or autumn), the type of water body (non-tidal, microtidal oligohaline / mesohaline / polyhaline, or microtidal euhaline), and the presence or absence of aquatic seagrasses (Catalano *et al.*, 2017; Zucchetta *et al.*, 2020).
Hence, the HFBI was calculated by station and sampling period. These values were first averaged by lagoon and then on annual basis in order to have a single HFBI per lagoon. The ecological status assessments were expressed in terms of five classes of ecological status (i.e. High, H; Good, G; Moderate, M; Poor, P; Bad, B; e.g. higher values of HFBI correspond to high quality class), as prescribed by the WFD (Catalano *et al.*, 2017; Zucchetta *et al.*, 2020).

Results

During the two sampling campaigns, a total of 22 fish species belonging to 13 teleost families were found (Table 1). The Mugilidae and Gobiidae families were the most represented in the fish assemblage, with 5 and 4 species, respectively.
Figure 2 shows the mean values of total abundances and total biomasses of the fish assemblage in each lagoon, comparing the two sampling seasons.
Large differences in both abundance and total biomass were observed between lagoons in spring and the differences were statistically significant ($p<0.01$). The highest values (427 individuals/100 m^2; 219 g/100 m^2), were observed in Scardovari, while markedly lower values were observed in the remaining lagoons; the minimum total abundances and biomasses were found in Caleri (15 individuals/100 m^2; 7.2 g/100 m^2).

Table 1 - List of species recorded during the two sampling campaigns; for each species, the classifications into Estuarine Use Functional Guilds (EUFG) and Feeding Mode Functional Guilds (FMFG) are reported. The presence of each species during the two seasonal samplings is also reported. EUFG Legend (Scapin et al., 2019): ES = resident taxa with also marine or freshwater populations; ESs = resident taxa solely estuarine; ME-D = marine estuarine-dependent migrants; ME-O = marine estuarine-opportunist migrants; MS = marine stragglers; FS = freshwater stragglers. FMFG Legend (Catalano et al., 2017): Bmi = microbenthivores; Bma = macrobenthivores; HZ = hyperbenthivores/zooplanktivores; HP = hyperbenthivores/piscivores; Dv = detritivores; PL = planktivores; Ov = omnivores.
Tabella 1 – Lista delle specie rinvenute durante le due campagne di campionamento; per ogni specie è riportata la classificazione in guild di utilizzo degli estuari (EUFG) e in guild di modalità di foraggiamento (FMFG). È riportata anche la presenza di ogni specie durante ognuna delle due campagne di campionamento stagionali. Legenda EUFG (Scapin et al., 2019): ES = taxa residenti aventi anche popolazioni marine o di acqua dolce; ESs = taxa residenti esclusivamente estuarini; ME-D = migratori marini dipendenti dagli estuari; ME-O = migratori marini opportunisti; MS = marini occasionali; FS = di acqua dolce occasionali. Legenda FMFG (Catalano et al., 2017): Bmi = microbentivori; Bma = macrobentivori; HZ = iperbentivori/zooplanctivori; HP = iperbentivori/piscivori; Dv = detritivori; PL = planctivori; Ov = onnivori.

Family	Species	Label	EUFG	FMFG	Spring	Autumn
Atherinidae	*Atherina boyeri*	**ABO**	ES	HZ	x	x
Callionymidae	*Callionymus risso*	**CRI**	MS			x
Clupeidae	*Sardina pilchardus*	**SPI**	ME-O	PL	x	
Clupeidae	*Sprattus sprattus*	**SSP**	ME-O	PL	x	
Cyprinodontidae	*Aphanius fasciatus*	**AFA**	ESs	Bmi, OV	x	x
Gobiidae	*Knipowitschia panizzae*	**KPA**	ESs	Bmi, HZ	x	x
Gobiidae	*Pomatoschistus canestrinii*	**PCA**	ESs	Bmi, HZ	x	x
Gobiidae	*Pomatoschistus marmoratus*	**PMA**	ES	Bmi, HZ	x	x
Gobiidae	*Pomatoschistus minutus*	**PMI**	ME-O	Bmi, HZ	x	
Moronidae	*Dicentrarchus labrax*	**DLA**	ME-O	HZ, HP	x	
Mugilidae	*Chelon auratus*	**CAU**	ME-D	HZ, DV	x	x
Mugilidae	*Chelon labrosus*	**CLA**	ME-O	HZ, DV	x	
Mugilidae	*Chelon ramada*	**CRA**	ME-D	HZ, DV	x	x
Mugilidae	*Chelon saliens*	**CSA**	ME-D	HZ, DV	x	x
Mugilidae	*Mugil cephalus*	**MCE**	ME-O	HZ, DV	x	x
Pleuronectidae	*Platichthys flesus*	**PFL**	ME-D	Bmi, Bma, HP	x	
Poeciliidae	*Gambusia* gr. *holbrooki*	**GHO**	FS			x
Scophthalmidae	*Scophthalmus rhombus*	**SRH**	MS		x	
Soleidae	*Solea solea*	**SSO**	ME-O	Bmi, Bma	x	
Sparidae	*Sparus aurata*	**SAU**	ME-D	Bmi, Bma, HZ	x	
Syngnathidae	*Syngnathus abaster*	**SAB**	ESs	Bmi, HZ	x	x
Syngnathidae	*Syngnathus taenionotus*	**STA**	ES	HZ	x	

Multiple comparison of the mean ranks following the Kruskal-Wallis test results showed statistically significant differences ($p<0.05$) between the Caleri and Scardovari lagoons in terms of density; in terms of biomass, the differences between Caleri and Scardovari and between Caleri and Canarin lagoons were significant.
In autumn, total abundances and biomasses were higher in Caleri (272 individuals/100 m^2; 156 g/100 m2) and Scardovari (286 individuals/100 m^2; 155 g/100 m^2), than in the other lagoons, with the lowest values in Barbamarco (24 individuals/100 m^2; 19.1 g/100 m^2). But in autumn the differences observed between lagoons were not statistically significant, both in terms of density and biomass.

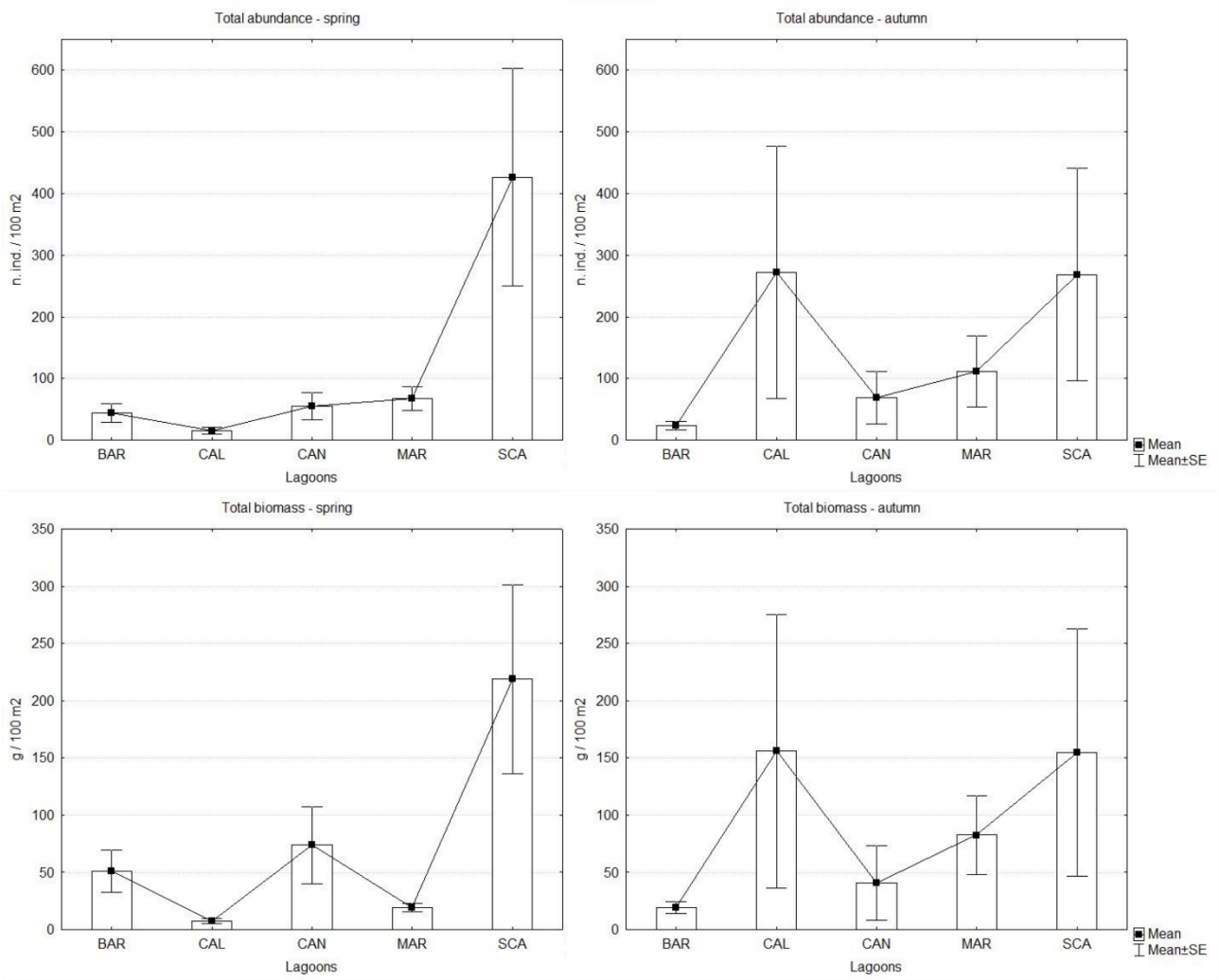

Figure 2 - Total abundance (number of individuals/100m^2; above graphs) and Total Biomass (g/100m^2; below graphs) of the fish assemblage in five lagoons of the Po Delta (CAL = Caleri; MAR = Marinetta; BAR = Barbamarco; CAN = Canarin; SCA = Scardovari), in Spring (left graphs) and Autumn (right graphs).
Figura 2 – Abbondanza totale (numero di individui/100m^2; grafici sopra) e biomassa totale (g/100m^2; grafici sotto) del popolamento ittico nelle cinque lagune del Delta del Po (CAL = Caleri; MAR = Marinetta; BAR = Barbamarco; CAN = Canarin; SCA = Scardovari), in Primavera (grafici a sinistra) ed in Autunno (grafici a destra).

In terms of species composition, a differentiation between spring and autumn samples is observed as shown by the nMD (Figure 3). The multivariate statistical analysis conducted on the species allowed to separate the species into three groups: a first group, consisting of *Chelon auratus*, *C. ramada*, *Sparus aurata* and *Sardina pilchardus* characterizes the spring samples; a second group represented by *C. saliens*, *Atherina boyeri*, *Pomatoschistus marmoratus*, *Aphanius fasciatus* and *Knipowitschia panizzae* characterizes the autumn samples; finally, *Solea solea* and *P. minutus* were found only in spring, but with very low abundance values.
Functional structure of the fish assemblage, calculated on percentage biomass by EUFG category, is showed in Figure 4. In spring, the fish assemblage was dominated, in terms of biomass, by the categories of marine migrants (especially ME-D, but also ME-O) in all the investigated lagoons. The contribution of resident species (ESs and ES) seemed completely negligible, with the only exception of Caleri and, to a lesser extent, Marinetta. Compared to spring, in autumn the resident species were more important in characterizing the fish community in terms of biomass, both in Caleri (ESs) and in the Marinetta and Barbamarco (ES). In Canarin and Scardovari, on the other hand, the population was dominated even in autumn by ME-D (Figure 4).

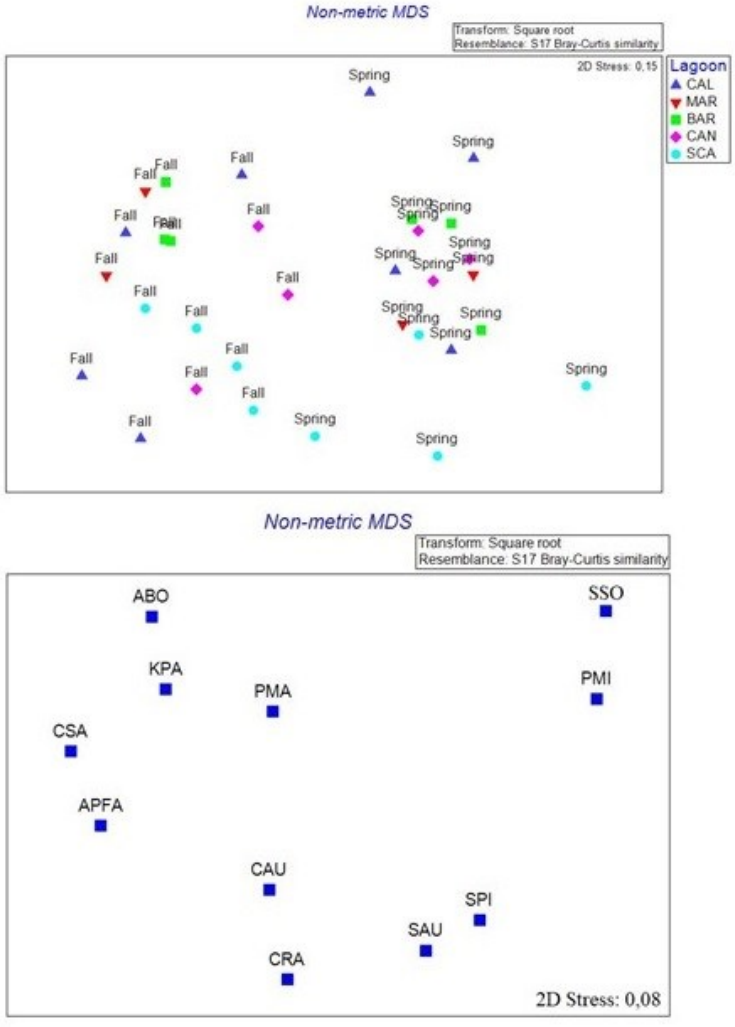

Figure 3 – Non metric Multi Dimensional Scaling on density data of the most abundant species (>98% of the total); above: sample point are displayed; belove: species point are displayed. (Legends: Spring = spring; Fall = autumn; CAL = Caleri; MAR = Marinetta; BAR = Barbamarco; CAN = Canarin; SCA = Scardovari; for the abbreviations of species, see Table 1).
Figura 3 –Non metric Multi Dimensional Scaling sui dati di densità delle specie più abbondanti (>98% del totale); grafico superiore: ordinamento dei punti-campione, grafico inferiore: ordinamento dei punti-specie. (legenda: Spring= primavera, Fall = autunno, CAL = Caleri, MAR = Marinetta, BAR = Barbamarco, CAN = Canarin, SCA = Scardovari; per le sigle delle specie, vedi Tabella 1).

Figure 4 – Percentage EUFG guild structure of fish assemblage in five lagoons of Po Delta (CAL = Caleri; MAR = Marinetta; BAR = Barbamarco; CAN = Canarin; SCA = Scardovari), based on biomass (g/100 m^2), in spring (above) and autumn (below). EUFG labels are reported in Table 1.
Figura 4 – Struttura percentuale in EUFG del popolamento ittico in cinque lagune del Delta del Po (CAL = Caleri; MAR = Marinetta; BAR = Barbamarco; CAN = Canarin; SCA = Scardovari), basata sulla biomassa (g/100 m2), in Primavera (sopra) ed in Autunno (sotto). Per le sigle delle categorie EUFG, vedi Tabella 1.

To correctly apply the HFBI, the resident fish (ES and ESs) and marine migrants (ME-D and ME-O) guilds were also classified into FMFG (Table 1). However, the classification is not unique, given that in many cases a species can be attributed to different categories (Catalano *et al.*, 2017). Overall, 14 species contribute to the guild of hyperbenthivores/zooplanktivores (HZ), 9 to that of microbenthivores (Bmi) and 5 that of detritivores (DV), while the remaining guilds were less represented in terms of number of species.
Figure 5 shows the scores of the six metrics composing the HFBI. Considering the species representing 90% or more of the total biomass, different patterns were observed in the studied lagoons: in Caleri and Marinetta the diversity was bigger in spring than in autumn, while in Scardovari and, above all, in Canarin the opposite was observed; finally, in Barbamarco, the relatively low diversity values were comparable in the two seasonal sampling campaigns. The

diversity of marine migrants (ME-D and ME-O together) was bigger in spring than in autumn, with the only exception of the Canarin lagoon, where the values were comparable in the two sampling seasons.
Different patterns were also observed for the metric calculated by the ratio between total biomass and total abundance (B/N) (Figure 5). The ratios were higher in autumn than in spring in Caleri and, above all, in Marinetta, while the opposite pattern was observed in Barbamarco and Canarin; in Scardovari, the B/N values were comparable in the two seasons.
Even in the case of metrics relating to FMFG, differences between lagoons can be observed in the seasonal pattern (Figure 5). The biomass of the benthivorous species (Bmi and Bma considered together) was higher in autumn in Caleri, Marinetta and Scardovari, while an opposite pattern was observed in Barbamarco and Canarin. The diversity of both benthivores and detritivores was the highest during spring in Caleri and Marinetta and during autumn in Barbamarco and Canarin; in Scardovari, these two metrics were not substantially different in the two considered seasons (Figure 5).

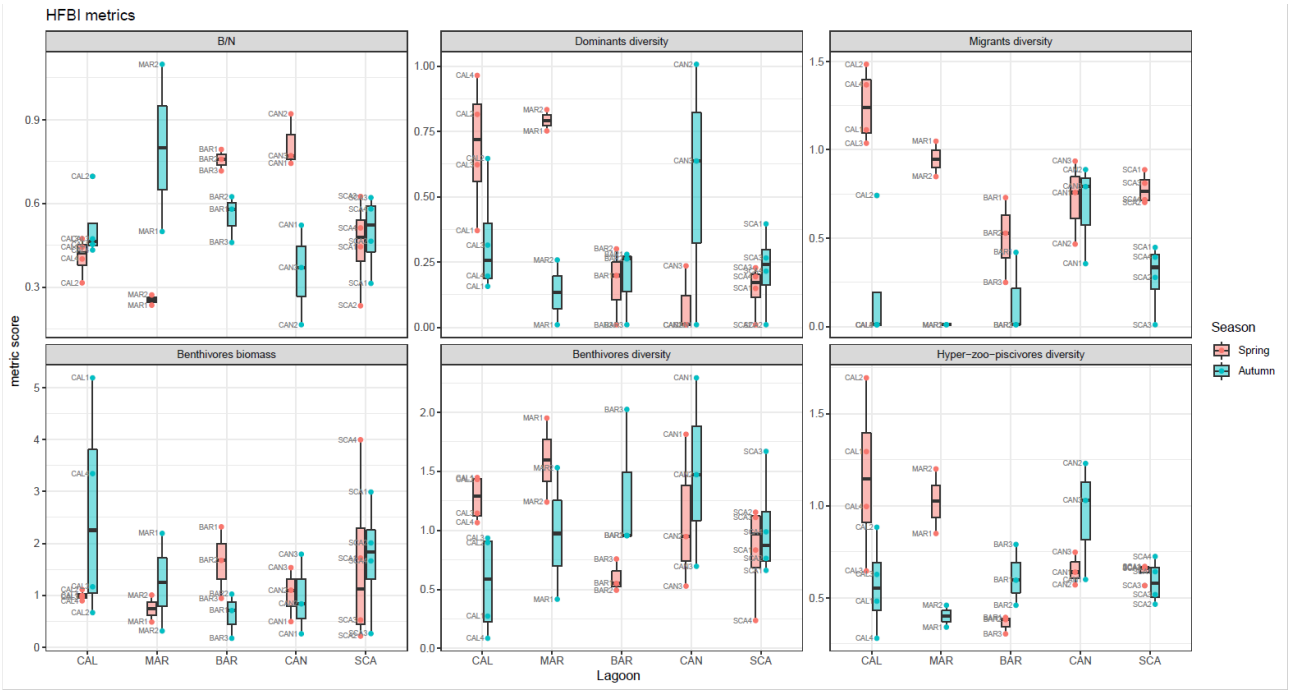

Figure 5 – HFBI metrics calculated by sampling station and season. The boxplots represent the median scores, the first and third quartiles, the highs and lows values for each lagoon and season. Lagoons are abbreviated as follows: CAL: Caleri; MAR: Marinetta; BAR: Barbamarco; CAN: Canarin; SCA: Scardovari.
Figura 5 – Metriche dell'indice HFBI, calcolate separatamente per stazione di campionamento e stagione. I boxplot rappresentano il valore mediano, il primo e terzo quartile, ed i valori massimi e minimi per ogni laguna e stagione. Le lagune sono abbreviate come di seguito: CAL: Caleri; MAR: Marinetta; BAR: Barbamarco; CAN: Canarin; SCA: Scardovari.

The six metric values were combined to calculate the HFBI (Figure 6). In all lagoons, a wide variability in HFBI scores between stations and seasons was detected, indicating the wide variability in environmental, ecological and anthropogenic pressures that characterizes these systems. The HFBI scores calculated by combining the spring and autumn scores (Catalano *et*

al., 2017) showed marked differences between the 5 water bodies. Caleri was the only lagoon in good ecological status in the survey's year, albeit with marked differences in the index values between stations and between seasons. Marinetta was found to be in moderate condition, even with an average score just below the threshold of "good" ecological status. In this lagoon, the ecological status resulted markedly better in spring than in autumn (Figure 6). The Canarin lagoon was also found to be in a moderate ecological status, but in this case an improvement can be observed from spring to autumn. Finally, an overall poor ecological status was observed both in Scardovari and in Barbamarco. However, while in Scardovari a high variability of the index scores was observed, both between seasons and between stations, in Barbamarco a lower variability was recorded and with no value, calculated by sampling station, in good condition (Figure 6).

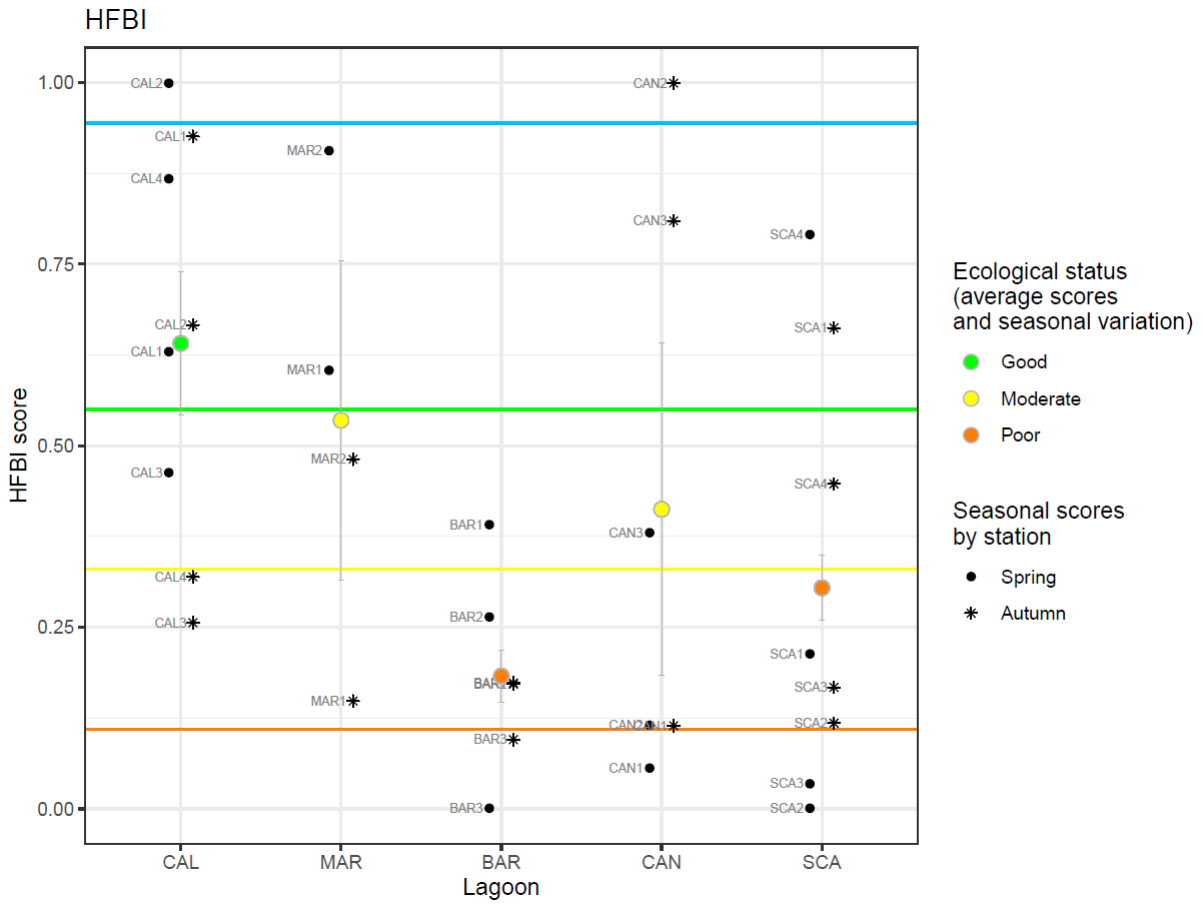

Figure 6 – HFBI scores of each station and ecological status classification. Lagoons are abbreviated as follows: CAL: Caleri; MAR: Marinetta; BAR: Barbamarco; CAN: Canarin; SCA: Scardovari.

Figura 6 – Valori HFBI di ogni stazione e classificazione in stato ecologico. Le lagune sono abbreviate come di seguito: CAL: Caleri; MAR: Marinetta; BAR: Barbamarco; CAN: Canarin; SCA: Scardovari.

Discussion and Conclusions

The shallow water fish assemblage: differences between lagoons and seasons

Overall, the survey of the shallow water fish fauna of the Po Delta's lagoons made it possible to highlight both similarities and differences between the 5 water bodies. Marked differences

in the composition of the fish assemblage between the two seasonal surveys can be seen in all the lagoons investigated. This is in accordance with what is known about the seasonal dynamics of fish assemblages in the transitional environments of the Northern Adriatic (Rossi, 1986; Franzoi *et al.*, 1989a; Franco *et al.*, 2006). In the development phase of the HFBI index, these seasonal differences in the species composition and functional structure of the shallow-water fish community were in fact taken into account (Catalano *et al.*, 2017; Zucchetta *et al.*, 2020). During the spring sampling campaign in all the investigated lagoons, the fish assemblage was dominated by the component of marine migrants (Franzoi *et al.*, 2010), present on the shallow waters as fry or juveniles. As already observed in the past (Rossi, 1986; Franzoi *et al.*, 1989b, Franzoi & Trisolini, 1991), the species present with the widest distribution and with the highest density values were the mullets *Chelon auratus* and *Chelon ramada*, and the sea bream *Sparus aurata*. From a qualitative point of view, the results of this study are substantially in agreement with what was observed in the 1980s of the past century in the Sacca di Scardovari (Rossi, 1986; Franzoi *et al.*, 1989b). Overall, the Scardovari lagoon was the one with the greatest spring presence of fish fry in ascent by the sea, both in terms of number of species and density of individuals, among all investigated lagoon environments.
In autumn, the marine migrant guild was formed by the mugilids *Chelon saliens*, *C. auratus* and *Mugil cephalus*. During this season, *C. saliens* was found in all investigated environments. Also in this case, results of this study were in accordance with what is reported in the literature (Rossi, 1986; Franzoi *et al.*, 1989b; Franzoi & Trisolini, 1991).
Lagoon residents are more represented in the fish assemblage in autumn than in spring. Even in the case of the component of lagoon residents, the comparison is possible only in relation to the Scardovari Lagoon. Compared to what was observed in the 1980s (Franzoi *et al.*, 1989a, 1989b), no important differences can be observed in the list of species found overall. However, it should be considered that the pipefish *Syngnathus abaster* and *S. taeniotus*, which contributed to characterize the fish community of the lagoon in the 1980s (Franzoi *et al.*, 1989a, 1993), were caught only occasionally and only with very few individuals in 2021.
The fish assemblage of Po Delta's lagoons therefore appears to be characterized by greater stability and greater resilience than that observed for freshwater Delta environments (Lanzoni *et al.*, 2010). In particular, Po Delta's lagoons seem to play an important role as nursery areas for juveniles of marine migrant species, as already highlighted in previous studies (Rossi, 1986; Franzoi *et al.*, 1989a, 1989b).

The assessment of the ecological status of fish fauna: the implementation of the HFBI

The results of the application of the multimetric index HFBI showed a greater variability of the ecological status of delta lagoons than what emerged in previous studies on the environmental quality of these water bodies. In particular, a study conducted in 2008 on the state of aquatic vegetation had found, in all the lagoon environments considered in this study, the total absence of phanerogams and a negligible and sporadic presence of a few macroalgal taxa sensitive to environmental quality (Sfriso *et al.*, 2016). The application of the MaQI index, which evaluates the ecological quality of the aquatic macrophyte vegetation of Italian transitional water bodies (Sfriso *et al.*, 2007, 2009, 2014), had highlighted poor to bad ecological status conditions for all lagoons of the Po Delta (Sfriso *et al.*, 2016). On the other hand, in the present study marked differences in the ecological quality between lagoons considered were highlighted. These differences can be at least partially explained in the light of the different physical (hydrodynamics and granulometric textures of the seabed) and morphological features of the lagoons investigated (Consorzio di Bonifica Delta Po Adige - CFR, 2015; Stefani *et al.*, 2018).

Only the Caleri lagoon was found to be in good condition based on the application of the HFBI, and this agrees with the potential good quality measured on the basis of hydrodynamics, bathymetry and sediment grain size. Furthermore, this lagoon is the most influenced by the inflows of sea water, receiving fewer freshwater inputs from the Po River and the surrounding area than in the other water bodies investigated; the exchange of water within the lagoon is guaranteed by a channel about 150 m wide in direct communication with the sea in front (Verza & Cattozzo, 2015; Mascolo *et al.*, 2019; Franzo *et al.*, 2019). On the contrary, the lagoon that presented the worst classification ("poor"), Barbamarco, is also the one with the lowest potential quality based on its physical characteristics (Consorzio di Bonifica Delta Po Adige - CFR, 2015). The shallow-water fish assemblage of this lagoon is the one that, on the whole, has presented the lowest values of abundance, biomass and biodiversity.
The interpretation of the ecological status classification of the other three lagoons seems more difficult (Marinetta and Canarin classified in "moderate" ecological status; Scardovari classified, as Barbamarco, in "poor" ecological status) on the basis of the environmental potential assessed only on the basis of their physical characteristics. In fact, all these three lagoons have both areas with low environmental potential and areas with high environmental potential (Consorzio di Bonifica Delta Po Adige - CFR, 2015). In the case of the Scardovari lagoon, moreover, the sampling of fish fauna was conducted in the southern area (called *Sacca di Bottonera*) which corresponds to the area with the greatest environmental potential. It is therefore evident the need for further investigations to highlight all environmental factors that contribute to determining the functional characteristics of the fish community and therefore the classification of the ecological status. In particular, it would be necessary to apply an approach to identify the main anthropogenic pressures that insist on the transitional water bodies of the Po Delta and the impacts that these determine on the lagoon shallow fish fauna, through the study of the relationships between anthropogenic pressure indicators and characteristics (metrics) of the fish assemblage, as highlighted in other studies (Zucchetta *et al.*, 2020; Zucchetta *et al.*, 2021).

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