APPLICAZIONE DELL'HABITAT FISH BIO-INDICATOR A DUE CORPI IDRICI DELLA LAGUNA DI VENEZIA: RUOLO DELLA VARIABILITÀ AMBIENTALE NELLA VALUTAZIONE DELLO STATO ECOLOGICO DELL'EQB FAUNA ITTICA

IMPLEMENTATION OF THE HABITAT FISH BIO-INDICATOR IN TWO WATER BODIES OF THE VENICE LAGOON: THE ROLE OF SEASONAL AND ENVIRONMENTAL VARIABILITY IN THE ASSESSMENT OF ECOLOGICAL STATUS OF FISH FAUNA

FRANZOI PIERO[1*], SCAPIN LUCA[1], REDOLFI BRISTOL SIMONE[1], ZUCCHETTA MATTEO[1]

1. *Dipartimento di Scienze Ambientali, Informatica e Statistica (DAIS) – Università Ca' Foscari di Venezia, Via Torino 155, 30170 Mestre-Venezia, *corresponding author pfranzoi@unive.it, 0412347734*

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Riassunto
Nell'ambito della Direttiva Quadro sulle Acque (WFD; Dir. 2000/60/CE), i pesci costituiscono uno degli Elementi di Qualità Biologica che devono essere inclusi nella valutazione dello Stato Ecologico dei corpi idrici di transizione. L'Habitat Fish Bio-Indicator (HFBI), un indice multi-metrico costituito da sei descrittori basati su caratteristiche funzionali della comunità ittica, è stato recentemente sviluppato per valutare le acque di transizione italiane. In questo lavoro l'HFBI è stato impiegato, seguendo il protocollo ufficiale di applicazione, in due sottobacini della laguna di Venezia caratterizzati da diverse caratteristiche fisico-chimiche di acqua e sedimento e da differenti habitat. L'analisi evidenzia che, oltre alla stagionalità, anche la variabilità ambientale lungo i gradienti di confinamento e la presenza ed estensione di praterie di angiosperme acquatiche influenzano in maniera rilevante le sei metriche, il punteggio di HFBI e, di conseguenza, il risultato della valutazione. La selezione del numero e della posizione delle stazioni di campionamento deve pertanto basarsi sulle caratteristiche ambientali e sulla variabilità di ciascun sottobacino, e costituisce una fase di cruciale importanza nella valutazione dello Stato Ecologico negli ecosistemi di transizione.

Abstract
Under the Water Framework Directive (WFD; Dir. 2000/60/EC), fish are one of the Biological Quality Elements that need to be taken into account when evaluating the Ecological Status of transitional water bodies. The Habitat Fish Bio-Indicator (HFBI), a multi-metric index composed of six descriptors based on functional traits of fish assemblages, was recently developed to assess Italian transitional waters. In this study, HFBI was applied, following the national application protocol, to two water bodies in the Venice lagoon featuring different water and sediment physco-chemical properties and habitat distribution. The analysis highlighted that, in addition to seasonality, environmental variability along confinement gradients and

presence and extent of seagrass meadows strongly influence the six metrics, the HFBI score and the subsequent assessment outcome. The selection of number and location of sampling sites must then be based on environmental characteristic and variability found within each water body, and is therefore a crucial step in the evaluation of Ecological Status in transitional water ecosystems.

Introduction

The assessment and monitoring of the ecological status of transitional waters, understood as an "expression of the quality of the structure and functioning" of these ecosystems, is regulated by the Community Directive 2000/60/EC, also known as "Water Framework Directive" (WFD). For the assessment of the ecological status of these water bodies the WFD requires the use not only of physico-chemical and hydromorphological factors, but also of biological elements, one of which is represented by the composition and abundance of fish fauna. In fact, fish fauna is an important component of the biodiversity that characterizes coastal lagoons and estuarial zones: some important ecosystem processes and services are mediated by fish fauna, such as regulation of trophic webs and the connectivity between different parts of the ecosystem and between transitional and coastal marine ecosystems (Kneib, 2000; McLusky e Elliott 2004). Several fish indices have been proposed to assess the quality of European estuaries and lagoons (Franco et al. 2009; Perez-Dominguez et al., 2012).

The Habitat Fish Bio-Indicator (HFBI), a multi-metric index composed of six descriptors based on functional traits of fish assemblages (Franco et al., 2008, 2009), was recently developed to assess Italian transitional waters (Catalano et al., 2017). The six metrics considered in the HFBI are: Margalef index assessed on the biomass of the dominant species (species representing 90% of the total fish biomass), $\mathbf{D_{dom}}$; Individual average weight, **B/N** (in g); Margalef index assessed on the biomass of migratory species, $\mathbf{D_{migr}}$; Biomass density of bentivorous species, $\mathbf{B_{bv}}$ (in g/100m^2); Margalef index evaluated on biomass of bentivorous species, $\mathbf{D_{bv}}$; Margalef index assessed on the biomass of the hyperbentivorous/zooplanctivorous/piscivorous species, $\mathbf{D_{hzp}}$. The HFBI application protocol prescribes fish sampling during both spring and autumn in shallow water areas by means of a beach seine net with defined and standardised length, height and mesh size, and accounts for different reference conditions according to season (spring, autumn), water body type (non tidal, microtidal oligo-/meso-/polyhaline, microtidal euhaline/hyperhaline) and habitat (vegetated, unvegetated).

The aim of the study was to link HFBI and its components to seasonality and main environmental variability in transitional waters, in order to highlight the role of number and location of monitoring sites in protocols for the WFD assessment of ecological status of fish fauna. HFBI was applied to two water bodies in the Venice lagoon featuring different water and sediment physico-chemical properties and habitat distribution.

Materials and methods

The study was carried out during spring (April and May) and autumn (October) 2016 in two sub-basins of the northern Venice lagoon (Figure 1). The sub-basins feature different water and sediment characteristics and habitat distribution, as well as multiple confinement gradients. Fish sampling and the subsequent application of HFBI followed the protocol for the evaluation of ecological status of fish fauna in Italian transitional waters (Catalano *et al.*, 2017). Fish were sampled at 14 sites by means of a small seine net of standard dimensions (Franco *et al.*, 2006); all specimens were identified at the species level, and total abundance and biomass were calculated and standardised over 100 m^2. During each sampling occasion, a set of environmental variables were also collected: water parameters (temperature, salinity, dissolved

oxygen saturation, turbidity) were registered with a multi-parametric probe; seagrass cover was estimated by visual census following the Braun-Blanquet method (Braun-Blanquet, 1972), and subsequently expressed as percent cover; finally, sediment granulometry (percentage of sand in the surface layer) was derived for each site from thematic maps (ARPAV, 2012).
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 occasion and site, and the HFBI seasonal scores were then derived. Annual HFBI scores were calculated for each site by averaging seasonal scores, and Ecological status classification was finally obtained for each site sub-basin by averaging annual HFBI scores. While the protocol requires the Ecological Status assessment to be carried out at the sub-basin level (Catalano *et al.*, 2017), for the purposes of this study also annual HFBI scores at each site were classified.
In order to highlight any influence of environmental variability on the results of the index application, seasonal values of the metrics and HFBI scores were linked to physico-chemical parameters and seagrass cover by means of a distance-based redundancy analysis (dbRDA) using Euclidean distance. The significance of each eigenvalue, hence the strength of the overall relationship between explanatory and response matrices, was inferred by means of a pseudo-F test. In addition, a Mantel test was employed to assess the correlation between the response matrix and each environmental variable considered.

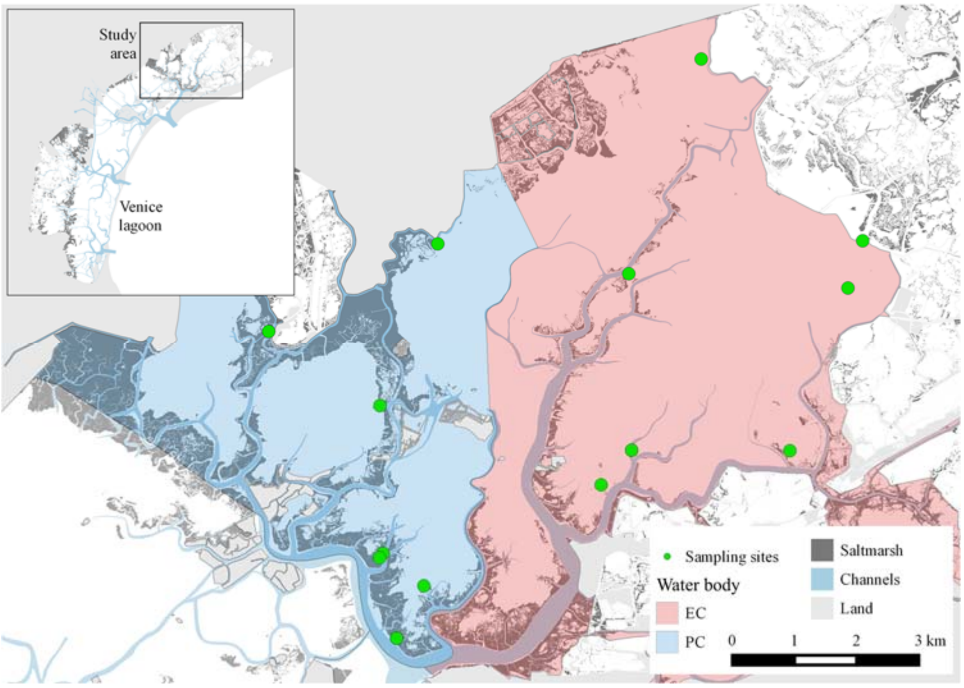

Figura 1. Area di studio. Per ciascuno dei corpi idrici considerati (EC: eualino confinato; PC: polialino confinato) sono illustrate le stazioni di campionamento della fauna ittica
Figure 1. Study area. For each water body considered (EC: euhaline confined; PC: polyhaline confined), fish sampling sites are shown

Results

The six metrics included in the HFBI varied between spring and autumn as well as among sampling sites and between the two sub-basins (Figure 2). Overall, average individual weight and diversity of migrants were higher during spring, while density of benthivores and diversity of hyper-zoo-piscivores showed higher values during autumn. On average, the PC sub-basin featured higher values of average individual weight, diversity of migrants and diversity of benthivores, whereas EC showed higher values of diversity of dominant species and density of benthivores.

Only the first two axes of the dbRDA were significant (p-value <0.05), and allowed to explain 83% of total variability in metric values and HFBI scores (Figure 3). The first axis (57.5% of variation explained) was positively correlated with salinity, sand content in sediments and seagrass cover, hence identifying a major spatial gradient from euhaline areas (mostly included in the EC sub-basin), featuring sandier sediments and larger portions of seagrass habitat, to more brackish ones (included in PC), characterised in turn by finer sediments and bare substrata. The second axis (25.6% of variation explained) was positively related to turbidity, and represented a spatial gradient of water transparency. Temperature also played a major role in explaining the variation of metric and HFBI values, and was mainly correlated (positively) with the second axis. This variable separated the spring from the autumn surveys, the former characterised by overall lower temperatures. Some diversity metrics, such as diversity of dominant species, as well as HFBI scores, were overall associated to less confined sites with higher salinity and sand content values, while diversity of migrants and average individual weight were higher at lower salinity. The Mantel test did not highlighted any significant correlation between the index matrix and any of the environmental variables considered (p-value >0.05). Despite the absence of correlation, the multivariate analysis highlighted that both HFBI scores and values of high-weight metrics respond to overall variations in water and sediment characteristics. In particular, a large proportion of overall variability in index outcomes could be explained by the existence of a salinity gradient.

The Ecological Status classification resulting from the application of the HFBI protocol varied markedly among sites of both the sub-basins (Figure 4). In EC, one site was classified as “poor”, four as “good” (HFBI scores ranging from 0.64 to 0.81) and two as “high”. In PC, one site was classified as “poor”, five as “good” (HFBI scores ranging from 0.58 to 0.80) and one as “high”. Despite the large variability among sites, the overall classification was consistent between the two sub-basins, with both of them being classified as “good” (HFBI scores of 0.74 and 0.65 for EC and PC respectively).

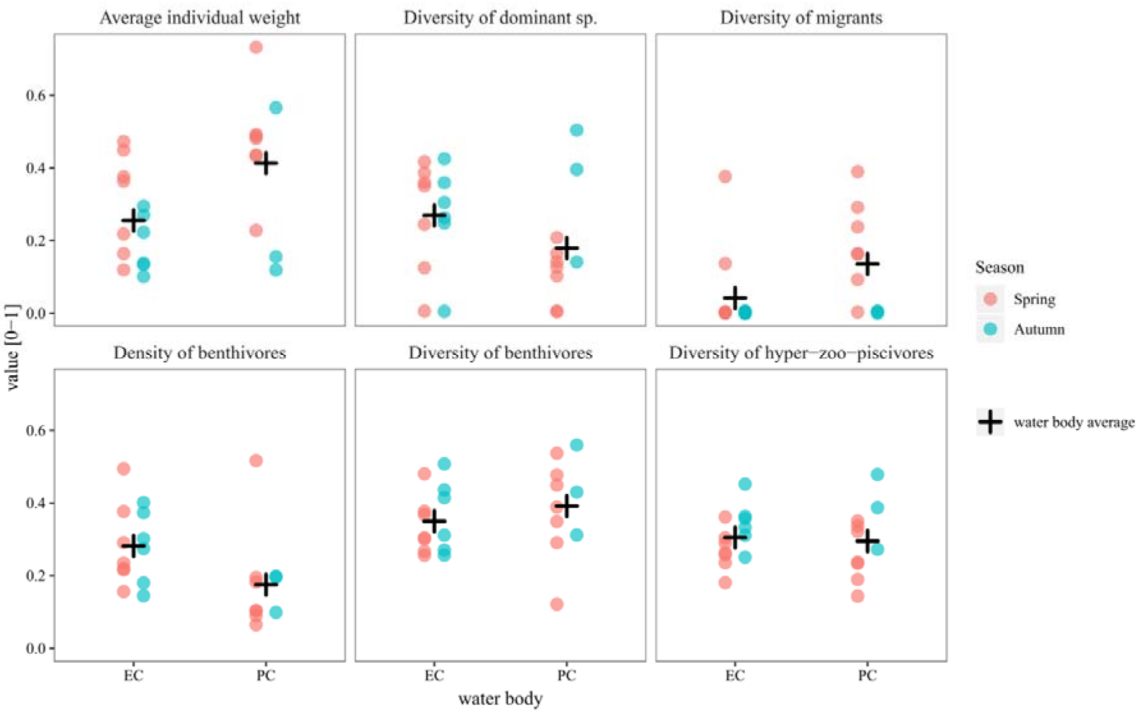

Figura 2. Valori stagionali delle sei metriche incluse nell'HFBI, espressi come rapporti di qualità ecologica. Per ciascun corpo idrico è inoltre riportato il valore medio di ciascuna metrica
Figure 2. Seasonal values of the six metrics included in HFBI, expressed as ecological quality ratios. For each water body the average value of each metric is also shown

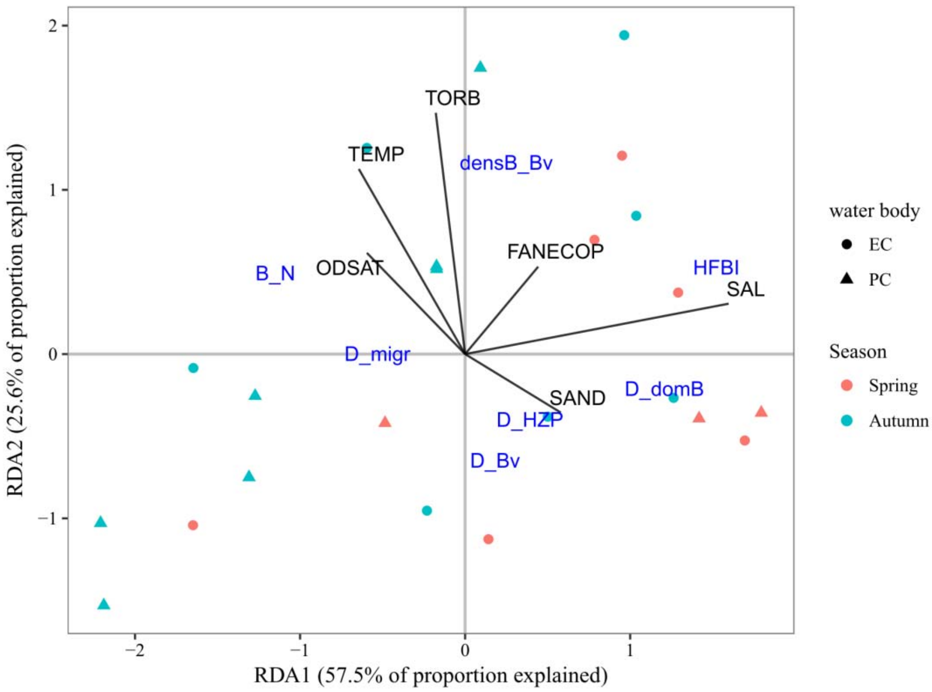

Figura 3. dbRDA calcolata sulla matrice delle metriche e dei punteggi stagionali di HFBI. I predittori ambientali inclusi nell'analisi sono abbreviati come segue: TEMP: temperatura; SAL: salinità; ODSAT: ossigeno disciolto; TORB: torbidità; SAND: percentuale di sabbia nel sedimento; FANECOP: copertura di fanerogame. Si veda il testo per l'abbreviazione delle metriche
Figure 3. dbRDA performed on metrics and HFBI seasonal scores matrix. Environmental predictors included in the analysis are abbreviated as follows: TEMP: temperature; SAL: salinity; ODSAT: dissolved oxygen; TORB: turbidity; SAND: percentage of sand in sediment; FANECOP: seagrass cover. See text for metrics abbreviations

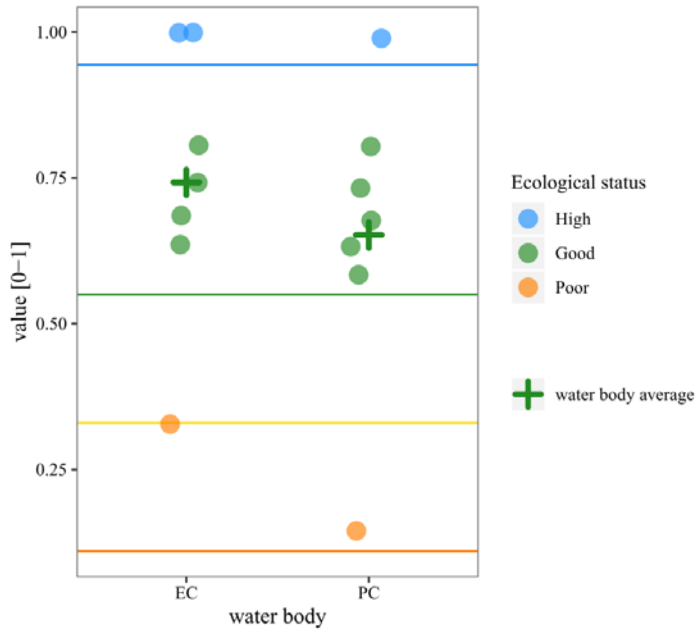

Figura 4. Punteggi medi di HFBI e relativa classificazione in stato ecologico per stazione di campionamento. Per ciascun corpo idrico è riportato il punteggio medio di HFBI. Sono inoltre indicate le soglie tra le classi di stato ecologico
Figure 4. Average HFBI scores and ecological status classification for sampling site. For each water body the average HFBI score is shown. Limits among classes of ecological status are also indicated

Discussion and Conclusions

Fish assemblages in Italian transitional waters exhibit marked seasonal variations in their structure and species composition (Franco *et al.*, 2006). As a result, the Ecological Status assessment of fish fauna in these ecosystems is carried out by performing spring and autumn surveys (Franco *et al.*, 2009; Catalano *et al.*, 2017). This work highlights that also environmental variability within sub-basins plays a major role in affecting the outcomes of the assessment.

In the Venice lagoon the degree of confinement and the resulting gradients in environmental parameters shape the structure and composition fish assemblages, with different taxa exhibiting preferences for different ranges of e.g. salinity, temperature, turbidity, trophic state and sediment granulometry (Malavasi *et al.*, 2005; Franzoi *et al.*, 2010). Habitat heterogeneity also contributes to the variability of fish assemblages, and affected some of the HFBI metrics analysed in this study. Seagrass meadows, for instance, host higher fish densities and overall more diverse communities, featuring large proportions of microbenthivorous and hyperbenthivorous-zooplanctivorous-piscivorous estuarine residents (Franco *et al.*, 2006; Malavasi *et al.*, 2007).

Due to such environmental influence on HFBI, the outcome of the assessment at the site level can be highly variable within each sub-basin, even if the index application at the sub-basin level would result in the same classification. Subsequently, the selection of the number of monitoring sites, as well as their location along environmental gradients and across the habitat mosaic, are critical. While HFBI accounts for salinity differences at the sub-basin scale (Franco *et al.*, 2009; Catalano *et al.*, 2017), this study highlights that finer-scale multiple environmental gradients are also important. From a management perspective this suggests that, if a reliable and realistic assessment of the ecological status of fish fauna is expected, a sufficient number of sampling sites located along the environmental gradient should be designed or, alternatively, sub-basin boundaries should be re-defined in order to take into account smaller-scale variability.

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