

Body condition in fish as a tool to detect the effects of anthropogenic pressures in transitional waters

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Abstract In the last years, increasing interest has been dedicated to the quality assessment of brackishwater systems. Traditionally, fish community is an important biological element used to assess the quality status of transitional water bodies. In this study, we analysed the effect of anthropogenic pressures on the population of a small teleost, the sand smelt Atherina boyeri, in a Mediterranean lagoon by means of body condition. Fish were sampled once a year during the period 2010-2012, in 32 sampling sites, and for each specimen individual body condition factor was estimated. A negative significant correlation was found between condition factor and pressures related to alteration of the hydrographic regime, while a significant positive correlation was found with trophic status indicators and fishery activities. Therefore, morphological and hydrological alteration of coastal lagoons, modifying the quality and the availability of resources, seems to influence the health of resident populations.

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N. Bettoso · A. D'Aietti · L. Faresi ARPA FVG, Regional Agency for Environmental Protection in the Friuli Venezia Giulia region, Via Cairoli, 14, 33057 Palmanova, UD, Italy **Keywords** Atherina boyeri · Bioindication · Condition factor · Coastal lagoon

Introduction

The contribution of biological elements to the assessment of ecological status in transitional waters is a relevant issue (Cabral et al. 2012). In the last decade, many papers dealt with this problem by many points of view. In particular, a great effort has been devoted to develop good methods able to define and assess the ecological status of these aquatic ecosystems, especially in Europe for the implementation of the Water Framework Directive (WFD). For the northern Atlantic region, official methodologies using fish as BQE (Biological Quality Elements) have been developed and intercalibrated (Lepage et al. 2016), but for the Mediterranean transitional water systems an official methodology is still lacking, even if different tools have been proposed (e.g. Franco et al. 2009; Drouineau et al. 2012).

Estuaries and coastal lagoons are widely known to be elective habitats for abundant and diversified fish populations (Blaber 1997; Franco et al. 2008; Able and Fahay 2010; Potter et al. 2015). The high productivity, coupled with physicochemical and morphological habitat features, allows these environments to play several ecological functions, such as feeding grounds or spawning and nursery areas for many fish species (Elliott and Hemingway 2002; McLusky and Elliott 2004; Franco et al. 2006; Rountree and Able 2007; Perez-Ruzafa et al. 2013), some of which are of conservation or economic value. On the other hand, fish fauna is a key component of aquatic ecosystems, mainly for its important ecological role in aquatic food webs, the significant lifespan, which integrates the effects of environmental pressures on a relative long timescale, and for its economical values as food resource (Whitfield and Elliott 2002; Van Der Oost et al. 2003; Harrison and Whitfield 2004; Cabral et al. 2012).

The increasing attention paid to the quality of lagoons and estuaries (Gilliers et al. 2004), which are subjected to high anthropogenic pressures (Kennish 2002; Airoldi and Beck 2007), raised the issue of evaluating and quantifying environmental quality using the health status of fish populations. One of the first attempts to deal with this issue involved the introduction of body condition factors at the beginning of the twentieth century (Froese 2006). These indices, firstly developed for fish, are simple and concise indicators that, integrating several aspects of fish biology, can be good predictors of individual fitness, thus giving a comprehensive picture of the status of natural populations (Vila-Gispert and Moreno-Amich 2001). Indeed, different factors contribute to the body condition of an individual, such as general conditions of the environment, food quality, quantity and availability. For these reasons, fish condition can be considered an integrative measure of environmental quality, summarising the health and physiological state of a population (Vila-Gispert and Moreno-Amich 2001; Jakob et al. 1996). Indeed, it integrates the effects of both biotic and abiotic environmental influences on fish (La Peyre et al. 2007; Searcy et al. 2007; Zapfe and Rakocinski 2008), thus allowing the comparison of habitat quality in different environments (Amara et al. 2007; Haas et al. 2009; Vasconcelos et al. 2009).

Individual health, quantified by means of condition indices, gives a snapshot of the physiological state of an animal (Jakob et al. 1996), hence providing information about the entire population (Stevenson and Woods 2006). Recently, Perez-Ruzafa et al. (2018) stressed the importance of analysing the physiological mechanisms involved in the allocation of energy resources by individuals to obtain information at the population level. This would help to evaluate the response of organisms and populations to human-induced alteration of ecosystems.

Among morphometric indices, one of the most commonly used is Fulton's condition factor. However, it presents several issues, in particular when the assumption of isometric growth is not met (Jones et al. 1999). This drawback may be overcome by using the Kn index (Blackwell et al. 2000), calculated as the ratio between the measured weight and the theoretical weight predicted according to the length-weight regression. Kn values are not influenced by body shape, and the index can be used for the comparison of fishes having different lengths. Moreover, it showed to be influenced by environmental factors (Blackwell et al. 2000).

In the fields of fishery management and environmental monitoring policy, the link existing between the health of fish populations, and the effect of human activities on such populations, is of particular interest (Leamon et al. 2000; Ciotti et al. 2013). Many works used small-sized fish in the context of environmental monitoring activities, since these species are relatively abundant, can be easily captured and show a certain degree of site fidelity (Leamon et al. 2000; Galloway and Munkittrick 2006). Furthermore, in some cases, the use of the body condition indices allowed to detect the impacts of anthropogenic pressures on fish populations (Efitre et al. 2009; Piazza and La Peyre 2010).

Estuarine residents are the elective guild of transitional water habitats, and they can be used to assess the quality of these ecosystems. Their entire life cycle is indeed spent within transitional waters, and thus, they integrate all the stimuli coming from such environment within their biological cycle (Bortone et al. 2005; Fiorin et al. 2007; Zucchetta et al. 2012). Despite that, the difficulties in the evaluation of anthropogenic pressures in transitional water ecosystems are exacerbated by the so-called Estuarine Quality Paradox (Elliott and Quintino 2007). Organisms inhabiting such environments, and resident species in particular, are well adapted to naturally stressing environmental conditions, and at the same time, these areas are often subjected to many different pressures deriving from human activities (Franco et al. 2009; Cavraro et al. 2017). Thus, determining and quantifying the health status of a population in relation to man-induced pressures it is not always an easy task in transitional water ecosystems. In this context, a particular key point is disentangling the relative contribution of natural variability and anthropogenic pressures in shaping both biological and life-history traits of a species (Cavraro et al. 2013, 2014).

In this study, we used the body condition of a small estuarine resident teleost, the sand smelt *Atherina boyeri* Risso, 1810, to evaluate the effects of anthropogenic pressures in a Mediterranean coastal lagoon. In particular, the aims of this study were to: (1) analyse the spatiotemporal variability of the body condition index, taking into account the natural heterogeneity that characterises coastal lagoons, and (2) verify whether this indicator might be sensitive to environmental alterations deriving from anthropogenic pressures and thus its contribution to the assessment of ecological status.

Materials and methods

Study area

The Marano and Grado Lagoon belongs to the extended transitional system network of the northern Adriatic Sea basin (Fig. 1). It is located between the Tagliamento and Isonzo River deltas, and it extends over approximately 160 km². The Lagoon is one of the best conserved wetlands of the whole Mediterranean area. This system is a coastal microtidal lagoon of large dimensions protected by the Ramsar Convention since 1971, and it is a Site of Community Importance at European level.

Based on geomorphological and hydraulic differences, two main basins are identifiable: Marano and Grado (De Vittor et al. 2012). The first one (western sector) shows few areas above the mean sea level and several channels linking the plain spring rivers flowing into the lagoon basin with the sea. The second one (eastern sector) is shallower (< 1 m, on average) with a complex hydrographical network including tidal flats, tidal channels and sub-tidal zones. Besides its natural significance, the lagoon and the neighbouring mainland host several socio-economic activities (e.g. industrial sites, commercial harbours, marinas, fishing, fish and clam farming, and tourism; Ramieri et al. 2011), which pose various environmental concerns (e.g. in terms of priority pollutants).

Field samplings

Preliminary studies conducted in the lagoon highlighted the presence of three water body types (mesohaline, polyhaline and euhaline) and 13 river mouths. Considering some specific descriptors such as geographical location, geomorphology, tides and surface salinity, 16 water bodies (WBs) were identified by local administration, three of them being heavily modified. The latter represent areas subjected to substantial changes due to anthropogenic activities (e.g. significant hydraulic regime changes and ex fish farms).

А programme three-year fish monitoring (2010–2012) was carried out on 32 stations (Fig. 1), where samples were collected by means of fyke nets. Four traps were used in each station. This gear is commonly used by traditional fishery throughout the northern Adriatic lagoons (Zucchetta et al. 2016). The fyke net consists of a barrier (about 60 m in length and 1.3 m in height) with a 0.7-cm mesh size, leading the nektonic organisms towards four cone-shaped, unbaited traps, of the same mesh size. Sampling was performed twice a year during spring and autumn with the aid of local fishermen, and all samples were collected after 24 h from the setting of the fyke nets. A. boyeri samples were preserved at -20° C.

Data collection and analysis

Fish were measured to the nearest 0.1 mm (total length, TL) and weighed to the nearest 0.1 g (total fresh weight, TW). In the samples exceeding 100 individuals, 100 specimens were randomly selected for the measurements. A preliminary check of the population structure, using the length-frequency histograms of the sampled fish ("Appendix A"), was performed, on both spring and autumn samples, with the Bhattacharya method. Results of this analysis (not reported in the present study) showed that, in autumn, most of the individuals sampled belonged to the same cohort (0+). Since the species spawns during spring and summer, after September, females' ovaries are empty (Boscolo 1970). Therefore, only fish sampled in autumn were considered in this analysis, to avoid the influence of gonad development on total weight. Individual condition was calculated as the relative condition factor, according to the equation Kn = W/W', where W is the total weight of an individual fish



Fig. 1 Study area with highlighted (black dots), the sampling points and the partition in water bodies (*FM* heavily modified, *TEU* euhaline, *TPO* polyhaline, *TME* mesohaline). The dashed line separates the Marano basin (left) from the Grado basin (right)

and W' is the length-specific weight predicted by the linear regression calculated for the relationship $\log TL - \log TW$ (Blackwell et al. 2000). For each sampling station, mean values of TL, TW and Kn were then calculated, as well as mean salinity. Anthropogenic pressures were estimated for each water body (N = 16) as in Zucchetta et al. (2016), adapting the

approach proposed by Aubry and Elliott (2006) (Table 1, "Appendix B"). An analysis of variance (ANOVA) was performed to test for differences in biological, environmental and pressure variables between the two sub-basins of Marano and Grado, using water bodies as replicates. The same data set was used to calculate Spearman correlations among Kn,

Pressure category	Indicator	Measure
Morphology	Intertidal	% of intertidal area lost between 1954 and 2006 (Fontolan et al. 2012)
	Coastline	Relative change in bottom depth between 1927 and 2002 (Sarretta et al. 2010), taking a variation of ± 0.75 m as minimum significant change
	Hydro	% of area affected by interferences with the hydrographic regime
Use	Aquaculture	% of area covered by clam farming
	Fishery	number of traps km^{-2}
	Marinas	number of berths km ⁻²
	Port	Extent of ports
Quality	Sediment	Mean hazard quotient (mHQ)
	Benthos	M_AMBI index level (Muxika et al. 2007)
	DIN	$\mu g L^{-1}$ of dissolved inorganic nitrogen
	RP	$\mu g L^{-1}$ of reactive phosphorous
	Chl-a	$\mu g L^{-1}$ of chlorophyll-a
	DO	% saturation of dissolved oxygen

Table 1 Description of the anthropogenic pressures analysed in the present work, adapting the scheme proposed by Aubry and Elliott (2006), based on the ARPA FVG data set for WFD monitoring programme

salinity and the pressures. To test for the effect of salinity and pressures on Kn, a multiple regression approach was adopted, considering the interaction of each variable with the sub-basin. Collinear predictor variables were identified and removed from the model when the variance inflation factor resulted higher than 5 (Montgomery and Peck 1992).

Results

Overall, total anthropogenic pressure level of intensity, calculated as the average of the three pressure categories, did not differ between the two sub-basins (Fig. 2, Table 2). The Marano sub-basin was subjected to significantly higher pressure related to the use of lagoon resources and to environmental quality, while morphological alteration significantly affected the Grado sub-basin more strongly, mainly due to the alteration of the hydrographic regime (Fig. 2, Table 2). Within the pressure category related to the use of the lagoon, fishery effort and the extent of ports in particular were significantly higher in Marano compared to Grado. Regarding the pressure category related to environmental quality, despite the significantly lower values of the biotic index for the macrozoobenthic community and the dissolved oxygen concentration in the Marano sub-basin, Grado showed an overall lower intensity of pressure,

probably as the result of one indicator, the low DIN values recorded (Fig. 2).

Significant differences (Table 2) in average salinity were found between the two sub-basins (Fig. 3). In the Grado sub-basin, salinity was on average 10 points higher than in Marano. Furthermore, a substantial spatial homogeneity was observed in the former subbasin, while Marano showed a clear sea-lagoon gradient (Fig. 4b).

Significant differences were found also in average length and weight of *A. boyeri* (Fig. 3), with fish in Marano being longer and heavier compared to those sampled in Grado. On the contrary, in terms of condition factor (Fig. 3) no significant difference was found between the two basins (Table 2). Finally, no significant relationship was found between size and condition factor.

Significant correlations were found among *A. boyeri* condition, salinity and the indicators/pressures considered in the two parts of the lagoon (Table 3). In the Grado sub-basin, no significant correlation of salinity was found with the condition factor or any indicator/pressure. In turn, a significant negative correlation was found between condition factor and morphological pressures (-0.34) and, in particular, between condition factor and pressure deriving from the alteration of the hydrographic regime (-0.42). On the contrary, a positive correlation between condition factor and pressure deriving from the alteration of the hydrographic regime (-0.42). On the contrary, a positive correlation between condition factor and DIN was found (0.45).



Fig. 2 Comparison of mean values (±SE) of the pressures/indicators considered between the two sub-basins of Marano and Grado

Conversely, the condition factor in the Marano basin showed a significant negative correlation with salinity (-0.60). Indeed, moving from the sea inlets to the inner parts of the lagoon, higher values of Kn were observed (Fig. 4). Also, in the Marano sub-basin some indicators/pressures resulted to be correlated with salinity. When an indicator/pressure showed a positive correlation with salinity (thus decreasing along the sea-lagoon gradient), the possible effect of this indicator/pressure on fish cannot be distinguished from the natural gradient's effect. Therefore, the attention was focused only to the indicators/pressures showing no significant correlation, or a negative correlation, with salinity. In this context, in both sub-basins, a negative correlation was found between condition factor and the alteration of the hydrographic regime (-0.41), and a positive correlation was found not only with dissolved nitrogen concentration (0.72)but also with reactive phosphorous concentration (0.46). Furthermore, a significant positive correlation was found between fishery and condition factor (0.43).

At first, multiple regression analysis considered as predictors all the pressure variables and salinity in the two sub-basins. Once collinear variables were excluded, the model included only the sub-basin, the alteration of the hydrographic regime and concentrations of dissolved oxygen and reactive phosphorous. Among predictors, only alteration of the hydrographic regime resulted to be significant (Table 4), with a negative relationship with condition factor in both sub-basins (Fig. 4).

Looking at spatial variability in condition factor (Fig. 5), it is clear that this indicator showed higher values in the more confined water bodies of Marano sub-basin, following the salinity gradient (Fig. 5). This gradient was absent in Grado, while the condition factor showed higher values near the sea inlets and lower values in proximity of the mainland. Higher values of morphological and, in particular, hydrological pressures were found in Grado (Fig. 2), particularly in the eastern part of the sub-basin (Fig. 5c), while Marano showed a higher intensity of pressure

	Lago	oon	Resid	luals
	df	Mean sq.	df	Mean sq
Total length	1	2.05	46	0.13
Total weight	1	2.01	46	0.13
Condition factor	1	0.03	46	0.01
Salinity	1	1248.80	46	31.30
Morpho	1	40.35	46	2.99
Use	1	48.00	46	2.38
Quality	1	0.27	46	0.07
Total pressures	1	0.14	46	0.97
Intertidal	1	3.00	46	8.48
Coastline	1	15.19	46	4.00
Hydro	1	180.19	46	11.01
Aquaculture	1	15.19	46	5.21
Fishery	1	147.00	46	2.02
Marina	1	27.00	46	12.39
Port	1	42.19	46	4.69
Sediment	1	3.00	46	1.50
Benthos	1	6.75	46	0.88
DIN	1	18,920.00	46	566.00
RP	1	0.31	46	0.44
Chl-a	1	0.71	46	0.38
DO	1	279.29	46	54.37

Table 2 Results of ANOVA (mean sq.) performed to compare the mean values of three *Atherina boyeri* traits (total length, total weight, condition factor), salinity and the total and indicators/pressures correlated with *A. boyeri* condition between the two sub-basins of Marano and Grado

Values in bold are significant for P < 0.05

fishery activities (Table 2; Fig. 5d). Considering the water quality indicators, an east–west gradient of DIN was observed (Fig. 5e), with significant higher values in Marano; no statistical difference was found for RP (Table 2).

Discussion

Estuarine resident fish species are known to be well adapted to stressful environments such as coastal lagoons (Franco et al. 2008). This guild is the most abundant in lagoon shallow-water habitats, accounting for about 90% of the total fish abundance (Franzoi et al. 2010). *A. boyeri* is one of the most abundant resident fish in Mediterranean coastal lagoons



Fig. 3 Comparison between the two sub-basins of Marano and Grado in terms of salinity and total length, total weight and condition factor of *Atherina boyeri*. Values presented are mean \pm SE



Fig. 4 Relationships between condition factor Kn and alteration of the hydrographic regime in the two sub-basins of Marano and Grado

(Franzoi et al. 1989; Malavasi et al. 2004; Manzo 2010; Perez-Ruzafa et al. 2007a; Maci and Basset 2010; Verdiell-Cubedo et al. 2012), with a key role in estuarine food web being one of the main links between primary benthic and planktonic consumers and the higher trophic levels (Bartulovic et al. 2004). Although *A. boyeri* is not a sedentary benthic species, it is characterised by a certain degree of site fidelity, in

Grado Marano Kn salinity Kn salinity Salinity 0.01 1.000 - 0.60 1.000 0.670 Intertidal 0.38 0.119 - 0.61 Coastline -0.38-0.1120.03 0.180 Hydro -0.420.010 - 0.41 0.368 Fishery -0.09-0.2460.43 - 0.409 -0.037Marina -0.27- 0.45 0.566 -0.26-0.222-0.12Benthos 0.168 DIN 0.45 -0.2880.72 - 0.612 RP 0.20 0.287 0.46 - 0.465 DO 0.03 0.190 - 0.60 0.445 -0.330.265 Aquaculture 0.32 0.082 Port 0.00 0.137 - 0.45 0.566 Sediment 0.32 0.082 0.00 0.289 Chl-a -0.310.002 0.29 - 0.419 Morphology - 0.34 -0.071- 0.59 0.665 -0.12-0.0560.319 Use -0.28Quality -0.24-0.2930.08 -0.055- 0.31 0.523 Total -0.041- 0.48

Table 3 Results of the Spearman correlations for the condition factor of *Atherina boyeri* with salinity, the three pressures and their indicators in the 3 years analysed in the two lagoons

Values used in the correlation analysis are the average for each water body. Values in bold are significant for P < 0.05

Table 4 Results of the multiple regression analysis performedamong Kn and salinity and the indicators/pressures, taking intoaccount the two sub-basins of Marano and Grado

Variable	df	Mean sq.	F	Р
Hydro	1	0.101	11.209	< 0.05
Rp	1	0.025	2.730	NS
Do	1	0.026	2.832	NS
Sub-basin	1	0.000	0.000	NS
Hydro × sub-basin	1	0.001	0.143	NS
$Rp \times sub-basin$	1	0.015	1.617	NS
Residuals	41	0.009	0.009	

Values in bold are significant for P < 0.05

particular to spawning grounds, resulting in semiisolated populations (Koutrakis et al. 2004). Nevertheless, the plasticity of this species allows the colonisation of nearly all any shallow-water habitats (Leonardos 2001; Koutrakis et al. 2004), coping with the often strong and rapid variations in temperature, salinity and dissolved oxygen (Perez-Ruzafa et al. 2007b). In transitional water systems, salinity is one of the main drivers of biological communities (Attrill 2002; McLusky and Elliott 2004), influencing species abundance and distribution. In the Adriatic lagoon of Acquatina, for example, Maci and Basset (2010) analysed the populations of A. boyeri along a decreasing salinity gradient, observing higher densities and a better body condition moving from the sea to the inner portion of the lagoon. A similar pattern was found also in the present study, but with a distinction between the two sub-basins investigated. Indeed, a clear salinity gradient was present only in Marano, while it was absent in Grado (Ferrarin et al. 2010). Overall, the mean size of A. boyeri was higher in Marano, probably due to the higher trophic status of this basin. The significant river discharge that characterises this part of the lagoon not only determines the freshwater input that generates the observed salinity gradient, but also causes a relevant input of nutrients, i.e. nitrogen, deriving from agricultural activities and untreated domestic sewage (Acquavita et al. 2015). Thus, A. boyeri can take advantage of the high productivity of lagoon waters: despite the stressful environmental conditions often found in transitional water ecosystems, lagoon fish species were found to adopt life-history strategies that allow them to cope with natural stress (Perez-Ruzafa et al. 2013). The differences in size and weight found between the two sub-basins should not be ascribed to differences in age, as reported in Materials and Methods. This would be in accordance with the biology of A. boyeri: other authors (Fernandez-Delgado et al. 1988; Bartulovic et al. 2004) found a short life cycle, with only a small number of individuals older than 1 +age class, with most of the spawners dying after the reproductive season. Anyway, further studies are needed to highlight if some of the differences found between the two sub-basins could depend on differences in life-history traits related to growth and reproduction.

In coastal lagoons, a high nutrient enrichment may be detrimental to the ecological status, since it can be the cause of dystrophic crisis (Solidoro et al. 2010). Usually, these events occur during the summer period, when the high temperatures cause mass mortality of the accumulated biomass of macroalgae (Sfriso et al. 2003). In the studied lagoon, such events are usually rare and occur on a small spatial scale, since the



Fig. 5 Variation among the 16 water bodies of **a** *Atherina boyeri* condition factor, **b** salinity, **c**–**f** pressures correlated with *A. boyeri* condition. The dashed line separated the two sub-basins of Marano (left) and Grado (right). Black arrows indicate the sea inlets

system is phosphorous limited, and the water renewal through the sea inlets guarantees a dilution of nutrients (Acquavita et al. 2015). This would explain the positive correlation found between the condition factor and water concentration of nitrogen and phosphorous, although no significant differences in Kn were found between the two sub-basins. The higher trophic status stimulates the productivity of the basin, maintaining a trophic network that allows secondary consumers like *A. boyeri* to find more food, thus reaching a larger size.

Also the intensity of fishing activity was positively linked to the body condition of *A. boyeri*. In the lagoon, the main fishing activity is represented by artisanal fishery carried out by means of fyke nets (Bettoso et al. 2013), with a mean fishing effort higher in Marano than in Grado (25 fishermen in Marano, 5 in Grado). Usually, fishing results in a reduction in the mean individual size in natural populations (Berkeley et al. 2004), but in the present study *A. boyeri* in Marano were significantly larger than in Grado. Artisanal fishery, such as that carried out in North Adriatic lagoons, is thought to be a sustainable activity. For example, in a similar ecosystem (the Venice lagoon), minor effects of artisanal fishery were observed on the fish community analysed by Zucchetta et al. (2016) and only the 6% of the catches, in terms of biomass, is discarded (Pranovi et al. 2013). Furthermore, fishing effort is usually concentrated in the areas that guarantee the maximum yield. According to the findings of Zucchetta et al. (2016) for the Venice lagoon, also in the present study the positive correlation observed between fishery pressure and body condition of A. boyeri might be explained by a sustainable fishing effort focused on the most productive areas of the lagoon, which therefore host fish populations in better conditions.

While pressures deriving from nutrient concentrations and fishing effort did not cause significant negative impacts on the lagoon ecosystem, a different pattern emerged considering the morphological alterations. In the last 60 years, severe morphological changes deeply altered the lagoon landscape (Fontolan et al. 2012). Drowning (the combined effect of eustatism, subsidence and autocompaction) and erosion by vessel-generated waves are two of the main causes of the reduction in salt marsh extension (144 ha, -16%). Also submerged habitats, such as tidal and sub-tidal flats, are affected by erosion processes. The loss of fine sediments is determining a general deepening of the lagoon and a progressive shift into a marine embayment (Fontolan et al. 2012; Ferrarin et al. 2016). In the present study, a negative correlation was found between the intensity of morphological pressures and the body condition of A. boyeri. This picture is quite clear in the Grado basin, where no salinity gradient was observed and the intensity of morphological pressures was higher. On the other hand, in the Marano basin the positive correlation between salinity and some of the indicators of pressure (i.e. intertidal, marina, DO and port) does not allow to assess whether the decreasing values of body condition observed moving from the inner lagoon towards the sea inlets are due to a reduction in the pressure intensity or whether it is a natural decreasing linked to the salinity gradient. Considering the single-pressure indicators, alteration of the hydrological regime gives a clearer picture. In both basins, no significant correlation with salinity was found, while body condition showed a significant negative correlation with the pressure indicator. As an example of alteration to the hydrographic regime, the eastern part of the lagoon is physically divided into two sections by the bridge connecting the main land to the city of Grado (Ferrarin et al. 2010). Considering the water bodies, such bridge separates TEU1 and TPO1 from FM2 and FM3. The latter were indeed classified as heavily modified water bodies in which the sediment particles entering the lagoon via the easternmost inlet remain trapped due to the presence of the bridge (Ferrarin et al. 2016).

A clearly negative response of fish community to morphological alterations was also found by Zucchetta et al. (2016) in the Venice lagoon. In that study, effects of pressures on the lagoon morphology were stronger for resident species. Thus, in coastal lagoons it seems that morphological pressures, and the alteration of the hydrodynamic conditions in particular, might play a key role, affecting those species which spend all the life cycle within transitional waters.

Conclusions

An official method based on fish fauna for the assessment of ecological status in Mediterranean coastal lagoons is still lacking, although a multimetric index has been proposed by Franco et al. (2009). Multimetric indices are usually focused on the whole fish assemblage, considering metrics related to species/guilds abundance and biomass, but also information about disturbance-sensitive species might be used as supporting information to assess the ecological status of aquatic environment. An approach considering the health status of individuals of key species might provide complementary information on environmental conditions, although reliable predictions and assessments of cause-effect relationships should rely on a good knowledge about the links between biological and physiological parameters with the health status of individuals (Perez-Ruzafa et al. 2018). In some cases, the combined effect of natural variability and environmental parameters could have prevented to directly identify the impacts on fish caused by anthropogenic pressures. Despite this, results of the present study showed how even a simple index like the body condition factor could be a good indicator of the health status of A. boyeri populations affected by anthropogenic pressures, in particular for those pressures deriving from the morphological (hydrological) alteration of the environment. The extension of this approach to other lagoons, possibly using different species or indicators [i.e. the Wr proposed by Blackwell et al. (2000) or other physiological indices], could support the fish-based assessment of ecological status on a broader geographical scale.

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Appendix A



Pressure	Indicator	Measure			Degree of chan	lges					Vote
				1	No change (0)	Very low (1)	Low (3)	Medium (;	5) High (7)	Very high (9)	
Coastline morphological change	Intertidal	% of interti between 1 (Fontolan	dal area los 1954 and 20 et al. 2012)	t] 06	Increase	No change	< 1% los since 1.	st $\geq 1\%$ and 850 $< 5\%$ lo	$ \geq 5\% \acute{e} $ st $< 10\% $ lost	$\geq 10\%$ I lost	Tixed in time
	Coastline	Relative ch depth betv (Sarretta e variation (minimum	ange in bott ween 1927 a 2t al. 2010), of ± 0.75 m significant	tom 1 nd 2002 taking a as change	No development	< 5% of the coastline impaci by industrial or urban activities	≥ 5% an < 30%	d ≥ 30% an < 60%	d ≥ 60% and < 90%	≈ 90%	
	Hydro	% of area a interferen hydrograp	uffected by ces with the hic regime	-	No construction	< 5% of the intertidal and su tidal area affect	>=5% ar bb- < 10% ed	id >=10% an < 20%	id >=20% and < 40%	>=40%	
				No resource use (0)	Very low	(1)	Low (3)	Medium (5)	High (7)	Very high (9)	
Resource Aqu use change	aculture	% of area c clam farn	overed by ning	No fish farming	< 1% of tl sub-tidal	he intertidal and area covered	≥ 1% and < 10%	≥ 10% and < 30%	≥ 30% and < 50%	≥ 50%	Fixed in time
Fish	eries	number of km^{-2}	traps	No fishery activities	< 10% of affected	the surface of WB by fishery	$\geq 10\%$ and $< 30\%$	$\geq 30\%$ and $< 60\%$	≥ 60% and < 90%	≥ 90%	
Mar	inas	number of km ⁻²	berths	No marina	1 < 100 bert	ths in marina	≥ 100 and < 150 berths	\geq 150 and $<$ 300 berths	≥ 300 and < 500 berths	≥ 500 berths	
Inter de	nsity of port velopments	Extent of p	orts	No harbou	ur < 500 m c	of quays	≥ 500 and < 2 km	≥ 2 and < 5 km	≥ 5 and < 10 km	$\geq 10 \text{ km}$ of quays	
				V	ery low (1)	Low (3)	Medium (5)	High (7)	Very high (9)		
Environmental S quality and its perception	sediment Se	diment chemical status based m decree 56/2009	100% of substances below the (Environm Quality Standard)	EQS cental	ne not priority substance fails to comply with EQSs	More than one not priority substance fails to comply with EQSs	One priority substance fails to comply with EQSs	More than one priority substance fails to comply with EQSs	More than one substance and than one not I substance fail with EQSs	priority one or more priority to comply	Fixed in time

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Appendix B

			Very low (1)	Low (3)	Medium (5)	High (7)	Very high (9)	
Benthos	M_AMBI index level (Muxika et al. 2007)	Normal	Normal	Normal	Recovering or deteriorating	Modified	Severely modified	Fixed in time
DIN	$\mu g L^{-1}$ of dissolved inorganic nitrogen	Pressure calculated as seasonal average of concentration on a monthly basis						
RP	$\mu g L^{-1}$ of reactive phosphorous	Pressure calculated as seasonal average of concentration on a monthly basis						
Chl-a	$\mu g L^{-1}$ of chlorophyll-a	Pressure calculated as seasonal average of concentration on a monthly basis						
DO	% saturation of dissolved oxygen	Pressure calculated as seasonal average of concentration on a monthly basis						

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