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 brackish-water 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.

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 & Fahay, 2010; Potter et al. 2015). The high productivity, coupled with physico-chemical 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 & Hemingway 2002; McLusky & Elliott 2004; Franco et al., 2006; Rountree & 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 life span, which integrates effects of environmental pressures on a relative long time scale, and for its economical values as food resource (Whitfield & Elliott, 2002; Van Der Oost et al., 2003; Harrison & 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 & 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 XX 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 & 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 & 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 & 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 (Jacob et al., 1996), hence providing information about the entire population (Stevenson & 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 & 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 & 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

& 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 quantifing 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 spatio-temporal variability of the body condition index, taking into account the natural heterogeneity that characterises coastal lagoons; and 2) verify if 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 (Figure 1). It is located between the Tagliamento and Isonzo River deltas and it extends over approximately 160 km^2. 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.

Fig. 1 The 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)

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 subtidal zones. Besides its natural significance, the lagoon and the neighboring mainland host several socio-economic activities (e.g., industrial sites, commercial harbors, 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, euhaline) and 13 river mouths. Considering some specific descriptors such as geographic 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, ex fish farms).

A three-year fish monitoring program (2010-2012) was carried out on 32 stations (Figure 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 hours 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 and W' is the length-specific weight predicted by the linear regression calculated for the relationship logTL-logTW (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 & 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 dataset was used to calculate Spearman correlations among Kn, 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 (Figure 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 (Figure 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 (Figure 2).

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

Significant differences (Table 2) in average salinity were found between the two sub-basins (Figure 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 sub-basin, while Marano showed a clear sea-lagoon gradient (Figure 4b).

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

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 ± S.E.

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 DIN was found (0.45).

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

(Figure 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 to 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 (Figure 4).

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

Looking at spatial variability in condition factor (Figure 5), it is clear that this indicator showed higher values in the more confined water bodies of Marano sub-basin, following the salinity gradient (Figure 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 (Figure 2), particularly in the eastern part of the sub-basin (Figure 5c), while Marano showed a higher intensity of pressure fishery activities (Table 2; Figure 5d). Considering the water quality indicators, an east-west gradient of DIN was observed (Figure 5e), with significant higher values in Marano; no statistical difference was found for RP (Table 2).

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

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 (Franzoi et al. 1989; Malavasi et al., 2004; Manzo, 2010; Perez-Ruzafa et al., 2007a; Maci & Basset, 2009; 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 particular to spawning grounds, resulting in semi-isolated 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 & Elliott, 2004), influencing species abundance and distribution. In the Adriatic lagoon of Acquatina, for example, Maci & Basset (2009) 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 the 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 for 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 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 of 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) do not allow to assess if the decreasing values of body condition observed moving from the inner lagoon towards the sea inlets is due to a reduction of the pressure intensity or if it is a natural decreasing linked to the salinity gradient. Considering the single pressure indicators, alteration of the hydrologic 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 multimetrics index has been proposed by Franco et al. (2009). Multimetrics 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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References

Acquavita A, Aleffi IF, Bencia C, Bettoso N, Crevatina E, Milania L, Tamberlicha F, Toniatti L, Barbieri P, Licen S, Mattassi G (2015) Annual characterization of the nutrients and trophic state in a Mediterranean coastal lagoon: The Marano and Grado Lagoon (northern Adriatic Sea). Reg Stud Mar Sci 2:132-144.

Airoldi L, Beck MW (2007) Loss, status and trends for coastal marine habitats of Europe. Oceanogr Mar Biol: Ann Rev 45:345-405.

Amara R, Maziane T, Gilliers C, Hrmel G, Laffargue P (2007) Growth and condition indices in juvenile *Solea solea* measured to assess the quality of essential fish habitat. Mar Ecol Prog Ser 351:201-208.

Attrill MJ (2002) A testable linear model for diversity trends in estuaries. J Anim Ecol 71:262-269.

Aubry A & Elliott M (2006) The use of environmental integrative indicators to assess seabed disturbance in estuaries and coasts: application to the Humber Estuary, UK. Mar Poll Bull 53:175–185.

Bartulovic V, Glamuzina B, Conides A, Ducic J, Lucic D, Njire J, Kozul V (2004) Age, growth, mortality and sex ratio of sand smelt, *Atherina boyeri* Risso, 1810 (Pisces: Atherinidae) in the estuary of the Mala Neretva River (middle-eastern Adriatic, Croatia). J Appl Ichthyol 20:427-430.

Berkeley SA, Hixon MA, Larson RJ, Love MS (2004) Fisheries Sustainability via Protection of Age Structure and Spatial Distribution of Fish Populations. Fisheries 29:23-32.

Bettoso N, Acquavita A, D'Aietti A, Matassi G (2013) The Marano and Grado lagoon: a brief synopsis on the aquatic fauna and fisheries resources. Ann Ser Hist Nat 23:135-142.

Blaber SJM (1997) Fish and Fisheries of Tropical Estuaries (Fish and Fisheries Series 22). Chapman and Hall, London, 367 pp.

Blackwell BG, Brown ML, Willis DW (2000) Relative Weight (Wr) Status and Current Use in Fisheries Assessment and Management. Rev Fish Sci 8:1-44.

Bortone SA, Dunson WA, Greenawalt JM (2005) Fishes as Estuarine Indicators. In "Estuarine Indicators", Bortone (ed), CRC Press, Boca Raton, Florida, USA.

Boscolo L (1970) Osservazioni sulla biologia e sulla pesca dell'*Atherina boyeri* Risso 1810 (Osteichthyes, Atherinidae) vivente nelle acque dell'Alto Adriatico. Boll pes piscicol idrobiol 25:61-79.

Cabral HN, Fonseca VF, Gamito R, Gonçalves CI, Costa JL, Erzini K, Gonçalves J, Martins J, Leite L, Andrade JP, Ramos S, Bordalo A, Amorim E, Neto JM, Marques JC, Rebelo JE, Silva C, Castro N, Almeida PR, Domingos I, Gordo LS, Costa MJ (2012) Ecological quality assessment of transitional waters based on fish assemblages in Portuguese estuaries: The Estuarine Fish Assessment Index (EFAI). Ecol Indic 19:144-153.

Cavraro F, Torricelli P, Franzoi P, Malavasi S (2013) Productivity in natural and artificial habitats in brackish water systems: an example from Aphanius fasciatus populations. Trans Wat Bull 7:23-31.

Cavraro F, Daouti I, Leonardos I, Torricelli P, Malavasi S (2014) Linking habitat structure to life history strategy: Insights from a Mediterranean killifish. J Sea Res 85:205-213.

Cavraro F, Zucchetta M, Malavasi S, Franzoi P (2017) Small creeks in a big lagoon: The importance of marginal habitats forfish populations. Ecol Eng 99:228-237.

Ciotti BJ, Targett TE, Nash RDM, Burrows MT (2013) Small-scale spatial and temporal heterogeneity in growth and condition of juvenile fish on sandy beaches. J Exp Mar Biol Ecol 448:346-359.

De Vittor C, Faganeli J, Emili A, Covelli S, Predonzani S, Acquavita A (2012) Benthic fluxes of oxygen, carbon and nutrients in the Marano and Grado Lagoon (northern Adriatic Sea, Italy). Est Coast Shelf Sci 113:57-70.

Drouineau H, Lobry J, Delpech C, Bouchoucha M, Mahévas S, Courrat A, Pasquaud S, Lepage M (2012) A Bayesian framework to objectively combine metrics when developing stressor specific multimetric indicator. Ecol Indic 13, 314-321.

Efitre J, Chapman LJ, Murie DJ (2009) Fish condition in introduced tilapias of Ugandan crater lakes in relation to deforestation and fishing pressure. Environ Biol Fish 85: 63-75.

Elliott M & Hemingway KL (2002) Fishes in Estuaries. Blackwell, Oxford.

Elliott M & Quintino V (2007) The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Mar Poll Bull 54:640-645.

Fernandez-Delgado C, Hernando JA, Herrera M, Bellido M (1988). Life-history patterns of the sand smelt *Atherina boyeri* Risso, 1810 in the estuary of the Guadalquivir river, Spain. Est Coast Shelf Sci 27: 697-706.

Ferrarin C, Umgiesser G, Bajo M, Bellafiore D, De Pascalis F, Ghezzo M, Mattassi G & Scroccaro I (2010) Hydraulic zonation of the lagoons of Marano and Grado, Italy. A modeling approach. Est Coast Shelf Sci 87:561-572.

Ferrarin C, Umgiesser G, Roland A, Bajo M, De Pascalis F, Ghezzo M, Scroccaro I (2016) Sediment dynamics and budget in a microtidal lagoon – A numerical investigation. Mar Geol 381:163-174.

Fiorin R, Malavasi S, Franco A, Franzoi P (2007) Comparative energy allocation in two sympatric, closely related gobies: the black goby *Gobius niger* and the grass goby *Zosterisessor ophiocephalus*. J Fish Biol 70:483-496.

Fontolan G, Pillon S, Bezzi A, Villalta R, Lipizer M, Triches A, D'Aietti A (2012) Human impact and the historical transformation of saltmarshes in the Marano and Grado Lagoon, northern Adriatic Sea. Est Coast Shelf Sci 113:41-56.

Franco A, Franzoi P, Malavasi S, Riccato F, Torricelli P, Mainardi D (2006) Use of shallow water habitats by fish assemblages in a Mediterranean coastal lagoon. Est Coast Shelf Sci 66:67-83.

Franco A, Elliott M, Franzoi P, Torricelli P (2008) Life strategies of fishes in European estuaries: the functional guild approach. Mar Ecol Progr Ser 354:219-228.

Franco A, Torricelli P, Franzoi P (2009) A habitat-specific fish-based approach to assess the ecological status of Mediterranean coastal lagoons. Mar Poll Bull 58:1704-1717.

Franzoi P, Trisolini R, Carrieri A, Rossi R (1989) Caratteristiche ecologiche del popolamento ittico ripario della sacca di Scardovari (Delta de Po). Nova Thalassia, 10: 399-405.

Franzoi P, Franco A, Torricelli P (2010) Fish assemblage diversity and dynamics in the Venice lagoon. Rend Lincei, 21: 269-281.

Froese R (2006) Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. J Appl Ichthyol 22:241-253.

Galloway BJ & Munkittrick KR (2006) Influence of seasonal changes in relative liver size, condition, relative gonad size and variability in ovarian development in multiple spawning fish species used in environmental monitoring programmes. J Fish Biol 69:1788-1806.

Gilliers C, Amara R, Bergeron J-P, Le Pape O (2004) Comparison of growth and condition indices of juvenile flatfish in different coastal nursery grounds. Env Biol Fish 71:189-198.

Haas HL, Freeman CJ, Logan JM, Deegan L, Gaines EF (2009) Examining mummichog growth and movement: are some individual making intra-season migrations to optimize growth? Journal of Experimental Mar Biol Ecol 369:8-16.

Harrison TD & Whitfield AK (2004) A multi-metric fish index to assess the environmental condition of estuaries. J Fish Biol 65:683-710.

Jakob EM, Marshall SD & Uetz GW (1996) Estimating fitness: a comparison of body condition indices. Oikos, 77: 61-67.

Jones RE, Petrell RJ & Pauly D (1999) Using modified length-weight relationships to assess the conditio of fish. Aquac Eng 20:261-176.

Kenneth WA, Michael PF (2010) Ecology of Estuarine Fishes: Temperate Waters of the Western North Atlantic. Johns Hopkins University Press. 566 p.

Kennish MJ (2002) Environmental threats and environmental future of estuaries. Env Cons 29:78-107.

Koutrakis ET, Kamidis NI, Leonardos ID (2004) Age, growth and mortality of a semi-isolated lagoon population of sand smelt, *Atherina boyeri* (Risso, 1810) (Pisces: Atherinidae) in an estuarine system of northern Greece. J Appl Ichthyol 20:382-388.

La Peyre MK, Gossman B, Nyman JA (2007) Assessing functional equivalency of nekton habitat in enhanced habitats: comparison of terraced and unterraced marsh ponds. Est Coast 30:526-536

Leamon JH, Schultz ET, Crivello JF (2000) Variation among four health indices in natural populations of the estuarine fish, *Fundulus heteroclitus* (Pisces, Cyprinodontidae), from five geographically proximate estuaries. Env Biol Fish 57:451-458.

Leonardos I (2001) Ecology and exploitation pattern of a landlocked population of sand smelt, *Atherina boyeri* (Risso 1810), in Trichonis Lake (western Greece). J Appl Ichthyol 17:262-266.

Lepage M, Harrison T, Breine J, Cabral H, Coates S, Galván C, García P, Jager Z, Kelly F, Mosch EC, Pasquaud S, Scholle J, Uriarte A, Borja A (2016) An approach to intercalibrate ecological classification tools using fish in transitional water of the North East Atlantic. Ecol Indic 67:318-327.

Maci S, Basset A (2010) Spatio-temporal patterns of abundance, size structure and body condition of *Atherina boyeri* (Pisces: Atherinidae) in a small non-tidal Mediterranean lagoon. Est Coast Shelf Sci 87:125-134.

Malavasi S, Fiorin R, Franco A, Franzoi P, Granzotto A, Riccato F, Mainardi D (2004) Fish assemblages of Venice Lagoon shallow waters: an analysis based on species, families and functional guilds. J Mar Sys 51:19-31.

Manzo C (2010) Fish assemblages in three Mediterranean coastal lagoons: structure, functioning and spatio-temporal dynamics. PhD thesis, 105 pp.

McLusky DS, Elliott M (2004) The Estuarine Ecosystem – Ecology, Threats and Management, third ed. Oxford University Press, New York, p. 214.

Montgomery DC, Peck EA (1992). Introduction to Linear regression analysis 2nd edition. John Wiley, New York, p. 544.

Muxika I, Borja A, Bald J (2007) Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. Mar Poll Bull 55:16-29.

Perez-Ruzafa A, Marcos C, Perez-Ruzafa IM, Barcala E, Hegazi MI, Quispe J, (2007b) Detecting changes resulting from human pressure in a naturally quick-changing and heterogeneous environment: spatial and temporal scales of variability in coastal lagoons. Est Coast Shelf Sci 75:175-188.

Perez-Ruzafa A, Mompean MC, Marcos C (2007a) Hydrographic, geomorphologic and fish assemblage relationships in coastal lagoons. Hydrobiologia 577:107:125.

Perez-Ruzafa A, Marcos C, Perez-Ruzafa IM, Perez-Marcos M (2013) Are coastal lagoons physically or biologically controlled ecosystems? Revisiting *r vs. K* strategies in coastal lagoons and estuaries. Est Coast Shelf Sci 132: 17-33.

Perez-Ruzafa A, Perez-Marcos M, Marcos C (2018) From fish physiology to ecosystems management: Keys for moving through biological levels of organization in detecting environmental changes and anticipate their consequences. Ecol Indic 90: 334-345.

Piazza BP, La Peyre MK (2010) Using *Gambusia affinis* growth and condition to assess estuarine habitat quality: a comparison of indices. Mar Ecol Prog Ser 412: 231-245.

Potter IC, Tweedley JR, Elliott M, Whitfield AK (2015) The ways in which fish use estuaries: a refinement and expansion of the guild approach. Fish and Fisheries 16, 230-239

Pranovi F, Caccin A, Franzoi P, Malavasi S, Zucchetta M, Torricelli P (2013) Vulnerability of artisanal fisheries to climate change in the Venice lagoon. J Fish Biol 83:847-863.

Ramieri E, Barbanti A, Picone M, Menchini G, Bressan E, Dal Forno E (2011) Integrated plan for the sustainable management of the Lagoon of Marano and Grado. Littoral 2010, 05008.

Rountree RA, Able KW (2007) Spatial and temporal habitat use patterns for salt marsh nekton: implications for ecological functions. Aquat Ecol 41:25-45.

Sarretta A, Pillon S, Molinaroli E, Guerzoni S, Fontolan G 2010. Sediment budget in the Lagoon of Venice, Italy. Cont Shelf Res 30:934-949.

Searcy SP, Egglestone DB, Hare JA (2007) Is growth a reliable indicator of habitat quality and essential fish habitat for a juvenile estuarine fish? Can J Fish Aquat Sci 64: 681-691

Sfriso A, Facca C, Ghetti PF (2003) Temporal and spatial changes of macroalgae and phytoplancton in a Mediterranean coastal area: The Venice lagoon as a case study. Mar Env Res 2003; 56: 617- 636

Solidoro C, Bandelj V, Aubry Bernardi F, Camatti E, Ciavatta S, Cossarini G, Facca C, Franzoi P, Libralato S, Melaku Canu D, Pastres R, Pranovi F, Raicevich S, Socal G, Sfriso A, Sigovini M, Tagliapietra D, Torricelli P (2010) Response of Venice Lagoon Ecosystem to Natural and Anthropogenic Pressures over the Last 50 Years. In: Hans Paerl & Mike Kennish (eds) Coastal Lagoons: Systems of Natural and Anthropogenic Change, CRC press, Taylor and Francis, pp. 483-511.

Stevenson RD & Woods WA (2006) Condition indices for conservation: new uses for evolving tools. Int Comp Biol 46:1169-1190.

Van Der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers risk assessment: a review. Env Tox Pharm 13:57-149.

Vasconcelos RP, Reis-Santos P, Fonseca V, Ruano M, Tanner S, Costa MJ, Cabral HN (2009) Juvenile fish condition in estuarine nurseries along the Portuguese coast. ECSS 82: 128-138

Verdiell-Cubedo D, Torralva M, Andreu-Soler A, Oliva-Paterna FJ (2012) Effects of shoreline urban modification on habitat structure and fish community in littoral areas of a Mediterranean coastal lagoon (Mar Menor, Spain). Wetlands 32: 631-641.

Vila-Gispert A & Moreno-Amich R (2001) Mass-length relationship of Mediterranean barbel as an indicator of environmental status in South-west European stream ecosystems. J Fish Biol 59:824-832.

Whitfield AK, Elliott M (2002) Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. Journal of Fish Biology, 61: 229-250.

Zapfe GA, Rakocinski CF (2008) Coherent growth and diet patterns in juvenile spot (*Leiostomus xanthurus* Lacepede) reflect effects of hydrology on access to shoreline habitat. Fish Res 91:107-111.

Zucchetta M, Cipolato G, Pranovi F, Antonetti P, Torricelli P, Franzoi P, Malavasi S (2012) The relationships between temperature changes and reproductive investment in a Mediterranean goby: Insights for the assessment of climate change effects. Est Coast Shelf Sci 101:15-23.

Zucchetta M, Scapin L, Cavraro F, Pranovi F, Franco A, Franzoi P (2016) Can the Effects of Anthropogenic Pressures and Environmental Variability on Nekton Fauna Be Detected in Fishery Data? Insights from the Monitoring of the Artisanal Fishery Within the Venice Lagoon. Est Coasts, 39: 1164-1182.

Table 1. Description of the anthropogenic pressures analysed in the present work, adapting the scheme proposed by Aubry & Elliott (2006), based on the ARPA FVG dataset for WFD monitoring program

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	n° of traps km^{-2}
	marinas	n° 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 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 $P < 0.05$.

	Lagoon		Residuals	
	d.f.	Mean sq.	d.f.	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	**18920.00**	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

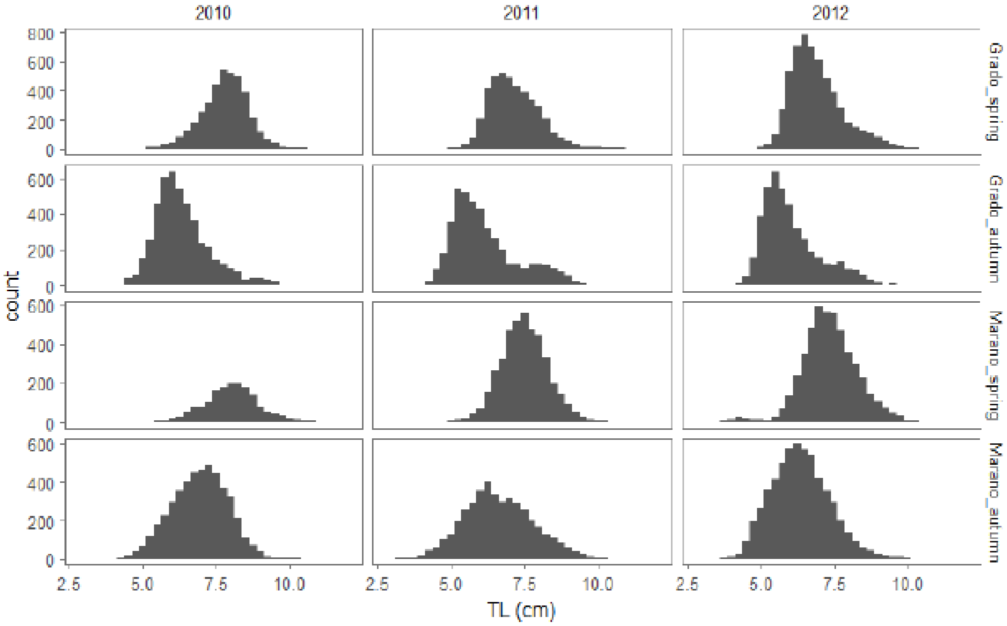
Table 3. Results of the Spearman correlations for the condition factor of *Atherina boyeri* with salinity, the three pressures and their indicators in the three years analysed in the two lagoons. Values used in the correlation analysis are the average for each water body. Values in bold are significant for $P < 0.05$.

	Grado		Marano	
	Kn	salinity	Kn	salinity
Salinity	0.01	1.000	**-0.60**	1.000
Intertidal	0.38	0.119	**-0.61**	**0.670**
Coastline	-0.38	-0.112	0.03	0.180
Hydro	**-0.42**	0.010	**-0.41**	0.368
Fishery	-0.09	-0.246	**0.43**	**-0.409**
Marina	-0.27	-0.037	**-0.45**	**0.566**
Benthos	-0.26	-0.222	-0.12	0.168
DIN	**0.45**	-0.288	**0.72**	**-0.612**
RP	0.20	0.287	**0.46**	**-0.465**
DO	0.03	0.190	**-0.60**	**0.445**
Aquaculture	0.32	0.082	-0.33	0.265
Port	0.00	0.137	**-0.45**	**0.566**
Sediment	0.32	0.082	0.00	0.289
Chl-a	-0.31	0.002	0.29	**-0.419**
Morphology	**-0.34**	-0.071	**-0.59**	**0.665**
Use	-0.12	-0.056	-0.28	0.319
Quality	-0.24	-0.293	0.08	-0.055
Total	**-0.31**	-0.041	**-0.48**	**0.523**

Table 4. Results of the multiple regression analysis performed among Kn and salinity and the indicators/pressures, taking into account the two sub-basins of Marano and Grado. Values in bold are significant for $P < 0.05$.

Variable	d.f.	Mean sq.	F	P
Hydro	**1**	**0.101**	**11.209**	**< 0.05**
Rp	1	0.025	2.730	*N.S.*
Do	1	0.026	2.832	*N.S.*
Sub-basin	1	0.000	0.000	*N.S.*
Hydro x sub-basin	1	0.001	0.143	*N.S.*
Rp x sub-basin	1	0.015	1.617	*N.S.*
Residuals	41	0.009	0.009	

Appendix A


2010
2011
2012
Grado_spring
Grado_autumn
Marano_spring
Marano_autumn
count
800
600
400
200
0
2.5
5.0
7.5
10.0
TL (cm)

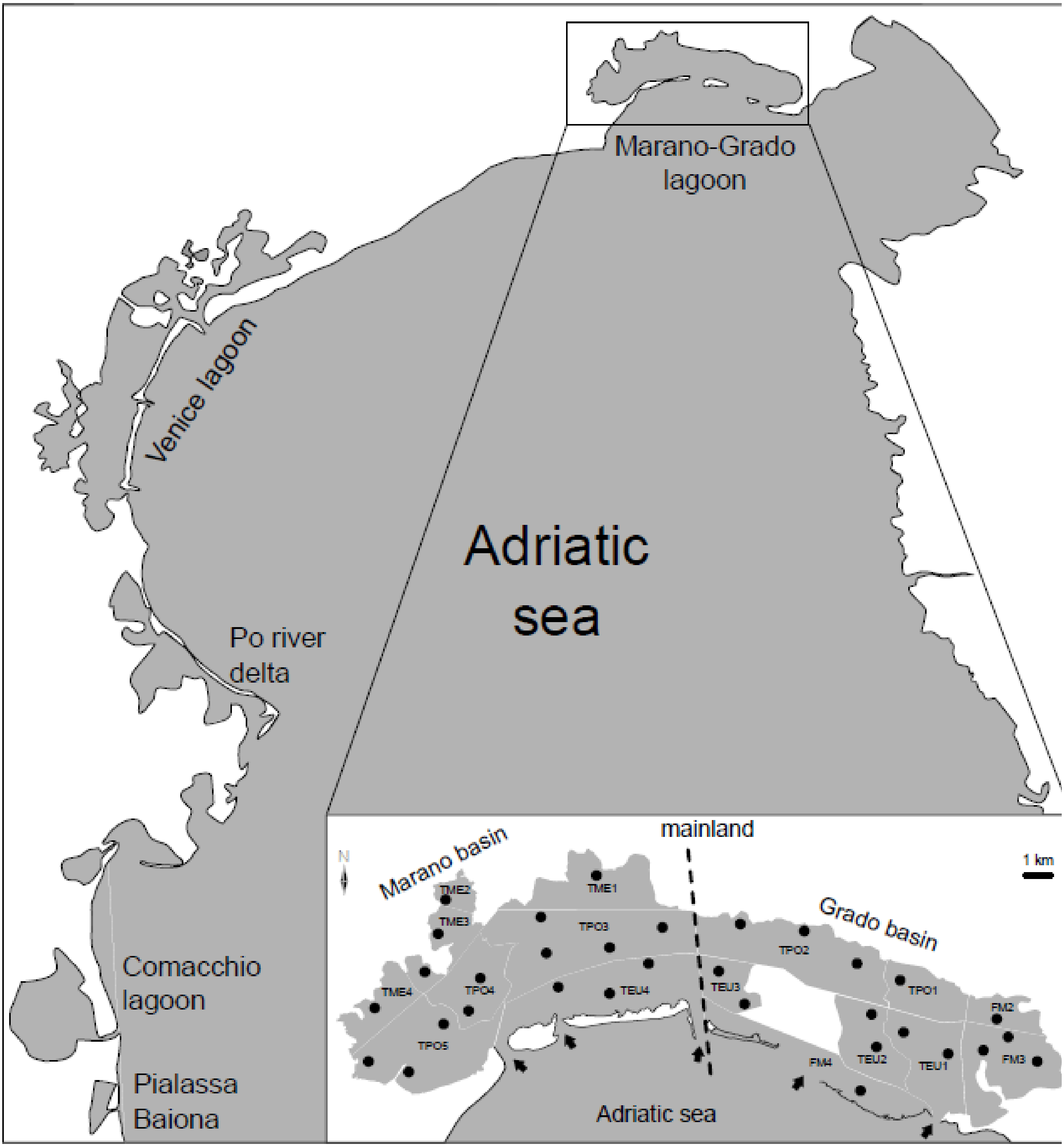
Appendix B

Pressure	Indicator	Measure	Degree of changes						NOTE
			No change (0)	**Very low (1)**	**Low (3)**	**Medium (5)**	**High (7)**	**Very high (9)**	
COASTLINE MORPHOLOGICAL CHANGE	Intertidal	% of intertidal area lost between 1954 and 2006 (Fontolan et al., 2012)	Increase	No change	<1% lost since 1850	≥1% & <5% lost	≥5% é <10%lost	≥ 10% lost	fixed in time
	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	No development	<5% of the coastline impacted by industrial or urban activities	≥5% & <30%	≥30% & < 60%	≥60% & < 90%	≥ 90%	

	Hydro	% of area affected by interferences with the hydrographic regime	No construction	<5% of the intertidal and subtidal area affected	>=5% and <10%	>=10% and <20%	>=20% and <40%	>=40%	
			No resource use (0)	**Very low (1)**	**Low (3)**	**Medium (5)**	**High (7)**	**Very high (9)**	
RESOURCE USE CHANGE	Aquaculture	% of area covered by clam farming	No fishfarming	<1% of the intertidal and subtidal area covered	≥1% & <10%	≥10% & <30%	≥30% & <50%	≥ 50%	fixed in time
	Fisheries	n° of traps km^{-2}	No fishery activities	< 10% of the surface of WB affected by fishery	≥10% & <30%	≥30% & <60%	≥60% & <90%	≥ 90%	
	Marinas	n° of berths km^{-2}	No marina	< 100 berths in marina	≥100 & <150 berths	≥150 & <300 berths	≥300 & <500 berths	≥ 500 berths	

	Intensity of port developments	extent of ports	No harbour	<500 m of quays	≥500 & <2 km	≥2 & <5 km	≥5 & <10 km	≥ 10 km of quays	
				Very low (1)	**Low (3)**	**Medium (5)**	**High (7)**	**Very high (9)**	
ENVIRONMENTAL QUALITY AND ITS PERCEPTION	sediment	sediment chemical status based on Decree 56/2009	100% of substances below the EQS (Environmental Quality Standard)	One 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 priority substance and one or more than one not priority substance fail to comply with EQSs	fixed in time

	benthos	M_AMBI index level (Muxika et al., 2007)	Normal	Normal	Normal	Recovering or deteriorating	Modified	Severely modified	fixed in time
	DIN	µg L^{-1} of dissolved inorganic nitrogen	pressure calculated as seasonal average of concentration on a monthly basis						
	RP	µg L^{-1} of reactive phosphorous	pressure calculated as seasonal average of concentration on a monthly basis						
	Chl-a	µg 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						


Marano-Grado lagoon
Venice lagoon
Adriatic sea
Po river delta
Comacchio lagoon
Pialassa Baiona
mainland
N
Marano basin
1 km
TME2
TME1
TME3
TPO3
Grado basin
TPO2
TME4
TPO4
TEU4
TEU3
TPO1
FM2
TPO5
FM4
TEU2
TEU1
FM3
Adriatic sea

Figure

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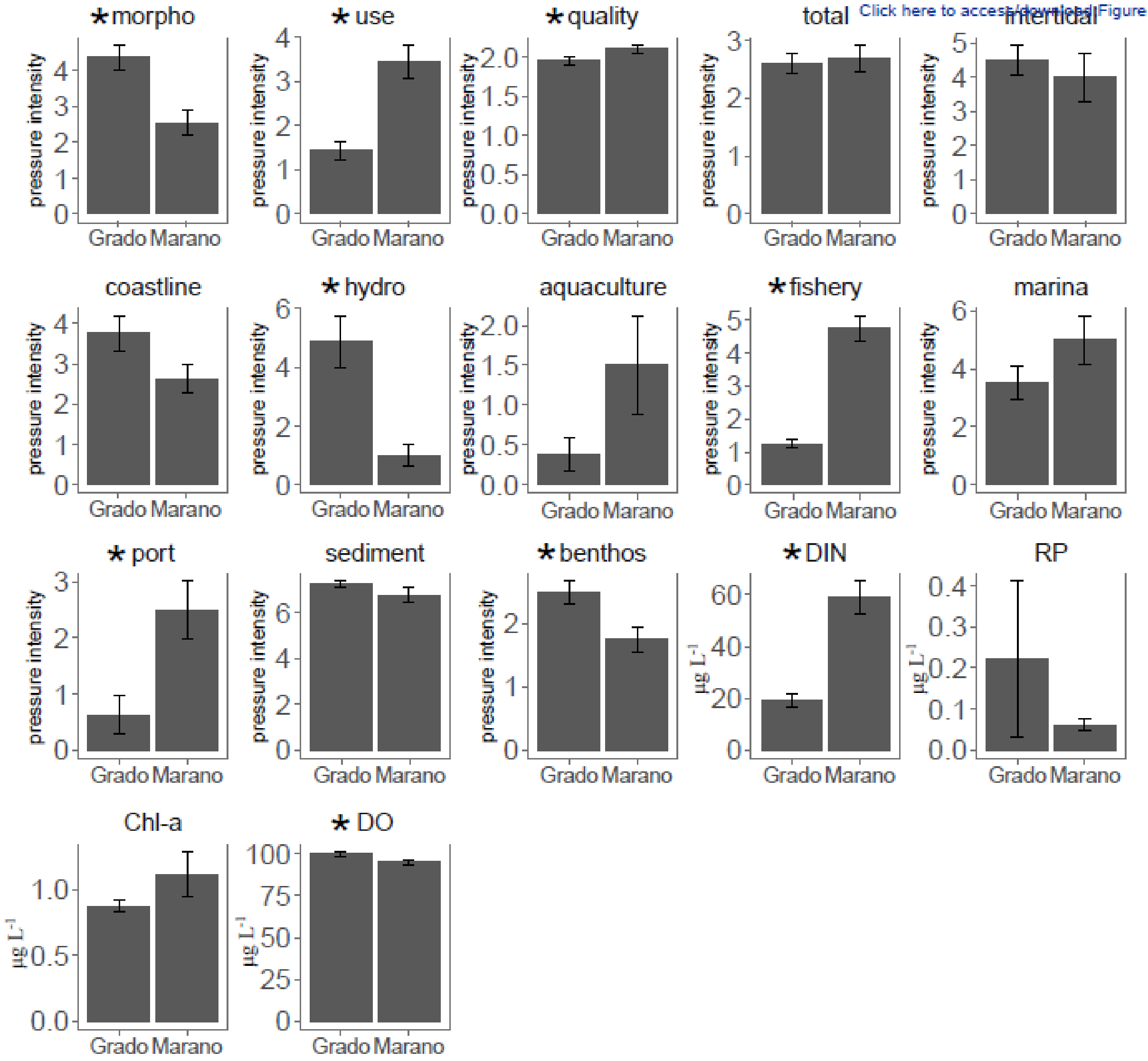
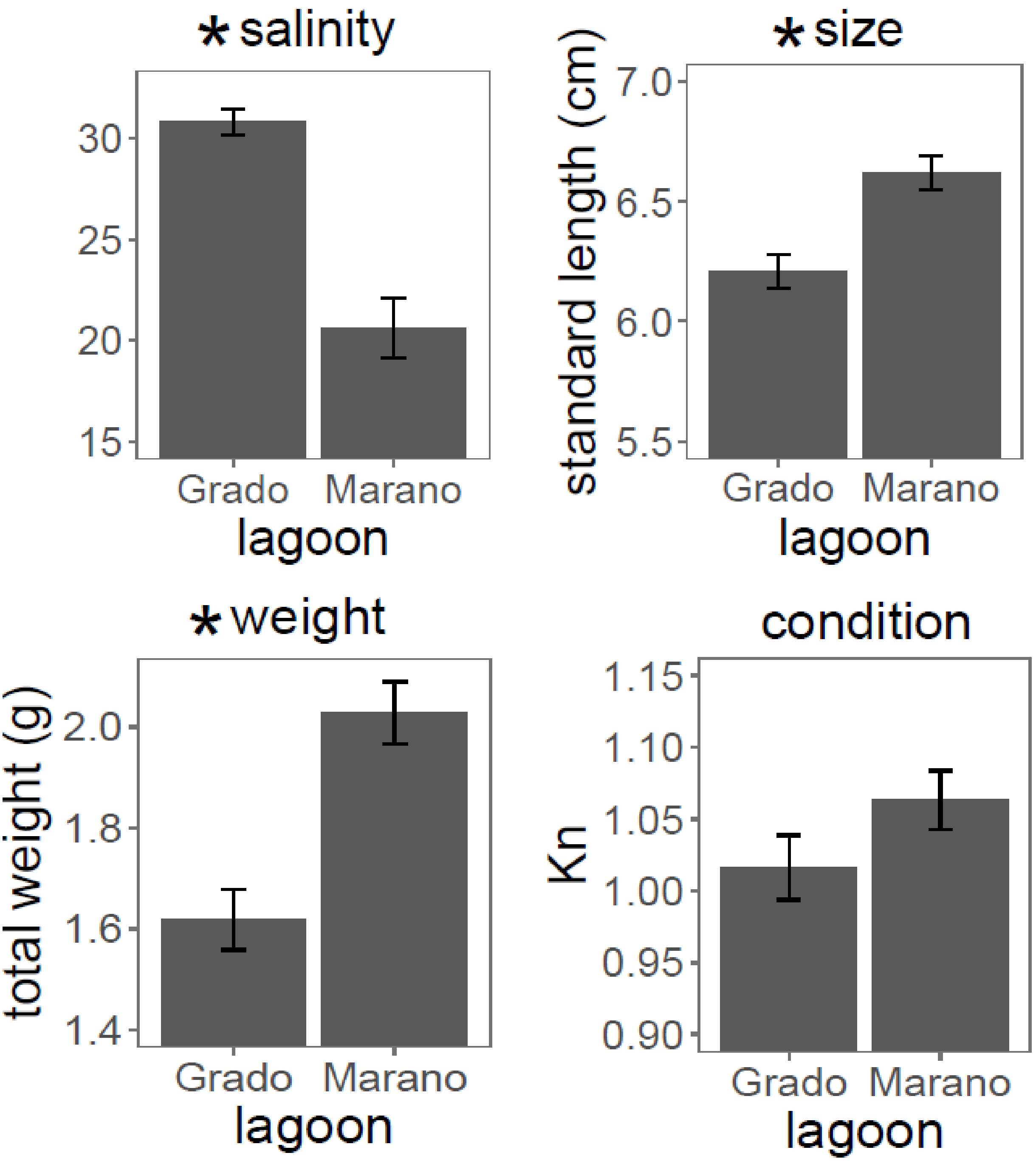
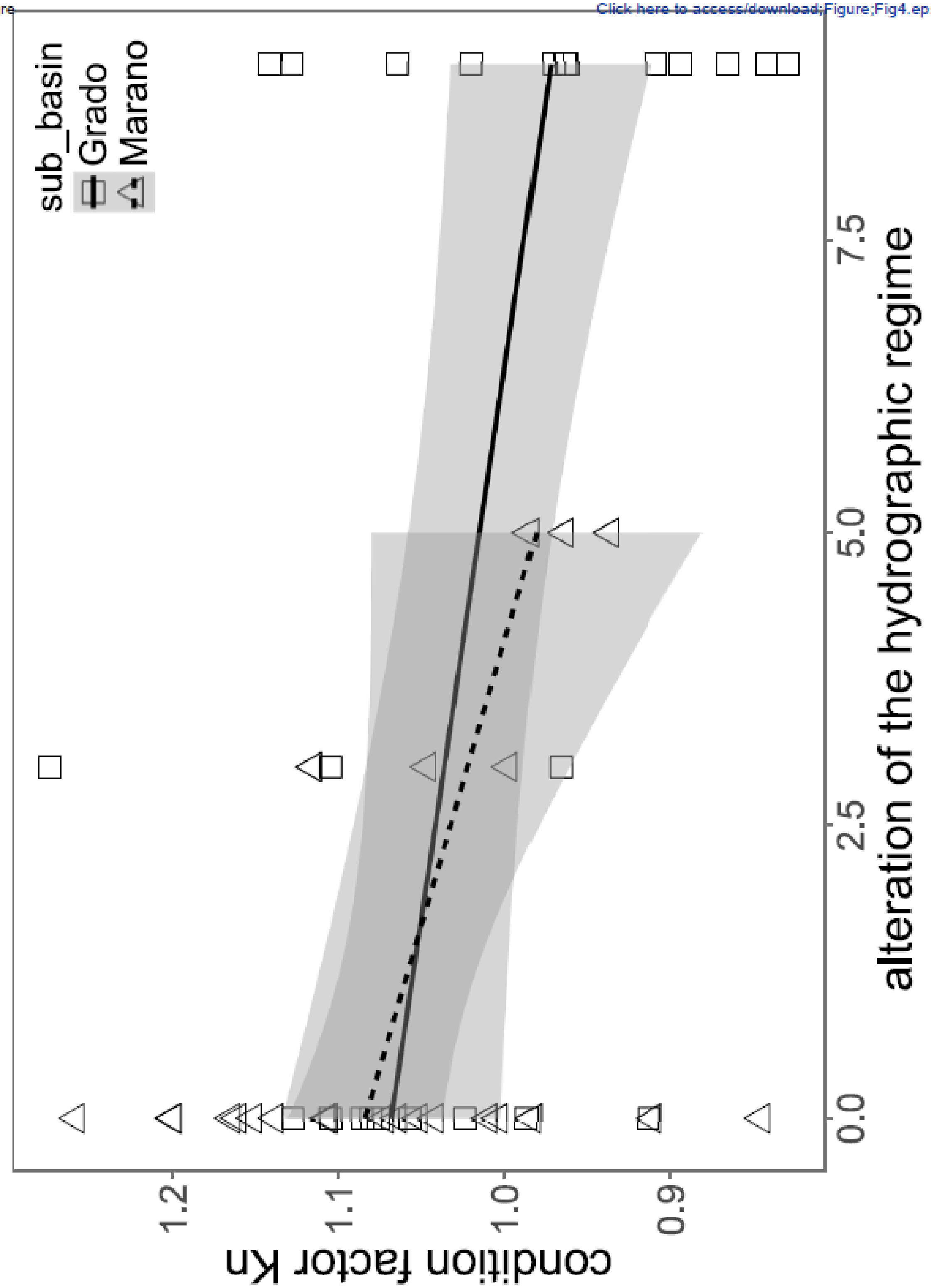
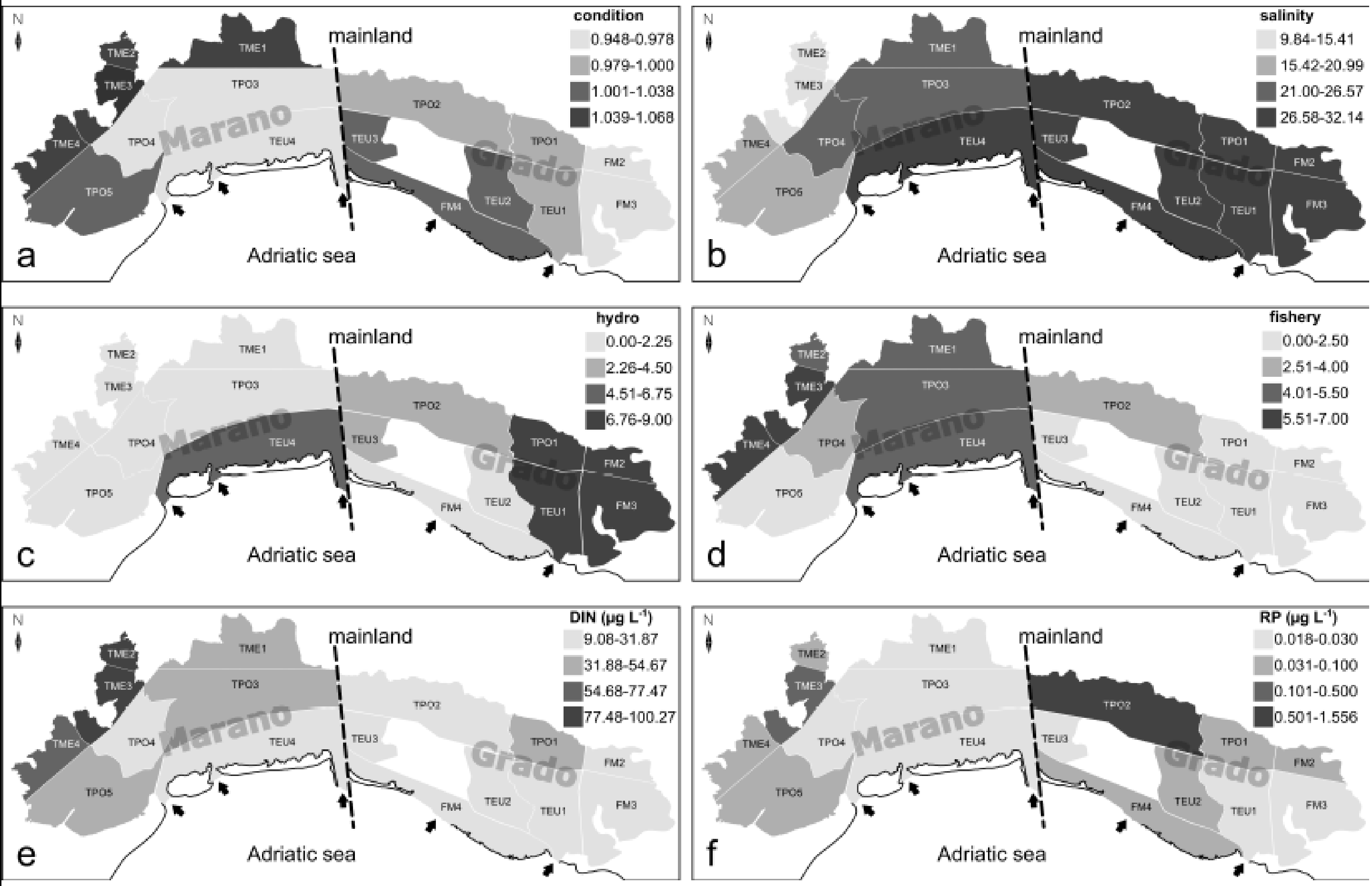



*salinity
30
25
20
15
Grado
Marano
lagoon
*size
standard length (cm)
7.0
6.5
6.0
5.5
Grado
Marano
lagoon
*weight
total weight (g)
2.0
1.8
1.6
1.4
Grado
Marano
lagoon
condition
Kn
1.15
1.10
1.05
1.00
0.95
0.90
Grado
Marano
lagoon


sub_basin
Grado
Marano
condition factor Kn
0.9
1.0
1.1
1.2
alteration of the hydrographic regime
0.0
2.5
5.0
7.5


N
mainland
Adriatic sea
Marano
Grado
TME1
TME2
TME3
TME4
TPO1
TPO2
TPO3
TPO4
TPO5
TEU1
TEU2
TEU3
TEU4
FM2
FM3
FM4
a
condition
0.948-0.978
0.979-1.000
1.001-1.038
1.039-1.068
b
salinity
9.84-15.41
15.42-20.99
21.00-26.57
26.58-32.14
c
hydro
0.00-2.25
2.26-4.50
4.51-6.75
6.76-9.00
d
fishery
0.00-2.50
2.51-4.00
4.01-5.50
5.51-7.00
e
DIN (μg L-1)
9.08-31.87
31.88-54.67
54.68-77.47
77.48-100.27
f
RP (μg L-1)
0.018-0.030
0.031-0.100
0.101-0.500
0.501-1.556