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Fish morphological and parasitological traits as ecological indicators of habitat quality in a Mediterranean coastal lagoon

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Abstract

1. Coastal lagoons are key 'transitional' aquatic environments for biodiversity conservation. Ecological indicators are useful tools for the management of water resources in the European Union. Among different biological communities, fish are a very sensitive tool to assess environmental integrity. Indeed, their parasites can be used as complementary indicators of habitat quality. Yet there is still a deep lack of information on ecological assessment using fish (along with their parasites) for coastal lagoons, particularly for the Iberian Peninsula.
2. The aim of the present study was to assess the use of fish morphology and their parasite communities as ecological indicators of anthropogenic impact within the Mar Menor coastal lagoon (SE Spain), a Mediterranean area of special conservation concern for European/Iberian biodiversity.
3. Fish samples (black-striped pipefish *Syngnathus abaster* and marbled goby *Pomatoschistus marmoratus*) were collected in August 2022 from the Littoral (high level of nutrient enrichment) and Barrier habitats. Physical status (both external and internal indices), asymmetry (level of developmental instability), parasite load, diversity and life-cycle complexity were compared between habitat types.
4. Body condition and mainly the health assessment index were better in the Barrier habitat. Except for pectoral fins in pipefish, the fluctuating asymmetry was statistically greater in the Littoral habitat (i.e. with eutrophication leading to poorer fish development). The parasite load was higher in the Littoral habitat for both host fish species. However, the diversity and life-cycle complexity of parasite communities were statistically lower in the Barrier habitat (a structurally simpler environment) only for gobies.
5. This study demonstrates an elevated potential for certain fish morphological and parasitological traits to be considered as good ecological indicators of environmental health. This could help environmental managers and policy makers to design effective monitoring programmes to detect impacts within valuable areas for conservation, such as the Mar Menor coastal lagoon.

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KEYWORDS

anthropogenic impact, aquatic conservation, body condition, eutrophication, fluctuating asymmetry, Iberian Peninsula

1 | INTRODUCTION

Coastal lagoons are shallow brackish, marine or hypersaline water bodies, being partly separated from the open ocean by a land barrier. These habitats are 'transitional' environments widely recognized as key ecosystems for supporting biodiversity and providing multiple services (Elliott & Whitfield, 2011). However, coastal lagoons are currently among the most threatened aquatic environments at the global scale (Kennish & Paerl, 2010), owing to a variety of anthropogenic pressures (e.g. agricultural, urban, industrial development), particularly around the Mediterranean Sea (Pérez-Ruzafa, Marcos & Pérez-Ruzafa, 2011; Pérez-Ruzafa, Pérez-Marcos & Marcos, 2020a). In a global context of biodiversity crisis, there is a need to design assessment protocols to effectively quantify the environmental health of coastal lagoons (Kennish & Paerl, 2010). Because of this conservation concern, environmental managers have been using community traits (e.g. species richness, diversity, abundance) as ecological indicators of the particular status within these transitional ecosystems (Hallett et al., 2012; Facca et al., 2020). These community-based indicators can be obtained from a variety of biological assemblages inhabiting the aquatic ecosystems, such as diatoms, macrophytes, benthic invertebrates and fish (Karr, 1991; Hering et al., 2006). These communities can respond to a wide range of environmental stressors, reflecting changes in the availability of critical habitats or food web effects (Hallett et al., 2012; Facca et al., 2020).

In the European Union (EU), the Water Framework Directive and the Marine Strategy Framework Directive regulate the management of water resources and requires the Member States to assess the ecological status of their water bodies (European Commission: EC, 2000). Indeed, both Directives promote the specific use of fish to estimate ecological integrity (Karr, 1981; Hering et al., 2006; Vidal-Abarca et al., 2016), as this taxonomic group is very sensitive to common anthropogenic disturbances, including hydrological alteration, habitat degradation, loss of water quality and land-use change (Aparicio et al., 2011; Almeida et al., 2017). The particular impacts of these disturbances on fish assemblages usually involve changes in taxonomic composition, abundance or physical condition. This is because many fish species have relatively long life-spans and generation times, as well as large home ranges compared with most other aquatic taxa, making them more appropriate to detect alterations at large spatial and temporal scales, e.g. 'old' disruptive events (Karr, 1981; Simon, 1999). In the last decades, fish-based indices have been widely developed and applied to transitional water bodies (see Souza & Vianna, 2020 for a systematic review). Despite some surveys having been carried out in Mediterranean areas from Europe (see Franco, Torricelli & Franzoi, 2009 for the Venice lagoon, Italy; and Sapounidis & Koutrakis, 2021 for a few shallow lagoons in

northern Greece), there is still a large knowledge-gap on the ecological assessment for coastal lagoons, which is of particular conservation concern for the EU Directives (Tagliapietra & Ghirardini, 2006). Indeed, there is a compelling case for the development of new and more sensitive ecological indices, incorporating diverse aquatic communities (not only fishes).

Over recent years, the use of 'alternative' and 'complementary' bio-indicators (see examples of 'typical' biological assemblages above) has increased for environmental assessment, such as parasite communities in aquatic habitats. Most of this research has been performed in fresh waters (see Lafferty, 1997; Blonar et al., 2009; Biswal & Chatterjee, 2020 for extensive reviews), although some valuable studies on parasites have highlighted the pivotal role of Marine Protected Areas for the whole ecosystem health, using invertebrates (Lamb et al., 2016; Davies, 2021) and fish (Sasal, Faliex & Morand, 1996) as the host organisms. However, such an approach (i.e. the use of parasites as indicators) is still under-developed, particularly in transitional waters (but see Blonar, Marcogliese & Couillard, 2011 as one of few examples). One of the reasons for using parasites is that certain taxa are more sensitive to environmental disturbances than their host species (e.g. fish) at higher levels of ecosystem organization, such as within the structure of trophic webs (Marcogliese, 2005), contributing to the ecological role as 'One Health Sentinels' (Jenkins et al., 2015). Also, parasite community traits can reflect the physiological status of hosts (e.g. fish) owing to disruptions in the immune system under conditions of environmental stress, i.e. more easily developing a parasitic disease (Petitjean et al., 2020; Sueiro et al., 2020). Thus, these biotic relationships (i.e. parasite vs. fish hosts) would be modulated depending on the allocation of energy resources, in the context of Dynamic Energy Budget theory, as shown by Pérez-Ruzafa, Pérez-Marcos & Marcos (2018) after analysing fish and environmental data from coastal lagoons in the Atlanto-Mediterranean region.

A particular disruptive factor of habitat quality in aquatic ecosystems is eutrophication, this being one of the major global threats to ecological status of transitional waters (Lemley, Adams & Strydom, 2017), including Mediterranean coastal lagoons (Pérez-Ruzafa et al., 2019; Pérez-Ruzafa et al., 2020b). This environmental disturbance consists of an increase in phytoplankton productivity within a body water owing to nutrient enrichment (usually nitrogen and phosphorous). Such an alteration in habitat quality can change the abundance of intermediate hosts and the susceptibility of definitive hosts to infection and disease, and the new environmental conditions can even affect free-living stages of parasites (Pietrock & Marcogliese, 2003; Sures et al., 2017; Falkenberg et al., 2019). Ultimately, the overall composition of fish parasite species and community structure will change accordingly (Valtonen, Holmes &

Koskivaara, 1997; Zargar et al., 2012). Consequently, the combined analysis of parasite communities within resident populations of host fish, along with their physical status, could reveal diverse aspects of ecosystem health, providing a more efficient and integrated assessment of the aquatic habitat. Given that fish are host to a wide diversity of parasite taxa (e.g. >30,000 helminth species; see Williams & Jones, 1994), these biological communities have a great potential for the assessment of ecological status, but this potential is deeply under-developed. Particularly for the Iberian eco-region, to the best of the authors' knowledge, the use of fishes and their parasites as bio-indicators of environmental quality in coastal lagoons (and other water bodies) has been virtually undescribed.

In light of this dearth of information, the aim of the present study was to assess the use of fish morphology and their parasite communities as ecological indicators of anthropogenic impact in Iberian transitional waters. Thus, a variety of parameters were compared in two target fish species between contrasting habitats within the Mar Menor coastal lagoon (Spain). This water body was selected because it is a Mediterranean area of special relevance for biodiversity, e.g. acting as a stop-over site for migratory birds and a nursery area for fisheries. This coastal lagoon is also of high conservation concern because of long-term human impacts, e.g. strong urban pressure and agricultural exploitation (see below a detailed description of the 'Study Area'). Specifically, the following parameters were analysed: fish physical status, both by calculating external and internal indices, asymmetry as an indication of developmental instability, and parasite load, diversity and life-cycle complexity. The hypotheses tested were that: (1) the study fish species will display lower physical condition in more impacted sites (e.g. Cavraro et al., 2019); (2) morphological traits on a long-term basis (i.e. asymmetry levels) will also reflect the reduction in habitat quality (Almeida et al., 2008; Almeida & Grossman, 2014; Lutterschmidt, Martin & Schaefer, 2016); and (3) parasite communities will be more abundant, but less diverse, under poor environmental conditions (Chapman et al., 2015; Reshu et al., 2022).

2 | MATERIAL AND METHODS

2.1 | Study area

The Mar Menor is the largest coastal lagoon in the western Mediterranean Sea (approximate surface area = 135 km², mean depth = 4 m, maximum depth = 6 m). This hypersaline (45 g L⁻¹) water body is located in the south east of the Iberian Peninsula (Murcia Region, Spain), which is separated from the Mediterranean Sea (salinity = 38 g L⁻¹) by a sand-bar (mean length = 21 km, mean width = 500 m), the so-called 'La Manga' (in Spanish). Three inlets connect the lagoon to the open sea, which are approximately located in the north, the middle and the south (Figure 1). This area is characterized by a semi-arid Mediterranean climate, with rainfall concentrated in autumn–winter (20–30 mm) and intense summer drought (<5 mm). The average annual temperature typically ranges between 15 and 20°C. The lowest temperatures occur in January

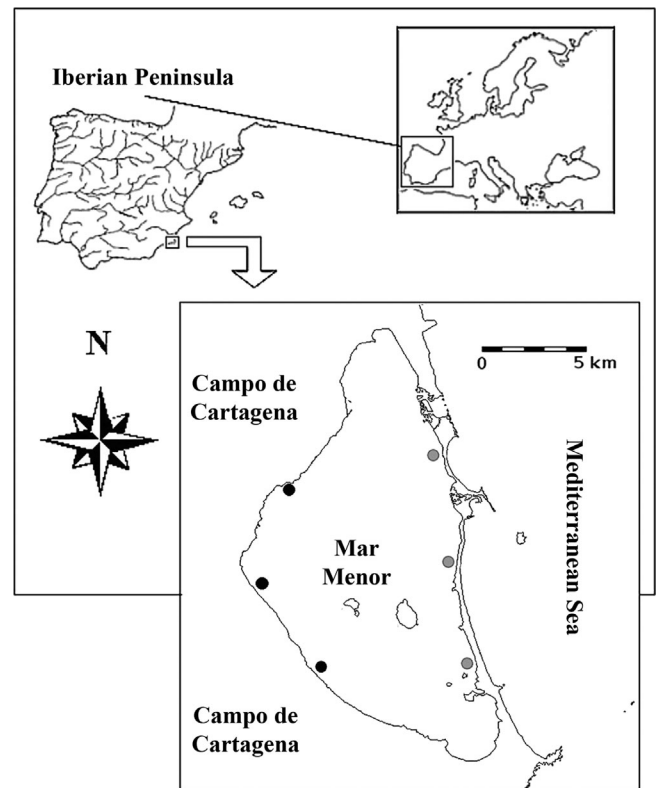


FIGURE 1 Map of the study area showing the geographic location for the Mar Menor coastal lagoon (south-east Spain). Black dots: sampling sites in the Littoral habitat. Grey dots: sampling sites in the Barrier habitat. The geographic location of 'Campo de Cartagena' County is also shown.

(down to 3°C) and the highest in August (>40°C). Minimum water temperature is observed in January (10°C) and maximum in August (30°C). The geological substratum in the study area consists of an extensive sedimentary cover from the Quaternary, which is formed by black and red silts. Climatic and environmental data were retrieved from the Ministry of Environment Spain (in Spanish, 'Ministerio para la Transición Ecológica y el Reto Demográfico', 'MITECO' hereafter; MITECO, 2023a) and the Water Agency of the River Segura Basin (in Spanish, 'Confederación Hidrográfica del Segura', 'CHS' hereafter; CHS, 2023). The Mar Menor is a very important wetland area for biodiversity conservation (e.g. see Martínez-Fernández et al., 2014). Specifically regarding aquatic vegetation, the littoral shallow waters are mostly occupied by the seagrass *Cymodocea nodosa*, which also forms mixed meadows with *Ruppia cirrhosa*. The fish fauna is also highly diverse and has been previously well described by Oliva-Paterna et al. (2006). The shallow areas of the Mar Menor are extremely important as settlement areas and juvenile refuges for several fish species, because of its ecological role as a nursery area. In addition, this coastal lagoon is home to important populations of highly threatened fish species, such as the European eel *Anguilla anguilla* and the Iberian toothcarp *Aphanius iberus* (Verdiell-Cubedo et al., 2013; Guerrero-Gómez et al., 2022). The lagoon is also a breeding site for threatened bird species, such as Audouin's gull *Ichthyaeetus audouinii* and the

slender-billed gull *Chroicocephalus genei*. The Mar Menor and its surrounding areas are recognized for their ecological importance, and up to 10 approved environmental protection areas converge here. For instance, it is classified as a Special Area of Conservation and Special Protection Area for Birds in the Natura 2000 Network. Also, the Mar Menor has been a Wetland of International Importance since 1994, according to the Ramsar Convention, and is also a Specially Protected Area of Mediterranean Importance, included in the Barcelona Convention (Kukkala et al., 2016; Guaita-García et al., 2022).

According to the contrasting anthropogenic impact owing to nutrient runoff, two habitat types of shallow waters are highlighted within this coastal lagoon (from west to east): the 'Littoral' and the 'Barrier' (Figure 1). The Littoral is directly fed by the discharges from small water courses that drain through the so-called 'Campo de Cartagena' County, an area of mainly agricultural land use. In fact, this is one of the most intensive areas for crop production in Spain and the EU, according to Martínez-Fernández et al. (2014) and the Ministry of Agriculture Spain (in Spanish, 'Ministerio de Agricultura, Pesca y Alimentación', 'MAPA' hereafter; MAPA, 2023). In the Littoral zone, intense summer drought and evaporation levels promote strong increases in nutrient concentrations (e.g. nitrates $>100 \text{ mg L}^{-1}$ and phosphates $>5 \text{ mg L}^{-1}$; CHS, 2023; MITECO, 2023b). Consequently, this chemical context, along with the high temperatures, triggers large algal blooms, with eutrophication episodes being commonly observed in this coastal lagoon (see a comprehensive analysis on nutrient dynamics in Pérez-Ruzafa et al., 2019). Historically, the Mar Menor has been considered as an oligotrophic body mass (Pérez-Ruzafa et al., 2020b), which is why nutrient runoff is of particular conservation concern (Álvarez-Rogel et al., 2020), as episodes usually involve massive fish mortalities owing to the resulting oxygen depletion below 1 m depth.

2.2 | Field sampling

Fish were collected in mid-August 2022, after the breeding season (spawning until July) for the two target species (see details below) in the study area (Guerrero-Gómez et al., 2022), thereby avoiding any effect of this physiological (i.e. reproductive) status on data. Moreover, the selected sampling period (i.e. summer) allowed environmental differences to be more contrasting between habitat types, with higher chemical and ecological disturbances (i.e. eutrophication) in the Littoral habitat. The year 2022 is considered to have been hydrologically 'average' in the study area (MITECO, 2023a). As a result, the effects of particularly dry or wet years on morphological and parasitological traits are avoided within the study period, which allows the data to be considered representative for these two fish species and their parasites in the Mar Menor. According to the CHS (2023) records, a massive fish mortality event was observed in August 2021 (4.5 tons, southern area), but only one small event (3 kg, western area) occurred in May 2022. Therefore, no catastrophic episode was close to the sampling dates, with no influence being detected on the present data. For a

better spatial representativeness, three sampling sites were selected for each habitat type (i.e. $n = 6, 3 \times \text{Littoral} + 3 \times \text{Barrier}$), which were separated by $>5 \text{ km}$ (shoreline) to minimize spatial data dependence (Figure 1). Also, sampling sites were located in the vicinity of similar environmental surroundings per habitat type, with regards to vegetation, and far from the influences of main tributaries, towns and fishing areas. Each site was randomly sampled on different dates during 10 days, alternating between habitat types. Fish collection was carried out at 10:00 (solar time) at each site, and weather conditions (e.g. temperature, humidity, visibility) were similar among the sampling dates. A consistent sampling protocol was followed by wading according to the European legislation (Comité Européen de Normalisation/International Organization for Standardization; EC, 2014). Also, the sampling effort was similar per site, in terms of time (1.5 h) and research team (the same three people, see 'Author contributions' below), to ensure comparability between habitat types. Adapted from Verdiell-Cubedo et al. (2013), the catch method consisted of hauling a beach-seine net ($10 \times 2 \text{ m}$, 2 mm mesh size) along a 20 m section of shoreline. Five replicates (i.e. $5 \times 20 \text{ m section} = 100 \text{ m shoreline}$) were collected at each sampling site. The area covered by each haul was approximately 175 m^2 (total hauled area = 875 m^2 per site).

All of these sampling conditions allowed the collection of a representative and comparable sample of the target fish species at each site and habitat type. In particular, black-striped pipefish *Syngnathus abaster* Risso, 1827 ('pipefish', hereafter) and marbled goby *Pomatoschistus marmoratus* (Risso, 1810) ('goby', hereafter) were collected. Pipefish and goby are among the most common and representative fishes in the Mar Menor shallow areas (Verdiell-Cubedo et al., 2013). These small-bodied species were selected because they display a very phylopatric distribution pattern (i.e. low dispersion capacity), which allows a more effective assessment of the main impacts within their territories (e.g. a higher nutrient runoff in the Littoral). Moreover, environmental requirements differ among the study species, thus allowing a better integrated assessment of each habitat type: the pipefish is a zooplanktivorous species (swimming through aquatic vegetation), while the goby is chiefly a benthic invertivorous fish (being more static on the bottom) (Doadrio et al., 2011).

After each survey was concluded per site, captured fish were identified and target species (i.e. pipefish and goby) were separately placed in tanks with cooled water (using cooling accumulators) and supplied oxygen (two battery-operated aerators with portable pumps) to reduce stress conditions during handling/transportation. To minimize the effect of body size on data and ensure an age sufficient to effectively detect developmental stress (see details below), the largest individuals of each target species were visually selected within the complete fish sample per site for subsequent examination (no significant differences were found for size between habitat types, see below). This 'largest size' sub-sample was transported to a laboratory facility at the University of Murcia, which is located $<1 \text{ h}$ from the study area.

All field procedures complied with the animal use and care regulations of Europe and Spain (specific licences were granted for Scientific Field Research in Murcia, Spain). Fish were collected by

trained personnel (i.e. the holder of the licences, F.J. Oliva-Paterna). Thus, no adverse effects were caused to the wildlife in the study habitats and all native fish fully recovered from the collection methods. Except for the target specimens, fish were released after recovery at the same collection sites.

2.3 | Morphological examination

On arrival at the laboratory, two 50 L aquaria (i.e. one per species) were filled with water collected from the same site of the Mar Menor lagoon. Aquaria were equipped with a constant oxygen supply, water filter, temperature/pH controller and environmental enrichment (e.g. similar substrate, such as sand, gravel and pebbles, along with submerged vegetation as per each site), according to Näslund & Johnsson's (2016) recommendations. Fish were allowed to acclimatize for a 30 min period by swapping water between the laboratory aquaria and the transportation tank, before being completely released into aquaria. All fish individuals collected at one particular site ($n = 33\text{--}34$ per species, see total sampling size below) were processed on the same date of capture within 6 h of aquarium release (and 8 h after collection, see time schedule above for 'Field Sampling'). This overall procedure helped to: (1) minimize the effect of stress on the fishes; (2) reduce contagion transmission among fish; and (3) reduce disturbance of their parasite infra-communities (e.g. specimen loss). This is a more appropriate alternative to maintaining fish in aquaria for more than 24 h (see a comprehensive review on fish captivity in Näslund & Johnsson, 2016). Fish were individually euthanized by immersion in an overdose solution of anaesthetic (dropwise addition of clove oil) for 5 min (adapted from Chapman et al., 2015), followed by severance of the spinal cord. Immediately after euthanasia, fish were measured for standard length (SL, ± 1 mm). As per Latorre et al. (2020), this particular fish length was selected because it avoids potential 'noise' given by variation of caudal fin length not related to body size (e.g. wounds and cuts in the fish skin and rays). In total, $N = 200$ individuals per fish species ($33\text{--}34 \times 3$ sites $\times 2$ habitat types) were examined. For pipefish, the size range was 80–123 (mean = 88.8, standard error of the mean, abbreviated as SEM = 1.3) and 81–125 (mean = 92.7, SEM = 1.5) mm SL from the Littoral and the Barrier, respectively (analysis of variance, ANOVA: $F_{1,198} = 0.81$, $P = 0.369$). For gobies, the size range was 30–45 (mean = 32.9, SEM = 0.5) and 31–47 (mean = 34.2, SEM = 0.6) mm SL from the Littoral and the Barrier, respectively (ANOVA: $F_{1,198} = 0.64$, $P = 0.425$). After SL measurements, fish were dissected and the sex determined.

Body condition ('BC', hereafter) is basically any index calculated by using the body mass relative to size, and then compared with a reference value. Therefore, the BC concept emerges as the plumpness, fatness or well-being of the animal (Jakob, Marshall & Uetz, 1996; García-Berthou, 2001). Indeed, a BC index can be also used as a morphological approach to measure the environmental impact on physical health of fish on a short-term scale (e.g. weeks or months; Le Cren, 1951; Nyboer & Chapman, 2017). In this study, eviscerated body mass (eBM, ± 1 mg) was measured, using an electronic balance, to provide an integrated quantification of BC,

avoiding bias from gonad mass and gut content. Then, data on fish size (SL) were used for the calculation of BC (see details in Section 2.5). For pipefish, the eBM range was 177–765 and 210–801 mg from the Littoral and the Barrier, respectively. For gobies, the eBM range was 351–640 and 383–674 mg from the Littoral and the Barrier, respectively.

Adapted from Adams, Brown & Goede (1993), a Health Assessment Index ('HAI', hereafter) was computed. This is another approach to relate the physical health of resident fish with environmental impact in a particular habitat type. Unlike other 'external' condition indices (e.g. BC), a variety of internal organs are usually examined to compute the HAI (see details below). Thus, the simultaneous use of both BC and HAI (i.e. on the same fish individuals) provides a better understanding of the environmental and physiological processes related to fish physical status. Moreover, HAI helps to complement data on parasite infra-communities (see details below in Section 2.4) and it can also reveal the effect of poor environmental conditions over short periods (e.g. young-of-the-year, abbreviated as YOY fish; see Chapman et al., 2015). Specifically, skin (fins and scales), eyes and gills were externally examined for abnormalities. Then, major organs were internally examined to assess the colour, size and condition: alimentary tract, heart, liver, spleen and gonads. Special care was taken to avoid damaging the parasite fauna during this health assessment (see below). Scores for each examined category ranged from 0 to 30 points and they were combined to compute a particular HAI value per individual. An HAI score of zero indicates a fish that did not have any appreciable abnormalities, whereas a high score reflects an individual perceived to be unhealthy. Theoretically, HAI could range between 0 and 240 points (i.e. 30×8 anatomical regions/organs) in this study; however, no fish exceeded a total score of 100 points. This index was calculated according to the following formula:

$$\text{HAI} = \sum_i^n \text{HS}_i,$$

where HS_i is the health score of the anatomical region/organ i (range 0–30 points) and n is the total number of examined anatomical regions/organs ($n = 8$) on a particular fish individual.

Given that most morphological and parasitological traits can significantly vary after relatively recent disturbances (see Chapman et al., 2015 for an example in parasites of juvenile fish), it is appropriate to use other parameters to detect environmental impacts over a longer time-scale. In this respect, fluctuating asymmetry ('FA', hereafter) is considered a morphological trait that acts as a good indicator of environmental instability, promoting developmental stress throughout an animal's life-span (Leary & Allendorf, 1989; Parsons, 1992; Allenbach, 2010). This approach has been successfully applied for more integral evaluation of pollution effects on water quality using selected fish taxa. In terms of conservation biology, more impacted sites will be characterized by a greater number of individuals displaying high levels of FA (e.g. Leary & Allendorf, 1989; Almeida et al., 2008). Specifically, the study species reach a maximum age of 4 years in the Mar Menor (F.J. Oliva-Paterna, pers. observ. from tagged fish). To test for differences in FA between habitat types,

two morphometric traits were measured: eye diameter and pectoral fin length. These bilateral traits were chosen because they commonly respond to developmental stress in fish (Almeida et al., 2008; Almeida & Grossman, 2014; Cruz et al., 2022). To obtain more accurate morphometric measurements ($\pm 1 \mu\text{m}$), bilateral traits (i.e. eyes and fins) were examined using a dissecting microscope ($40\times$) with integrated Camera (Leica Microsystems EZ4; Vista, CA, USA). Images were then processed and paired traits measured by using the software Aperio ImageScope v.12 (Aperio Technologies; Vista, CA, USA). All measurements were taken by the same person (D. Almeida).

2.4 | Parasitological examination

Ecto- and endo-parasites were identified and quantified per fish individual during the HAI examination (i.e. the same anatomical regions and organs, see above). In this study, the number of fish examined for parasites was much higher than that in other studies, where species accumulation curves were applied to calculate representative sample size (e.g. see Figure 1 in Chapman et al., 2015; <20 fish individuals per site). The sample size (>30 individuals per site) in the present work achieved a good representativeness of parasite infra-communities in each of the two habitat types (see another example of this procedure in Cruz et al., 2022). Fish were examined under a dissecting microscope ($40\times$) for the presence of parasites according to standard protocols (e.g. Weber & Govett, 2009; Stoskopf, 2010). Apart from the dissecting microscope, parasites were also identified using a light microscope ($1000\times$) equipped with phase contrast and differential interference contrast. Parasites were excysted, when necessary, using fine forceps. Occasionally, parasites were fixed in 70% ethanol for later staining (e.g. Iron Aceto-Carmine, abbreviated as IAC) and identification. Parasites were identified (and counted) using the keys/databases in Brewster (2016), Bruno, Nowak & Elliot (2006) and Falaise (2017). For particular parasite taxa, Family or Genus was the lowest practical taxonomic level, since these parasite categories provide enough information to assess their ecological role as potential bio-indicators (Ondračková et al., 2012; Chapman et al., 2015). After examinations, specimens were deposited in the Parasitological Collection of the Department of Basic Medical Sciences, USP-CEU University, Spain.

2.5 | Data analyses

For the FA analysis, statistical techniques followed Almeida & Grossman (2014, and see a comprehensive list of references therein). Briefly, paired measurements of bilateral traits were transformed into signed asymmetry values according to the formula: right minus left ($R - L$) sides. Then, the unsigned (absolute) values, i.e. $|R - L|$, were used to assess the FA levels (μm) between habitat types. This FA level was calculated according to the following formula:

$$FA = \sum_i^n |R_i - L_i| / n,$$

where R and L are the morphometric measurements (in μm) from the right and left sides of a particular bilateral trait for the fish individual i , and n is the total number of examined fish individuals.

Quantitative descriptors were used to properly analyse the variation in parasitological traits between habitat types. Three parameters of parasite infra-communities were calculated for each fish individual (adapted from Bush, Lafferty & Lotz, 1997): 'total load' (TL, total number of parasite specimens detected from the examined anatomical regions/organs), 'diversity' (Shannon index H' , calculated by using \log_2 ; Shannon & Weaver, 1949) and a 'life-cycle complexity index' (LCI). The latter descriptor was calculated according to the following formula:

$$LCI = \sum_i^n (l_i \times h_i) / \sum_i^n l_i,$$

where l_i is the load of the parasite taxon i , h_i is the number of different hosts in the life-cycle of i and n is the total number of parasite taxa on a particular fish individual. The number of hosts was allocated to each parasite taxon according to the available literature (see identification keys above). This number ranged from one to three hosts for the parasite taxa found in the present study, and consequently, the descriptor LCI also varied within this range. This index can be regarded as a proxy of general biodiversity in the ecosystem, with high values indicating an elevated abundance of heteroxenous parasites that need several ecologically diverse hosts (e.g. zooplankton, molluscs, fish, birds or even aquatic mammals) to complete their life-cycle. Additionally, the 'prevalence' (the proportion of examined hosts infected with parasites) was also calculated.

A chi-square test with Yates correction was used to compare the prevalence between habitat types. One-way ANOVA was used to ensure that similar fish sizes (SL) were selected between habitat types (see results above). Despite no differences being found in fish length, the effect of body size was controlled for subsequent comparisons using analysis of covariance (ANCOVA) to avoid any subtle interaction. In particular for BC, ANCOVA allowed the comparison of eBM between habitat types, after controlling for fish size (covariate: SL). Similarly to the procedure with BC, ANCOVA (covariate: eBM) was used to test for significant differences between habitat types for the response variables HAI and the three parasite descriptors (TL, H' and LCI). FA was also assessed by means of ANCOVA, with 'character size' (i.e. the average between both sides, $(R + L)/2$) as the covariate (Fessehaye et al., 2007; Almeida et al., 2008).

Data were transformed by using $\log_{10}(x + 1)$. In particular for absolute FA distributions, these data were normalized using the two-parameter Box-Cox transformation (Swaddle, Witter & Cuthill, 1994), which consists of the form $(FA + a)^b$, where 'a' and 'b' are two coefficients obtained through iteration. This transformation is often suitable for normalizing skewed positive data (e.g. a half-normal distribution containing zeroes). Assumptions of normality of distributions and homogeneity of variances were verified through the Shapiro-Wilks and Levene tests, respectively. Statistical analyses were performed with R v. 3.6.3 (R Core Team, 2020). The significance level was set at $\alpha = 0.05$. The values reported in the results are means \pm SEM.

3 | RESULTS

After controlling for fish size (SL), adjusted eBM values (i.e. BC) were higher in the Barrier habitat for both fish species, although this difference was marginally non-significant ($P = 0.065$) for gobies (Table 1). After controlling for fish mass (eBM), adjusted HAI values were higher in the Littoral habitat, with these differences being very significant (P -values < 0.01) for both fish species (Table 1). Significant differences were found between habitat types for FA, except for pectoral fins in the pipefish ($P \approx 0.07$). FA levels were always higher in the Littoral habitat (Figure 2).

Both fish species showed a higher percentage of parasite prevalence in the Littoral habitat (72% for pipefish; 81% for gobies) relative to the Barrier habitat (62% for pipefish; 67% for gobies), although this difference was statistically significant only for gobies ($\chi^2 = 2.26$, $P = 0.133$ for pipefish; $\chi^2 = 4.39$, $P = 0.036$ for gobies). Thirteen parasite taxa (family, genus or species level) were identified for pipefish and 15 taxa for gobies. As higher hierarchical taxonomic groups, parasites ranged from single-cell organisms to metazoan: Ciliophora, Cestoda, Monogenea, Digenea, Nematoda, Acanthocephala (only in pipefish) and Crustacea (Tables 2 and 3). Digenean species were the most diverse and abundant parasites on both fishes (six taxa for pipefish; seven taxa for gobies). In particular, the most common parasite taxon for pipefish (with higher frequency in the Littoral) was the taxonomic family Cyathocotylidae (Table 2), whereas *Bucephalus minimus* (Stossich, 1887) Nicoll, 1914 was most common for gobies (similarly with more parasite load in the Littoral) (Table 3). All monogenean parasites belonged to the genus *Gyrodactylus*, being found in both habitat types and host species, with a higher load in the Littoral habitat. All Ciliophora parasites belonged to the genus *Trichodina*, although a different species was identified for each fish host (Tables 2 and 3). Cestodes were more diverse in gobies, with four different Genera (*Acanthobothrium*, *Bothriocephalus*, *Ligula* and *Proteocephalus*; Table 3), whereas only one genus (*Proteocephalus*) was found in pipefish (Table 2). Of the total parasite taxa identified in

pipefish (13 categories), three digenean (*Acanthostomum*, *Diplostomum* and *Metagonimus*) and one acanthocephalan (*Acanthocephalus*) were not found in the Barrier habitat (Table 2). Of the total parasite taxa identified in gobies (15 categories), five (two cestodes, two digenean and one crustacean) were not found in the Barrier habitat, whereas two (cestode *Bothriocephalus scorpii* (Müller, 1776) Cooper, 1917 and digenean *Proserhynchoides gracilescens* (Rudolphi, 1819) Stunkard, 1976) were not found in the Littoral habitat (Table 3). The digenean *Cryptocotyle concavum* (Creplin, 1825) Lühe, 1899 was the only species present in both fish hosts. This parasite was relatively frequent (chiefly in gobies) and with more load in the Littoral habitat (Tables 2 and 3). Crustacean species were ecto-parasites, taxonomically very different, depending on fish host, with the copepod *Ergasilus ponticus* Markevich, 1940 being found in pipefish (slightly more abundant in the Littoral habitat, Table 2) and the isopod *Mothocya epimerica* Costa, 1851 in gobies (absent on fish from the Barrier habitat, Table 3). Regarding the three parameters of parasite infra-communities (TL, H' , LCI), adjusted values were always higher in the Littoral habitat, after controlling for eBM (Table 4). However, differences were marginally non-significant ($P \approx 0.08$) for H' and clearly non-significant ($P > 0.10$) for LCI in the pipefish (Table 4).

4 | DISCUSSION

Information about fish parasites of the Mar Menor coastal lagoon is scarce; the existing few studies are focused on particular fish species of great socio-economic interest (e.g. fisheries, aquaculture), such as European eel (Mayo-Hernández et al., 2015) or the sparid sharpnose seabream *Diplodus puntazzo* (Alama-Bermejo, Raga & Holzer, 2011; Sánchez-García et al., 2015). Some of these studies calculated parameters of the parasite infra-community, such as richness and diversity, but only for specific parasite taxa (e.g. helminths in Mayo-Hernández et al., 2015; Myxozoa in Alama-Bermejo, Raga & Holzer, 2011). In the present study, a greater variety of parasites (i.e. protozoans, helminths, crustaceans) were analysed and three community parameters were calculated per fish individual (i.e. abundance, diversity and life-cycle complexity). Also, samples were collected from representative sites with different levels of impact, although a true undisturbed reference habitat was not included, which is a potential limitation of this study. However, it was easily possible to identify the least degraded habitat (i.e. the Barrier), in terms of nutrient runoff, using scientific literature and data from Spanish agencies and ministries (CHS and MITECO). Given that the main goal of this study was to assess whether fish and their parasites can act as potential bio-indicators, relative changes in morphological and parasitological traits between habitats (Littoral vs. Barrier) would be clearly indicative of the different levels of habitat quality. Another key point of the research design consisted of selecting two fish species abundant in the study area, which display contrasting requirements, in terms of metabolism, behaviour and micro-habitat (Verdiell-Cubedo et al., 2012). Thus, all these approaches are relevant for a more integrated assessment of

TABLE 1 Morphological traits (body condition, BC, and health assessment index, HAI) for black-striped pipefish *Syngnathus abaster* and marbled goby *Pomatoschistus marmoratus*.

<i>S. abaster</i>	Littoral	Barrier	$F_{1,197}$	P
BC (mg)	290 ± 16	429 ± 20	5.18	0.024
HAI (points ^a)	38.6 ± 1.7	23.2 ± 1.4	7.07	0.008
<i>P. marmoratus</i>	Littoral	Barrier	$F_{1,197}$	P
BC (mg)	419 ± 17	577 ± 23	3.45	0.065
HAI (points ^a)	32.1 ± 1.5	19.0 ± 1.1	9.67	0.002

Note: Results are (adjusted) means ± SEM, reported per habitat type in the Mar Menor coastal lagoon. F -ratios, degrees of freedom and significance levels (P -values) are presented, after ANCOVA (covariates: standard length, SL/visceral body mass, eBM; see Section 2.5 for details). Significant results are highlighted in bold.

^aSpecific range: 0–100 points (see Section 2.5 for detailed formula and explanation).

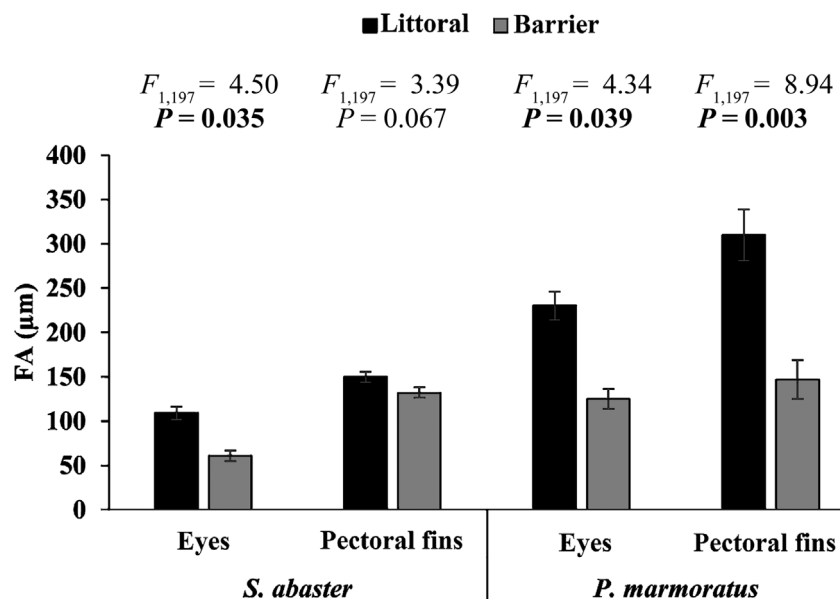


FIGURE 2 Fluctuating asymmetry (FA) levels (μm) of two bilateral and morphometric characters in black-striped pipefish *Syngnathus abaster* and marbled goby *Pomatoschistus marmoratus*. Results are (adjusted) means \pm standard error of the mean (SEM), reported per habitat type in the Mar Menor coastal lagoon. F -ratios, degrees of freedom and significance levels (P -values) are presented, after analysis of covariance (ANCOVA) (covariate: character size, see Section 2.5 for details). Significant results are highlighted in bold.

TABLE 2 Parasite list found in black-striped pipefish *Syngnathus abaster* from the Mar Menor coastal lagoon.

Parasite (taxon)	Parasite (family/genus/species)	Littoral	Barrier
Ciliophora	<i>Trichodina partidisci</i>	0.7	0.6
Cestoda	<i>Proteocephalus</i> sp.	0.5	0.4
Monogenea	<i>Gyrodactylus</i> sp.	1.3	0.9
Digenea	<i>Acanthostomum imbutiformis</i>	0.9	—
	<i>Apopodocotyle atherinae</i>	2.5	3.0
	<i>Cryptocotyle concavum</i>	3.3	2.5
	Cyathocotylidae	7.5	5.8
	<i>Diplostomum</i> sp.	1.1	—
	<i>Metagonimus</i> sp.	0.5	—
Nematoda	<i>Contracaecum microcephalum</i>	0.1	0.2
	<i>Acanthocephalus incrassatus</i>	0.7	—
Acanthocephala	<i>Pomphorhynchus laevis</i>	0.8	0.7
Crustacea	<i>Ergasilus ponticus</i>	1.6	1.2

Note: Mean values of parasite loads (i.e. abundances, unit: number of specimens) are shown per family, genus or species and habitat type.

ecological status as a whole, beyond particular biological populations, with these 'fish' results probably being a reflection of community responses from a wider range of aquatic species at large scales. In this regard, Pérez-Ruzafa, Pérez-Marcos & Marcos (2018) also highlighted the importance of mechanisms and processes acting from the (fish) physiological level to the ecological level of biological organization. Thus, the assessment of parameters at the individual level, such as morphology and parasitology in the present work, may help with understanding the consequences at the community level in response to habitat features. These features could turn into conditions of environmental stress after anthropogenic pressures. Consequently, such disturbances may promote an unbalanced allocation of energy budget and lead to a significant loss of fish condition/fitness, along with disruption of the entire ecosystem structure/function through trophic 'cascades'.

With respect to body condition, partial support was found for the first hypothesis of this study, as the same pattern (i.e. a lower BC in the Littoral habitat) was observed for both fish species, although a statistically significant difference was only found in pipefish. This finding suggests a close species-specific response under eutrophic conditions, according to the contrasting ecological traits. As another example, Cavraro et al. (2019) showed that the sand smelt *Atherina boyeri* (a small pelagic/limnetic teleost) was a useful tool to detect the effects of anthropogenic pressures in transitional waters (specifically a lagoon draining to the Adriatic Sea). In particular, BC was lower in impacted sites by alteration of the hydrographic regime, whereas a significant positive correlation was found with trophic status. The sand smelt is a fish species also present in the Mar Menor coastal lagoon, but it was not selected for this study because of its higher locomotive capacity than the target fishes for the present work. For

TABLE 3 Parasite list found in marbled goby *Pomatoschistus marmoratus* from the Mar Menor coastal lagoon.

Parasite (taxon)	Parasite (family/genus/species)	Littoral	Barrier
Ciliophora	<i>Trichodina heterodontata</i>	6.0	3.5
Cestoda	<i>Acanthobothrium</i> sp.	0.7	0.3
	<i>Bothriocephalus scorpii</i>	–	0.1
	<i>Ligula intestinalis</i>	0.1	–
	<i>Proteocephalus</i> sp.	0.1	–
Monogenea	<i>Gyrodactylus</i> sp.	0.5	0.2
Digenea	<i>Bucephalus baeri</i>	0.3	–
	<i>Bucephalus minimus</i>	14.9	11.0
	<i>Cryptocotyle concavum</i>	10.1	8.7
	<i>Cryptocotyle lingua</i>	6.2	5.4
	<i>Podocotyle atomon</i>	0.3	–
	<i>Proserhynchoides gracilescens</i>	–	0.1
	<i>Timoniella</i> sp.	4.8	2.8
Nematoda	Acuariidae	2.7	2.2
Crustacea	<i>Mothocya epimerica</i>	0.1	–

Note: Mean values of parasite loads (i.e. abundances, unit: number of specimens) are shown per family, genus or species and habitat type.

TABLE 4 Parasitological traits (total load, TL, diversity, H' , and life-cycle complexity index, LCI) for black-striped pipefish *Syngnathus abaster* and marbled goby *Pomatoschistus marmoratus*.

<i>S. abaster</i>	Littoral	Barrier	$F_{1,131}$	P-value
TL (number of specimens)	21.5 ± 1.2	15.3 ± 1.0	4.57	0.034
H' (bits of information)	2.21 ± 0.11	1.66 ± 0.12	3.07	0.082
LCI (number of host species ^a)	1.96 ± 0.09	1.57 ± 0.10	2.41	0.123
<i>P. marmoratus</i>	Littoral	Barrier	$F_{1,145}$	P-value
TL (number of specimens)	46.8 ± 2.0	34.3 ± 1.8	5.75	0.018
H' (bits of information)	2.61 ± 0.13	1.81 ± 0.12	5.67	0.019
LCI (number of host species ^a)	2.32 ± 0.08	1.84 ± 0.09	4.24	0.041

Note: Results are (adjusted) means ± SEM, reported per habitat type in the Mar Menor coastal lagoon. F -ratios, degrees of freedom and significance levels (P -values) are presented, after ANCOVA (covariate: eviscerated body mass, eBM; see Section 2.5 for details). Significant results are highlighted in bold.

^aNumber of host species in the parasite life-cycle, averaged per fish individual from its particular infra-community, and weighted by load of each parasite species; specific range: 1–3 host species (see Section 2.5 for detailed formula and explanation).

the smelt species, it would be difficult to estimate the movement rates from impacted sites. This may be the reason why the 'smelt' response was contrary to the expectations of the present research, in terms of eutrophication (i.e. negative association between BC and elevated trophic status in the Littoral habitat). Mechanistically, two potential scenarios can be considered to explain the eutrophication effects on fishes. As a direct effect, oxygen depletion (mainly at the bottom) poses a strong stress on fish physiology (see Mishra et al., 2022 for a dramatic example), with hypoxia affecting feeding habits, and subsequent loss of energy reserve or muscle mass, i.e. decrease in growth and body condition (Cottingham et al., 2018; Abdel-Tawwab et al., 2019). Indirect effects are also possible, in terms of 'bottom-up' control of the food web (e.g. Bell, Garnier & Huss, 2022), as zooplankton grazers (e.g. copepods) have been observed to undergo massive increases in their abundances within the Mar Menor

(Gilbert, 2001), which can occur after proliferation of phytoplankton in transitional waters (e.g. Dimitrieva & Semenova, 2012). Taking into account both (direct and indirect) effects, the present results on BC were slightly puzzling, as gobies should clearly have displayed a worse body condition in the Littoral habitat, because of its benthic habits (i.e. more exposed to hypoxia at the bottom). To try to explain this finding, some gobiids (including the genus *Pomatoschistus*) are known for their tolerance to oxygen depletion (Petersen & Petersen, 1990), with some species becoming highly invasive owing to this key trait (see the case of Ponto-Caspian round goby *Neogobius melanostomus* in Dickey et al. [2021]). Given that the pipefish species selected for this study chiefly feeds on copepods in Iberian waters (Doadrio et al., 2011), a positive (bottom-up) effect on body condition could have been expected in the Littoral habitat, because of abundant food supply through the water column (where the effect of

hypoxia is lower). However, the diversity of zooplankton may also decrease in lagoons after a eutrophication event (e.g. Akbulut & Tavşanoğlu, 2018; Papanitiou et al., 2020). In fact, an explosive increase in copepods from the Mar Menor was detected by Gilbert (2001) mainly for one species (*Oithona nana*). Syngnathids can display a strong prey selection (see Castro-Fernández et al., 2020 for a study on four syngnathid species, including *S. abaster*) and this may be the reason, along with a potential decrease in copepod diversity, why the observed effect on the examined pipefish species was negative in this study (i.e. lower body condition in the Littoral sites).

Statistically speaking, HAI was a good (very significant) indicator of health status, both for fish and for water quality. In particular, environmental conditions were clearly more suitable in the Barrier habitat for the two study fish species (i.e. lower HAI values), in accordance with the first hypothesis. In this respect, Chapman et al. (2015) found worse fish health, using HAI, in YOY pumpkinseed *Lepomis gibbosus* in more eutrophic water courses. Apart from freshwater fish inhabiting fluvial habitats, the present study shows that this health index also appears to be a useful tool for environmental assessment in transitional waters. Such findings demonstrate the importance of using both 'external' and 'internal' indices of fish physical status, with different levels of sensitivity, to detect disturbances (e.g. Cruz et al., 2022). Eutrophication may be directly affecting fish organs owing to histo-pathological effects from nutrient enrichment (i.e. the toxicity of nitrates and phosphates), such as tissue inflammation, hypertrophy and necrosis (Pereira et al., 2017; Wagenaar & Barnhoorn, 2018; Rodgers et al., 2021). Additionally, HAI could also reflect physiological/histological effects of infectious diseases (e.g. parasitism on gills and guts by ecto-/endo-helminths), as this health index can be positively related to parasite load because of eutrophication (Crafford & Avenant-Oldewage, 2009; Chapman et al., 2015). A potential explanation for this relationship is that there may be a disruption of the immune response of fishes at the molecular (immuno-globulins) and cellular (lymphocytes) levels (e.g. see a comprehensive immuno-ecological study on common carp *Cyprinus carpio* in Rohlenová et al. [2011]) under eutrophic conditions, i.e. leading to a decrease in 'resistance', in the terms of Blanchet et al. (2009) and Blanchet, Rey & Loot (2010). These authors suggest that hosts can protect themselves against the impact of parasites by actively reducing parasite burden (i.e. resistance) or by limiting the damage caused by parasites (i.e. tolerance). Consequently, TL can be considered as a proxy for 'resistance', whereas HAI can be considered as a proxy for 'tolerance' in the present study. Both parameters (i.e. resistance and tolerance) were higher in the Barrier habitat, with pipefish and gobies displaying a lower parasite abundance and better health index, respectively.

As mentioned already, BC and HAI can rapidly change (within months or even weeks) after habitat alterations (e.g. Chapman et al., 2015; Nyboer & Chapman, 2017). Thus, FA results helped to build a more realistic 'picture' of environmental processes operating throughout fish development (Almeida et al., 2008; Almeida & Grossman, 2014; Cruz et al., 2022). Even FA levels can reflect a strong exposure to parasitism on a long-term basis (see a study on

common carp and goldfish *Carassius auratus* in Almeida et al. [2008]). Partial support was found for the second hypothesis of this study, as fishes were more asymmetric in the Littoral habitat, i.e. more impacted sites (Lutterschmidt, Martin & Schaefer, 2016). However, FA of pectoral fin length did not reach a significant difference between habitat types for pipefish. The variation pattern was in accordance with expectations for FA of eye diameter (i.e. higher values in the Littoral habitat) in both fish species. This could be related to the selected morphological trait, according to its functional importance. Thus, both species are highly dependent on the use of sight for catching prey and avoiding predators (Doadrio et al., 2011). Eye development implies a tight control for fishes, in terms of endocrine and genetic regulation (Richardson et al., 2017; Baumann et al., 2019). Such organ development may be more sensitive to environmental stress, for example the reduced water clarity owing to an increase of suspended matter (e.g. phytoplankton) under conditions of eutrophication (Evans, 2004; Taipale et al., 2018). Regarding pectoral fins, these appendages are surely more important for manoeuvrability in gobies, as they are proportionally larger (~20% fish total length) than in pipefish (<5%). In pipefish, the dorsal fin is the main means of locomotion, beating at high oscillatory frequencies (13–26 Hz; see Ashley-Ross, 2002). This was probably the reason why FA level of this bilateral character (i.e. pectoral fins) was significantly much higher in the Littoral habitat only for gobies.

Parasite taxa recovered from pipefish and gobies were similar to those found in previous research on the same (or phylogenetically close) fish species, although the richness was slightly lower (e.g. Malek, 2004; Ondračková et al., 2012). This finding (i.e. a lower richness) may be due to the particular hypersaline conditions within the Mar Menor lagoon. Elevated salinity commonly affects the intermediate hosts of plankton (e.g. copepods) or benthos (e.g. gastropods), with fish parasites displaying complex life-cycles being more disturbed (see an example for a pupfish–snail–trematode interaction with salinity in Rogowski & Stockwell [2006]). Thus, digeneans and cestodes with more than one host species were the specific parasite taxa with lower diversity, in comparison with previous studies (Malek, 2004; Ondračková et al., 2012). Focusing on habitat types, parasite prevalence was higher in the Littoral sites, although this disparity was only clearly detected in gobies. A similar pattern (i.e. higher values in the Littoral habitat) was also observed for parasite load, including both fish species. These results related to prevalence and parasite burden were also revealed by Chapman et al. (2015) in riverine habitats with elevated eutrophication. High concentrations of nitrates and phosphates can constitute a general physiological stressor in fish, particularly impacting the immune system (e.g. Yu et al., 2021). Additionally, summer hypoxia could also contribute to the reduction of water quality at particular sites within the Mar Menor coastal lagoon. As observed for other fishes by Poulin (1992) and Abdel-Tawwab et al. (2019), the toxicity of excessive nutrients and low oxygen levels affect the immune-competence of the study fish species, facilitating penetration and migration of parasites through host tissues (Khan, 2004; Inendino et al., 2005), the effect of which was also reflected in the HAI. Overall, these

environmental, physiological and parasitological mechanisms may act in a synergistic way, increasing the prevalence and load of diseases in the Littoral habitat, according to the third hypothesis.

As already mentioned, one of the main goals of this paper was to identify parasite communities as potential bio-indicators of habitat quality in the Mar Menor coastal lagoon. In particular, taxa that change their abundances or even disappear between contrasting habitats are especially relevant (Chapman et al., 2015). Also, species commonly found in a variety of fish hosts would make any assessment easier. Taking into account these features, there are promising parasite indicators in the genera *Trichodina* (Ciliophora), *Proteocephalus* (Cestoda) and *Gyrodactylus* (Monogenea) and the species *Cryptocotyle concavum* (Digenea), which were found in both target host fish species and were always more abundant in the Littoral habitat. Another feature that enhances their potential use as a bio-indicator species is their ease of identification with a standard microscope. Furthermore, these parasites are extremely different in their life-cycles and transmission pathways. *Trichodina* (single-cell organism) and *Gyrodactylus* (metazoan helminth) are monoxenous ecto-parasites with direct cycles, i.e. they are transferred from fish to fish. However, *Proteocephalus* and *Cryptocotyle concavum* (metazoan helminths) display heteroxenous cycles, involving copepods and snails as intermediate hosts, respectively. Moreover, *Cryptocotyle concavum* reproduces in aquatic birds (cormorants, gulls and herons), as definitive hosts, with encysted metacercaria larvae sheltering in fish muscle/skin. This parasite stage changes fish behaviour to make them more vulnerable to predation by piscivorous birds (e.g. swimming close to the surface; see Barber, 2003 for a comprehensive review). The parasitised organs and tissues are also diverse among these parasite taxa, with *Trichodina* being common on skin, *Gyrodactylus* on gills, *Proteocephalus* in the gut and *Cryptocotyle concavum* on external muscle tissue. Such a variety of parasites would also contribute to a more integrated assessment of fish health and subsequent ecosystem status (Marcogliese & Pietrock, 2011). In terms of effort, another more complex approach would be to use host-specific parasite taxa. Particularly for the pipefish and goby, the taxonomic Family Cyathocotylidae and the species *Bucephalus minimus* showed the highest abundances and greater differences between habitats. Both parasite types display a life-cycle involving three hosts: (1) intermediate molluscs (snail or bivalve with miracidium larvae); (2) fishes (pipefish or goby with metacercaria larvae); and (3) definitive hosts (piscivorous fishes or birds with adult parasites). Alternatively, the crustaceans *Ergasilus ponticus* (Copepoda) and *Mothocya epimerica* (Isopoda) may also have the potential to be appropriate bio-indicators for the study fish species, respectively, since their larger sizes would facilitate detection and counting (see an example for the copepod *Lamproglana clariae* in Pretorius & Avenant-Oldewage [2022]). Thus, these specific parasites (both digenean and crustacean) highlighted that fish living in sub-optimal areas, such as the Littoral zone, displayed higher rates of infestation when compared with those residing at locations with more suitable environmental conditions, i.e. the Barrier habitat.

Regarding the H' and LCI results (i.e. higher values in the Littoral habitat), parasite diversity and life-cycle complexity increased in more eutrophicated sites for both fish species, although this was statistically significant only in gobies, which was contrary to the third hypothesis of this study. Chapman et al. (2015) showed a negative association between parasite diversity and eutrophication. These authors found a specific mean value of phosphate concentration = $82 \mu\text{g L}^{-1}$ for the most eutrophicated study creek (parasite $H' = 1.49$) and $54 \mu\text{g L}^{-1}$ for the best preserved creek (parasite $H' = 2.03$). To put this in context, the Mar Menor coastal lagoon was an oligotrophic water body during the 1980s, with a mean concentration of phosphate less than $160 \mu\text{g L}^{-1}$, but nowadays the mean phosphate concentration is almost $1,000 \mu\text{g L}^{-1}$ (usually higher in the Littoral habitat) (Pérez-Ruzafa et al., 2020b). Mechanistically, Chapman et al. (2015) and also Reshu et al. (2022) suggested that the increase in parasite abundance is not even across the parasite species, with certain taxa being more favoured at the impacted sites (e.g. see Shah et al., 2013 for an example of the digeneans *Posthodiplostomum* spp. because of a particular increase in its intermediate host). Therefore, the present results were puzzling according to the expectations, as either no differences were observed (pipefish) or parasite communities were more diverse and complex (gobies) in the Littoral habitat. At these sites, such findings may be reflecting a higher habitat heterogeneity, including extensive patches of terrestrial-bank vegetation (e.g. *Arundo donax*, *Juniperus phoenicea*, *Pinus halepensis*, *Tamarix boveana*; see Azuara et al., 2020). Thus, the ecosystem complexity could provide more refuges for a variety of piscivorous birds (e.g. Ardeidae species, such as grey heron *Ardea cinera*, cattle egret *Bubulcus ibis* or little egret *Egretta garzetta*; see Farinós-Celdrán et al., 2017) in the Littoral habitat, which are not directly affected by poor water quality from eutrophication, and with many of them being definitive hosts for fish parasites. Thus, the associated increase in host variety, apart from fishes, would contribute to a higher parasite diversity and complexity of life-cycles. The Barrier habitat is more homogeneous, with large expanses of sandy soils and typical coastal vegetation of grasses and bushes (e.g. *Asparagus macrorrhizus*, *Calystegia soldanella*, *Pancratium maritimum* or *Salicornia europaea*; see Azuara et al., 2020) adapted to those harsh conditions (salt-tolerant and xerophytic plants), which are not suitable to sustain populations of larger bird species (Farinós-Celdrán et al., 2017). With respect to fishes, the patchy distribution of seagrass species (e.g. *Cymodocea nodosa* and *R. cirrhosa*) in the Littoral area (D. Almeida, pers. observ.) may provide diverse refugia for benthos, including intermediate hosts for goby parasites, such as snails. However, zooplankton (as an intermediate host) may be a more homogeneous food supply for pipefish (see explanation above on the lower diversity of zooplankton under eutrophication conditions). This may be the reason why statistical differences were not found for parasite H' and LCI in pipefish. According to these results, the use of these two descriptors means a certain degree of confusion, since they do not depend solely on environmental 'health', but also on ecological complexity.

In conclusion, this study demonstrated good potential for certain fish morphological (e.g. HAI, FA level) and parasitological (e.g. TL)

traits to be considered as reliable ecological indicators of conservation status within the Mar Menor coastal lagoon. The present results underpin the use of individual level metrics, such as fish condition and parasite burden, for the evaluation of habitat quality and overall ecosystem health (Cooke & Suski, 2008; Marcogliese & Pietrock, 2011). To provide more accurate data for environmental assessment and to overcome potential limitations of this study, future research should focus on different fishes displaying a wider range of ecological traits (e.g. large-bodied, predatory or euryhaline migrating species). Also, parasite taxa especially sensitive to eutrophication should be identified to measure response patterns, such as community composition, after changes in environmental conditions (see an example on mixozoan species related to eutrophication in Marcogliese & Cone 2021). In terms of knowledge transfer to other areas, a good 'starting point' would be lagoons under eutrophication pressure across the Mediterranean Basin, where the same (or closely related) fish target species can also be found (i.e. pipefish and gobies). Indeed, some examples of fish assemblages have already been used as ecological indicators to assess the lagoon conservation status. In particular, Cavraro et al. (2019), Franco, Torricelli & Franzoi (2009) or Sapounidis & Koutrakis (2021) analysed a variety of fish metrics for a wide range of Mediterranean lagoons. Thus, FA measures and parasite examinations may be applied to those study areas to complement their existing 'fish' data. If specific ecological, physiological or parasitological patterns are revealed and they are common among Mediterranean lagoons, this overall information would be useful to establish a community baseline and focal species for the design of particular indices applied to coastal lagoons at a broader scale. In fact, geographically closer Temperate regions should be targeted for such applications (e.g. see Repečka, 2003 for a study in the Curonian lagoon from the Baltic Sea). Subsequently, such methodologies could be transferred to 'fill' the important knowledge gap on fish-based indices for lagoons located in Tropical regions, as was highlighted by Souza & Vianna (2020) after a comprehensive review (>70 years, 324 publications). By adding information from fish morphology and parasites as new metrics, more integrated indices could help environmental managers and policy makers to design effective monitoring programmes that promptly detect impacts within valuable areas for conservation under strong socio-economic pressures, such as agricultural exploitation around coastal lagoons (Cooke & Suski, 2008; Kennish & Paerl, 2010).

AUTHOR CONTRIBUTIONS

David Almeida: Conceptualization and methodology. **Alejandra Cruz:** Methodology. **Carla Llinares:** Methodology. **Mar Torralva:** Methodology and conceptualization. **Esther Lantero:** Methodology. **David H. Fletcher:** Writing—review and editing. **Francisco J. Oliva-Paterna:** Conceptualization; methodology and writing—review and editing.

David Almeida, Mar Torralva and Francisco J. Oliva-Paterna contributed to the study design. David Almeida, Mar Torralva and Francisco J. Oliva-Paterna carried out the field sampling. David Almeida, Carla Llinares, Alejandra Cruz, Mar Torralva and

Esther Lantero carried out the laboratory work. David Almeida, Carla Llinares, Alejandra Cruz, Esther Lantero and David H. Fletcher performed the statistical analyses. Mar Torralva and Francisco J. Oliva-Paterna were responsible for the acquisition of funds. David Almeida and David H. Fletcher led the writing of the manuscript, and Mar Torralva and Francisco J. Oliva-Paterna contributed substantially to revisions of the manuscript. All authors gave final approval for submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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