Mediterranean Marine Science

Vol 25, No 1 (2024)

VOL 25, No 1 (2024)

The black goby Gobius niger Linnaeus, 1758 in the Marchica Lagoon (Alboran Sea, Morocco): spatio-temporal distribution, its environmental drivers, and the site-related footprint

AMAL LAMKHALKHAL, MOHAMED SELFATI, IMANE RAHMOUNI, NASSIR KADDOURI, BOUABID BADAOUI, ANTOINE PARISELLE, ABDELAZIZ BENHOUSSA, MARCELO KOVAČIĆ, NIKOL KMENTOVÁ, MAARTEN P.M. VANHOVE, HOCEIN BAZAIRI

doi: 10.12681/mms.33849

To cite this article:

LAMKHALKHAL, A., SELFATI, M., RAHMOUNI, I., KADDOURI, N., BADAOUI, B., PARISELLE, A., BENHOUSSA, A., KOVAČIĆ, M., KMENTOVÁ, N., VANHOVE, M. P., & BAZAIRI, H. (2024). The black goby Gobius niger Linnaeus, 1758 in the Marchica Lagoon (Alboran Sea, Morocco): spatio-temporal distribution, its environmental drivers, and the site-related footprint. *Mediterranean Marine Science*, *25*(1), 28–43. https://doi.org/10.12681/mms.33849

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
www.hcmr.gr
DOI: http://doi.org/10.12681/mms.33849

The black goby *Gobius niger* Linnaeus, 1758 in the Marchica Lagoon (Alboran Sea, Morocco): spatio-temporal distribution, its environmental drivers, and the site-related footprint

Amal LAMKHALKHAL[1], Mohamed SELFATI[2], Imane RAHMOUNI[1], Nassir KADDOURI[1], Bouabid BADAOUI[1], Antoine PARISELLE[1,3], Abdelaziz BENHOUSSA[1], Marcelo KOVAČIĆ[4], Nikol KMENTOVÁ[5], Maarten P.M. VANHOVE[5] and Hocein BAZAIRI[1,6]

[1] Mohammed V University in Rabat, Faculty of Sciences, Laboratory of Biodiversity, Ecology and Genome, 4 Avenue Ibn Battouta, B.P. 1014 RP, Rabat, Morocco
[2] Laboratoire Santé Et Environnement, Faculté des Sciences Aïn Chock, Hassan II University of Casablanca, B.P 5366 Maarif, 20100 Casablanca, Morocco
[3] ISEM, CNRS, Université de Montpellier, IRD, Montpellier, France
[4] Natural History Museum Rijeka, Lorenzov Prolaz 1, 51000 Rijeka, Croatia
[5] Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity and Toxicology, Agoralaan Gebouw D - B-3590 Diepenbeek, Belgium
[6] Natural Sciences and Environment Research Hub, University of Gibraltar, Europa Point Campus, Gibraltar

Corresponding author: Hocein Bazairi; hocein.bazairi@fsr.um5.ac.ma

Contributing Editor: Paraskevi K. KARACHLE

Received: 09 March 2023; Accepted: 18 December 2023; Published online: 07 February 2024

Abstract

Gobies (Gobiidae) are well represented in the Marchica Lagoon on the Moroccan Mediterranean coast, both in terms of species richness and in terms of abundance, with the black goby, *Gobius niger* Linnaeus, 1758, being the dominant species. The present study aims to examine (1) the spatial and temporal distribution of *G. niger* and its environmental drivers in the lagoon and (2) the potential lagoon-related footprint using morphometric, genetic, and parasitological proxies. Systematic monthly sampling covering the whole lagoon basin performed between October 2015 and September 2016 revealed a year-long presence of *G. niger* throughout the lagoon with significantly low densities in winter. The highest abundances were recorded in the shallow bottoms of the lagoon's inner margins on a variety of substrates (mud, muddy-sand, sandy-mud, and fine sand) mostly covered by macroalgae and/or seagrass meadows. Depth was revealed to be the most important predictor of the distribution of the black goby in the lagoon. It should, however, be regarded as a variable that acts synergistically with other factors, such as temperature, vegetation cover, and the sediment's mean grain size. Comparison of black goby populations from the lagoon with their conspecifics from the adjacent Mediterranean coast of Morocco revealed that specimens caught at sea are larger than those of the lagoon population. Of the 180 gobies investigated, not a single one hosted the parasites we targeted in the parasitological approach, monogenean flatworms. The absence of population structuring, low genetic diversity, and the presence of common haplotypes indicate no apparent restriction in the gene flow between the two populations. Therefore, the observed morphometric differences seem to be due to external environmental conditions rather than genetic differences.

Keywords: Transitional waters; Mediterranean Sea; abundance; Gobiidae; morphometry; parasites; genetics.

Introduction

Coastal lagoons are aquatic ecosystems at the terrestrial and marine interface, occupying approximately 13% of the world's coastline (Kjerfve, 1994). Due to the multiple ecosystem services they provide (Levin *et al.*, 2001) (e.g. shoreline protection, fishery resources, and nursery area), lagoons are considered one of the most valuable coastal habitats on the planet (Pérez-Ruzafa *et al.*, 2019). However, combined natural and man-made stressors make them among the most heavily exploited and threatened natural systems worldwide (Eisenreich, 2005; Newton *et al.*, 2018).

Fish play a fundamental role in ecological processes, through trophic relationships with other biotic components (Stein *et al.*, 1995; Vanni, 2002), and are essential for the functioning and resilience of lagoon ecosystems (Koutrakis *et al.*, 2005; Franco *et al.*, 2006; Aliaume *et al.*, 2007). They are highly valuable for the local human population as a food supply and for providing livelihood

(Holmlund & Hammer, 1999; Lopes & Videira, 2013; Newton *et al.*, 2014). Moreover, fish are relevant biotic indicators of the biodiversity and ecological status of ecosystems (Whitfield & Elliott, 2002; Breine *et al.*, 2010). Therefore, reliable scientific data on the fish fauna and abiotic components of lagoon ecosystems are of particular importance for effective management (Vasconcelos & Galyean, 2007), ensuring the sustainability of ecosystem functions and services.

With 2,949 currently recognized species, Gobiidae is the most species-rich family of fishes (Fricke *et al.*, 2023; Renoult *et al.*, 2022). Gobies are found in both marine and freshwater environments (Renoult *et al.*, 2022). They show the highest species richness in warm temperate and tropical seas, on the continental shelf, mostly in the shallow part. Typically, gobies are small and have short lifespans, with the majority residing within the substrate or concealed within various hidden spaces. Among them are epibenthic, hyperbenthic, and cryptobenthic species, while some gobiid species are nektonic (Kovačić & Patzner, 2011). Gobies have a crucial trophic function by linking benthic invertebrates to larger predatory fish (Casabianca & Kiener, 1969; Miller, 1988; Raffaelli *et al.*, 1989; Tiralongo *et al*., 2021). Despite their low commercial value, gobies play an important role as food resources for many commercially important species.

In the Mediterranean basin, 78 species of Gobiidae are currently known to occur (Kovačić *et al.*, 2022; Mavruk *et al.*, 2022). In the Mediterranean, 249 fish species were listed as inhabiting estuaries and/or lagoons, including both resident fishes (euryhaline species, which spend their entire life cycle inside lagoons and estuaries) and migratory fishes (species that, after spending some time in lagoons, are obliged to return to their marine or river environment to complete their life cycle) (Kara & Quignard, 2019). Gobiidae (at least 11 species) constitutes the most represented family of resident fish in Mediterranean lagoons (Kara & Quignard, 2019).

The Marchica Lagoon (35.16° / -2.85°), situated on the Moroccan Mediterranean coast, is known to host a diverse fish fauna supporting important fishing activities (Selfati, 2020). Since the first inventory in 1911 (Oden, 1914), Gobiidae has been found to be well represented in the Marchica Lagoon, with the black goby, *Gobius niger* Linnaeus, 1758, as the dominant species in the fish fauna (Selfati *et al.*, 2020). However, very little information exists on the ecological characteristics of this species, in particular its spatio-temporal structure in relation to environmental factors.

Resident animals, such as some gobies, are the most suitable ones to be used as indicators of lagoon environmental conditions (Bortone *et al.*, 2005). Their presence and abundance may provide important indications of the status of coastal lagoon habitats (Facca *et al.*, 2020). Assuming that the black goby *G. niger,* the most common species of goby in the Marchica Lagoon, is a resident fish in coastal lagoons (Franco *et al.*, 2008a, 2008b, 2012; Kara & Quignard, 2019; Selfati *et al.*, 2019), our study aims (1) to analyse the spatial and temporal distribution of *G. niger* and its environmental drivers using systematic sampling covering the whole lagoon basin and (2) to examine the potential lagoon-related footprint, i.e. whether the supposed resident population of the black goby in the Marchica Lagoon differs from black gobies from the adjacent Mediterranean coast of Morocco by considering morphometric, genetic, and parasitological proxies.

Materials and Methods

Study area

The Marchica Lagoon (Fig. 1) (35.16° / -2.85°), also called the lagoon of Nador, is one of the largest coast-

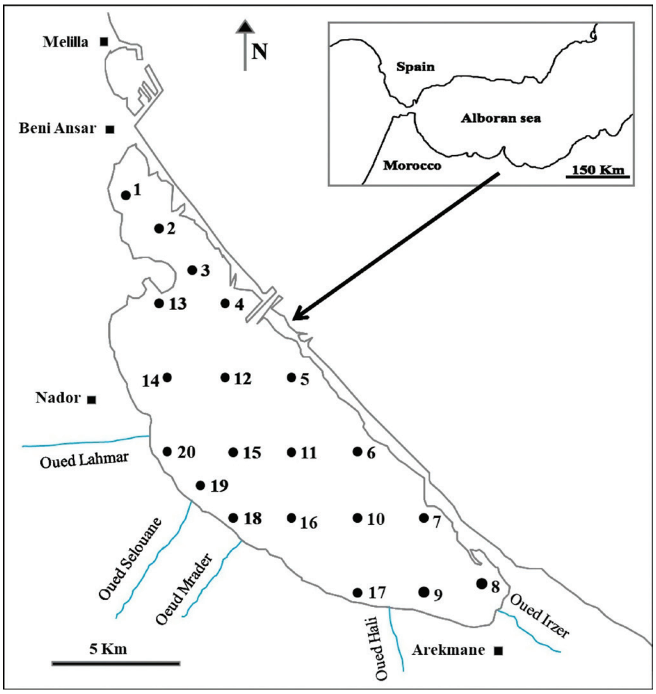

Fig. 1: Map showing the geographical localization of the Marchica Lagoon and the sampling stations of *Gobius niger*.

al lagoons in the Mediterranean (115 km², 25 km long, and 7.5 km wide) and the only one on the Mediterranean coast of Morocco (Selfati, 2020). The maximum depth is approximately 8 m, and the lagoon is separated from the sea by a 25-km-long sandbar (Lido), with one artificial opening (300 m wide and 6 m deep) that allows water exchange. Despite its ecological value (Site of Biological and Ecological Interest since 1996; RAMSAR site since 2005) and socio-economic value (mainly artisanal fisheries), the lagoon is under pressure from a complex mixture of human-mediated stressors (urbanization, pollution, overfishing, tourism, and wastewater, among others) (Selfati *et al.*, 2017; El Kamcha *et al.*, 2020).

Sampling design and environmental data

To examine the spatial and temporal variations in species abundance of *G. niger* and its environmental drivers in the Marchica Lagoon, data on monthly abundances were obtained by monitoring the fish fauna in the lagoon between October 2015 and September 2016. The monitoring was carried out according to an optimized network of 20 stations (S1–S20) covering the whole lagoon (Fig. 1). The collecting gear was a purse seine of about 110 m in length and 11 m in height, with a mesh size of 6 mm. The catches were expressed per unit area. The seine described a circle on the surface of the water with an estimated radius of 16 m, so the encircled surface was 800 m², which was regarded as the unit area (UA). Environmental parameters were measured at each station. Water temperature (T), salinity (S), and conductivity (Cond, mS/cm) were measured *in situ* using a conductivity meter (Cond 315i/SET), and depth (D) was recorded using an LCD Digital Sounder (HONDEX PS-7). The water pH was measured in the laboratory from water samples collected in the field using a pH meter (IONOMETER-EUTECHINSTRUMENTS-CYBERSCAN-PH-510). The measurement of suspended matter (SM, mg/l) in water samples was performed in the laboratory by the 0.45-μm membrane filtration method. The percentage of vegetation cover (VC: combined seagrass and macroalgae) and the nature of the substratum expressed as mean grain size (MGS, μm) were derived from the literature (Najih *et al.*, 2016, 2017). Data were obtained by overlapping the fish sampling points on the vegetation cover map and on the distribution map of surface sediment facies.

To examine the potential lagoon-related footprint, i.e. whether the supposed resident population of the black goby in the Marchica Lagoon differs from black gobies from the adjacent Mediterranean coast of Morocco considering morphometric, genetic, and parasitological proxies, 120 black gobies from the Marchica Lagoon and 60 black gobies from the adjacent Mediterranean coast of Morocco were collected as bycatch of fishing boats in July 2020. All the specimens were stored in separate plastic bags, transported in a portable freezer (Engel MT45), and then stored in the laboratory in a freezer (-20 °C) for further study. After thawing, the fish were numbered, labelled, and photographed. A piece of the pectoral fin of each fish was taken and kept in an Eppendorf tube filled with 96% ethanol for molecular characterization.

Species identification

Many ichthyologists agree that species-level identification of members of Gobiidae is challenging (Kovačić, 2008). Given the small size and the very high species richness in Gobiidae, we have applied two protocols to identify our species in the present work. Firstly, we have used a simpler determination inspired by the determination protocols provided by Brownell & Collignon (1978), Bauchot & Pras (1980), and Bauchot (1987) to determine the genus, and the species was identified by a minimum combination of characters that positively identify the specimens of *G. niger* among species of Gobiidae in the Mediterranean (Kovačić, 2020): (1) suborbital sensory papillae of the head lateral line system without suborbital row *a*, (2) all three head canals of the head lateral line system present, (3) anterior dorsal row *g* of sensory papillae ending behind or on the lateral end of row *o*, (4) six suborbital transverse rows *c* of sensory papillae, (5) anterior oculoscapular head canal with pore *α* at the rear of the orbit, (6) oculoscapular row x^1 of sensory papillae ending forward behind pore *β*, (7) longitudinal scale count < 50, (8) predorsal area scaled, and (9) suborbital row *d* of sensory papillae continuous.

Spatial and temporal distribution of G. niger *in the Marchica Lagoon and environmental drivers*

Spatial and temporal variations in abundance of *G. niger* (expressed as densities per 800 m²) were illustrated on maps using 11 classes of abundance based on the Sturges rule (Sturges, 1926). The spatial pattern of abundances (expressed as abundance per month and per station) of *G. niger* in the Marchica Lagoon was explored to identify affinity groups of stations. To achieve this, a hierarchical cluster analysis associated with a similarity profile test (SIMPROF) was used. The analysis was conducted on a transformed fourth-root abundance similarity matrix, aiming to mitigate the impact of highly abundant occurrences on the final results (Clarke, 1993). The similarity matrix was based on the Bray–Curtis similarity measure, which is robust in cases of concurrent absences (Field *et al.*, 1982). Differences between sampling stations and between seasons (winter: December, January, and February; spring: March, April, and May; summer: June, July, and August; autumn: September, October, and November) were tested with a two-way crossed PERMANOVA design, followed by post hoc pairwise tests.

Distance-based linear modelling (DISTLM) was performed to identify the key environmental drivers of the black goby's distribution pattern in the Marchica Lagoon. The best overall model was selected using the BEST selection procedure with the Akaike information criterion (AIC) in order to reveal the significant variables influencing the observed patterns in spatial abundance (Akaike, 1973; Anderson *et al.*, 2008). A distance-based redundancy analysis (dbRDA; Legendre & Anderson, 1999; McArdle & Anderson, 2001) was used to illustrate the results graphically. The draftsman plots, and the associated correlation matrix between all pairs of variables, were examined for evidence of collinearity (Clarke *et al.*, 2014). All the multivariate analyses were performed using the PRIMER 6 software (Clarke & Gorley, 2006).

Characterization and comparison of goby populations from the Marchica Lagoon and adjacent Mediterranean Sea

Morphometric and meristic analysis

Based on the literature (Gaamour *et al*., 2001), six morphometric characters were measured on each specimen with a 1-mm resolution using a Vernier calliper: total length (TL), standard length (SL), head length (HL), snout length (SnL), body height (BH), and eye diameter (ED). Moreover, five meristic characters were considered: number of rays in the first dorsal fin (DF1), number of rays in the second dorsal fin (DF2), number of rays in the anal fin (AF), number of rays in the pectoral fin (PF), and number of rays in the ventral fin (VF) (Gaamour *et al*., 2001) (Fig. 2).

In order to characterize specimens of *G. niger* collected from Marchica Lagoon and the Mediterranean Sea and to describe the relative variations in the different parts of their body, the length–length relationship was estimated using the following expression: $\mathrm{Log}\ y = a\ \mathrm{Log}\ x + \mathrm{Log}\ b$, between the standard length (SL) and the total length (TL), the head length (HL), and the body height (BH), as well as between the head length (HL) and the eye diameter (ED) and the snout length (SnL). All the parameters of the length–length relationship were estimated by linear regression analysis using STATISTICA software.

Statistical analyses (Student's t-test) were performed to detect differences in terms of size between specimens from two locations (Marchica Lagoon and adjacent sea). In order to explore whether there is a clustering based on the origin of *G. niger*, a principal component analysis (PCA) was performed using morphometric and meristic characters, after a transformation ($\mathrm{Log}_{10}X + 1$) of the raw measurements in order to linearize the allometries (Huxley, 1932) and to roughly equalize the variances (Jolicoeur, 1963). All morphometric and meristic analyses were performed in the PAST v4.03 software (Hammer *et al*., 2001).

Genetic differentiation

DNA extraction of 120 specimens (60 from the Marchica Lagoon and 60 from the adjacent Mediterranean coast) was performed according to the protocol of Aljanabi & Martinez (1997). Approximately 50 µg of a pectoral fin fragment was digested at 55 °C overnight with 20 µl of proteinase *K* (20 mg/ml) and 180 µl of extraction buffer (0.4 M NaCl, 1 M Tris, 2 mM EDTA, and 40 µl of 20% SDS). The extracted DNA was suspended in 150 µl of sterile double-distilled water and stored at -20 °C until amplification by PCR. We targeted a fragment of the 16S rRNA gene, since mitochondrial ribosomal sequences are well represented in the genetic literature on Mediterranean and European gobies. As they capture interspecific and intraspecific diversity in these fishes, they can be considered potential barcoding markers (Vanhove *et al.,* 2012, 2016, 2022, and references therein). Amplification of the 16S rRNA gene was performed in a final volume of 20 µl, containing 4 µl buffer (10X Standard *Taq* Reaction Buffer), 1 µl of 10 mM dNTPs, 0.8 µl of 10 µM forward primer 16SH (5'-CGCCTGTTTATCAAAAACAT-3'), 0.8 µl of 10 µM reverse primer 16SL 5'-CGCCTGTTTATCAAAAACAT-3') (Palumbi *et al.*, 1991), 0.4 µl (2 units) of *Taq* polymerase, 1 µl of genomic DNA, and 12 µl of nuclease-free water. Amplification reactions were performed in a thermal gradient PCR according to the following program: initial denaturation at 94 °C for 3 min, followed by 40 cycles each with denaturation for 30 sec at 94 °C; a hybridization step for 30 sec at 55 °C and an elongation phase for 1 min at 72 °C; and, at the end, a final elongation phase for 10 min at 72 °C. The PCR products were checked on a 1% agarose gel and sent to the National Center for Scientific and Technical Re-

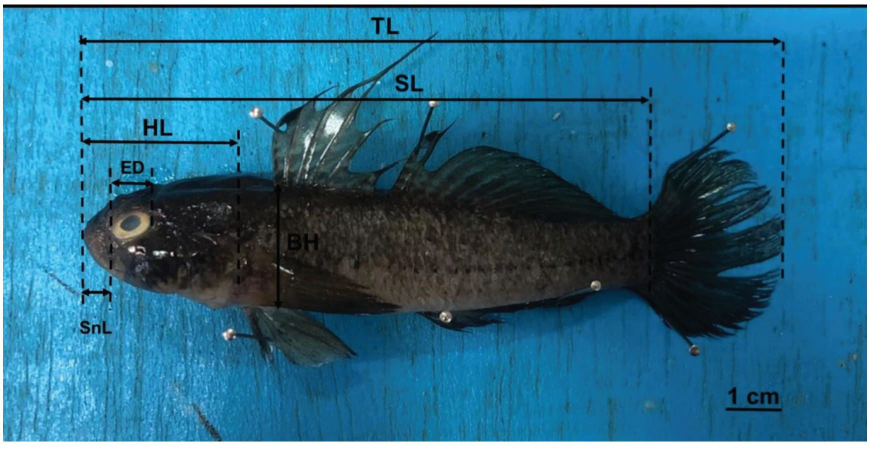

Fig. 2: Picture of *Gobius niger* from the Marchica Lagoon showing the main measurements taken: total length (TL), standard length (SL), head length (LT), snout length (SnL), body height (BH), and eye diameter (ED).

search (CNRST) in Rabat, where they were sequenced by a Genomix sequencer (MGX) using the same forward and reverse primers as for the PCR.

Each DNA sequence obtained in both directions was cleaned and checked in MEGA X (Kumar *et al.*, 2018) to assemble the corresponding consensus sequence. The obtained sequences were aligned with the CLUSTAL W algorithm (Thompson *et al.,* 1994), and then each sequence was blasted (Altschul *et al.*, 1990) with the 16S rDNA sequences of *G. niger* available in NCBI GenBank to check for possible matches.

To make sure that all of our 16S sequences, both the newly obtained Moroccan sequences and those we included from other studies, cluster monophyletically without representatives of other species, a phylogenetic tree was built including all other species of *Gobius* that are represented in the GenBank by the targeted fragment of the 16S rRNA gene (see Supplementary Material Table S1). The phylogenetic tree was constructed with MEGA X using the neighbour-joining algorithm and the Kimura 2-Parameter substitution model (Kimura, 1980) with 1000 bootstrap replicates.

All sequences were confirmed to belong to *G. niger* (Supplementary Material Figure S1) and hence were included in a maximum parsimony analysis in the software package FITCHI (Matschiner, 2016) in order to visualize the genealogical relationships across the geographical range of *G. niger*.

DNASP v6.12.03 (Rozas *et al.*, 2017) was used to calculate molecular diversity indices: number of segregating sites (K), number of haplotypes (H), haplotype diversity (h), and nucleotide diversity (π). Moreover, Fu and Li's F and Tajima's neutrality tests were performed to check for possible selection or change in population demography. The pairwise fixation index (F_{ST}) was calculated in ARLEQUIN v3.5 (Excoffier & Lischer, 2010) using 100 permutations to express the degree of genetic differentiation among sets of individuals.

A matrix correlation analysis (Mantel test; Mantel, 1967), permuting a morphological distance matrix against a genetic distance matrix, was performed using the R v3.5 software package ape (Paradis *et al.*, 2004), running 1000 permutations.

Parasitological screening

Each specimen was placed in plastic bags with tap water, and then the bags were vigorously shaken to detach the parasites from the skin. After that, the external surface of the skin, the fins, and the holding water were examined. Gill arches on the right side of each specimen were removed through ventral and dorsal sections, placed in a petri dish, and rinsed with a rinsing bottle filled with tap water. Then, the water and gill arches were examined under a stereoscope (Wild M8).

Results

Spatial and temporal distribution of **G. niger** *in the Marchica Lagoon and its environmental drivers*

Cluster analysis based on abundances of *Gobius niger* in the 20 sampled stations in the Marchica Lagoon, supported by a SIMPROF test, separated the samples into two large groups, labelled as G1 and G2, in addition to three atypical stations (S4, S5, and S12), located near the pass (Fig. 3). The G1 group, in which abundances were high (mean = 87.3 ± 166 ind/UA), concerns the north-west (S1 and S2) and south-east extremities of the lagoon (S7, S8, and S9), as well as the west, inner coast around Oued Selouane (S17, S18, S19, and S20). Stations S1 and S17, corresponding to the north-west and south-east extremities, stand out as a subgroup within G1. The G2 group corresponds roughly to the centre of the lagoon, where the goby was overall less abundant (mean = 10 ± 18.5 ind/UA).

The DISTLM marginal tests assessed the importance of each variable separately. They indicated that depth, temperature, and substratum had a significant effect ($p < 0.05$), explaining respectively 49.6%, 22.5%, and 21.3% of the spatial variations of *G. niger* in the Marchica Lagoon (Table 1). Salinity and vegetation cover were close to the limit of significance, and each explained about 13% of the total variance. The remaining variables, pH and suspended matter, did not show any significant impact, and each accounted for less than 1% of the variability. The best model was obtained by a combination of depth, salinity, and suspended matter, which accounted for 58.89% of the variability in the data. In the dbRDA performed on the selected model, the first two axes accounted for 57.9% of the total variability (dbRDA1 = 56.6%; dbRDA2 = 1.3%) (Fig. 4). Depth was the main contributor to the first axis (loading dbRDA = 0.91), while the main contributors to the second factor were salinity and suspended matter (loading dbRDA = 0.57 and -0.64, respectively). Furthermore, depth was negatively correlated with all parameters considered in the present study, with significance for temperature (Spearman's rho; $r = -0.65$, $p < 0.05$) and substrate (Spearman's rho; $r = -0.52$, $p > 0.05$).

The black goby was present in the Marchica Lagoon throughout the sampling year, both over space and time but with variable abundance (Fig. 5). Overall, the monthly abundance in all stations fluctuated between zero and a maximum of 643 individuals. The mean abundance (± SD) was 44.13 (± 88) individuals per station (Table S2). The PERMANOVA results showed significant differences in total abundance between seasons and stations (Table 2). Interactions between the two factors were not significant (Pseudo F = 1.072, Pperm > 0.05). A posteriori pairwise comparison revealed that the black goby was significantly less abundant in winter than in the other seasons (Table 3). Regarding the 'station' factor, most of the significant differences concern the combinations formed by the peripheral and central stations (Table S3).

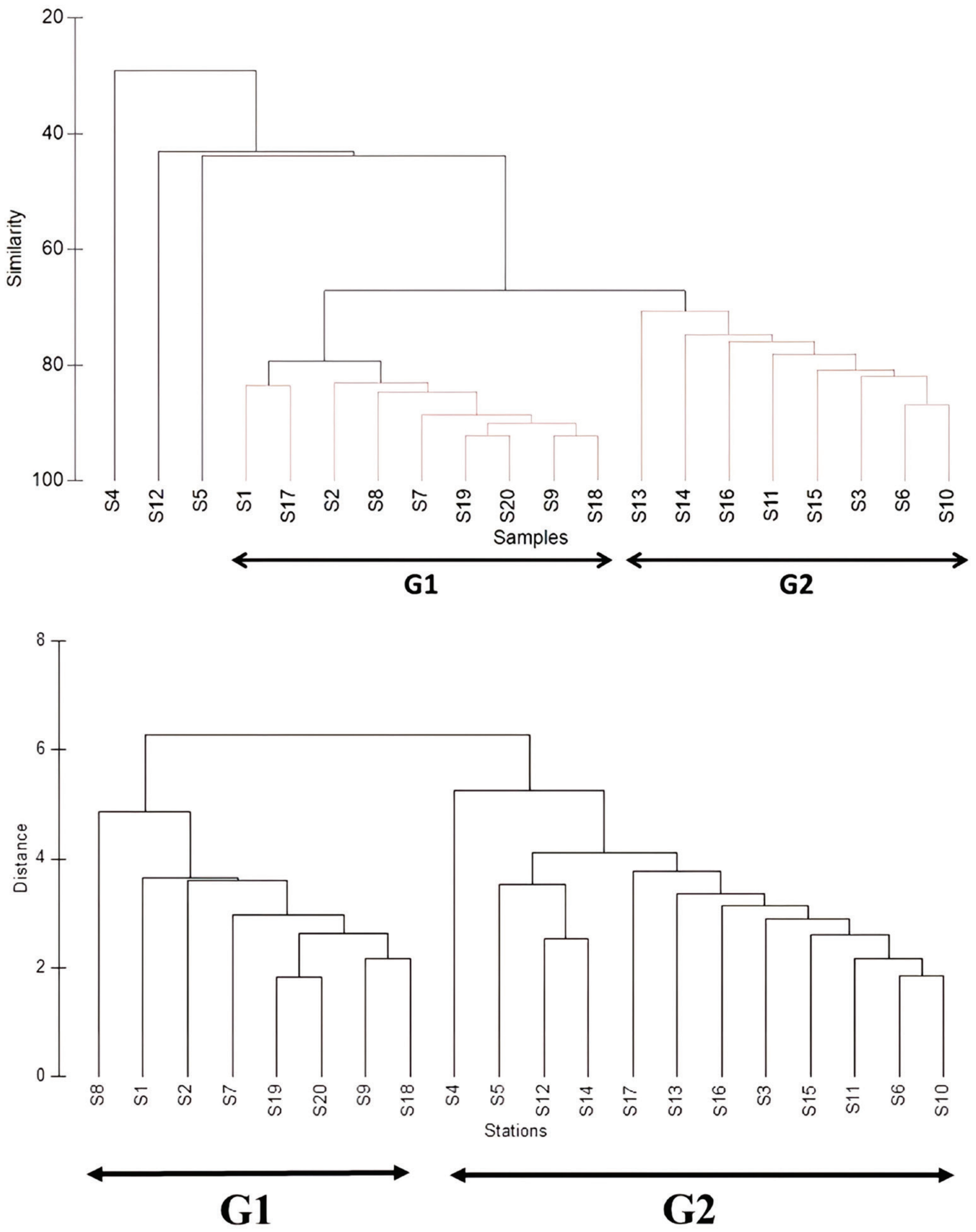

Fig. 3: Cluster analysis associated with the similarity profile test (SIMPROF), based on abundances of *Gobius niger*, reveals reciprocal relations among the 20 sampled stations in the Marchica Lagoon using the Bray–Curtis distance.

Table 1. DISTLM marginal test results: significance (p-value) of the relationship between abiotic factors and monthly abundances of *Gobius niger*. Prop. (%): relative contribution of each environmental variable to the variation in spatial structure.

Variable	SS (trace)	Pseudo-F	p-value	Prop. (%)
Temperature	63.356	5.2469	0.01*	0.2257
Depth	139.49	17.781	0.001*	0.49694
Salinity	38.621	2.8717	0.073	0.13759
pH	25.637	1.8092	0.138	9.13E-02
SM (suspended matter)	14	0.94485	0.348	4.99E-02
VC (vegetation cover)	36.035	2.651	0.072	0.12837
MGS (sediment mean grain size)	60.034	4.8969	0.012*	0.21387

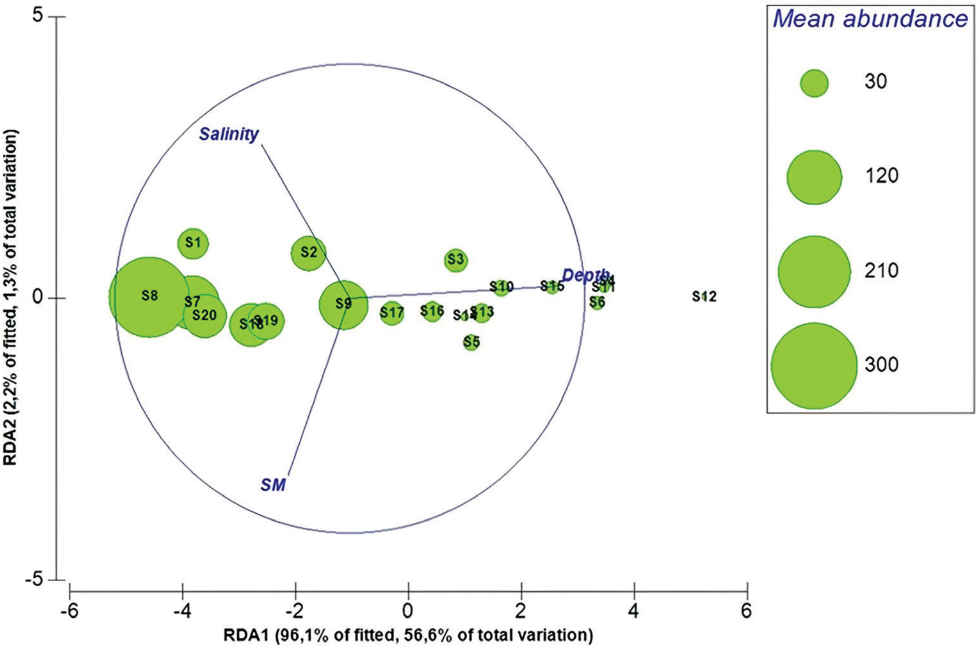

Fig. 4: Two-dimensional redundancy analysis (RDA) ordination representing the spatial distribution of *Gobius niger* related to the predictor variables selected through the best linear models based on distance (DISTLM). SM: suspended matter.

Characterization and comparison of goby populations from the Marchica Lagoon and adjacent Mediterranean Sea

Morphometric and meristic analysis

Data from meristic and morphometric characters of *G. niger* sampled at sea and in the Marchica Lagoon are reported in Tables S4 to S7, respectively. Overall, specimens caught in the sea (110.2 ± 11.2 mm) differ significantly ($t = -12.46$; $p < 0.05$) from the ones caught in the lagoon (84.8 ± 11.1 mm).

The length–length relationships ($\text{Log } Y = a \text{ Log}X + \text{Log}b$) between the standard length (SL) and the total length (TL), the head length (HL), and the body height (BH), as well as between the head length (HL) and the eye diameter (ED) and the snout length (SnL), are summarized in Tables S8 and S9. The results of the morphological analysis indicate significant differences between morphological characteristics (SL and TL, HL, BH; HL and ED, SnL) in the two sites.

The first PCA (PC1 vs. PC2) performed on the morphometric and meristic data allowed the identification of a distinct stratification based on the origin of the individuals. The first two axes are the most informative, representing respectively 78.67% and 9.75% of the total inertia, thus 88.42% of the total variation (Fig. 6). PC1 was strongly correlated with SL (Pearson correlation 0.962) (Fig. 7). PC2 and PC3 were not correlated with SL (Pearson correlation -0.022 and -0.004, respectively), indicating that among-group differences on this axis are independent of specimen size and are directly interpretable as differences in shape. The first axis was correlated with morphometric characteristics, while the second axis was correlated with meristic characteristics.

Genetic differentiation

From the 120 individuals of *G. niger* from Morocco, 88 sequences were obtained. The alignment of the portion of the 16S rRNA gene was 547 base pairs long and contained 26 polymorphic sites; a total of 31 haplotypes were found.

Diversity measures, calculated for the two groups of *G. niger* (Marchica Lagoon and the adjacent sea), are reported in Table 4.

The pairwise F_{ST} comparisons show a low value; the F_{ST} p-value is not significant. This implies that there is not a considerable degree of genetic differentiation between the different groups of *G. niger* from Marchica Lagoon and the adjacent Mediterranean coast of Morocco.

The maximum parsimony network shows three ancestral haplotypes (numbers 22, 29, and 41 in Fig. 8). There is no visible geographical segregation except for the sequences from Turkey and Greece whose haplotypes were not shared with the other individuals. The Western and Central Mediterranean and the Atlantic Ocean populations share haplotypes, while the Eastern Mediterranean population does not.

The result of the Mantel test indicates no correlation between the genetic and morphological distance matrices ($r = -0.11$; $p = 0.97$), suggesting no isolation by distance and no limitation of gene flow between the lagoon and the sea (Fig. 9).

Monogenean parasites from G. niger

Of the 180 specimens of *G. niger* examined, no fish was parasitized by monogenean flatworms, either in the sea or in the Marchica Lagoon.

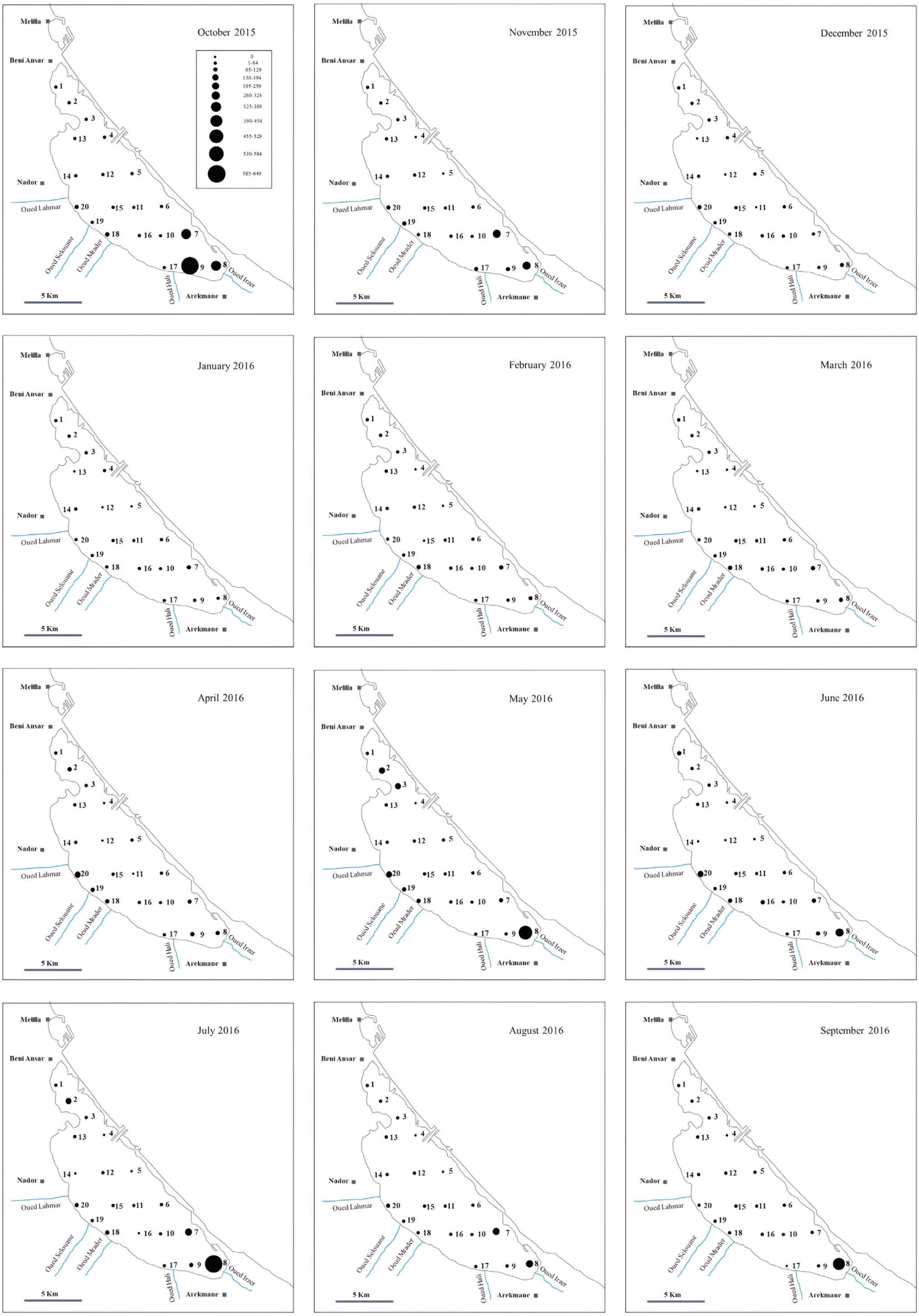

Fig. 5: Spatial and temporal distribution of *Gobius niger* in the Marchica Lagoon.

Table 2. Results of the multivariate permutational analysis (PERMANOVA) of *Gobius niger* considering season and station. Df: degrees of freedom; Pperm: level of significance.

	Sum sq	Df	Mean sq	F value	Pperm
Season	49908	3	16636	3.905	0.011*
Station	860873	19	45309	10.636	0.001*
Season : Station	260307	57	4567	1.072	0.302
Residuals	681627	160	4260		

Table 3. Seasonal mean abundance of *Gobius niger* with pairwise comparisons. The compact letter display 'a' and 'b' indicates significant mean abundance differences.

	Autumn	Summer	Winter	Spring
Abundance (mean ± SD)	57.1 ± 117^{a}	53.8 ± 102^{a}	20.3 ± 27.1^{b}	45.3 ± 75.1^{a}

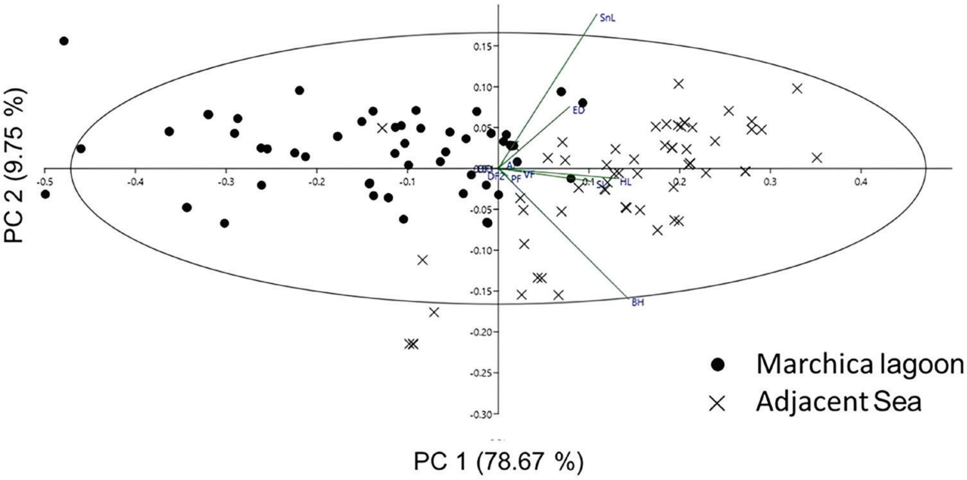

Fig. 6: PCA of the morphological variables of *Gobius niger* (standard length, SL; body height, BH; head length, HL; snout length, SnL; eye diameter, ED; first dorsal fin, DF1; second dorsal fin, DF2; anal fin, AF; pectoral fin, PF; ventral fin, VF) with projection of phenotypic groups. PC1 vs. PC2 and PC2 vs. PC3. The percentage of variation explained by each PC axis is given within parentheses.

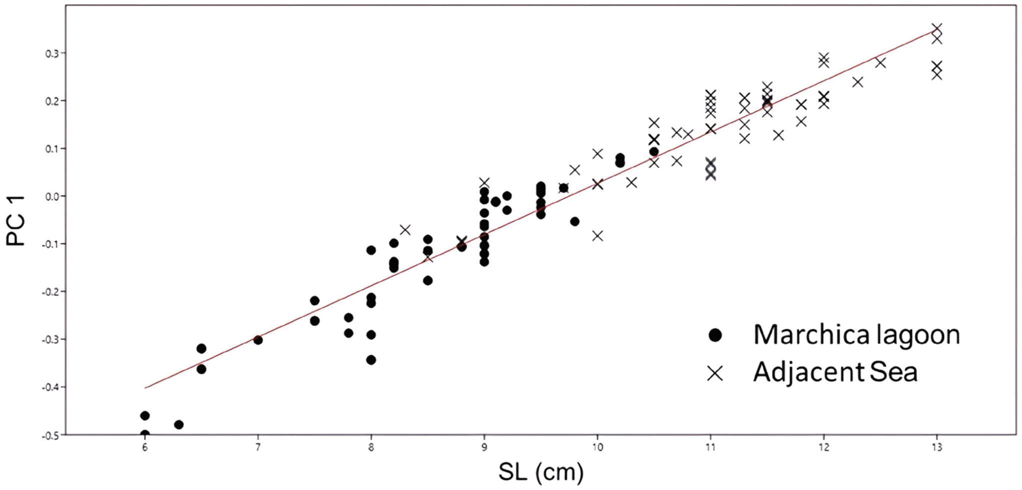

Fig. 7: Linear regression of the principal component score axis (PC1) from morphometric measurements on the log standard length of *Gobius niger* with projection of phenotypic groups.

Table 4. Results of the haplotype diversity analysis of the two groups of *Gobius niger*. N: number of sequences; S: number of segregating sites; Hn: number of haplotypes; Hd: haplotype diversity; π: nucleotide diversity. Significant values at α ≤ 0.05.

Groups	N	S	Hn	Hd	π	F_{ST}	p-value	Fu & Li's F	Tajima's D
Sea	50	20	21	0.85878	0.00413	0.01286	0.09180 ± 0.0088	-2.53253	-1.70437
Marchica Lagoon	38	16	17	0.93741	0.00462				

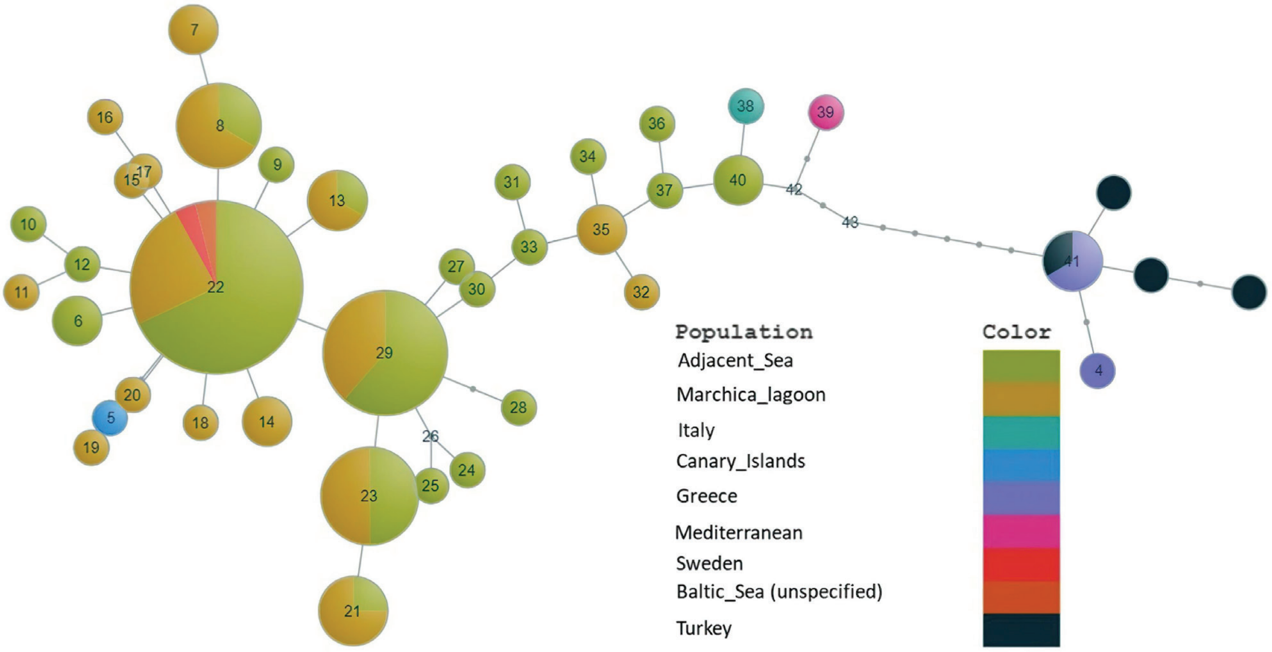

Fig. 8: Haplotype network constructed from 16S rDNA sequences of *Gobius niger*. The size of a particular circle reflects the haplotype frequency. The numbers indicate the nodes.

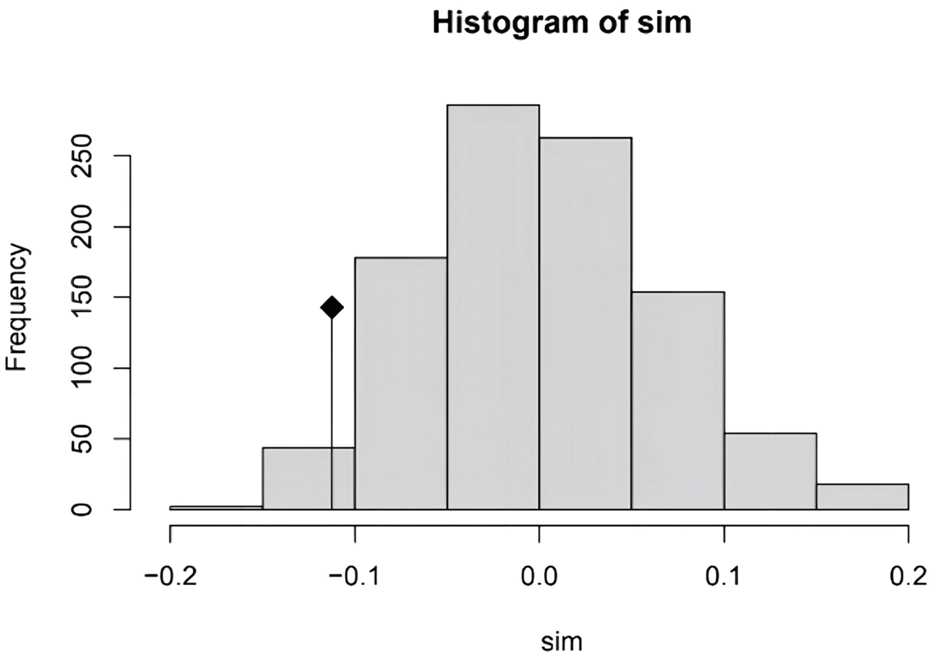

Fig. 9: Histogram of the Mantel test assessing the relationship between genetic and morphologic distance for *Gobius niger*. Sim: simulations; Frequency: frequency values of the correlation between the genetic and morphologic distances. The dot represents the original value of the correlation between the distance matrices.

Discussion

Spatial and temporal distribution of **G. niger** *in the Marchica Lagoon and environmental drivers*

The spatial and temporal distribution of *G. niger* evidenced that the species is permanently present in the Marchica Lagoon, with higher abundances in the peripheral shallow bottoms of the lagoon on a variety of substrates (mud, muddy-sand, sandy-mud, and fine sand) mostly covered by macroalgae and/or seagrass meadows (Najih *et al*., 2016, 2017). This is consistent with the ecology of the species in other Mediterranean coastal lagoons, where it prefers shallow habitats (depth < 1.5 m) on sandy and sandy-muddy vegetated beds (Kara & Quignard, 2019). Moreover, its permanent and abundant presence in the Marchica Lagoon supports its status as resident (Franco *et al*., 2008a, 2008b, 2012; Kara & Quignard, 2019; Selfati *et al*., 2019) and a very frequent fish in Mediterranean coastal lagoons (Kara & Quignard, 2019). *Gobius niger* plays a key eco-trophic role by providing a link between benthic invertebrates and large predators (Casabianca & Kiener, 1969; Miller, 1988; Raffaelli *et al*., 1989). Therefore, the shallow beds of the lagoon, where the species is abundant, are key habitats in the Marchica Lagoon and need to be considered in all management plans aiming at the conservation of biodiversity and ecological processes. Moreover, being a benthic and resident fish, *G. niger* is a suitable candidate for monitoring the ecological status of the Marchica Lagoon, especially in its most disturbed peripheral areas (Ben Hassine *et al*., 1999). Indeed, *G. niger* has been used in many pollution monitoring studies (Fossi *et al*., 1989; Katalay & Parlak, 2002; Migliarini *et al*., 2005; Maradonna *et al*., 2004; Barucca *et al*., 2006; Maradonna & Carnevali, 2007; Ramšak *et al*., 2007).

Overall, depth seems to be the most important predictor variable explaining the distribution of *G. niger* in the lagoon. This is in accordance with the distribution patterns of fish assemblages in the Marchica Lagoon, where a spatial gradient in the bentho-demersal component of the fish assemblage structure was observed, with more marine species occurring near the sea inlet and more resident species in the inner margins of the lagoon (Selfati *et al*., 2019). Moreover, depth is a variable that acts synergistically with other factors, such as vegetation cover, pH, temperature, dissolved oxygen, salinity, and suspended matter (Selfati *et al*., 2019). Furthermore, depth is negatively correlated with all parameters considered in the present study, with significance for temperature (Spearman's rho; $r = -0.65$, $p = 0.001$) and type of substrate (Spearman's rho; $r = -0.52$, $p = 0.01$). This indicates that the shallower areas of the lagoon present different conditions in terms of temperature, illumination, and other factors that support primary productivity. This is in line with the ecological requirements of the black goby, particularly in terms of reproduction, feeding, and shelter from predation. The black goby requires coarse substrates that act as shelters for nest construction (Vaas *et al.,* 1975; Wiederholm, 1987; Mistri *et al.,* 2000; Leatemia *et al.,* 2017). The vegetated habitats are important for their abundance of marine macroinvertebrates, which are the main prey resource for the black goby (Hajji *et al.,* 2013; Matern *et al.,* 2021). Vegetation cover also limits predation by increasing the complexity of the habitats (Heck & Orth, 1980; Kulczycki *et al.,* 1981). The significant decrease in *G. niger* during winter could be related to the low temperatures during this season, leading to migration of the species to deeper marine waters (Nash, 1984). Another explanation proposed by Arruda *et al*. (1993) in the Ria de Aveiro Lagoon (Portugal) is the massive mortality following the spawning period.

Characterization and comparison of goby populations from the Marchica Lagoon and adjacent Mediterranean Sea

Morphometric and meristic patterns

In the Moroccan Mediterranean, the black goby has previously been reported to occur both in the lagoon and in the open sea (Aloncle, 1961; Selfati *et al*., 2019). The work of Böhlke & Robins (1968), Hoese (1971, 1983), Bath (1973), Akihito (1986), Gill *et al.* (1992), and, more recently, Bouchereau *et al.* (2000) and Kovačić & Golani (2007) has shown that the standard length (SL) and head length (HL) are very useful in the classification of genera and species of Gobiidae. The phenotypic traits are under the influence of natural selection and the composite effects of environmental factors, such as temperature, depth strata preference, salinity, and trophic groups, in addition to genetic and ontogenetic factors (Bakhshalizadeh *et al*., 2022). The difference in size between individuals from the lagoon and their marine conspecifics is probably due to the depth difference between the two sites. Indeed, the majority of fishes show a size positively correlated with depth (Harvey & Stewart, 1991). Fish are exposed to the risk of predation; to reduce it, they choose deeper habitats and therefore will have a longer life span, which should be reflected to some extent in a larger body size (Harvey & Stewart, 1991). This difference in size can also be related to temperature. According to the gill oxygen limitation theory (GOLT), there is a negative correlation between the size of fish and the temperature of their habitat; smaller fish may have an advantage in warmer waters because they can extract more oxygen from the water per unit of body mass (Pauly, 2021).

Genetic structure: lagoon and open sea

The genetic structure of marine fish populations reflects the historical and contemporary interaction between a complex set of ecological, demographic, behavioral, genetic, oceanographic, climatic, and tectonic processes. The combined effect of these mechanisms, acting on a range of spatial and temporal scales, determines the rates and patterns of the dispersal of gametes, zygotes, larvae, and adults (Giovannotti *et al*., 2009).

Our study revealed the presence of common haplotypes shared by two groups from different habitats. According to Bortone *et al.* (2005), *G. niger* is considered a resident species in the Marchica Lagoon (Selfati, 2020). However, the results of our study suggest recurrent migrations between the groups of the sea and lagoon. Indeed, the haplotype network reconstruction revealed no apparent population subdivision and no geographical segregation between the lagoon and the sea. F_{ST} values are used to determine the level of gene flow (Chanthran *et al.*, 2020). According to Wright (1965), populations with F_{ST} values of 0–0.05 show a small differentiation, values of 0.05–0.15 a moderate differentiation, values of 0.15–0.25 an important differentiation, and values greater than 0.25 a very important differentiation. The occurrence of common haplotypes between the groups and a small to moderate differentiation based on the F_{ST} results suggests unrestricted gene flow with no relation to habitat.

Parasites of G. niger

Monogenean parasites are one of the largest groups of Platyhelminthes characterized by high species diversity and high host specificity (Gusev, 1995; Kearn, 1994; Poulin, 1998). The most abundant genus of monogeneans in gobies as well as in many other fishes is *Gyrodactylus* von Nordmann, 1832. According to Huyse & Volckaert (2005), species of *Gyrodactylus* parasitize on 19 orders of fresh and marine bony fishes. To date, three *Gyrodactylus* spp. parasitizing on *G. niger* have been recorded: *G. niger* sp. (*sensu* Huyse *et al.* 2003) from the North Sea, *Gyrodactylus flesi* Malmberg, 1957, and *G. proterorhini* Ergens, 1967 (Huyse *et al.*, 2003; Harris *et al.*, 2004).

The absence of monogenean parasites on the gills and the skin of *G. niger* from the Marchica Lagoon and sea may be related to environmental conditions. Another explanation for this absence is the immune system of fishes. Indeed, Zander *et al.* (1993) and Zander *et al.* (1999) showed that in the Baltic Sea, *Podocotyle atomon* (Rudolphi, 1802) is present in high abundances; however, in *G. niger*, this species was often absent, which was explained by its immune system efficiency.

Conclusion

Gobius niger was revealed to be present year-round in the Marchica Lagoon with higher abundances recorded in the shallow bottom of the lagoon's inner margins on a variety of mostly vegetated substrates and with a general decrease in abundances during winter. Depth is a key factor for understanding the spatial patterns of *G. niger* in the Marchica Lagoon, but it should be regarded as a variable that acts synergistically with other factors, such as vegetation cover, type of substrate, and temperature. Thus, the shallow beds of the lagoon, where the species is abundant, are key habitats in the Marchica Lagoon and need to be considered in all management plans aiming at the conservation of biodiversity and ecological processes. Comparison of black goby populations from the Marchica Lagoon with their conspecifics from the adjacent Mediterranean coast of Morocco revealed that specimens caught in the sea are significantly bigger than those from the lagoon. Moreover, the absence of population structuring and common haplotypes between the two populations indicates no apparent restriction in the gene flow between the two populations.

Acknowledgements

This research was supported by the Special Research Fund of Hasselt University (BOF23BL07 to A.L.; BOF21PD01 to N.K.; BOF20TT06 to M.P.M.V.; BOF21INCENT09) and by research grant 1513419N of the Research Foundation – Flanders (FWO-Vlaanderen). The sampling survey was undertaken in the framework of an international cooperation between Le Conservatoire du Littoral, Agence de l'Eau Rhône-Méditerranée-Corse, the Ecocean Society, University Mohammed V in Rabat, Institut National de Recherche Halieutique, Observatoire de la Marchica, and Fondation Mohammed VI pour la Protection de l'Environnement. M.S. thanks the Agence de l'Eau Rhône Méditerranée Corse and the Ecocean Society for their financial support. The authors are very grateful to fishers for providing *G. niger* samples from both the Marchica Lagoon and the adjacent Mediterranean coast of Morocco. Dr. Lukas Rüber (Natural History Museum of Bern, Switzerland) is thanked for curatorial services.

References

Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. p. 716-723. In: *2nd International Symposium of Information Theory*. Petrov, N., Csaki, F. (Eds). Akademia Kiado, Budapest.

Akihito, P., 1986. Some morphological characters considered to be important in gobiid phylogeny. p. 629-630. In: *Indo-Pacific Fish Biology*: *Proceedings of 2nd International Conference on Indo-Pacific Fishes*. Uyeno, T., Arai, R., Taniuchi, T., Matsuura, K. (Eds). Ichthyological Society of Japan, Tokyo.

Aliaume, C., Do Chi, T., Viaroli, P., Zaldívar, J.M., 2007. Coastal lagoons of Southern Europe: recent changes and future scenarios. *Transitional Waters Monographs*, 1 (1), 1-12.

Aljanabi, S.M., Martinez, I., 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25, 4692-4693.

Aloncle, H., 1961. La pêche dans la «Mar Chica» de Mellila. *Bulletin de l'Institut des Pêches Maritimes du Maroc*, 7, 13-32.

Altschul, S.F., Gish,W., Miller,W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403-410.

Anderson, M.J., Gorley, R.N., Clarke, K. R., 2008. *PERMANOVA+ for PRIMER. Guide to software and statistical methods*. Primer-E: Plymouth, UK.

Arruda, L. M., Azevedo, J., Neto, A. I., 1993. Abundance, age-structure and growth and reproduction of gobies (Pisces, Gobiidae) in the Ria de Aveiro Lagoon (Portugal). *Estuarine, Coastal and Shelf Science*, 37, 509-523.

Bakhshalizadeh, S., Abbasi, K., Rostamzadeh, L.A., Bani, A., Pavithran, A. *et al*., 2022. Morphometric analyses of phenotypic plasticity in habitat use in two Caspian Sea mullets. *Journal Marine Science Engineering*, 10, 1398.

Barucca, M., Canapa, A., Olmo, E., Regoli, F., 2006. Analysis of vitellogenin gene induction as a valuable biomarker of estrogenic exposure in various Mediterranean fish species. *Environmental Research*, 101, 68-73.

Bath, H., 1973. Wiederbeschreibung der Grundelart *Gobius macrocephalus* Kolombatovic aus dem Mittelmeer und Aufstellung einer Gattung *Millerigobius* (Teleostei: Gobioidea: Gobiidae). *Senckenberg. Biologica*, 54, 303-310.

Bauchot, M.L., Pras, A., 1980. *Guide des poissons marins d'Europe*, *Les guides du naturaliste*. Delachaux & Niestle (Eds), 427 pp.

Bauchot, M.L., 1987. Poissons osseux. p. 891-1421. In: *Fiches FAO d'identification pour les besoins de la pêche. (rev. 1). Méditerranée et mer Noire. Zone de pêche 37. Vol. II.* Fischer, W., Bauchot, M.L., Schneider, M. (Eds). Commission des Communautés Européennes and FAO, Rome.

Ben Hassine, O.K., Benmansour, B., Neifar, L., Bahri, L., Hajji, T. *et al.*, 1999. L'état de la biodiversité dans les sites à activité halieutique du littoral est de la Tunisie. L'Homme et la mer. *Cahiers du C.E.R.S., série Géographique*, 21, 277-333.

Böhlke, J.E., Robins, C.R., 1968. Western Atlantic seven-spined gobies, with description of ten new species and a new genus, and comments on Pacific relatives. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 120 (3), 45-174.

Bortone, S.A., Dunson, W.A., Greenawalt, J.M., 2005. Fishes as estuarine indicators. p. 381-389. In: *Estuarine Indicators.* CRC Press: Boca Raton, FL, USA.

Bouchereau, J.L., Durel, J.S., Guelorget, O., Louali, L.R., 2000. L'ichtyofaune dans l'organisation biologique d'un système paralique: la lagune de Nador, Maroc. *Cybium,* 10 (1-2), 69-76.

Breine, J., Quataert, P., Stevens, M., Ollevier, F., Volckaert, F.A. *et al.*, 2010. A zone-specific fish-based biotic index as a management tool for the Zeeschelde estuary (Belgium). *Marine Pollution Bulletin*, 60 (7), 1099-1112.

Brownell, C., Collignon J., 1978. Catalogue raisonné des poissons des mers marocaines. Sous-ordre des Gobioides (famille des Gobiidae). *Bulletin de l'Institut des Pêches Maritimes du Maroc*, 139-156.

Casabianca, M.L., Kiener, A., 1969. Gobiidés des étangs corses: systématique, écologie, régime alimentaire et position dans les chaînes trophiques. *Vie et Milieu*, 20 (3), 611-634.

Chanthran, S.S.D., Lim, P.E., Li, Y., Liao, T.Y., Poong, S.W. *et al*., 2020. Genetic diversity and population structure of *Terapon jarbua* (Forskål, 1775) (Teleostei, Terapontidae) in Malaysian waters. *ZooKeys*, 911, 139-160.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, 18(1), 117-143.

Clarke, K.R., Gorley, R.N., 2006. *PRIMER v6: user manual/tutorial (Plymouth routines in multivariate ecological research)*. PRIMER-E, Plymouth, UK.

Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M., 2014. *Change in marine communities: an approach to statistical analysis and interpretation (3rd edition).* PRIMER-E: Plymouth, UK.

Eisenreich, S.J., 2005. *Climate changes and the European water dimension*. *A report to the European Water Directors*. EUR 21553 EN. IES-JRC, European Commission, Ispra, Italy, 253 pp.

El Kamcha, R., Bououarour, O., Boutoumit, S., Bazairi, H., 2020. Occurrence of the invasive *Caprella scaura* Templeton, 1836 (Amphipoda: Caprellidae) in the Marchica coastal lagoon (Alboran Sea, Morocco). *BioInvasions Records*, 9 (4), 763-771.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564-567.

Facca, C., Cavraro, F., Franzoi, P., Malavasi, S., 2020. Lagoon resident fish species of conservation interest according to the habitat directive (92/43/CEE): a review on their potential use as ecological indicator species. *Water*, 12, 2059.

Field, J.G., Clarke, K.R., Warwick, R.M., 1982. A practical strategy for analysing multispecies distribution patterns. *Marine Ecology Progress Series*, 37-52.

Fossi, C., Leonzio, C., Focardi, S., 1989. Regulatory detoxication responses in Gobiidae experimentally exposed to PCBs. *Ecotoxicology and Environmental Safety*, 18 (1), 11-14.

Franco, A., Franzoi. P., Malavasi, S., Riccato, F., Torricelli, P., 2006. Fish assemblages in different shallow water habitats of the Venice Lagoon. *Hydrobiologia,* 555 (1), 159-174.

Franco, A., Elliott, M., Franzoi, P., Torricelli, P., 2008a. Life strategies of fishes in European estuaries: the functional guild approach. *Marine Ecology Progress Series*, 354, 219-228.

Franco, A., Franzoi, P., Torricelli, P., 2008b. Structure and functioning of Mediterranean lagoon fish assemblages: a key for the identification of water body types. *Estuarine, Coastal and Shelf Science*, 79 (3), 549-558.

Franco, A., Pérez-Ruzafa, A., Drouineau, H., Franzoi, P., Koutrakis, E.T. *et al.*, 2012. Assessment of fish assemblages in coastal lagoon habitats: effect of sampling method. *Estuarine*, *Coastal and Shelf Science*, 112, 115-125.

Fricke, R., Eschmeyer, W.N., Fong, J.D., 2023. *Eschmeyer's Catalog of Fishes: Genera/Species by Family/Subfamily*. http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp (Accessed 9 March 2023).

Gaamour, A., Missaoui, M., El Abed, A., Deniel, Ch., 2001. Age et croissance de l'allache ou sardinelle ronde (Valenciennes, 1847) dans la région du Cap Bon (Tunisie). *Bulletin de l'Institut National des Sciences et Technologies de la Mer de Salammbô*, 28, 23-35.

Gill, H.S., Bradley, J.S., Miller, P.J., 1992. Validation of the use of cephalic lateral-line papillae patterns for postulating relationships among gobioid genera. *Zoological Journal of the Linnean Society*, 106, 97-174.

Giovannotti, M., Mario, L.M., Vincenzo, C., 2009. Life style and genetic variation in teleosts: the case of pelagic (*Aphia minuta*) and benthic (*Gobius niger*) gobies (Perciformes: Gobiidae). *Marine Biology*, 156 (3), 239-252.

Gusev, A.V., 1995. Some pathways and factors of monogenean microevolution. *Canadian Journal of Fisheries and Aquatic Sciences*, 52 (S1), 52-56.

Hajji, F., Ouannes-Ghorbel, A., Ghorbel, M., Jarboui, O., 2013. Feeding habits of the black goby, *Gobius niger* (Linnaeus, 1758), in the Gulf of Gabes (Southern Tunisia). *Cahiers de Biologie Marine*. 54 (1), 133-141.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4, 1-9.

Harris, P.D., Shinn, A.P., Cable, J., Bakke, T.A., 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. *Systematic Parasitology*, 59 (1), 1-27.

Harvey, B.C., Stewart, A.J., 1991. Fish size and habitat depth relationships in headwater streams. *Oecologia*, 87 (3), 336-342.

Heck, K.L., Orth, R.J., 1980. Seagrass habitats: the roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. *Estuarine Perspectives*, 449-464.

Holmlund, C.M., Hammer, M., 1999. Ecosystem services generated by fish populations. *Ecological Economics*, 29, 253-268.

Hoese, D.F., 1971. A revision of the eastern Pacific species of the Gobiid fish genus *Gobiosoma*, with a discussion of relationships of the genus. Ph.D. Thesis. University of California, San Diego, USA. 213 pp.

Hoese, D.F., 1983. Sensory papilla patterns of the cheek lateralis system in the Gobiid fishes *Acentrogobius* and *Glossogobius*, and their significance for the classification of gobioid fishes. *Records of the Australian Museum*, 35, 223-229.

Huxley, J.S., 1932. *Problems of relative growth*. The Dial Press, New York, 308 pp.

Huyse, T., Audenaert, V., Volckaert, F.A.M., 2003. Speciation and host–parasite relationships in the parasite genus *Gyrodactylus* (Monogenea, Platyhelminthes) infecting gobies of the genus *Pomatoschistus* (Gobiidae, Teleostei). *International Journal for Parasitology,* 33 (14), 1679-89.

Huyse, T., Volckaert, F.A.M., 2005. Comparing host and parasite phylogenies: *Gyrodactylus* flatworms jumping from goby to goby. *Systematic Biology*, 54 (5), 710-718.

Jolicoeur, P., 1963. The degree of generality of robustness in Martes americana. *Growth*, 27, 1-27.

Kara, M.H., Quignard J.P., 2019. *Fishes in lagoons and estuaries in the Mediterranean 2: sedentary fish. Ecological Science Series*. ISTE Ltd and John Wiley & Sons, Inc., 423 pp.

Katalay, S., Parlak, H., 2002. Su kirliliğinin, *Gobius niger* Linnaeus, 1758 (Pisces: Gobiidae) in kan parametreleri üzerine etkileri. *Ege Journal of Fisheries and Aquatic Sciences*, 19 (1), 115-121.

Kearn, G.C., 1994. Evolutionary expansion of the Monogenea. *International Journal for Parasitology*, 24, 1227-1271.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-120.

Kjerfve, B., 1994. Coastal lagoons. *Elsevier Oceanographic Series*, 1-8.

Koutrakis, E.T., Tsikliras, A.C., Sinis, A.I., 2005. Temporal variability of the ichthyofauna in a Northern Aegean coastal lagoon (Greece). Influence of environmental factors. *Hydrobiologia*, 543 (1), 245-257.

Kovačić, M., Golani, D., 2007. First record of *Papillogobius melanobranchus* in the Mediterranean Sea and new data on geographic distributions, bathymetric ranges and morphology of several small benthic fishes in the Levant. *Cybium*, 31 (4), 417-425.

Kovačić, M., 2008. Live colouration, morphology and habitat of *Vanneaugobius dollfusi* (Gobiidae) in the northern Adriatic Sea. *Journal of Fish Biology*, 73 (4), 1019-1023.

Kovačić, M., Patzner, R.A., 2011. North-Eastern Atlantic and Mediterranean Gobies. p. 177-206. In: *The biology of gobies*. Patzner, R.A., Van Tassell, J.L., Kovačić, M., Kapoor, B.G. (Eds). Science Publishers, Jersey, British Isles.

Kovačić, M., 2020. Checklist of gobies (Teleostei: Gobiidae) of the Mediterranean Sea and a key for species identification. *Zootaxa*, 4877 (1), 75-101.

Kovačić, M., Renoult, J.P., Pillon, R., Svensen, R., Bogorodsky, S. *et al*., 2022. Identification of Mediterranean marine gobies (Actinopterygii: Gobiidae) of the continental shelf from photographs of “in situ” individuals. *Zootaxa*, 5144, 1-103.

Kulczycki, G.R., Virnstein, R.W., Nelson, W.G., 1981. The relationship between fish abundance and algal biomass in a seagrass-drift algae community. *Estuarine, Coastal and Shelf Science,* 12 (3), 341-347.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35(6), 1547-1549.

Leatemia, S.P., Kopalit, H., Pakilaran, E.L., 2017. Macrozoobenthos abundancy in vegetated (seagrass) and un-vegetated areas of Doreri Bay-Manokwari. *Jurnal Sumberdaya Akuatik Indopasifik,* 1, 15-26.

Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* 69, 24.

Levin, L.A., Boesch, D.F., Covich, A., Dahm, C., Erseus, C. *et al.*, 2001. The function of marine critical transition zones and the importance of sediment biodiversity. *Ecosystems*, 4, 430-451.

Lopes, R., Videira, N., 2013. Valuing marine and coastal ecosystem services: an integrated participatory framework. *Ocean & Coastal Management*, 84, 153-162.

Mantel, N., 1967. Ranking procedures for arbitrarily restricted observation. *Biometrics*, 23 (1), 65.

Maradonna, F., Polzonetti, V., Bandiera, S.M., Migliarini, B., Carnevali, O., 2004. Modulation of the hepatic CYP1A1 system in the marine fish *Gobius niger*, exposed to xenobiotic compounds. *Environmental Science & Technology*, 38 (23), 6277-6282.

Maradonna, F., Carnevali, O., 2007. Vitellogenin, zonaradiata protein, cathepsin D and heat shock protein 70 as biomarkers of exposure to xenobiotics. *Biomarkers*, 12, 240-255.

Matern, S., Herrmann, J.P., Temming, A., 2021. Differences in diet compositions and feeding strategies of invasive round goby Neogobius melanostomus and native black goby Gobius niger in the Western Baltic Sea. *Aquatic Invasions,* 16 (2), 314-328.

Matschiner, M., 2016. Fitchi: haplotype genealogy graphs based on the Fitch algorithm. *Bioinformatics*, 32 (8), 1250-1252.

Mavruk, S., Toslak, C., Yalim, F.B., Ütük, A.E., Kara, A. *et al.*, 2022. First record of darter goby, *Ctenogobius boleosoma* (Jordan & Gilbert, 1882) (Gobiiformes: Gobiidae), in the Mediterranean with notes on larval morphology and cryptic diversity. *Mediterranean Marine Science*, 23 (4), 900-906.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, 82, 290-297.

Migliarini, B., Campisi, A. M., Maradonna, F., Truzzi, C., Annibaldi, A. *et al.*, 2005. Effects of cadmium exposure on testis apoptosis in the marine teleost *Gobius niger*. *General and Comparative Endocrinology*, 142 (1-2), 241-247.

Miller, J.M. 1988. Physical processes and the mechanisms of coastal migration of immature marine fishes. *American Fisheries Society Symposium*, 3, 68-76.

Mistri, M., Fano, E.A., Rossi, G., Caselli, K., Rossi, R., 2000. Variability in macrobenthos communities in the Valli di Comacchio, Northern Italy, a hypereutrophized lagoonal ecosystem. *Estuarine*, *Coastal and Shelf Science*, 51 (5), 599-611.

Nash, R.D.M., 1984. Aspects of the biology of the black goby, *Gobius niger,* in Oslofjorden, Norway. *Sarsia*, 69 (1), 55-61.

Najih, M., Berday, N.B., Rezzoum, N.D., Mounir, F.D., Lamrini, A. *et al*., 2016. Evolution temporelle et spatiale de la macroflore benthique de l'écosystème lagunaire de Nador. *European Scientific Journal*, 12 (36), 355-377.

Najih, M., Driss, N., Nadia, B., Roberto, P., Abdeljaouad, L. *et al*., 2017. Charactérization of the new status of Nador lagoon (Morocco) after the implementation of the management plan. *Journal of Marine Science and Engineering*, 5 (1), 7.

Newton, A., Icely, J., Cristina, S., Brito, A., Cardoso, A.C. *et al.*, 2014. An overview of ecological status, vulnerability and future perspectives of European large shallow, semi-enclosed coastal systems, lagoons and transitional waters. *Estuarine, Coastal and Shelf Science*, 140, 95-122.

Newton, A., Brito, A., Icely, J., Derolez, V., Clara, I. *et al.*, 2018. Assessing, quantifying and valuing the ecosystem services of coastal lagoons. *Journal for Nature Conservation*, 44, 50-65.

Oden, B., 1914. Notas sobre Biologia y aprovechamiento de Mar Chica. Annuario le pesca y esladistico de la marina mercante y de la pesca del ano 1911. Dirección General de la Marina Mercante. Madrid, 1012, 427-432.

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L. *et al*., 1991. *The simple fool's guide to PCR*. Department of Zoology, University of Hawaii, Honolulu, 44pp.

Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20 (2), 289-290.

Pauly, D., 2021. The gill-oxygen limitation theory (GOLT) and its critics. *Science Advances*, 7 (2), eabc6050.

Pérez-Ruzafa, A., De Pascalis, F., Ghezzo, M., Quispe, J.I., Hernández-García, R. *et al.*, 2019. Connectivity between coastal lagoons and sea: asymmetrical effects on assemblages' and population's structure. *Estuarine, Coastal and Shelf Science*, 216, 171-186.

Poulin, R., 1998. Comparison of three estimators of species richness in parasite component communities. *Journal of Parasitology,* 84, 485-490.

Raffaelli, D., Conacher, A.M.H., Emes, C., 1989. The role of epibenthic crustacean predators in an estuarine food web. *Estuarine, Coastal and Shelf Science*, 28 (2), 149-160.

Ramšak, A., Stopar, K., Sepčić, K.B.Z.M., Bajt, O. *et al.*, 2007. Reflection of hydrocarbon pollution on hepatic EROD activity in the black goby (*Gobius niger*). *Environmental Toxicology and Pharmacology*, 24 (3), 304-310.

Renoult, J.P., Pillon, R., Kovačić, M., Louisy, P., 2022. Frontiers in fishwatching series - gobies of the North-Eastern Atlantic and the Mediterranean: *Gobius* and *Thorogobius*. p. 1-237. In: *Les Cahiers de la Fondation Biotope: Cayenne*. France.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P. *et al.*, 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology Evolution*, 34, 3299-3302.

Selfati, M., El Ouamari, N., Crocetta, F., Mesfioui, A., Boissery, P. *et al.*, 2017. Closing the circle in the Mediterranean Sea: *Bursatella leachii* Blainville, 1817 (Mollusca: Gastropoda: Anaspidea) has reached Morocco. *BioInvasions Record*s, 6 (2), 129-134.

Selfati, M., El Ouamari, N., Franco, A., Lenfant, P., Lecaillon, G. *et al.*, 2019. Fish assemblages of the Marchica lagoon (Mediterranean, Morocco): spatial patterns and environmental drivers. *Regional Studies in Marine Science*, 32, 100896.

Selfati, M., 2020. *Faune ichtyque de la lagune de Marchica: approche quantitative de la fonction de nourricerie et potentialités de restauration écologique*. Ph.D. Thesis. Université Mohammed V de Rabat, 217 pp.

Stein, R. A., DeVries, D. R., Dettmers, J. M., 1995. Food-web regulation by a planktivore: exploring the generality of the trophic cascade hypothesis. *Canadian Journal of Fisheries and Aquatic Sciences*, 52 (11), 2518-2526.

Sturges, H.A., 1926. The choice of a class interval. *Journal of the American Statistical Association*, 21 (153), 65-66.

Tiralongo, F., Messina, G., Lombardo, B.M., 2021. Invasive species control: predation on the alien crab *Percnon gibbesi* (H. Milne Edwards, 1853) (Malacostraca: Percnidae) by the rock goby, *Gobius paganellus* Linnaeus, 1758 (Actinopterygii: Gobiidae). *Journal of Marine Science and Engineering*, 9 (4), 393.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673-4680.

Vaas, K.F., Vlasblom, A.G., Koeijer, P. De., 1975. Studies on the black goby (*Gobius niger*, Gobiidae, Pisces) in the Veerse Meer, SW Netherlands. *Netherlands Journal of Sea Research*, 9, 56-68.

Vanhove, M.P.M., Economou, A.N., Zogaris, S., Larmuseau, M.H.D., Giakoumi, S. *et al.*, 2012. Phylogenetics and biogeography of the Balkan 'sand gobies' (Teleostei: Gobiidae): vulnerable species in need of taxonomic revision. *Biological Journal of the Linnean Society,* 105 (1), 73-91.

Vanhove, M.P.M., Giakoumi, S., Zogaris, D., Kovačić, M., Huyse, T., 2022. First Eastern Mediterranean record of *Xenoligophoroides cobitis*, the only dactylogyrid monogenean infecting Mediterranean gobies: just arrived or missed the boat? *Diversity*, 14 (8), 580.

Vanhove, M.P.M., Kovačić, M., Zogaris, S., 2016. A distinct island population of threatened freshwater fish: to split or lump? *Hydrobiologia,* 777 (1), 79-93.

Vanni, M. J., 2002. Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics*, 33 (1), 341-370.

Vasconcelos, J.T., Galyean, M.L., 2007. Nutritional recommendations of feedlot consulting nutritionists: the 2007 Texas Tech University survey. *Journal of Animal Science*, 85 (10), 2772-2781.

Whitfield, A.K., 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.

Wiederholm, A.M., 1987. Habitat selection and interactions between three marine fish species (Gobiidae). *Oikos,* 48, 28-32.

Wright, S., 1965. The interpretation of population structure by F-statistics with special regard to system of mating. *Evolution*, 19, 395-420.

Zander, C.D., Strohbach, U., Groenewold, S., 1993. The importance of gobies (Gobiidae, Teleostei) as hosts and transmitters of parasites in the SW Baltic. *Helgoländer Meeresuntersuchungen*, 47 (1), 81-111.

Zander, C.D., Reimer, W., Barz, K., Bight, È., 1999. Parasite communities of the Salzhaff (Northwest Mecklenburg, Baltic Sea) I. Structure and dynamics of communities of littoral fish, especially small-sized fish. *Parasitology Research*, 85, 356-372.

Supplementary Material

The following supplementary material is available for this article:

Fig. S1: A neighbour-joining tree including all the species of *Gobius* that are represented in the GenBank by a targeted fragment of the 16S rRNA gene. The tree was created in MEGA X using 1000 bootstrap replicas and the Kimura 2-P substitution model. The scale bar represents the number of expected substitutions per site.

Table S1. Summary of species included in the phylogenetic tree, with accession numbers and locations of each species.

Table S2. Mean abundance of *Gobius niger* at different stations.

Table S3. Pairwise comparisons of *Gobius niger* abundance in terms of factor station.

Table S4. Meristic characters of *Gobius niger* from the Mediterranean Sea. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Table S5. Meristic characters of *Gobius niger* from the Marchica Lagoon. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Table S6. Morphometric characters of *Gobius niger* from the Mediterranean Sea. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Table S7. Morphometric characters of *Gobius niger* from the Marchica Lagoon. N: number; m: mean; M: mode; S: standard deviation; Sm: standard error of the mean.

Table S8. Length–length relationships between various morphometric characters measured in *Gobius niger* from the Mediterranean Sea. TL: total length; SL: standard length; HL: head length; BH: body height; ED: eye diameter; SnL: snout length; N: number of specimens; a: intercept of relationship; b: slope of relationship; R^2: coefficient of determination.

Table S9. Length–length relationships between various morphometric characters measured in *Gobius niger* from the Marchica Lagoon. TL: total length; SL: standard length; HL: head length; BH: body height; ED: eye diameter; SnL: snout length; N: number of specimens; a: intercept of relationship; b: slope of relationship; R^2: coefficient of determination.