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Using multiple indicators to assess the environmental status in impacted and non-impacted bathing waters in the Iranian Caspian Sea

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ABSTRACT

Human activities have increased in the Caspian Sea in last decades, impacting the coastal zone ecosystems. One of the increasing activities is recreation, including bathing areas in the south of the Caspian Sea, which have been scarcely studied and assessed. Investigating the interactions between human activities and the resulting environmental status in bathing areas, by using adequate indicators and assessment methods, is necessary to undertake management measures for ecosystem restoration. In this study, for the first time, we use the Nested Environmental status Assessment Tool (NEAT) outside the European waters to assess environmental status in bathing waters, to differentiate areas impacted and non-impacted by bathing activities. We have assessed the status in winter and summer seasons, by combining multiple indicators from different ecosystem components (8 physico-chemical, 4 bacteria, 2 plankton, and 1 benthos indicators). Despite the interactions between season and human affection, NEAT determined that the Caspian Seas is not in good status, differentiating, in summer, between impacted and non-impacted bathing areas, with a significant correlation with the number of beach users. Accordingly, management measures should be taken in the southern Caspian Sea to improve the environmental status in general and that of bathing areas in particular.

1. Introduction

Human activities at sea, including both traditional (i.e. fishing, shipping) and emerging (i.e. renewable energy, deep-sea mining), are increasing dramatically worldwide in recent decades and can result in increasing pressures and impacts on marine ecosystems (Korpinen and Andersen, 2016). Among these activities, the use of seas for recreation is becoming more and more popular, being considered as a cultural ecosystem service (Hernández-Morcillo et al., 2013). Among these activities, the use of beaches for leisure, including sunbathing, water sports and bathing are the most common, and require ecosystem services such as clean bathing waters (Ghermandi et al., 2012).

The quality of bathing waters (i.e. those legally designed for human bathing) has been long-time monitored in many countries and under different legislation (e.g.: EEC (1976) and European Commission (2006), in Europe; US Government (2000), in USA; Health Canada (2012), in Canada) requiring, among others, the control of faecal bacteria (Salas, 1986). The bathing waters monitoring is just a control of the variables that can affect the activity itself (bacteria concentration), determining the opening or closing of the bathing areas, to avoid risks to human health. However, the activity (beach use, bathing) can also affect the quality of the bathing area in different ways, e.g. the need of beach nourishment, which can impact on beach biodiversity (Cooke et al., 2012; Vanden Eede, 2013).

In addition, the environmental status of the area in which the activity is undertaken could be already affected by other activities (e.g. waste water discharges, agricultural activities, etc.). Such activities may compromise the bathing waters quality and lead to risks to human health, but also may limit the environmental conditions, so that it becomes more prone to disturbance. Hence, an ecosystem-based management system for beaches would be needed to maintain the ecosystem integrity while enabling the sustainable use of ecosystem services (Sardá et al., 2015).

One of the problems when assessing the environmental status of marine waters is that methods able to include multiple ecosystem components in an integrative evaluation, as those used in ecosystem-based management, were not available until recently or that they had major statistical or other flaws preventing their use (Borja et al., 2009).

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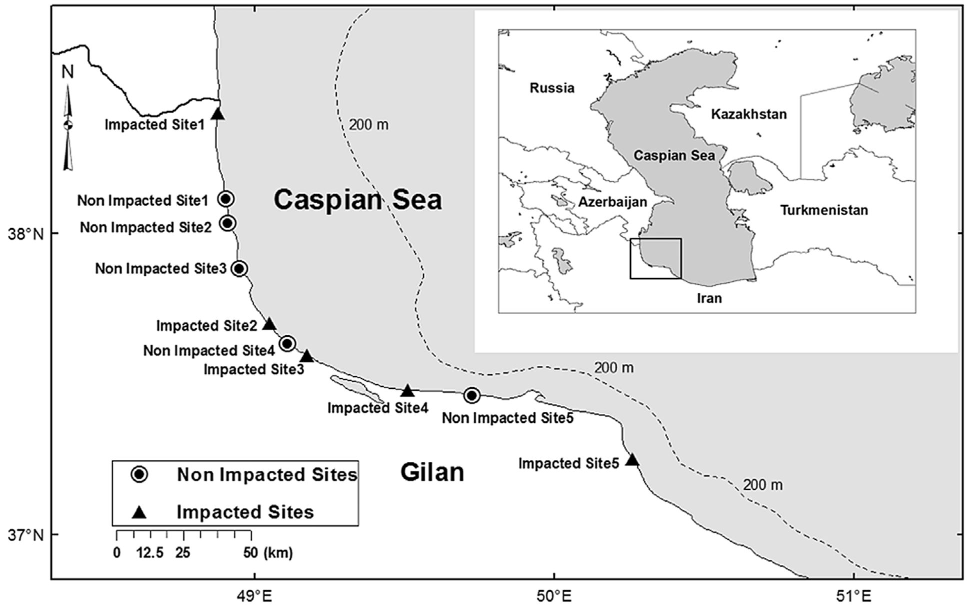

Fig. 1. Study area within the Caspian Sea.

Some of the available methods include, among others (see Borja et al., 2016a), the Ocean Health Index (Halpern et al., 2012), and recently the Nested Environmental status Assessment Tool (NEAT, Andersen et al., 2014; Borja et al., 2016b).

NEAT was primarily developed to assess the environmental status of marine waters within the European Marine Strategy Framework Directive (MSFD; 2008/56/EC, European Commission, 2008). This method has been successfully applied to all European Regional Seas (Uusitalo et al., 2016), but until now no application outside Europe has been undertaken.

Extending its use to other biogeographic regions, such as the Caspian Sea, could assist in demonstrating the applicability of NEAT under different geographic circumstances. The Caspian Sea is situated in Central Asia (Fig. 1) and is an enclosed water body that has supported decades of human activities (i.e. oil and gas extraction, fisheries, agriculture and tourism), which has resulted in a degradation of its environmental status (Barannik et al., 2004; Stolberg et al., 2006; UNEP, 2011), aggravated by decades of environmental mismanagement (Fendereski et al., 2014). Hence, the Caspian Sea ecosystem has changed dramatically (Karpinsky et al., 2005), with impacts on habitats, plankton and fish biomass, chlorophyll-a concentration, primary production and nutrient increase (Nasrollahzadeh, 2010; Shiganova, 2011), resulting in a eutrophic status (Leonov and Stygar, 2001; UNEP, 2011).

Despite this, trends indicate that bathing activities and recreation will continue to increase, but no coordinated bathing waters monitoring exists, and few research studies have been undertaken to assess the quality of bathing sites, in countries such as Iran and Turkmenistan (Pond et al., 2005; Binesh Barahmand et al., 2012).

Hence, the objective of this investigation is to check whether NEAT can be used in assessing the status of a sub-region of the Caspian Sea, in Iran, discriminating between areas impacted and non-impacted by bathing activities, and studying the potential interactions with other human activities in the area.

2. Methods

2.1. Study area and sampling design

The study area is located on the southwest coast of Caspian Sea in Gilan Province (Iran). Sampling was carried out at 10 sites: five sites were at recreational bathing areas (Impacted Sites 1–5), and five sites were not affected by bathing (Non-Impacted Sites 1–5) (Fig. 1). The bathing water areas present distinct use pressure, from absence of users (non-impacted sites), to low-moderate bathing practice (impacted sites 2 and 3, with an estimated number of swimmers between 15,000–20,000 swimmers per month), and high practice (sites 1, 4, and 5, with 25,000–40,000 swimmers per month). The number of swimmers per month was estimated during the summer sampling surveys since in winter there was no swimming activity. In addition, the bathing sites are subjected to regular beach nourishment to maintain the sand and to make the activity more pleasant. The assignment of sites to 'impacted' or 'non-impacted' was done only based on their use for recreation and bathing (and associated activities, such as beach nourishment). The sub-region studied presents also other additional pressures, such as runoff of polluted waters from rivers, rice agriculture inputs and industrial wastewater inputs (Zonn, 2005; Stolberg et al., 2006; UNEP, 2011), which could affect both impacted and non-impacted sites.

The sampling was undertaken in February 2015 (non-bathing period), and once a month from July to September 2015 (bathing period). Water surface temperature was measured on site using a digital thermometer.

Samples to analyse bacteria were collected in sterilized plastic bottles, at 10–20 cm below the surface (3 replicates per site), stored and transported in a cold box kept below 4 °C and analysed within 5–6 h of sampling (Clesceri et al., 1998).

Phytoplankton samples were collected with a Niskin bottle (3 replicates per site) (Venrick, 1978). The samples were preserved using buffered formaldehyde (4%). Conversely, zooplankton was sampled (3 replicates per site) filtering 1 m^3 per replicate through a plankton net (mesh size: 100 µm). Zooplankton samples were preserved in 4% formaldehyde (Harris, 2000).

Sediment samples were taken using a PVC Corer (3 replicates per site), to analyse Total Organic Matter (TOM) and particle size. Similarly, benthic macroinvertebrates were sampled by inserting a 20 cm wide 10 cm deep PVC Corer (5 replicates per site) into the sediment in the swash zone. Samples were then sieved through a 0.5 mm mesh. The retained macroinvertebrates were preserved in 10% buffered formalin/seawater.

2.2. Laboratory analyses

Water temperature was measured *in situ* with a digital thermometer. Samples for Dissolved Oxygen (DO) analysis were preserved after fixation (4500-O-B) and transported to the laboratory immediately. Oxygen saturation was calculated from DO data and temperature. Water physicochemical parameters (oxygen saturation, pH (4500-H^+-B), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), salinity, phosphate, nitrate, nitrite, Total Suspended Solids (TSS), and turbidity) were measured in the laboratory using standard methods, as described by Clesceri et al. (1998). For the TOM and grain size, the sediment samples were dried at 70 °C for 24 h and separately analysed (Gambi and Dappiano, 2004). The grain size of sediments was classified into seven fractions, from < 63 μm– > 2 mm, in ϕ scale.

In the laboratory, standard methods procedures for Total Coliforms (9221B), Faecal Coliforms (9221E), *Escherichia coli* (9221F) and *Staphylococcus aureus* (9213A) were used (Clesceri et al., 1998). The counting was performed using the Most Probable Number (MPN) method. The total number of positive tubes counted and considered for the calculation of MPN was using the standard method (Collins et al., 1989).

Phytoplankton samples were identified and counted by sedimentation in a separable (sedimentation cylinder) plankton chamber with the inverted microscope (Hasle, 1978). Zooplankton was identified in the laboratory with the Bogarov chamber, according to the standard method (Harris, 2000).

Phytoplankton, zooplankton and benthic macroinvertebrates were identified to species level when possible.

2.3. NEAT description and application

NEAT software (version 1.2) was downloaded from the DEVOTES project web page (www.devotes-project.eu/neat).

NEAT was designed based on the Ecosystem Approach, considering all available ecosystem components in the assessment. The first step of such evaluation is to determine the Spatial Assessment Units (SAU) in which the assessment is going to be undertaken. For this analysis, since the areas are linear and their size were approximately similar, we did not assign an area to each location. In addition, as distinguishing between impacted and non-impacted sites is aimed, each site was defined as a distinct SAU (Fig. 2). Also, we wanted to investigate if winter and summer survey results were different. Hence, they were considered as different sub-SAUs, to calculate NEAT values for each of them. Another alternative could be doing the winter and summer analyses separately, but this prevents a further aggregation of the information, for each location and the whole year. Within each SAU it is necessary to identify the habitats (for this study, benthic and pelagic) to which the indicators are associated (Fig. 2). Finally, indicators must be defined, requiring each of them a range of values, between bad and high status, and a target value for good condition, following the five quality classes considered by European directives (Borja et al., 2010), such as the Water Framework Directive (WFD, 2000/60/EC) or the MSFD: high, good, moderate, poor and bad status. In the NEAT calculations, the setting used were those set by default in the software (i.e. no weighting, no habitat priority, etc.).

The indicators, reference conditions and targets used in this study are shown in Table 1. Following the directives abovementioned, 'reference conditions' refer to those expected in absence of human pressure or less impacted, to which monitored data are compared to assess the status, whilst 'target' values are those determining the boundary between good and not good quality classes, representing the limit to take management actions (Borja et al., 2012). Most of them are based on the literature, using targets for similar salinity ranges when possible. In the case of coliforms, since no Iranian legislation exists, the European bathing waters directive (EEC, 1976) was used. Although a newer Directive exists (European Commission, 2006), this only includes *Escherichia coli* as indicator. Finally, for very few indicators, expert judgment of the local researchers was used (Table 1).

In NEAT, the worst and best indicator values are transformed to a scale ranging from 0 (bad status) to 1 (high status) (Borja et al., 2016b), and the target values for each indicator are standardized to a value of 0.6 (boundary between good (≥0.6) and not good (< 0.6) status). If no other boundary classes are defined, the software calculates the remaining boundaries automatically, by interpolating the intermediate values (Uusitalo et al., 2016).

2.4. Statistical methods

The Shannon index (Shannon and Weaver, 1963) for phytoplankton and zooplankton community was calculated using the PRIMER6 software (Clarke and Warwick, 2001). Using Statgraphics17, a multi-factorial ANOVA was performed to test the significance of differences in NEAT values between impacted and non-impacted sites (=affection, in the sense of disturbance produced by the activity), in winter and summer (=season). Such analyses were carried out both for final NEAT values as well as for the NEAT values corresponding to each indicator.

A Redundancy Analysis (RDA) was performed with CANOCO5 software (Ter Braak, 1988) to look for the environmental variables, which explained most of the variance of biological parameters. After square-root transformation, seven biological variables (Abundance of *Pontogammarus maeoticus*, Total Coliforms, Faecal Coliforms, *Escherichia coli*, *Staphylococcus aureus*, and Shannon indices for phytoplankton and zooplankton) were used in the ordination. A pre-selection of environmental variables was carried out by pair-wise Pearson's correlations between them, removing those showing high correlations. Hence, the RDA was applied to the dataset including six explanatory variables (nitrate, nitrite, TSS, phosphate, TOM and COD) and four supplementary variables (impacted, non-impacted, winter and summer), together with the abovementioned biological parameters. A manual forward selection process was used in CANOCO to select the subset of environmental variables with a significant effect on the ordination of samples based on biological parameters.

3. Results

The salinity average (± SE) values in the impacted and non-impacted sites were 10.93 ± 0.72 and 12.27 ± 0.14, respectively, ranging from 5.5 to 13.3 in the impacted sites and from 11.5 to 13.2 in non-impacted sites, being all of them mesohaline (Supplementary Material (SM), Table SM1). Minimum temperature was 9.2 °C, in winter, and maximum 34.7 °C, in summer. Average (± SE) pH value in sampling sites was 8.37 ± 0.03. Phosphate, nitrate, turbidity and TSS concentrations in winter were higher than in summer (Table SM1). In turn, COD and BOD5 values in winter were lower than in summer. In particular, nitrite concentrations at impacted sites were higher than in non-impacted ones.

Regarding sediments, sand was the predominant fraction (> 99%) at all sites. TOM ranged from 1.3% to 3.9% (Table SM1).

Mean values (± SE) for Total Coliforms, Faecal Coliforms, and *Escherichia coli* in summer (332.7 ± 46.2, 269.1 ± 44.2 and 45.7 ± 21.1 MPN 100 mL^{-1}, respectively) were higher than in winter (329.4 ± 78.3, 253.4 ± 57.3 and 21.6 ± 4.9 MPN 100 mL^{-1}, respectively). *Staphylococcus aureus* was an exception, with a higher mean value in winter (6.5 ± 1.0 MPN 100 mL^{-1}) than in summer (4.8 ± 1.8 MPN 100 mL^{-1}). The highest concentrations of Total Coliforms and Faecal Coliforms were observed in impacted Site1, whilst maximum concentrations of *Escherichia coli* and *Staphylococcus aureus* were found at impacted Site2 and 4, respectively. The lowest MPN of bacteria indicators were in non-impacted Site5 (Table SM1).

A total of 42 phytoplankton and 15 zooplankton species were identified. The mean abundance (± SE) of phytoplankton and zooplankton in impacted sites ($1.8 \times 10^8 \pm 3.2 \times 10^7$ cells m^{-3} and $7 \times 10^3 \pm 426$ ind m^{-3}, respectively) were higher than in non-impacted sites ($9 \times 10^7 \pm 1.1 \times 10^7$ cells m^{-3} and $2.7 \times 10^3 \pm 238$ ind m^{-3}, respectively) (Table SM1). Likewise, phytoplankton and zooplankton were more abundant in summer ($1.7 \times 10^8 \pm 2.2 \times 10^7$

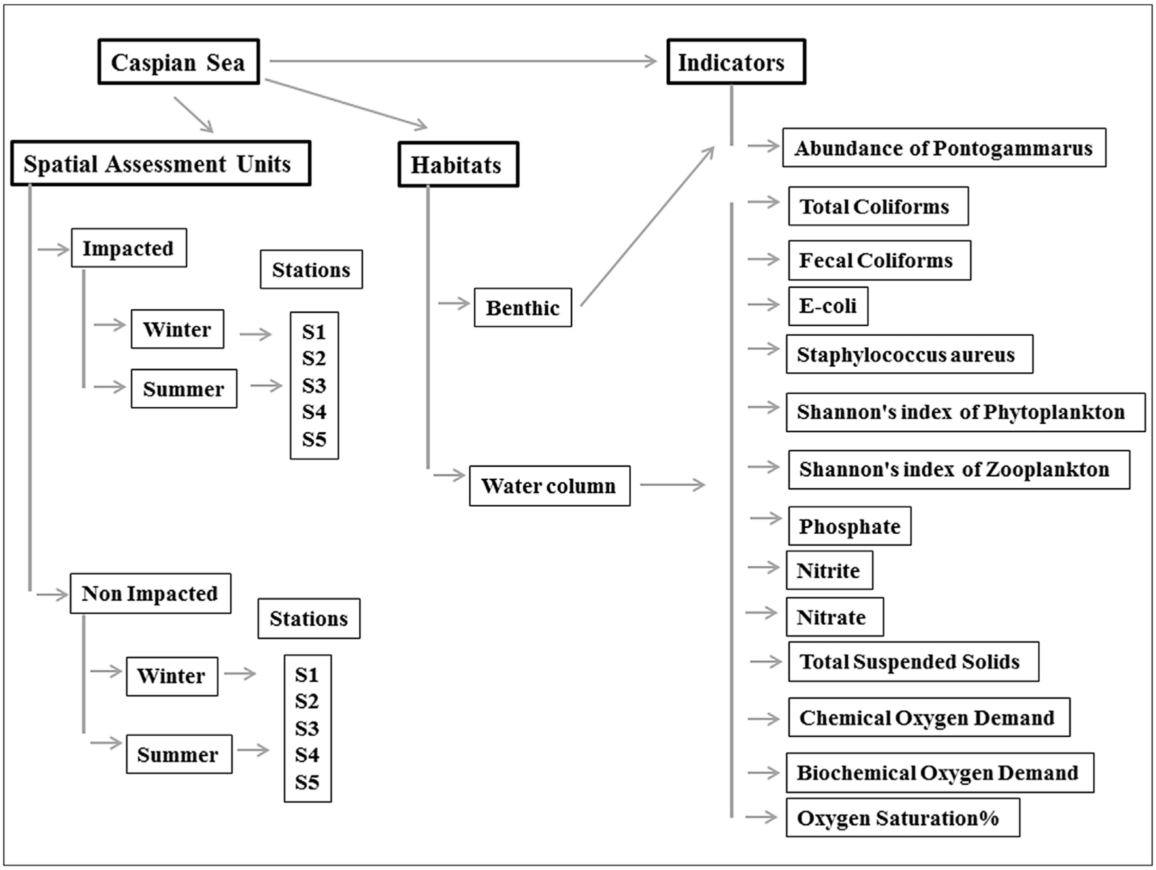

Fig. 2. Model of the design of the Nested Environmental status Assessment Tool (NEAT).

Table 1
Reference conditions of worst, target (good) and best quality status for each indicator. Key: Ab Po: Absolute abundance of *Pontogammarus maeoticus*; MPN: more probable number; TC: Total Coliforms; FC: Faecal Coliforms; EC: *Escherichia coli*; St: *Staphylococcus aureus*; H' PhP: Shannon index for phytoplankton; H' ZP: Shannon index for zooplankton; TSS: Total Suspended Solids; O2%: Oxygen Saturation; NO3: nitrate; NO2: nitrite; PO4: phosphate; COD: Chemical Oxygen Demand; BOD5: Biochemical Oxygen Demand.

Indicator	Units	Worst	Target (G/M)	Best	Reference
Ab Po	indm^{-2}	0	4000	19,000	Derived in this study
TC	MPN 100 mL^{-1}	1500	500	0	EEC (1976)
FC	MPN 100 mL^{-1}	1500	100	0	EEC (1976)
EC	MPN 100 mL^{-1}	500	100	0	EEC, 1976
St	MPN 100 mL^{-1}	50	2	0	Derived in this study
H' PhP	Bits cell^{-1}	0	1.4	2	Kitsiou and Karydis (2000); Balci and Balkis, (2017)
H' ZP	Bits ind^{-1}	0	1.75	2.5	Casé et al. (2008)
Turbidity	NTU	100	5	0	Bald et al. (2005)
TSS	mgL^{-1}	150	35	0	Borja et al. (2016b)
O2%	%	0	85	162	Derived from Best et al. (2007)
NO3	μmolL^{-1}	163.03	98	58.71	Bald et al. (2005)
NO2	μmolL^{-1}	10	0.45	0	Borja et al. (2016b)
PO4	μmolL^{-1}	10.58	4.7	1.06	Bald et al. (2005)
COD	mgL^{-1}	75	30	0	Derived in this study
BOD5	mgL^{-1}	35	7	0	Derived in this study

cells m^{-3} and $3.6 \times 10^3 \pm 315$ ind m^{-3}, respectively) than winter ($2.6 \times 10^7 \pm 5.4 \times 10^6$ cells m^{-3} and $1.9 \times 10^3 \pm 117$ ind m^{-3}, respectively) (Table SM1).

The amphipod *Pontogammarus maeoticus* was the only macroinvertebrate species identified. The abundance of *Pontogammarus maeoticus* showed an increase from winter to summer. The abundance of this species in impacted sites was lower than in non-impacted ones (Table SM1).

When undertaking the RDA analysis, after forward selection, nitrite and TSS were included in the model as significant environmental variables ($p < 0.05$). Nitrite was associated to impacted sites, with higher values (Fig. 3). In turn, TSS were associated with seasonal variability, with higher values in winter than in summer. The biotic-abiotic parameter correlations were 0.85 for the first axis and 0.6 for the second. Both axes together explained 72.2% of the variance in the biotic data.

A summary of the NEAT values, for each indicator and integrating all of them, for each location and season, for the impacted and non-impacted sites, and for the Caspian Sea sub-region studied, is presented in Table 2. When integrating all the information coming from the 15 indicators used in the assessment, the NEAT value (0.49) indicates a moderate status of this sub-region of the Caspian Sea. Both impacted and non-impacted sites are in moderate status; however, non-impacted are close to good status (NEAT value of 0.56, boundary between good and moderate being 0.6) and impacted sites are close to poor status (NEAT value of 0.41, being the boundary between moderate and poor 0.4). Impacted sites are, in general, in poor or moderate status, both in winter and summer (except Impacted Site3 in summer, which is in good status). In turn, non-impacted sites are in poor status in winter (except Site 5, which is in good status) and in good to high status in summer (Table 2). When comparing the number of beach users and NEAT values in summer (swimming period) there is a highly significant correlation (R^2: 0.75, $p < 0.01$) (Fig. 4).

When exploring the status of individual indicators, NEAT values for phosphate are always below good status, being both nitrate and oxygen

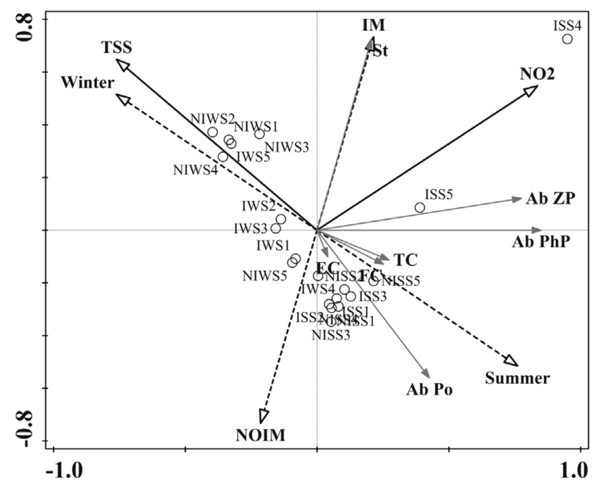

Fig. 3. The ordination diagram of Redundancy analysis (RDA) showing associations between environmental variables and spatial patterns in biological data in the study area. Supplementary variables: Impacted (IM), Non-Impacted (NOIM), Summer and Winter; biological parameters: Abundance of *Pontogammarus maeoticus* (Ab Po), Total Coliforms (TC), Faecal Coliforms (FC), *Escherichia coli* (EC), *Staphylococcus aureus* (St), and Shannon index for Phytoplankton (H' PhP) and for Zooplankton (H' ZP); environmental variables: nitrite (NO2) and Total Suspended Solids (TSS);○ = sampling sites: Impacted Sites in Winter (IWS1 to 5), Impacted Sites in Summer (ISS1 to 5), Non-Impacted Sites in Winter (NIWS1 to 5), Non-Impacted Sites in Summer (NISS1 to 5).

saturation in good status (Table 2), except the oxygen in non-impacted Site 4 in summer. NEAT values for Total Coliforms, Faecal Coliforms, *Escherichia coli* and *Staphylococcus aureus* follow the same pattern: these indicators show better status in non-impacted sites (usually in good status) than in impacted sites, especially in summer (Table 2). For planktonic indicators, in general, NEAT values for phytoplankton and zooplankton diversity indicate moderate or poor status (Table 2).

The NEAT values based on the abundance of *Pontogammarus maeoticus* in impacted sites show in general poor status, both in winter and summer. In turn, non-impacted sites are in good status in summer (Table 2).

The results of multifactorial ANOVA showed that the values of total NEAT, Total Coliforms, Faecal Coliforms and *Staphylococcus aureus* were significantly lower in the impacted sites than in non-impacted ($p < 0.05$). Also, in winter NEAT values of total NEAT, abundance of *Pontogammarus maeoticus*, turbidity, TSS, and nitrate were significantly lower than summer ($p < 0.01$). Interaction between factors (affection and season) was significant in the total NEAT and Turbidity ($p < 0.05$) (Table 2).

4. Discussion

The most recent and comprehensive studies of the Caspian Sea show a degraded environmental status, because of multiple human activities and a lack of adequate management (Barannik et al., 2004; Stolberg et al., 2006; UNEP, 2011; Fendereski et al., 2014), The sub-region studied here (in the South-Western part of the Caspian Sea, in Iran, and close to the Azerbaijan border) is polluted by contaminant inputs from rivers such as Kura (in Azerbaijan, but close to the Iranian border) and Sefid-Rud (in Iran, Gilan), and intense human activities, with concentrations of arsenic, mercury, copper, nickel, chromium and DDT above the Effects-Range Low in sediments (UNEP, 2011). In addition, the discharge of nutrients and organic matter, both from urban discharges and agriculture, has resulted in a eutrophic status in the area (Leonov and Stygar, 2001; UNEP, 2011).

After the RDA, TSS and nitrite are significantly related to the ordination of the samples based on biological parameters. Moreover, TSS is correlated to seasonality, whereas nitrite correlates to impact. Nitrate, TSS and turbidity are usually related to rainfall, land drainage and fresh

Table 2
NEAT values and results of multifactorial ANOVA analysis, calculated for each indicator, integrating all of them (NEAT column), for each location and season, integrating all impacted and non-impacted sites, and for the Caspian Sea sub-region studied. The status class for the integrated NEAT is also shown. Key: Ab Po: Abundance of *Pontogammarus maeoticus*; TC: Total Coliforms; FC: Faecal Coliforms; EC: *Escherichia coli*; St: *Staphylococcus aureus*; H' PhP: Shannon index for Phytoplankton; H' ZP: Shannon index for Zooplankton; TSS: Total Suspended Solids; O2%: Oxygen Saturation; NO3: nitrate; NO2: nitrite; PO4: phosphate; COD: Chemical Oxygen Demand; BOD5: Biochemical Oxygen Demand; *p < 0.05; **p < 0.01. Grey cells show sites with quality status lower than Good (i.e. moderate, poor and bad); White cells show sites with at least Good quality status (i.e. good and high).

Spatial Assessment Unit	Status class	NEAT	Ab Po	TC	FC	EC	St	H'PhP	H'ZP	Turbidity	TSS	O2%	NO3	NO2	PO4	COD	BOD5
Caspian Sea	moderate	0.49	0.33	0.75	0.61	0.90	0.72	0.56	0.54	0.50	0.68	0.84	0.88	0.63	0.26	0.63	0.47
Impacted	moderate	0.41	0.22	0.59	0.46	0.81	0.59	0.54	0.51	0.53	0.72	0.87	0.86	0.57	0.26	0.68	0.53
Non Impacted	moderate	0.56	0.44	0.91	0.76	0.99	0.84	0.58	0.57	0.48	0.64	0.81	0.90	0.69	0.26	0.59	0.42
Impacted-S1-Winter	poor	0.28	0.01	0.00	0.17	0.79	0.49	0.16	0.48	0.43	0.60	0.97	0.76	0.78	0.52	0.85	0.64
Impacted-S2-Winter	moderate	0.41	0.15	0.94	0.68	0.93	0.46	0.50	0.54	0.37	0.51	0.82	0.83	0.62	0.52	0.87	0.69
Impacted-S3-Winter	moderate	0.50	0.35	0.81	0.54	0.96	0.57	0.57	0.50	0.37	0.51	0.88	0.58	0.78	0.52	0.85	0.68
Impacted-S4-Winter	moderate	0.42	0.13	0.63	0.48	0.70	1.00	0.82	0.56	0.53	0.74	0.98	0.60	0.62	0.52	0.95	0.87
Impacted-S5-Winter	poor	0.33	0.01	0.63	0.57	0.86	0.44	0.51	0.53	0.43	0.30	0.88	0.80	0.62	0.52	0.99	0.97
Impacted-S1-Summer	poor	0.33	0.13	0.41	0.30	0.75	0.58	0.42	0.43	0.59	0.89	0.83	1.00	0.59	0.00	0.37	0.22
Impacted-S2-Summer	moderate	0.41	0.27	0.47	0.34	0.20	1.00	0.51	0.45	0.59	0.88	0.87	1.00	0.73	0.00	0.41	0.26
Impacted-S3-Summer	good	0.63	0.61	0.78	0.57	1.00	0.60	0.57	0.58	0.70	0.94	0.67	1.00	0.54	0.00	0.63	0.43
Impacted-S4-Summer	moderate	0.44	0.32	0.59	0.46	0.91	0.14	0.92	0.49	0.56	0.93	0.90	1.00	0.08	0.00	0.50	0.31
Impacted-S5-Summer	poor	0.40	0.21	0.68	0.54	0.99	0.60	0.47	0.55	0.68	0.91	0.90	1.00	0.30	0.00	0.37	0.19
Non Impacted-S1-winter	poor	0.36	0.02	0.98	0.91	0.99	1.00	0.73	0.48	0.14	0.29	0.86	1.00	0.62	0.52	0.77	0.55
Non Impacted-S2-Winter	poor	0.30	0.01	0.93	0.65	1.00	0.53	0.62	0.58	0.11	0.25	0.80	0.75	0.78	0.52	0.44	0.28
Non Impacted-S3-Winter	poor	0.36	0.04	0.93	0.65	0.96	0.53	0.84	0.64	0.27	0.33	0.83	0.80	0.48	0.52	0.88	0.70
Non Impacted-S4-Winter	poor	0.35	0.03	0.97	0.86	0.98	0.53	0.40	0.59	0.19	0.30	0.82	0.74	0.89	0.52	0.85	0.66
Non Impacted-S5-Winter	good	0.69	0.67	0.75	0.51	0.98	1.00	0.48	0.53	0.47	0.60	0.89	0.71	0.89	0.52	0.93	0.84
Non Impacted-S1-Summer	good	0.64	0.61	0.95	0.78	0.99	0.91	0.60	0.48	0.60	0.90	0.81	1.00	0.73	0.00	0.40	0.23
Non Impacted-S2-Summer	good	0.69	0.72	0.75	0.84	0.99	1.00	0.39	0.52	0.68	0.89	0.85	1.00	0.54	0.00	0.43	0.26
Non Impacted-S3-Summer	good	0.72	0.73	0.98	0.90	1.00	1.00	0.38	0.79	0.73	0.95	0.84	1.00	0.89	0.00	0.37	0.19
Non Impacted-S4-Summer	good	0.61	0.61	0.85	0.56	1.00	1.00	0.49	0.62	0.59	0.92	0.54	1.00	0.61	0.00	0.37	0.19
Non Impacted-S5-Summer	high	0.85	0.98	0.98	0.92	1.00	0.91	0.92	0.47	0.99	0.98	0.83	1.00	0.42	0.00	0.43	0.26
Main effects	A:Affection	0.016*	0.1393	0.0038**	0.0013**	0.0578	0.0295*	0.6802	0.1247	0.3924	0.0845	0.1916	0.2474	0.1713	NA	0.1103	0.0855
	B:Season	0.0051**	0.0005**	0.9008	0.7982	0.5976	0.3871	0.9608	0.7872	0**	0**	0.1257	0**	0.0651	NA	0**	0**
Interactions	AB	0.0468*	0.0788	0.9770	0.4724	0.5067	0.2370	0.5055	0.6659	0.0174*	0.0601	0.9624	0.2474	0.4090	NA	0.7140	0.6913

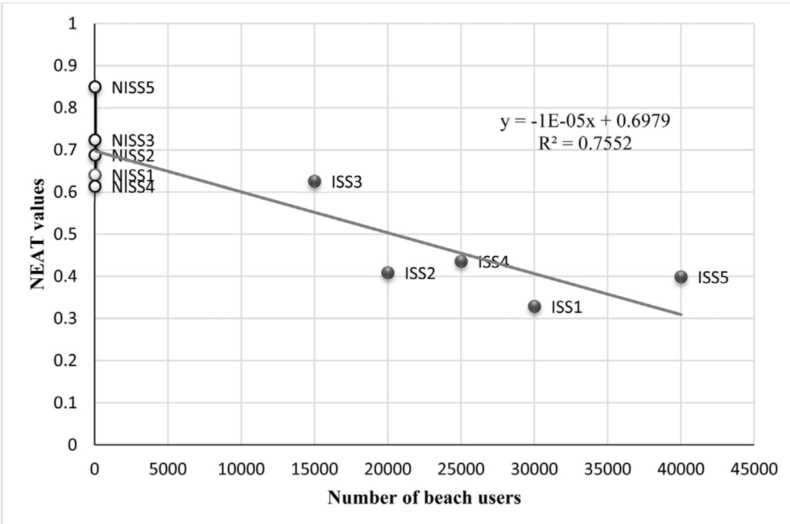

Fig. 4. Regression between the estimated number of beach users and NEAT values, in summer. ISS1 to 5 = Impacted Sites in Summer (black circles), NISS1 to 5 = Non-Impacted Sites in Summer (white circles).

water discharge, whilst nitrite and phosphate relate to deficient wastewater treatment (Turner et al., 2003; Borja et al., 2016c). Hence, the higher TSS and some nutrient values in winter could be related to the higher winter river runoff in the area (Kosarev, 2005), which is confirmed by the lower salinity values in winter. In turn, high nitrite concentrations could be responding to more intense wastewater discharges near impacted sites.

Most studies within the area show higher abundance of the planktonic community in summer than in winter (CEP, 2000), as shown also in our study. Although some studies found higher abundance in winter, the sampling depth was different (> 5 m) (Nasrollahzadeh et al., 2008; Nasrollahzadeh et al., 2014; Rowshan Tabari, 2013), being the swash zone (< 60 cm) never investigated before. Some of these differences could be explained also by higher TSS and turbidity values in the swash zone in winter.

In this study waters from areas impacted by bathing showed higher bacteria concentrations than non-impacted sites, in agreement with Binesh Barahmand et al. (2012), who found that total and faecal coliform concentrations were higher than standard levels in 24% of Gilan province bathing areas, being the most impacted sites they found the same we considered in our study. This can be related not only to the wastewater discharges near impacted sites, but also to the higher number of users, as we have found in this study.

In general, benthic communities are not very diverse in the Caspian Sea, with low richness (UNEP, 2011). In this study, *Pontogammarus maeoticus* was the only macroinvertebrate species in the sampling sites. On the Iranian coast of Caspian Sea *Pontogammarus maeoticus,* is the most abundant amphipod species, especially in the sublittoral parts of Gilan province (abundance > 90%) (Mirzajani, 2003; Mirzajani et al., 2011). Nemati et al. (2015) recorded more than 12 macroinvertebrate species in the southwest of Caspian Sea; however, the sampling depth was different (> 2 m). There is no previous record of the macroinvertebrate community structure in the swash zone of the southern part of Caspian Sea, but the presence of a single macroinvertebrate species could be an additional evidence of the alteration of the environmental status of this area, influenced by river runoff and intense human activities. However, we are aware that the swash zone tends to be poorly populated by benthic communities, and our interpretation could need further confirmation. One of the explaining activities can be the nourishment of bathing beaches, which is undertaken regularly in the studied sites, and has been described as a source of impact to benthic macroinvertebrates (Cooke et al., 2012; Schlacher et al., 2012; Vanden Eede, 2013; Wooldridge et al., 2016).

To assess the environmental status of any location, one of the most important tasks is to set targets and reference conditions (Borja et al., 2012). To set them, an important knowledge of the study area is necessary, together with long-term monitoring data of different ecosystem components (Borja et al., 2016b). However, the knowledge of the Caspian Sea, even having increased in recent years (Karpinsky et al., 2005; Barannik et al., 2004; Stolberg et al., 2006; UNEP, 2011; Fendereski et al., 2014), is still far from the minimum required to set adequate reference conditions and targets. Despite this, combining reference conditions from relatively comparable marine systems, the experience of local experts and some international legislation, targets and reference conditions have been set for the area, which have demonstrated to be adequate for a preliminary environmental assessment, in this case using NEAT. Thus, the general status in the area has been classified as moderate (i.e. less than good), meaning that management measures should be taken in the area to achieve good status (Borja et al., 2010).

Other important issue in the assessment of marine status, is the selection of indicators. This is mainly driven by the objectives of the assessment; however, in practice it is often constrained by the type of data available and sometimes on the knowledge at the time of monitoring (Teixeira et al., 2016; Uusitalo et al., 2016). Different indicators and aggregation can result in different assessment (Langhans et al., 2014). Although we used the best available data and indicators for the area, with further monitoring and increasing knowledge a re-evaluation of the initially indicators selected could be necessary.

The interaction between factors (affection and season) was significant in the total NEAT, probably because the complex relationships between multiple human activities and natural factors, such as runoff in winter, making difficult to quantify the multiple stress they can produce (Nõges et al., 2016). However, focusing only in summer (bathing season), NEAT was able to differentiate areas impacted and non-impacted by bathing, showing also the clear relationship between the level of human pressure (i.e. number of beach users) and the degradation of the sites. The areas non-impacted by bathing, which, in absence of additional pressures, should present NEAT values close to 1, show values in good status instead of high environmental status (see Fig. 4). This is indicating that there exists a general affection of the Caspian Sea by different human pressures, which can affect in the future some cultural ecosystem services, such as the provision of clean waters for summer bathing activity (Ghermandi et al., 2012). Hence, NEAT can be used not only for its primary development (i.e. application to the MSFD in Europe; Uusitalo et al., 2016), but also for new applications (i.e. detecting the status of bathing waters) outside Europe. In this case, combining multiple ecosystem components, including those traditionally used to assess the status of bathing waters, such as bacterial coliforms, NEAT has adequately determined the status, reinforcing the idea that multiple ecosystem components are more useful in assessing the ecological integrity than single components (Borja et al., 2009). This can assist policy-makers and managers in taking measures based upon an ecosystem approach to management (Borja et al., 2016b, 2016d).

5. Conclusions

The Caspian Sea, a landlocked water body, faces stress from anthropogenic activities. TSS values reflected the seasonality affecting biological data, whereas nitrite concentrations discriminated between impacted and non-impacted sites. NEAT indicated an alteration in sites impacted by bathing activity, with a global classification of moderate status for the region studied. However, an important effort should be done in increasing the knowledge of the Caspian Sea, from an environmental point of view, in order to improve the adequacy and accuracy of the preliminary boundaries used in this study, necessary for a future ecosystem based management.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2017.06.054.

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