

This discussion paper is/has been under review for the journal Biogeosciences (BG). Please refer to the corresponding final paper in BG if available.

Bio-geographic classification of the Caspian Sea

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Received: 29 January 2014 - Accepted: 4 March 2014 - Published: 20 March 2014

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Published by Copernicus Publications on behalf of the European Geosciences Union.

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Like other inland seas, the Caspian Sea (CS) has been influenced by climate change and anthropogenic disturbance during recent decades, yet the scientific understanding of this water body remains poor. In this study, an eco-geographical classification of the CS based on physical information derived from space and in-situ data is developed and tested against a set of biological observations. We used a two-step classification procedure, consisting of (i) a data reduction with self-organizing maps (SOMs) and (ii) a synthesis of the most relevant features into a reduced number of marine ecoregions using the Hierarchical Agglomerative Clustering (HAC) method. From an initial set of 12 potential physical variables, 6 independent variables were selected for the classification algorithm, i.e., sea surface temperature (SST), bathymetry, sea ice, seasonal variation of sea surface salinity (DSSS), total suspended matter (TSM) and its seasonal variation (DTSM). The classification results reveal a robust separation between the northern and the middle/southern basins as well as a separation of the shallow near-shore waters from those off-shore. The observed patterns in ecoregions can be attributed to differences in climate and geochemical factors such as distance from river, water depth and currents. A comparison of the annual and monthly mean Chl a concentrations between the different ecoregions shows significant differences (Kruskal-Wallis rank test, P < 0.05). In particular, we found differences in phytoplankton phenology, with differences in the date of bloom initiation, its duration and amplitude between ecoregions. A first qualitative evaluation of differences in community composition based on recorded presence-absence patterns of 27 different species of plankton, fish and benthic invertebrate also confirms the relevance of the ecoregions as proxies for habitats with common biological characteristics.

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The Caspian Sea (CS) is an enclosed water body that plays an important geopolitical role in the Central Asia region (Kosarev and Kostianov, 2005). During the last few decades, the joint action of natural and anthropogenic factors have been aggravating the environmental state in the CS (Kopelevich et al., 2004; Barale, 2008). Increasing human activities such as oil and gas industries, especially in the northern part of the CS, fisheries, agriculture and tourism (Kopelevich et al., 2004) together with decades of environmental mismanagement (Barale, 2008) have led to a severe degradation of the CS' water quality. The unintentional introduction of the ctenophore jellyfish *Mnemiopsis* leidvi in late 1999 (Shiganova et al., 2001) has added to the environmental problems. affecting a whole trophic level, since this organism feeds voraciously on zooplankton (Kidevs et al., 2008). The change in the trophic status of the CS from oligotrophic to eutrophic (Leonov and Stygar, 2001) is attributed partly to the effect of this jellyfish on the CS ecosystem (Saravi Nasrollahzadeh et al., 2008).

The inefficiency of management efforts focusing on single species only, or based on limited regions in marine environments is well known (Zacharias and Roff, 2000). For an ecosystem-based management approach, in which all the interactions between the CS ecosystem and its surrounding biotic and abiotic environment are considered (EPAP, 1996), managers need to deal with the challenge of mapping the physical and biological components of the whole ecosystem (Gregr and Bodtker, 2007). However, there is still no comprehensive archive of oceanographic information on the whole CS (Kosarev and Kostianov, 2005). The existing data on oceanographic observations in the CS are heterogeneously distributed in space and time and most of the observations stem from the northern and middle regions and are confined to the coastal parts of the CS. Moreover, most of the data date back to the Soviet era, not reflecting the substantial changes that have occurred since then (Kosarev and Kostianov, 2005).

In recent years, marine ecosystem classification has been greatly facilitated through the use of remote sensing techniques (Richardson and LeDrew, 2006; McDermid

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et al., 2005). Detailed broad scale spatial and temporal variability of several environmental variables, such as sea surface temperature and chlorophyll a concentration, can be captured by satellites (Robinson, 2004). Satellite data can be used for a biogeophysical classification of the CS, in a fashion similar to studies done in the European Seas (Hoepffner and DoWell, 2005), the North Atlantic (Roff et al., 2003; Devred et al., 2007), the North Pacific (Gregr and Bodtker, 2007; Kavanaugh et al., 2013), or the global ocean (Longhurst, 1998; Vichi et al., 2011; Spalding et al., 2012). In these studies, regions with similar environmental patterns are grouped into ecological units or so called "ecoregions". Each ecoregion may respond in a different way to environmental changes and management policies (Bailey, 1996).

The close association between the oceanographic and biological characteristics (Day and Roff, 2000) of the pelagic realm of the oceans has been described both for primary producers (e.g. Platt et al., 2005; Reynolds, 2006) as well as for the higher trophic levels of the food chain (e.g. Thrush and Dayton, 2010). The link between ocean physics and biology is of great significance, especially for the areas where the biological data are scarce. Different applications, such as the computation of primary production (Platt and Sathyendranath, 1999) or species habitat modeling (e.g. Valavanis, 2008; Irwin et al., 2012) have benefited from the association between physical/environmental factors and the biological responses. This association provided the basis for a number of studies in which bio-geographic maps of the oceans have been developed based on abiotic non-taxonomic oceanographic classifications (e.g. Dietrich et al., 1963; Longhurst, 1998; Day and Roff, 2000). Ecoregions reflect the diversity in physical habitat types and hence, indirectly, the range of conditions that influence species distribution and community composition in marine ecosystems (Bredin et al., 2001). These authors linked trawl surveys and observational data on a variety of rare and endangered fish and Cetacean species of the Bay of Fundy to the physicallydefined ecoregions for this area. They noticed the significance of some ecoregions for the distribution of specific species. Gregr and Bodtker (2007) assessed the biological relevance of their physical-based epi-pelagic ecoregions of the North Pacific

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using remote sensing measurements of chlorophyll a concentration (Chl a). Their results showed a significant difference in Chl a concentration between ecoregions and suggested the possibility of describing regions with different biological attributes using only physical variables.

In the present study, we developed an objective classification method to cluster the Caspian Sea into ecoregions based on a set of geo-physical data. The main objective of this study was to provide a map of the CS divided into different areas with homogeneous geophysical attributes, i.e., so called ecoregions to assist the effective management of the CS. We then tested for the biological significance of the obtained ecoregions using long-term satellite derived Chl a distribution and available species composition data. Throughout the manuscript, we use the terms ecoregion to imply a homogenous area of the CS with similar biogeophysical/environmental characteristics.

Methods

Study area

The CS (Fig. 1) is a land-locked water body with no major outlet to the ocean. Its surface area is about 371 000 km², with a length of about 1210 km and average width of 320 km from 37° N to 47° N and 47° E to 55° E (Barale, 2008). The CS is commonly divided into three basins, hereafter denoted as the northern Caspian Basin (NCB), the middle Caspian Basin (MCB) and the southern Caspian Basin (SCB), occupying 27%, 38 % and 39 % of the surface area of the CS, respectively. Progressing southward, the depth of the CS increases considerably, averaging from only about 5 m in the NC to 190 m in the MC and 330 m in the SC (UNEP, 2006). Around 60 % of the CS, mainly the area along the northern and eastern coasts, consists of shelf areas with less than 100 m depth (Fig. 1; Ibrayev et al., 2010). The Kara Bogaz Gol Bay, a hypersaline

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lagoon at the eastern slope of the CS Bay is not considered in this study (Kopelevich et al., 2008).

2.2 Data description

2.2.1 Abiotic environmental data for ecoregion classification

We identified 12 geo-physical variables as potential candidates for the ecoregion classification (Table 1). For five observables, we considered both the annual mean and their seasonal variations, forming 10 independent variables, while sea-ice and bathymetry constituted the remaining ones.

The satellite-based observables consisted of sea surface temperature (SST) from the AVHRR satellite, total suspended matter (TSM) from MERIS (Level-3 binned products), photosynthetic active radiation (PAR) from MODIS Aqua Level 3 products, and wind speed (WSP) from Quikscat. Following Bakun and Parrish (1991), we computed the wind generated turbulent mixing index as the cube of surface wind speed (WSP3). This represents the rate at which wind turbulent kinetic energy is injected into the ocean and becomes available for mixing the upper thermocline (Niiler, 1975). Bathymetry data based on interpolated echosounding measurements was extracted from the digitalized official CS navigation map (The Navigation Map, Navigation and Oceanography Department of the Defense Ministry of the USSR (DNO of the RF MD), scale: 1:1000000: 1987). We digitized monthly surface isohaline data of the CS to create sea surface salinity (SSS) maps (Kosarev and Tuzhilkin, 1995). Sea ice data, provided by the Centre for Ice Hydrometeorological Information at the Arctic and Antarctic Research Institute in St Petersburg (AARI) were used to create maps of percentage of sea ice coverage throughout the year (2004 to 2012). All data were interpolated onto a 0.1° longitude-latitude resolution grid, i.e. re-gridded onto a 120 x 98 grid with 11 760 pixels. The seasonal amplitudes of the physical variables, denoted here by DSST, DSSS, DTSM, DWSP, DWSP3 and DPAR, were computed by subtracting average values of the summer months (June, July, August) from those of the winter months (December, January, February).

2.2.2 Biological data for ecoregion validation

To validate the geo-physically based ecoregion classification, we used satellite chlorophyll data as well as in-situ species observations. The chlorophyll *a* concentration (Chl *a*) were obtained from ESA's GlobColour Project (http://www.globcolour.info/) as monthly mean standard mapped images with a spatial resolution of 4.6 km. The Glob-Colour data contain the merged product from three satellite sensors, namely SeaWiFS, MODIS-AQUA and MERIS (Maritorena et al., 2010). The satellite-derived Chl *a* concentration tends to be biased high in the CS (e.g. Kopelevich et al., 2004; Fendereski et al., 2010), but is unlikely to affect the relative spatial and temporal variability which is the critical property for the ecoregion classification (Kopelevich et al., 2004; Nezlin, 2005; Thomalla et al., 2011). We produced an annual mean climatology Chl *a* using data for the period January 2003 through December 2010 (Fig. 7), excluding data before 2003 in order to reflect the situation after the invasion of Mnemiopsis leidyi, which altered the Chl *a* pattern (Kopelevich et al., 2008).

For each ecoregion, in-situ observations on 36 different marine species of different trophic levels dwelling in whole water column were collected from caspianenvironment.org. The observations were based on the data from different cruises, compiled and mapped by the Caspian Environment Program (CEP) in 2002. We separated the species data into a water column group and into a benthic group, based on the observation that in shallow seas, the whole water column tends to be mixed, leading to only small differences in the vertical column (Nybaken, 2000). However, because of their high degree of dependency to topographic features, sediment particle size, etc. (Day and Roff, 2000), benthic species data were studied separately. Species for which sampling data were confined to a limited area in the CS were (also) discarded from further analysis. Finally, 27 species of one phytoplankton, two zooplankton, nine pelagic fish,

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Since abundance or biomass estimates were not available for all species, recorded observations were transformed into presence patterns, and subsequently grouped into ecoregions (Table S6). To this end, individual maps of species distribution throughout the CS were mapped onto the ecoregions. For each ecoregion, two vectors representing the presence of pelagic and benthic species were created. We defined presence of each species in ecoregions on the basis of the occurrence of their observational points on a given ecoregion. Species were assigned "1" for those ecoregions in which their observational points were solely located near/on the borders of that ecoregion. Ecoregions where the given species were not observed also assigned "NaN" for that species.

2.3 Selection of classification variables

A robust classification requires the input variables to be as independent as possible, which is often not the case with environmental data sets. To eliminate redundant (strongly correlated) variables (Raftery and Dean, 2006; May et al., 2011), the collinearity between the 12 potential input variables was investigated using Spearman's rank correlation coefficient (r_s ; Supplement, Sect. S1; Wheater and Cook, 2005). Variables were sorted and represented in a dendrogram, where the vertical axis represents the degree of similarity (σ) between variables based on their correlation coefficient ($\sigma = 1 - r_s$; Fig. 2; see Supplement, Sect. S1 for details).

We decided to cut the dendrogram at a dissimilarity of $\sigma = 0.25$ ($r_{\rm s} = 0.75$), which is a compromise between the retention of as many variables as possible and the avoidance of high correlations that would confound the interpretation of the resulting patterns (Fig. 2, dashed line on the dendrogram). From each branch, one representative variable was chosen as an independent variable. DSSS, TSM, Depth and DTSM were directly selected, while we chose ICE and SST from the two groups of highly correlated variables. A sensitivity test of classification output to the choice of representative

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Classification method

We followed a two step classification procedure, consisting of (i) a data reduction using self-organizing maps (SOMs) and (ii) a synthesis of most relevant features into reduced number of marine provinces using the Hierarchical Agglomerative Clustering (HAC) method. This two-step classification procedure has been successfully used in several previous studies (e.g. Saraceno et al., 2006; Leloup et al., 2007; Lachkar and Gruber, 2012).

Self-Organizing Maps (SOMs) 2.4.1

A batch-training algorithm was used to train the self-organizing maps (SOM toolbox package¹). Neurons were arranged on a hexagonal grid. This ensures that distance between neighboring neurons is the same in all directions. The strength of neuron connections was determined using a Gaussian neighborhood function, thus ensuring a smoother mapping and a higher generalization capability of the trained maps (Kohonen, 2000; Lachkar and Gruber, 2012).

The output of a self organizing neural network can be sensitive to the choice of additional training parameters such as the size of the map, i.e., the number of neurons of the map (e.g., Gonzalez et al., 1997). As yet, no standard, general principal exists for choosing these parameters (Chon, 2011; Lachkar and Gruber, 2012). To address this issue, the sensitivity of the SOM output to the neuron map size was quantified

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¹SomToolbox, Helsinky University of Technology available: www.cis.hut.fi/projects/ somtoolbox

2.4.2 Hierarchical ascending clustering (HAC)

We used the Hierarchical Agglomerative Clustering (HAC) method (Jain and Dubes, 1988) to cluster the resulting 400 neurons (Supplement, Fig. S3) into the major geophysical ecoregions of the CS in a bottom-up clustering procedure. A tree-like diagram (dendrogram) was used to display the HAC clustering (Supplement, Fig. S5). Cutting the hierarchical tree at different levels of similarity resulted in a different number of ecoregions. Since HAC is an unsupervised learning process, the appropriate number of classes was an unknown parameter that needed to be defined by the user.

2.4.3 Number of ecoregions

The choice of the final number of clusters highly affects the quality of the clustering (Hong et al., 2011). In this study, a 10-fold cross validation approach was conducted to determine the optimum number of ecoregions (De'ath and Fabricius, 2000). The data were divided into two unequal parts of 90 % (the training set) and 10 % (the validation set). The training data set was reduced to 400 classes (20 × 20 neurons) using the SOM (see Sect. 2.4.1 above). These 400 prototypes were further agglomerated using the HAC algorithm. Cutting the HAC dendrogram at different level of similarity for each cross validation fold, a total of 140 cross validation experiments were performed that clustered the 400 neurons into 2 to 15 ecoregions. Each observation from the validation set was then compared to the 400 neurons. The closest neuron on the map (using the Euclidean distance), also called best matching unit (BMU), was identified for each

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For each cross validation experiment, the clustering error was computed as the average distance of the validation observations to the center (average) of their respective ecoregions in the training set (Supplement, Sect. S4 for details). Final cross validation error for each experiment was calculated by averaging errors for all the ten given folds (Supplement, Table S3). The cross validation error was minimized with 11 ecoregions (Supplement, Fig. S4), which was identified as the optimal number of ecoregions in this study. Further analysis was performed to characterize the individual ecoregions. Since we were interested in large scale structures in the CS, we decided to absorb ecoregions that cover less than 3% of the CS into the statistically most similar neighboring ecoregion. This procedure resulted in a final classification of the CS into ten ecoregions.

We used a non-parametric, one-way analysis of variance by ranks (Kruskal–Wallis' H test) to test whether ecoregions differ in their environmental conditions (Zar, 1999). A significant result of the Kruskal–Wallis H test implies that at least one ecoregion differs from all others. We then applied a multi-step a posteriori pairwise testing procedure based on studentized range statistics (Dunn's procedure) to identify which ecoregions differ significantly from others (P < 0.05). Dunn's test is a non-parametric multiple comparison test, analogous to for example, a Tukey HSD test (Wheater and Cook, 2005).

2.5 Biological evaluation of the classes

To test whether physically different ecoregions may host different type of communities (Bredin et al., 2001), we compared the chlorophyll and ecosystem composition between ecoregions. Given the different nature of the data, two different methods had to be employed. For chlorophyll, a Kruskal–Wallis rank test was used to identify whether there are significant differences between the ten ecoregions in their spatial distribution of annual mean Chl a. Pair-wise comparisons of ecoregions were then conducted using the Dunn's test (see Sect. 2.4 above). For the community structure, the degree to

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which each pair of ecoregions were similar based on their pelagic and benthic species composition were measured using the Sørensen-Dice's similarity coefficient (Supplement, Sect. S5; Sørensen, 1948; Dice, 1948). This statistical measure for comparison of two samples was designed to compare presence-absence data. The Dice Coefficient ranges from 0 to 1, with 0 representing no overlap and 1 showing a complete agreement between two sets of samples, A and B. The resulting Dice coefficient matrix of all the potential pair-wise combinations of the different ecoregions were projected on a dendrogram (Fig. 10; see Supplement, Sect. S5 for details).

Results

Ecoregions of the CS

The ten identified geo-physically-based ecoregions form spatially coherent units and sub-divide the three basins in a natural manner (Fig. 3 and Table 2). Using names according to their geographical characteristics, we found five of the distinguished ecoregions in the NCB (NCB Ural Furrow (NCB-UF), NCB Western Shelf (NCB-WS), NCB Transition (NCB-T), NCB Easternmost Shelf (NCB-ES) and NCB River Outflows (NCB-RO)), three in the MCB (MCB Offshore (MCB-OS), MCB Transition (MCB-T) and MCB Coastal (MCB-C)) and two in the SCB (SCB Offshore (SCB-OS) and SCB Coastal (SCB-C)). In general, ecoregions in the NCB are smaller in size than those identified in the two other basins. The spatial coherence strongly support our classification method, as the geographical location of the individual pixels was not used in any steps of the classification procedure. Also a non-metric multidimensional scaling analysis (see Supplement, Sect. S6) supports our classification, but also highlights the existence of two clusters, i.e., one containing the ecoregions of the NCB, and the other containing those of the MCB and SCB.

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The maps of the environmental conditions that were clustered by our algorithm reveal how the different variables contributed to the classification. Most obvious is this with bathymetry, which separated the offshore MCB and SCB from the nearshore regions (Fig. 4). The mean conditions of the different variables differ substantially between the 10 regions (Fig. 6, Table S4) as revealed by a Kruskal–Wallis test (P < 0.05). Figure 5 depicts the results of the 45 pair-wise comparisons on six independent bio-geophysical variables for all ecoregions (Dunn's test; see Sect. 2.4; Wheater and Cook, 2005). For each variable, the same colors were assigned to those ecoregions that did not show significant differences in a given variable (P < 0.05). Overall, we failed to reject 7 of the 45 pair-wise comparisons for SST, 10 for DSSS, 12 for Depth, 5 for TSM, 7 for DTSM, and 12 for ICE (P > 0.05). Except for a few cases, significant differences between ecoregions the NCB and those in the MCB and SCB were detected for all six environmental variables.

3.2.1 Ecoregions in the MCB (MCB-OS, MCB-C and MCB-T) and SCB (SCB-OS and SCB-C)

Ecoregions located in the MCB and SCB are characterized by higher SST, but lower DSSS and TSM than ecoregions located in the NCB (Fig. 6 and Table S4). Ecoregions in the MCB and SCB differ significantly between each other in all of the environmental variables, except for ICE (P < 0.05; see Fig. 5). None of the ecoregions in the SCB and MCB are covered by sea ice (Fig. 6f). The exception is MCB-T in the northern part of the MBC which is (partly) ice covered during winter (Figs. 4f and 6f and Table S4).

Only MCB-OS and SCB-OS are characterized by open water areas, since 62 % of the CS consisted of the continental shelf (Fig. 6c; Ibrayev et al., 2010). These ecoregions are significantly deeper than other ecoregions in the MCB and SCB, located in the continental shelf of the CS (P < 0.05; Figs. 6c and 5 and Table S4). In terms of their annual mean SST, ecoregions in the SCB have significantly higher annual mean SST

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than other ecoregions in the CS (Figs. 5 and 6a and Table S4). This is because CS shows a large north-south gradient in SST during winter, while in summer there are no major differences in the SST throughout the CS (Ibrayev et al., 2010). In general, in the MCB and SCB, salinity is as high as 10–13 psu in ecoregions (Tuzhilkin et al., 2005), and these regions show significantly lower seasonal variabilities in their SSS (DSSS; Figs. 5 and 6b and Table S4). The elevated SSS values in summer compared to winter in ecoregions in these two basins (Fig. 6b and Table S4) can be attributed to their distance from major river outflows and higher rate of evaporation in summer. In the MCB and SCB, TSM in the continental shelf regions (MCB-C and SCB-C) is higher than in deep open waters (MCB-OS and SCB-OS, Figs. 5 and 6d and Table S4). However, in all of these ecoregions, TSM is higher in winter than in summer (Fig. 6e), with the highest variability in MCB-C.

3.2.2 Ecoregions in the NCB (NCB-UF, NCB-W, NCB-T, NCB-E, and NCB-RO)

Ecoregions located in the NCB are characterized by lower SST and higher DSSS and TSM than ecoregions located in the MCB and SCB. They are similar with each other in almost all of the environmental variables (Fig. 5 and Table S4). All of the ecoregions located in the NCB are covered in ice during winter. The percentage of ice cover in NCB-T is lowest compared to other ecoregions in the NCB (P < 0.05; Figs. 5 and 6f and Table S4). Other ecoregions in the NCB do not show significant differences in this variable (P < 0.05; Fig. 5).

The NCB has no open ocean ecoregion, as the entire NCB consists of continental shelf with an average water depth of only 5 m (Barale, 2008). In the NCB, SST is highest in the southern part (NCB-T) and lowest in the eastern part (NCB-ES; Table S4). Due to the spring floods from the Volga and to a lesser extent from Ural River to ecoregions in the NCB (Kosarev, 2005; Kara et al., 2010), high negative values for DSSS are observed in all ecoregions of the NCB (Fig. 6b). In NCB-WS, NCB-T and NCB-RO in the western part of the NCB, DSSS is significantly higher than those in the eastern part of this basin (NCB-UF and NCB-ES, P < 0.05; Fig. 5 and Table S4). Seasonal

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variability in SSS decreases southwards in the western part of the NCB (Table S4). In general, TSM in the shallow NCB was significantly higher than in the two other basins (P < 0.05; Figs. 6d and 5 and Table S4). Similar to SSS, distance from major river outflows was important in determining the patterns in TSM and its seasonal variation. In the NCB, TSM was higher in NCB-WS, NCB-ES and NCB-RO, while NCB-WS showed much smaller seasonal variations in comparison with the other two (Figs. 5 and 6e and Table S4).

Biological validation of ecoregions

To test the biological significance of ecoregions, we compared annual mean (2003-2011; Fig. 7) and monthly mean Chl a between the different ecoregions. We also attempted a first qualitative evaluation of differences in ecosystem composition based on recorded presence-absence patterns of 27 different species of plankton, fish and invertebrates.

3.3.1 Annual mean Chl a distribution in each ecoregion

Based on a Kruskal-Wallis test, the ten ecoregions are significantly different from one another in terms of their ChI a distributions (P < 0.05). This was supported by a Dunn's multiple comparison test, whose results are depicted in Fig. 8.

In general, only eight of the 45 pair-wise comparisons do not show significant differences in annual mean Chl a concentration (P > 0.05). However, results show significant differences in the annual mean Chl a concentration between ecoregions of the NCB and those of the MCB and SCB (Fig. 8). The highest Chl a concentrations are observed in the northern ecoregions of the NCB, i.e. NCB-WS and NCB-RO (P < 0.05; Fig. 8). These ecoregions are close to the mouth of the Volga River which is the main source of nutrients supply in the CS (80% of nutrients/river runoff; Tuzhilkin et al., 25 2005). High Chl a concentrations in the NCB are due to the high amount of nutrients supplied by the Volga and Ural Rivers (Tuzhilkin et al., 2005). On the west side of the

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NCB, surface Chl a concentrations gradually decrease southward. A pronounced eastwest and south-north increase in ChI a concentrations is also observed with increasing distance from the Volga River, so that the lowest Chl a concentration in the NCB is observed in ecoregions in the eastern (NCB-UF and NCB-ES) and southern part of this basin (NCB-T; Table S5). In the MCB and SCB, ecoregions located on the continental shelf (i.e. MCB-C and SCB-C) do not show significant differences in their annual mean Chl a distributions, but are statistically different from ecoregions comprising the open waters of these two basins (i.e. MCB-OS and SCB-OS, P < 0.05; Fig. 8). For open waters ecoregions of the MCB and SCB, MCB-OS has the lowest and SCB-OS the highest annual mean Chl a concentrations (Table S5).

Ecoregions were then analyzed in terms of the initiation, duration, and amplitudes of the phytoplankton bloom. The initiation of the net growth phase date was defined as the period of the year when an increase in Chl a concentration values was observed relative to the annual median (Thomalla et al., 2011). Two distinct periods are observed throughout the CS: a period of the net growth or bloom phase (bloom phase) in summer and fall and a period with low Chl a concentration during winter and spring (Fig. 9). In the NCB, the period of low Chl a concentrations during winter coincided with the period of formation of sea ice in that area, while in the two other basins, this period could be explained by lower temperature and light levels limiting phytoplankton growth (Reynolds, 2006). Since the last decade, phytoplankton biomass in the CS has been controlled by the invasive jellyfish, M. leidyi. This jellyfish causes phytoplankton biomass to increase through a top-down control on zooplankton biomass (Kideys et al., 2008). Decrease in jellyfish biomass during winter relieves zooplankton from the intensive grazing pressure and results in a low phytoplankton biomass (notice a decreasing trend in phytoplankton biomass in all of the ecoregions in the CS in winter; see Fig. 9).

Significant differences in the date of bloom initiation, its duration and amplitude were found between ecoregions (Fig. 9). In the NCB, the bloom starts between May (in NCB-

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Seasonal variations in Chl a concentration in ecoregions

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WS; Fig. 9b) and July (NCB-RO; Fig. 9a), which could be explained by the increase in temperature and light, in combination with the inflow of nutrient-rich waters after the ice melt in April and May, and spring floods of the Volga River between May and June (Kosarev, 2005). In all of the ecoregions in the NCB, the Chl a reach peak concentration 5 in July. The exception being NCB-WS, where phytoplankton biomass is low year-round and only shows a very small bloom in August and September (Fig. 9 and Table S5). Chl a concentrations in NCB-UF (Fig. 9c), NCB-WS and NCB-RO peak once more in November. The duration of the phytoplankton bloom in the NCB differs between ecoregions, lasting from five months in NCB-T (Fig. 9e) and NCB-RO (Fig. 9a) to seven months in NCB-UF (Fig. 9c) and NCB-WS (Fig. 9b; see Table S5). In the continental shelf of the SCB (SCB-C; Fig. 9i) and MCB-T (Fig. 9f), the bloom is initiated in July, while in the continental shelf of the MCB (MCB-C; Fig. 9g), the bloom starts in August. The bloom periods in open waters of the MCB and SCB (MCB-OS and SCB-OS; Fig. 9h and j, respectively) initiates with a delay of one month in comparison with the continental shelf part of the given basin (i.e. in September in MCB-OS and in August in SCB-OS) and open water blooms last shorter than those in shelf areas (Fig. 9). Within all ecoregions in the MCB and SCB, ChI a shows its maximum amplitude from the median in October, the exception being SCB-C, where the peak is observed earlier, in August. The increase in phytoplankton biomass in the MCB and SCB during summer, i.e. when a deep thermocline limits phytoplankton growth in these regions (Tuzhilkin and Kosarev, 2005) was explained by the increase in the biomass of M. leidyi with increasing SST (Kideys et al., 2008).

3.3.3 Differences in ecosystem composition between ecoregions

Based on the species data discussed in Sect. 2.5, we investigated differences in species composition between ecoregions. Almost all of the species under study were found in MCB-T, and most of the species are observed in NCB-T. These ecoregions act as a transition zone between the NCB and the MCB (Polovina et al., 2001).

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The agreement between each pair of ecoregions based on their pelagic and benthic species composition was measured using Dice similarity coefficient (see Methods, Sect. 2.5; Sørensen, 1948; Dice, 1948; Wheater and Cook, 2005). Figure 10 shows the dendrograms used to visualize the degree of similarity/dissimilarity between ecoregions based on their pelagic and benthic species composition data.

For pelagic species, NCB-RO differs to a great degree from other ecoregions in the CS. The other ecoregions split into two major groups: a group of ecoregions from the NCB (NCB-ES, NCB-WE, and NCB-UF) and a group of ecoregions from the SCB, MCB and southern part of the NCB (NCB-T, MCB-T, MCB-C, MCB-OS, SCB-C, and SCB-OS), which is visualized in the dendrogram in Fig. 10a. This is in accordance with the results of our physical classification, where ecoregions from the MCB and SCB were more similar in their physical attributes to each other than to those from the NCB (Fig. S6). The planktonic species in this study, i.e. R. *fragilissima*, E. *grimmi*, and *M. leidyi* and the brackish water fish subspecies, *Salmo trutta caspius* (Caspian brown trout) and *Clupeonella engrauliformis* were not observed in any of ecoregions in the NCB, except for NCB-T (Table S6). The presence of these species is known to be restricted by low salinity, which is a prominent feature of the NCB (CEP, 2002; Kara et al., 2010).

In ecoregions in the MCB, SCB and NCB-T, a higher number of species had been observed than in the other ecoregion of the CS. All planktonic species were observed in these ecoregions, but only in NCB-T, MCB-T, and MCB-C all the pelagic fish species in this study were observed (Table S6).

Based on their benthic species distribution, ecoregions could be grouped into three groups: NCB-ES and NCB-RO from the NCB, MCB-OS and SCB-OS from the deep open waters, and other ecoregions from all the three basins (i.e. NCB-UF, NCB-WS, NCB-T, MCB-T, MCB-C, and SCB-C; Fig. 10b). These results were not consistent with the physical classification results, where a strong separation was observed between ecoregions in the NCB and those in the MCB and SCB (Fig. 5). This is because other factors than the physical ones considered here control the distribution of ben-

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In this study, the CS was partitioned into ten distinct ecoregions using the annual mean climatologies and seasonal amplitudes of physical oceanographic variables together with bathymetry. Ecoregions are geographic regions with specific biotic and abiotic attributes (Day and Roff, 2000). Although the geographical coordinates of individual grid cells have not been included in the classification, the partitioning resulted in coherent, spatially uniform ecoregions. Ecoregions vary in size, with smaller ecoregions located in the NCB, which shows a higher spatial variability in the input variables. The MCB and SCB are relatively uniform containing three and two large ecoregions, respectively. In agreement with our findings, a greater level of biodiversity has also been reported for the northern part of the CS in comparison with the two other basins (UNEP, 2006).

4.1 Biological validation of CS ecoregions

Chl *a* is one of the few biological indicators that can be detected by satellite observations. The overall agreement between spatial distribution of Chl *a* in the CS and patterns captured in our classification indicates a biological significance of our ecoregions in terms of bulk phytoplankton biomass (Gregr and Bodtker, 2007). Furthermore, we found differences in the temporal variabilities in Chl *a* between ecoregions in terms of the date of bloom initiation, duration and amplitude. Differences between the NCB, MCB and SCB in terms of their Chl *a* seasonal variabilities have previously been documented in the literature (e.g., Kopelevich et al., 2004; Nezlin, 2005). Studies in other

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ocean regions confirm that seasonality of ChI a concentration varies between ecoregions (e.g. Vantrepotte and Melin, 2009 in the World's ocean and Thomalla et al., 2011 in the Southern Ocean). This implies that the seasonality in the phytoplankton biomass dynamics in ecoregions is mainly controlled by seasonality in the physical forcing (i.e. bottom-up control; Platt and Sathyendranath, 1999). Three major factors controlling phytoplankton growth, i.e. river runoff as a major source for nutrients, sea surface temperature, and wind energy (Nezlin, 2005), have been directly or indirectly, via depth, SSS and TSM, used in this classification. Since we use climatologies of the input physical variables, we are unable to capture inter-annual variabilities or long-term trends. Our ecoregions reflect persistent broad-scale patterns in the environmental parameters that influence phytoplankton growth in the CS.

Despite the observed consistency between Chl a and geo-physically-derived ecoregions, phytoplankton growth is also controlled by other natural and anthropogenic topdown and bottom-up factors, such as pollution, nutrient loading, grazing and overfishing. One of the most important of these natural controllers in the CS is M. leidyi (Kideys et al., 2008; Kopelevich et al., 2008). Since the invasion of this jellyfish, anomalous algal blooms (AABs) have been a common feature in satellite-based ChI a images in the late summer and the early fall in the SCB (Kopelevich et al., 2004, 2008; Nezlin, 2005). Although we have not used any data of the jellyfish distribution in our classification, the area where AABs are frequently reported has been separated in our classification (SCB-OS). This is in spite of the fact that some of the physical input variables lie in periods before the jellyfish invasion in the CS in 1999 (Shiganova et al., 2001). This may indicate that the physical forcing also controls jellyfish distribution patterns.

Hypothesizing that ecologically different regions support different species assemblages, we show that individual ecoregions show differences in species composition. Other studies, such as those presented by Bredin et al. (2001) in the Bay of Fundy and Verfaillie et al. (2009) in the Belgian part of the North Sea, have demonstrated a similar result. The observed consistency gives value to the ecosystem classification approach for its potentiality in developing management monitoring, especially in the areas where

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biological data are scarce. Due to a lack of comprehensive species data in the CS, our observations are limited to a small number of species and also to periods before the invasion of *M. leidyi*. Hence, updated information on the variety of species in the CS and its individual ecoregions would be useful to better quantify differences in their species composition.

4.2 Caveats of our approach

In analogy to persistent large-scale ecological patterns in terrestrial ecosystems (Bailey, 1996), we hypothesized that there are persistent ecological patterns in the oceans that can be summarized in a single classification based on annual mean and seasonal climatologies. Our static classification method, similar to the approach presented by Longhurst (1998) for classification of pelagic realm of the world ocean, has been criticized for its inability to reflect the highly dynamic nature of the oceans (Hardman-Mountford et al., 2008). Dynamic methods for delineation of ecological units in the oceans have been developed to address this issue (e.g. Devred et al., 2007; Gregr and Bodtker, 2007; Kavanaugh et al., 2013). The classification approach presented in this paper provides a description of the large-scale patterns of the CS ecosystem, which will be useful for many administrative purposes, such as longer-term planning and policy development (Spalding et al., 2012), management reporting and socio-economic statistics (Hoepffner and Dowell, 2005). However, for shorter-term and local applications, seasonal or even real-time classification methods, like those presented by Devred et al. (2007) and Gregr and Bodtker (2007), may result in a more detailed characterization.

We developed an objective classification approach in which physical input variables were selected based on statistical analyses. To be able to capture as many potential environmental patterns as possible (Verfaillie et al., 2009), we initially compiled a wide range of abiotic variables, known to be important for the phytoplankton growth (Fig. 2). Finally, we used an independent set of these variables for ecological partitioning of the CS. Despite the objective criteria applied here for the selection of input variables (see

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Sect. 2.3), we inevitably had to make subjective choices due to methodological constraint: the desired level of independency between input variables had to be specified, and representatives of each cluster/set of dependent variables had to be selected. In order to test the sensitivity of our results to these choices, we performed several sensitivity studies. The tests showed a minor effect of this subjective selection on the final classification output of the CS (see Supplement, Sect. S2). Investigating the sensitivity of classification to different levels of independence between environmental input variables was beyond the scope of the current study and should be considered in future studies.

In this study, we mainly used remotely-sensed physical and biogeochemical input variables for our classification. In addition to the factors investigated here, other factors that have been shown to exert a significant influence on the CS ecosystem composition, but could not be included in this analysis. These factors include human activities, such as pollution and overfishing (Kopelevich et al., 2004; Zonn, 2005), and other endogenous and exogenous natural factors, such as climate change, sea level fluctuations, mud volcanoes (Zonn, 2005) and especially the invasion of M. leidyi (Shiganova et al., 2001). Since Chl a is a proxy for the cumulative effect of both bottom-up and topdown controls, a classification of the CS based on Chl a only may yield ecoregions with a higher biological relevance than those obtained here based on physical parameters (Hardman-Mountford et al., 2008). Since we were interested in mechanistic relationships between physical forcing and biological response, we did not use Chl a as an input variable here for the classification.

Our ecoregion validation in terms of their biological composition confirmed to some extent the validity of the assumption of bottom-up control on species distribution patterns. However, due to the limited availability of in situ data, our biological validation of the ecoregions remains qualitative at best. A systematic data compilation for all major marine organisms inhabiting the CS, along with in-situ ChI a concentration would facilitate the determination of the ecological relevance of ecoregions based on physical drivers. Satellite can now detect phytoplankton functional groups from space (Raitsos

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In this paper, the Caspian Sea was partitioned into ten distinct ecoregions with similar annual mean climatologies and seasonal amplitudes of physical oceanographic variables using a neural network approach. The biological relevance of these ecoregions was verified using long-term satellite derived ChI *a* concentration and species distribution data. The results of the current research can assist policy makers and managers with a general outlook on the different major ecosystems of the surface waters of the CS. Researchers can benefit from these results in applications ranging from ecosystem conservation, sampling area selection and ecosystem modeling.

The approach developed in this paper is flexible in terms of input variables and spatial and temporal resolution and extent of the study area and observational data records. The method can be employed on updated datasets for improving the ecological classification of the CS. The method can also be applied in more specific applications in shorter-term or real-time scales and in a variety of spatial scales in favor of the objectives.

Supplementary material related to this article is available online at http://www.biogeosciences-discuss.net/11/4409/2014/bgd-11-4409-2014-supplement.pdf.

Acknowledgements. Forough Fendereski thanks the Iranian Ministry of Science and the Environmental Physics Group at the Institute for Biogeochemistry and Pollutant Dynamics at the Swiss Federal Institute of Technology for the funding for this project. Meike Vogt, Zouhair Lachkar and Nicolas Gruber acknowledge funding from ETH Zurich. Forough Fendereski

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Table 1. List of the potential variables used, their units, resolution and sources.

Variable	Sensor/providor	Units	Spatial resolution	Temporal extent	Source
Sea Surface Temperature (SST)	AVHRR	°C	0.08789°	1985-2003	http://apdrc.soest.hawaii.edu
Total Suspended Matter (TSM)	MERIS	gm^{-3}	0.04166°	2003-2010	http://www.globcolour.info/
Photosynthetic Active Radiation (PAR)	MODIS Aqua	Einstein m ⁻² day ⁻¹	0.04°	2003-2011	http://oceancolor.gsfc.nasa.gov
Wind Speed ¹ (Turbulence) (WSP)	Quikscat	m/s	0.25°	2000-2007	http://apdrc.soest.hawaii.edu
Bathymetry (Depth)	DNO of the RF MD	m	_	_	http://www.Caspianenvironment.org
Sea Surface Salinity (SSS)	CASPCOM ²	ppt	_	1995	http://www.Caspcom.com
Sea Ice (ICE)	AARI	%	_	2004-2012	http://wdc.aari.ru/datasets/

¹ QuikScat data are produced by Remote Sensing Systems and sponsored by the NASA Ocean Vector Winds Science Team. Data are available at www.remss.com.

² Committee on Hydrometeorology and Pollution Monitoring of the Caspian Sea.

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Ecoregion	Acronyms	Characteristics
NCB River Outflows	NCB-RO	Shallow, dominated by the Volga River outflows, high in SSS seasonality, high in TSM and its seasonality, covered in ice in winter
NCB Western shelf	NCB-WS	Shallow, low in SSS and high in its seasonality, high in TSM, covered in ice in winter
NCB Ural Furrow	NCB-UF	Shallow, low in SST, low in SSS and its seasonality, covered in ice in winter
NCB Easternmost shelf	NCB-ES	Shallow, low in SST, low in DSSS, high in TSM and its seasonality, highly ice covered in winter
NCB Transition	NCB-T	Shallow, high in SSS seasonality, partly ice covered in winter
MCB Transition	MCB-T	Medium SST, medium DSSS, medium TSM and DTSM, partly ice covered in winter
MCB Coastal	MCB-C	Continental shelf area, High in SSS and low in DSSS, low in TSM and its seasonality
MCB Offshore	MCB-OS	Deep, low in SST, low in TSM and its seasonality
SCB Coastal	SCB-C	Continental shelf area, High in SST and SSS, low in TSM and its seasonality
SCB Offshore	SCB-OS	Deep, high in SST, low in TSM and its seasonality

Table 2. Ecoregions and their characteristics in terms of the environmental variables.

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Table A1. Acronyms.

CS	Caspian Sea
SCB	South Caspian Basin
MCB	Middle Caspian Basin
NCB	North Caspian Basin
SCB-C	South Caspian Basin Coastal
SCB-OS	South Caspian Basin Offshore
MCB-C	Middle Caspian Basin Coastal
MCB-OS	Middle Caspian Basin Offshore
MCB-TR	Middle Caspian Basin Transition
NCB-TR	North Caspian Basin Transition
NCB-RO	North Caspian Basin River Outflows
NCB-WS	North Caspian Basin Western Shelf
NCB-ES	North Caspian Basin Easternmost Shelf
NCB-UF	North Caspian Basin Ural Furrow



Fig. 1. Map of CS showing the three sub-regions, the major rivers (dashed lines) and bathymetry (depth in meter).

Ural

Kazakstan

KaraBogazGol

Turkmenistan

54°0'0"

Ural

Furrow

Middle

Caspian Sea

South/

Caspian Sea

51°0'0"

U. . . .

Caspian Sea

50

100 300 500

700

Volga

10

Azerbaijan

Iran

48°0'0"

Russia

45°0'0"

42°0'0"

39°0'0"

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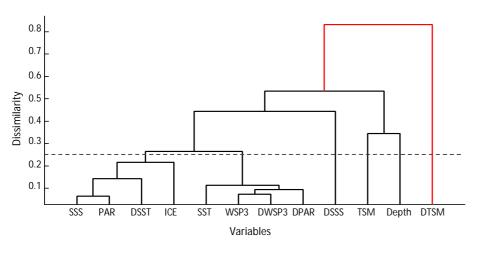


Fig. 2. Dendrogram of all the potential variables correlation matrix (annual means). The dashed line shows the sensitivity at which the dendrogram was cut. The abbreviations can be found in Table 1, with those start with "D" indicate seasonal variabilities.



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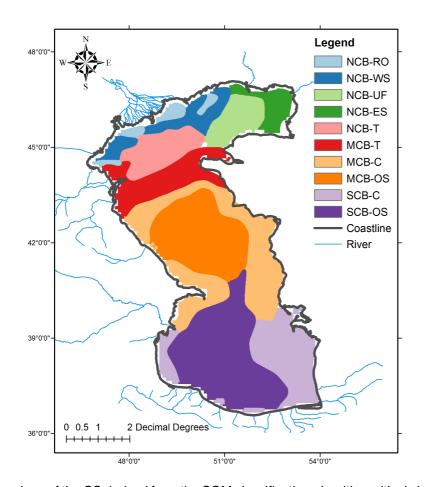


Fig. 3. Ecoregions of the CS derived from the SOM classification algorithm with six independent clustering variables (SST, ICE, DSSS, Depth, TSM and DTSM).

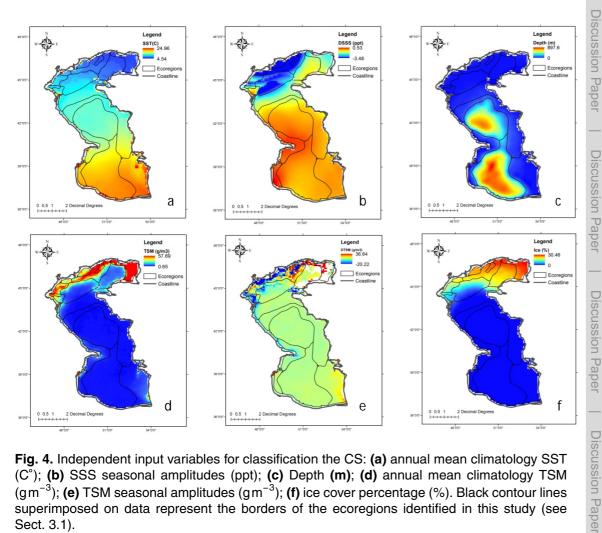


Fig. 4. Independent input variables for classification the CS: (a) annual mean climatology SST (C°); (b) SSS seasonal amplitudes (ppt); (c) Depth (m); (d) annual mean climatology TSM (gm⁻³); **(e)** TSM seasonal amplitudes (gm⁻³); **(f)** ice cover percentage (%). Black contour lines superimposed on data represent the borders of the ecoregions identified in this study (see Sect. 3.1).

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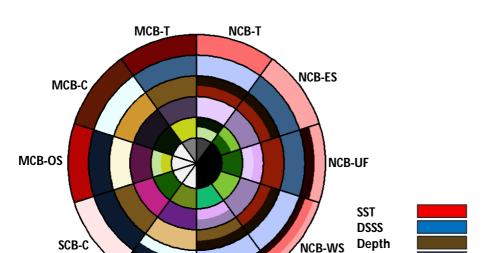


Fig. 5. Multiple comparison test between different ecoregions (1-10) for each physical variable; For each of the input variables, the degree of similarity in the saturation of the colors reflect the degree of similarity between the different ecoregions. Circles from outside: SST (red), DSSS (blue), Depth (brown), TSM (purple), DTSM (green), Sea Ice (grey). Circle segments divided into several rings with different colors depict variables that do not show significant differences (P < 0.05) to those of ecoregions with the corresponding colors.

NCB-RO

SCB-OS

TSM DTSM ICE **BGD**

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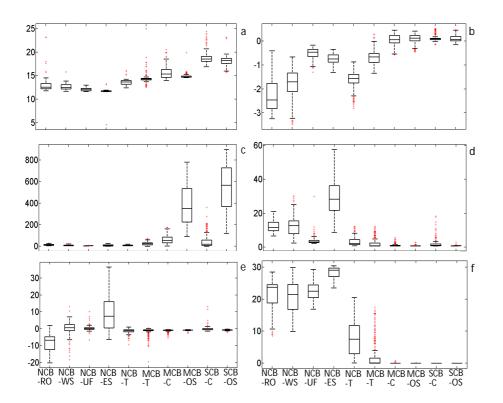


Fig. 6. Box plots of ecoregions (x-axis) against the physical variables (y-axis) (a: SST, b: DSSS, c: Depth, d: TSM, e: DTSM, and f: ICE). The line in the middle of each box is the ecoregion median. The tops and bottoms of each box are the 25th and 75th percentiles, respectively. The lines extending above and below each box, i.e. whiskers, have been drown from the ends of the inter-quartile ranges to the furthest observations within the whisker length. Red + signs are observations beyond the whisker length which are marked as outliers. Notches display the variability of the median between ecoregions. Box plots whose notches do not overlap have different medians at the 5% significance level.

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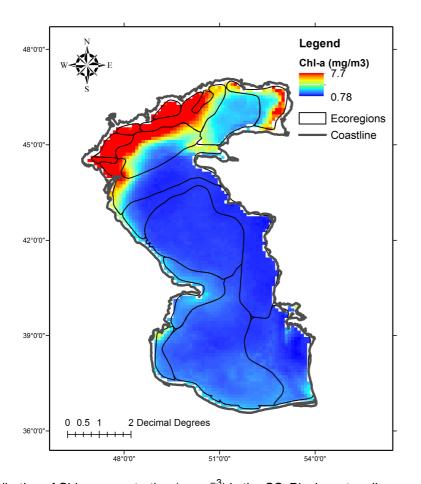


Fig. 7. Distribution of Chl a concentration (mg m⁻³) in the CS. Black contour lines superimposed on data represent the borders of the ecoregions identified in this study (see Sect. 3.1).



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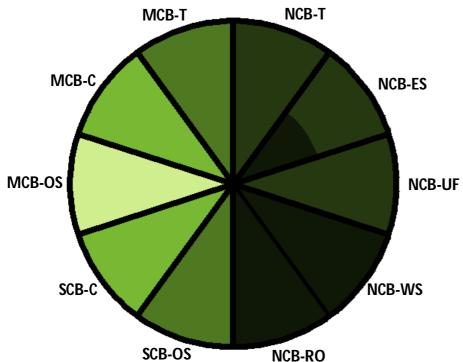


Fig. 8. Multiple comparison test result for annual mean Chl a concentration between different ecoregions (1-10). Semi-circles represent different ecoregions. There are no significant differences between semi-circles with the same color in their Chl a distribution (P < 0.05).

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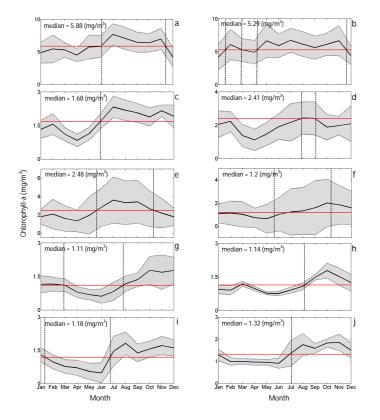


Fig. 9. Seasonal cycle of Chl a concentration from January 2003 to December 2010 in the ten ecoregions. (a) NCB RO, (b) NCB WS, (c) NCB UF, (d) NCB ES, (e) NCB T, (f) MCB T, (g) MCB C, (h) MCB OS, (i) SCB C, and (j) SCB OS. The red line represents the median of annual mean climatological Chl a in each ecoregion. The period when Chl a values are above the median was defined as the bloom phase. The dashed lines depict the duration of bloom in each ecoregion. The dashed blue line represents the spatial standard deviation of ChI a in each ecoregion.

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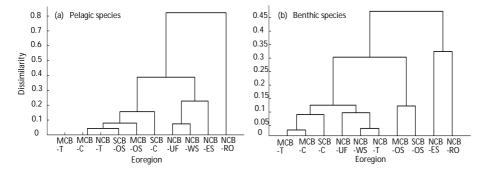


Fig. 10. Dendrogram of the similarity between ecoregions based on the available **(a)** pelagic and **(b)** benthic species composition data. Labels along the x-axis represent the ecoregions.

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