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Research Article

Predict the effects of climate change on primary production and vulnerability of fisheries species in coastal waters of the northern Persian Gulf

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Abstract

The objective of this study was to evaluate the effects of predicted climate changes on primary production and vulnerability of fish species in coastal waters of the northern Persian Gulf. Remote sensing data including chlorophyll a and sea surface temperature (SST) through using MODIS sensor images of the Terra satellite were obtained from the Google Earth Engine system. Net primary production per day was estimated through the VGPM model from the NASA Oceanographic Database (2000-2022). To predict climate change in The LARS-WG microscale model that is compared and evaluated under three scenarios RCP2.6, RCP4.5, and RCP8.5 during 2020-2080. These scenarios predict an increase in the average annual temperature rise of 2.4 to 5.3°C for the future of the region. It showed the inverse correlation between SST and chlorophyll a, and a direct correlation between primary production rate and marine trophic index. The annual comparison of total commercial fisheries catches shows that fish catch will decrease by 169, 185, and 386 kg in the RCP 2.6, 4.5, and 8.5 scenarios, respectively. Correlation of the target species from the total catch with primary production shows that demersal fish species such as *Pomadasys kaakan*, *Glaucostegus granulatus*, *Otolithes ruber*, *Atule mate* and large pelagic such as *Planiliza subviridis*, *Auxis thazard*, *Tenualosa ilisha*, *Thunnus tonggol* and small pelagic such as *Liza klunzingeri* species and also jellyfish group express a positive relationship which is an indication of increased vulnerability to climate changes compared to other fish species.

Introduction

Climate change is the most widespread threat to ocean ecosystems (Halpern *et al.*, 2008). As a result, oceans are becoming warmer and more acidic (Pachauri and Reisinger, 2007). Climate change also alters nutrient concentrations (Behrenfeld *et al.*, 2006). These environmental changes will directly affect the physiology of marine organisms (Pörtner and Farrell, 2008) and their population dynamics (Harley *et al.*, 2006). Changes in the biomass of a species alter ecological relationships and indirectly affect the entire webs of marine food chains (Schefferet *et al.*, 2005). Therefore, climate change affects all marine organisms and changes the composition of marine communities and ecosystem functions. Understanding the effects of climate change on marine food webs informs measures that help maintain biodiversity and support sustainable fisheries in the future. The largest impact of climate changes on marine ecosystems is the changes in the amount and distribution of primary production because primary production plays an essential role in the structure of marine food chains (Shurin *et al.*, 2005; Hunt and McKinnell, 2006). The main objective of this study was to evaluate the effects of climate change on the primary production and vulnerability of fish species in the coastal waters of the northern Persian Gulf. Biological and non-biological data were evaluated in past and present periods with the help of satellite images in addition to field sampling, to predict climate change and its effects on the LARS-WG microscale model that has been compared and evaluated from HadGEM2 global model data under three scenarios: RCP2.6, RCP4.5, and RCP8.5.

Google Earth Engine system (GEE) helps research in agriculture, natural resource management, disease outbreak prediction, natural resource management, etc. In recent years, by using GEE, it has been possible to study the earth's surface with high temporal resolution in the form of a time series. GEE facilitates the process of scientific discovery by providing users with free access to remote sensing data sets (Tamiminia *et al.*, 2020). In this approach, users access GEE through an Internet-based programming interface and an interactive web-based development environment (Gorelick *et al.*, 2017). The capabilities of this system provide unprecedented opportunities to use this platform to process and interpret large data in many fields (Amani *et al.*, 2019). In some cases, the GEE system contains more than 43 years of past, present, and future satellite images. These images, with the necessary tools and calculations for advanced spatial analysis, can lead researchers to meta-analytical studies (Tafte *et al.*, 2019).

Estimating primary production using satellites requires mathematical models. The simplest models only use chlorophyll a to estimate primary production (Balch *et al.*, 1989). In 1989, Morel and Berthon introduced advanced algorithms based on solar radiation as the second controlling factor of the primary output (Morel and Berthon, 1989). There are various empirical relationships to calculate primary production, which use factors such as chlorophyll concentration, light depth, and light adaptation parameters. Many models are also based on the optical-biological properties of the water. These models have used light characteristics such as light extinction coefficient and photosynthetically active rays in the water column (Balch *et al.*, 1989). The effects of climate change on marine ecosystems and fisheries can be broadly divided into observational studies of past and present effects of climate change and modeling studies of future effects. Some studies involve observation and modeling, and some models are empirically based on derived statistics. There is a lot of evidence of the impact of recent climate change on distribution, species composition, seasonality, and productivity in marine and freshwater systems (Brander, 2010).

Each species has special characteristics that show different performance depending on their flexibility and tolerance to environmental changes, which include growth, reproduction, mortality, and behavior to find food, avoid predators, and maintain themselves in favorable environments during their lifetime and their interactions with species and ecosystem processes (Jennings and Brander, 2010).

Materials and methods

Study area

The study comprised the coastal waters part of the northeastern Persian Gulf. Four transects perpendicular to the coastline in the area (fishing grounds of Lifeh, Buseif, Khur-e-musa Creek, and Bahrekan) and three fixed stations on each of the transects were established (Fig. 1).

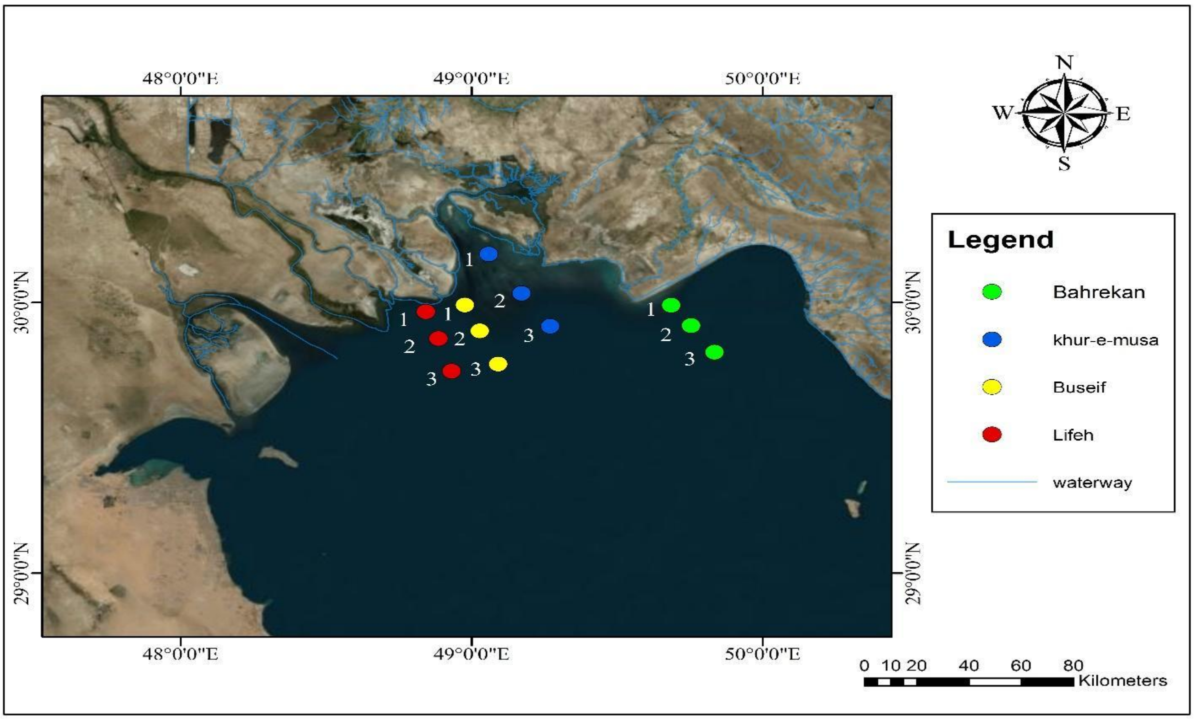


Figure 1: Sampling stations in the coastal waters of the northern Persian Gulf.

To predict climate change and its effects on primary production and fishery potential from the LARS-WG microscale model of HadGEM2 global model data were compared and evaluated under three scenarios RCP2.6, RCP4.5, and RCP8.5 in four time periods (2021-2040), (2031-2050), (2041-2060), (2051-2070), (2061-2080).

Satellite data

GEE is a new computational platform presented by Google, and it has enabled the production of global-scale data products by using a series of satellite imagery (DeVries *et al.*, 2020). In this study, the use of Modis satellite Terra sensor data is available in the GEE system (Zotarelli *et al.*, 2010). Various methods of time series processing were employed in GEE and spatial changes of water quality characteristics in the coastal waters during different periods were prepared as the input data. In this study, the SST algorithm of the region was used to measure the surface temperature, and the kd (490) experimental algorithms obtained in the Northwest of the Persian Gulf were used for the calibration of chlorophyll a value (Dehmordi *et al.*, 2016). Data collecting by use of satellite imagery of MODIS sensor in coastal water in the northern Persian Gulf for 23 years of observations (2000 to 2021) (*https://oceancolor.gsfc.nasa.gov/atbd/chlor_a)*.

Among the 36 bands of MODIS sensors, five bands (i.e., 20, 22, 23, 31, and 32) were used to estimate SST (MCSST model). This model is highly accurate due to satellite zenith angles and atmospheric correction using the difference between bands (Pakdaman et al., 2013). Earth series data is an important source for observing the dynamics and evolution of environmental phenomena, which are used to detect trends in long-term time series (Eastman, 2015).

Measurement chlorophyll a

To measure environmental chlorophyll a, one liter of water was collected from each middle layer and filtered with Millipore filter paper with a diameter of 0.45 microns. Then, filter papers along with their contents were stored in dark containers until the time of testing and extraction of pigments. Phytoplankton pigments were extracted with 90% acetone. The absorbance of the samples was read at the wavelengths of 750, 663, 645, and 630 nm, and then the amount of chlorophyll a was calculated using special equations(Grasshoff *et al.*, 2009).

Primary production model

The primary production model VGPM has been validated by thousands of real data on a large scale and in different areas for a long time, with accurate results. Daily primary productivity is calculated by the following equation (Behrenfeld and Falkowski, 1997):

$$P_p=0.66125\times P^B_{opt}\times(E_0/E_0+4.1)\times Z_{eu}\times C_{sat}\times DL$$

Where PP is integrated primary productivity in the euphotic zone (mgC m^{-2} d^{-1}), P^B_{opt} is the maximum rate of daily photosynthesis within a water column (mgC/mgChla/h), E_0 is daily PAR at the sea surface (Ein $m^{-2}d^{-1}$), Csat is the satellite-derived sea surface chlorophyll a concentration (mgm^{-3}), DL is the photoperiod (h), and Z_{eu} is the depth of the euphotic zone (m) defined as the depth that E_0 decreases by 1%, estimated by the sea surface chlorophyll a concentration using the following equations (Morel and Berthon, 1989):

Z_{eu}=(568.2(C_{tot}) $^{-0.746}$ when Zeu<102; 200.0(C_{tot})$^{-0.293}$ when Z_{eu} >102)

C_{tot}=(38.0(C_{sat})0.425 when C_{sat}<1.0, 40.2(C_{sat})0.547 when C_{sat} >1.0)

Considering the relationship between primary production and SST, Behrenfeld and Falkowski (1997) took the parameterization of P^B_{opt} as a seventh-order polynomial function of SST:

P^B_{opt} =1.2956+ 2.749×10^{-1} T+ 6.17×10^{-2} T ^{2} - 2.05×10^{-2} T ^{3} +2.462×10^{-3} T ^{4} - 1.348×10^{-4} T ^{5} +3.4132×10^{-3}

Data fish collection was carried out by the South Iran Aquaculture Research Institute by the statistics in the landing fish catch areas. These statistics were obtained at the place of catch collection by counting the weight and identifying the species in the fishing grounds of Lifeh, Buseif, Bahrekan, and Khur-e-musa Creek. Hierarchical Canonical Correlation Analysis (HCCA) was used to identify the correlation of SST with chlorophyll a, primary production, and commercial fisheries catch. Figure 2 illustrates the steps of running HCCA for four datasets. Data were computed using XLSTAT 2022(24.4.1379). Air temperature and SST, chlorophyll, primary production, and aquatic catch datasets use canonical correlation analysis (CCA) to combine datasets organized into hierarchies derived from datasets by calculating condition numbers (Fig. 2).

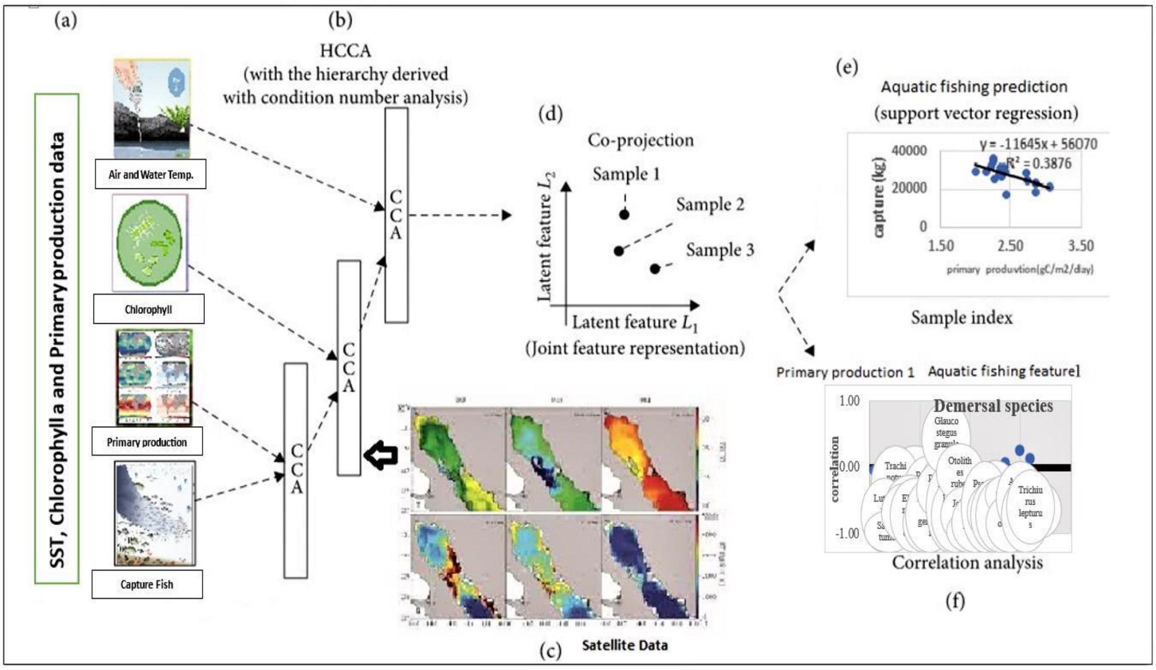


Figure 2: Overview of the workflow. (a) We first prepared the multiple remote sensing datasets. (b) HCCA is then applied to combine the information for finding a joint feature representation by the projection of the datasets in a hierarchy learned by analyzing the conditional numbers. (c) Optionally, the Satellite data can also be integrated with HCCA to learn a more joint feature representation with better functional coherence. (d) The projection of the integrated data provides the joint feature representation of the integrated datasets. (e) The joint feature representation is then used by the support vector regressor for catch-fishing prediction. (f) The joint feature representation is also analyzed for the association between primary production and the catch fishing features.

Results

The annual average air temperature in Abadan synoptic station is 26.98°C between the years 2000 and 2022. The maximum and minimum of this value are 43.3°C (2006) and 4.1°C (2007) (Fig. 3).

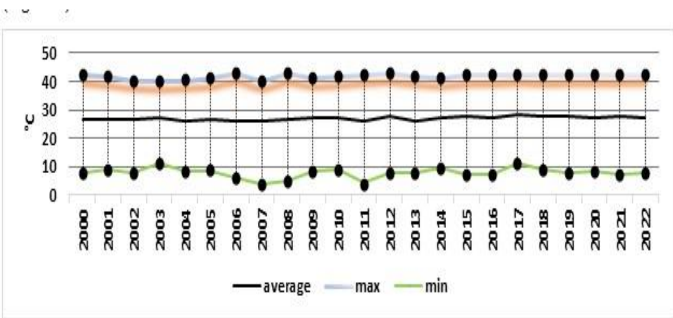
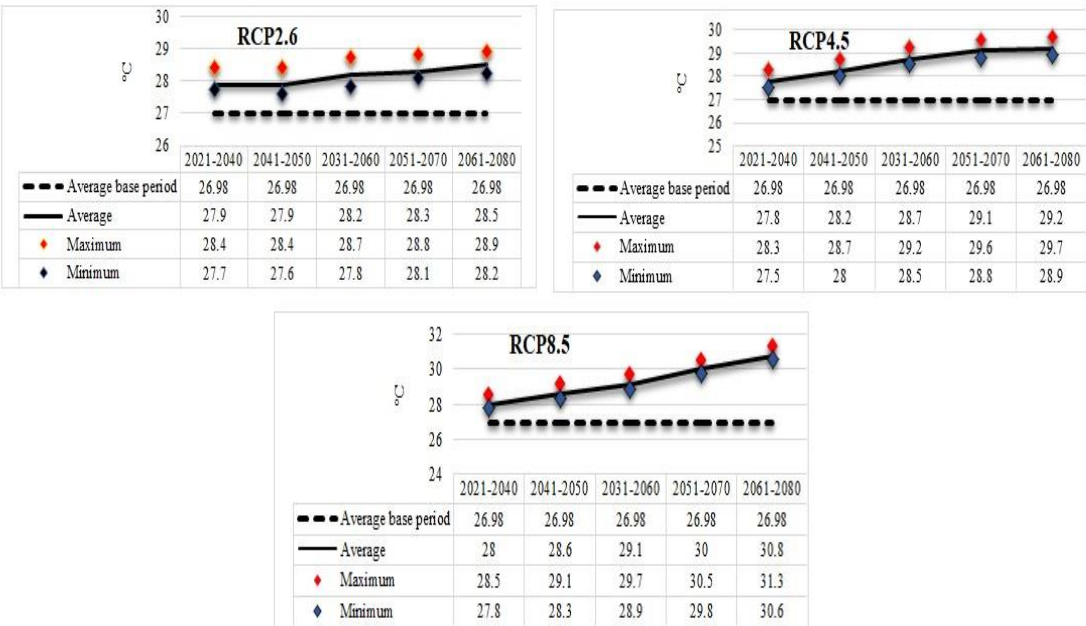


Figure 3: Air temperature changes of Abadan Synoptic station from 2000 to 2022.

The average predicted SST for five periods under RCP2.6, RCP4.5, and RCP8.5 scenarios are 27.9 to 28, 27.9 to 28.6, 28.2 to 29.1, 28.3 to 30, 28.5 to 30.8°C (2021-2080). Mean SST was 25.5°C over the period 2002 to 2022. The scenarios predicted an increase of 2.4 to 5.3°C in the future of the studied area (Fig. 4).



RCP2.6

	2021-2040	2041-2050	2031-2060	2051-2070	2061-2080
Average base period	26.98	26.98	26.98	26.98	26.98
Average	27.9	27.9	28.2	28.3	28.5
Maximum	28.4	28.4	28.7	28.8	28.9
Minimum	27.7	27.6	27.8	28.1	28.2

RCP4.5

	2021-2040	2041-2050	2031-2060	2051-2070	2061-2080
Average base period	26.98	26.98	26.98	26.98	26.98
Average	27.8	28.2	28.7	29.1	29.2
Maximum	28.3	28.7	29.2	29.6	29.7
Minimum	27.5	28	28.5	28.8	28.9

RCP8.5

	2021-2040	2041-2050	2031-2060	2051-2070	2061-2080
Average base period	26.98	26.98	26.98	26.98	26.98
Average	28	28.6	29.1	30	30.8
Maximum	28.5	29.1	29.7	30.5	31.3
Minimum	27.8	28.3	28.9	29.8	30.6

Figure 4: Annual comparison of predicted air temperature under all three scenarios using the LARS-WG model.

Chlorophyll a was negatively correlated with SST and positively correlated with net primary production. In other words, a negative correlation between SST and chlorophyll a indicates that chlorophyll a, and net primary production, is reduced. The annual comparison of chlorophyll a and primary production predicted under all three scenarios using the LARS-WG model shows a decreasing trend (Fig. 5).

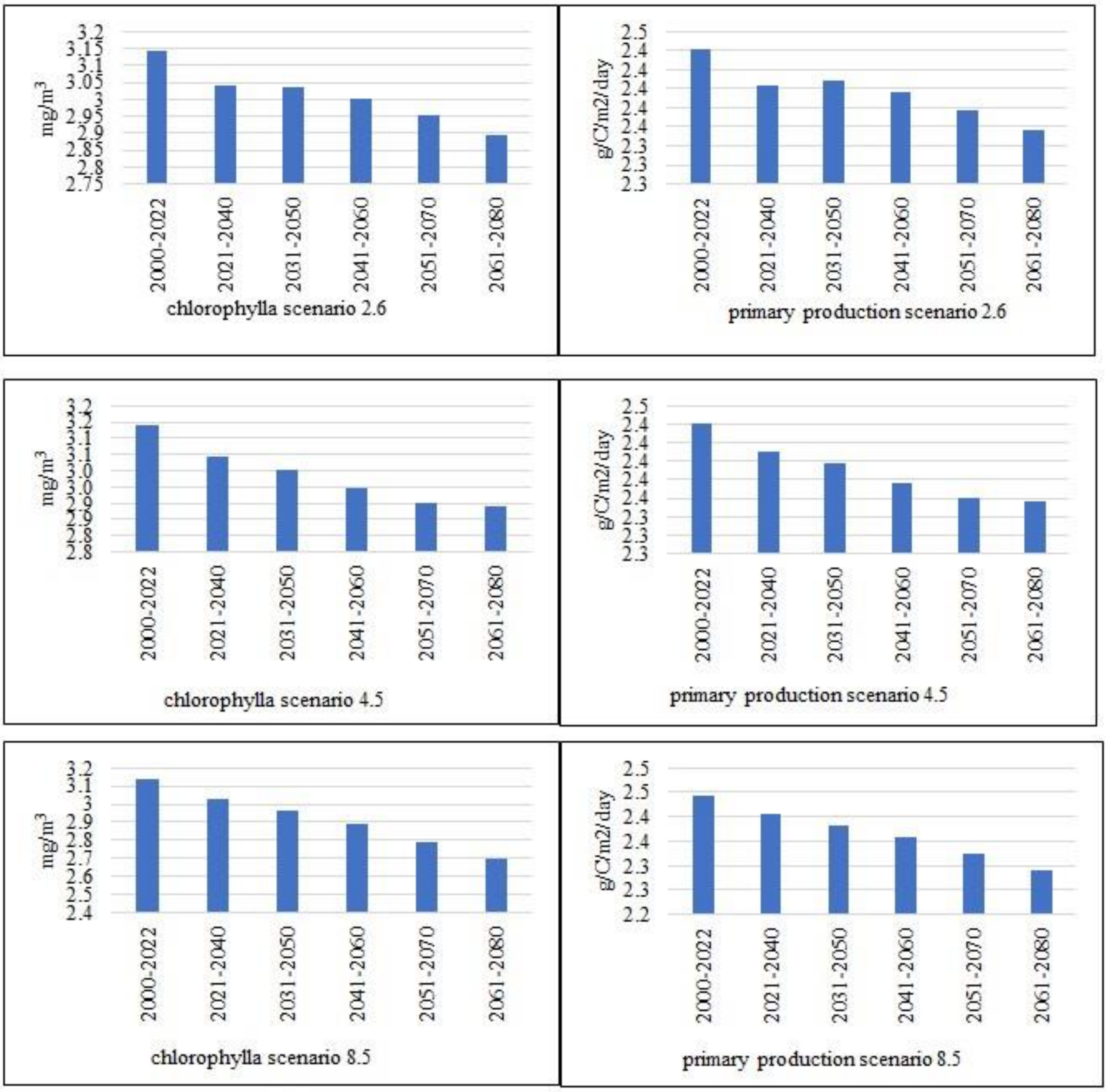


Figure 5: Annual comparison of chlorophyll a and predicted primary production under all three scenarios using the LARS-WG model.

Principal component analysis (PCA) of environmental and biological variables creates two principal components (PCs). F-1 and F-2 are the most important components representing 100% of the total abiotic and biotic factors. F-1 accounted for 57.76% of the total variance of environmental variables due to the negative load of air temperature (-0.97 %) and negative load of water temperature (-0.31%). F-2 accounted for 42.24% of the total variance of the environmental variables, which due to the positive load of water temperature (0.95%) and air temperature (0.24%) make up a percentage of the total. F-1 accounted for 57.76% of the total variance of the biological variables, which is due to the positive charge of chlorophyll (0.86%), primary production (0.83%), and the negative charge of aquatic life (-0.60%). F-2 accounted for 42.24% of the total variance of biological variables which due to the positive charge of chlorophyll (0.05%), the negative charge of primary production (-0.43%), total catch (-0.58%) make up the percentage of the total (Fig. 6).

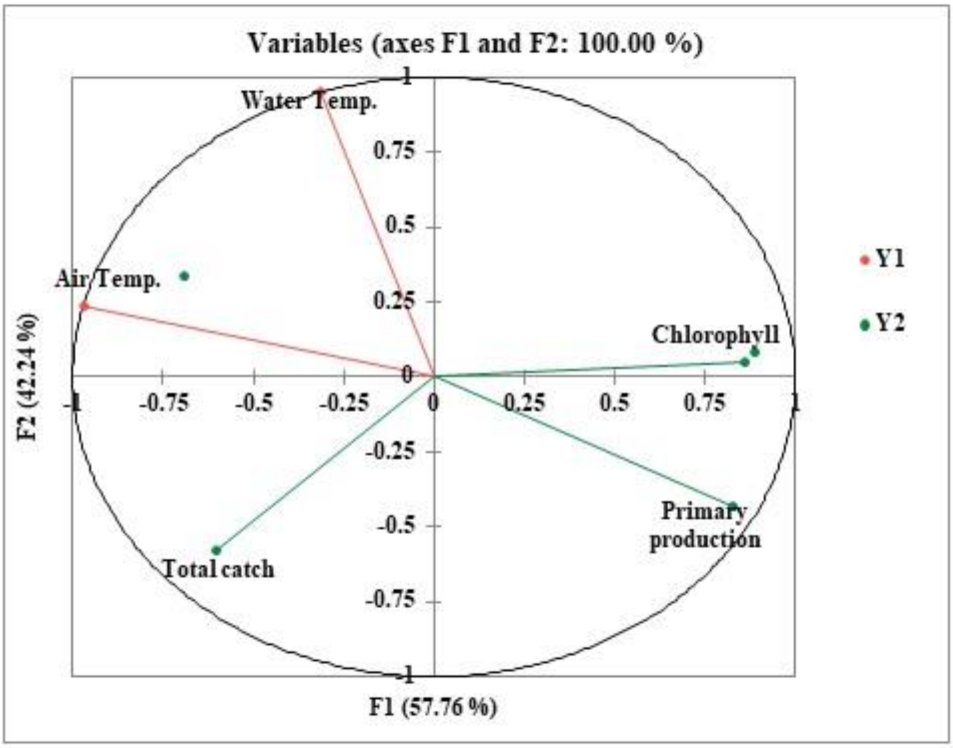


Figure 6: The relationship between environmental and biological factors with the first (F1) and second (F2) components.

Commercial fisheries catch data were classified into 5 groups, which include demersal, large pelagic, small pelagic, shrimps, and jellyfish. The most abundant are demersal fish 61%, large pelagic 20%, small pelagic 9%, shrimp 7%, and jellyfish 3%, respectively. It shows a maximum catch amount of 55509 kg in 2002 and a minimum of 29697 kg in 2022 during a period of twenty years (2002-2022). Most of the frequency demersal group(by-catch) is 3,933 kg during twenty years. From the demersal group *Chirocentrus dorab* species with 3668 and *Otolithes ruber* with 2757 kg per year and from the large pelagic group *Tenualosa ilisha* with 3,519, *Planiliza subviridis* with 1810 and *Scomberoides commersonnianus* with 1751 kg per year and from the small pelagic group *Liza klunzingeri* with 3132 kg per year, Shrimps and jellyfish were the most abundant aquatic species with 3279 and 2010 kg per year, respectively. The catch rate of demersal fish has been increased during this period. The catch of large pelagic fish fluctuates and these fluctuations are very noticeable in the small pelagic fish. The amount of shrimp caught also shows an upward trend.

The correlation between the primary production and the demersal, large pelagic fish and shrimps shows that with an increase of the primary production, the amount of the catches of these aquatics decreases, in other words, the covariance and the correlation coefficient is negative, indicating the changes of the two variables in opposite directions. However, the correlation between primary production and small pelagic fishes and jellyfish shows that with the increase in primary production, the amount of their catch increases and indicates the changes of two variables in the same direction (Fig. 7 and Table 1).

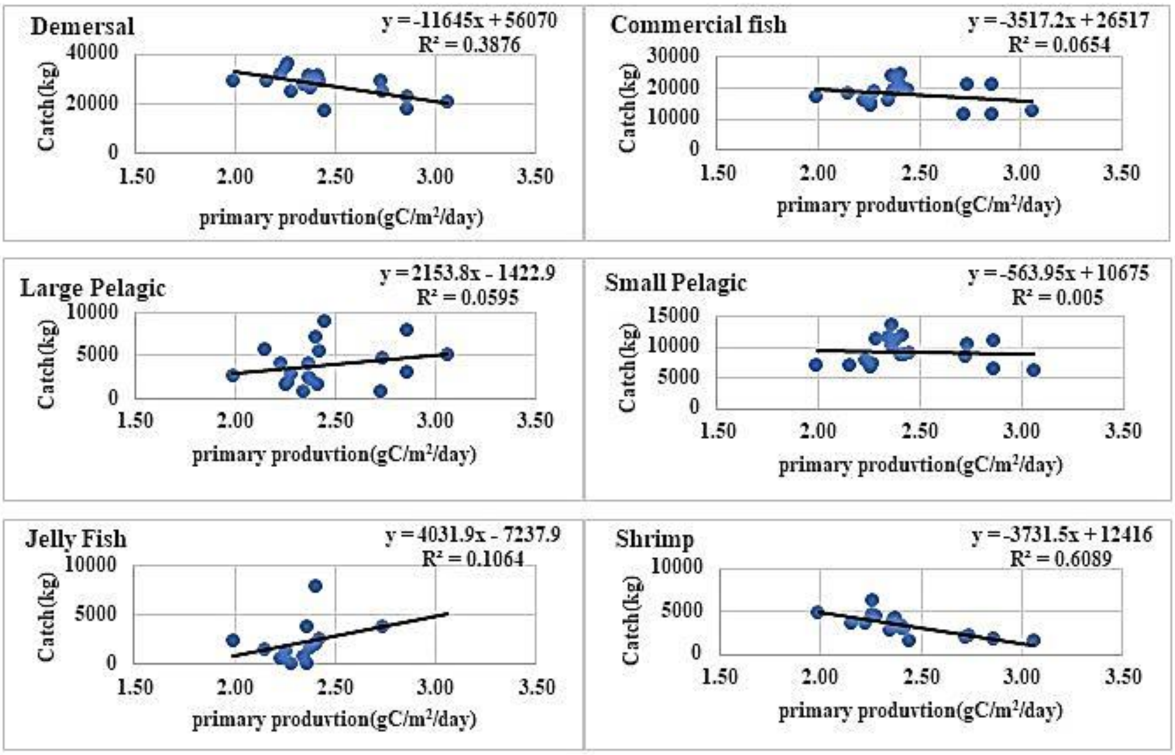


Figure 7: Correlation between primary production and Commercial fish catch, demersal, large and small pelagic, shrimp, and jellyfish.

Table 1: The Pearson Correlation Matrix for primary production and total catch of demersal, large, and small pelagic fish, shrimps, and jellyfish.

Variables	**Primary production**	**Commercial fish**	**Demersal**	**Large pelagic**	**Small Pelagic**	**Shrimp**	**Jellyfish**
Primary production	1						
Commercial fish	-0.26	1					
Demersal	-0.62	0.14	1				
Large pelagic	-0.07	0.72	0.05	1			
Small pelagic	0.24	0.31	-0.50	-0.12	1		
Shrimp	-0.78	0.08	0.76	-0.01	-0.44	1	
Jellyfish	0.33	0.75	0.15	0.34	-0.15	-0.42	1

The commercial species captured in the study area were divided into three different trophic levels, which include herbivorous and omnivorous species (species with a food level of less than 3.25), medium-level carnivorous species (species with a food level of more than 3.25 and less than 4), and high-level carnivorous species (species with food level more than 4).

The correlation between the total catch and the marine trophic index (MTI) shows that if the total catch increases, the amount of the marine trophic index increases at the same time. The correlation between primary production and MTI shows that if the amount of primary production increases, the MTI decreases at the same time, that is, the higher the amount of primary production increases, the more the MTI will decrease.

Discussion

In the current study, the average SST of the base period in the study area it was 25.5°C. The prediction of the results of the modeling conducted under the RCP scenario (2.6-4.5-8.5) shows that the average SST of the study area will increase between 2020-2080 compared to the average of the base years (2002-2022). The prediction of the results of the modeling conducted under the RCP scenario (2.6-4.5-8.5) shows that the average SST of the study area will increase between 2020-2080 compared to the average of the base years (2000-2022). Based on modeling results, the changes of SST between 2.4 to 5.3°C. The coastal waters of the studied region, which is northeast of the Persian Gulf, are shallow and have large SST changes in the future. The studies that other researchers have done on the numerical modeling of SST in many parts of the Persian Gulf show, showed that the amount of SST changes in the future will not happen in the same way in all parts of the Persian Gulf and the amount of temperature changes in shallow areas will be more (Farkhani and Hadjizadeh Zaker, 2020).

Our results showed a negative relationship between the chlorophyll a concentration and the SST in the study area. Researchers who examined chlorophyll a and SST for the Persian Gulf and Oman Sea, point out that no statistically significant correlation pattern was identified between chlorophyll a concentration and SST for the Persian Gulf and Oman Sea, except in North coastal areas where there is a negative relationship (Zotarelli *et al*., 2010; Khodam *et al.*, 2021). In the southern part of the Persian Gulf, there is a relatively good positive correlation between the above quantities, and in the northern coastal areas, which have a shallower depth than other areas, the chlorophyll concentration has an inverse relationship with SST (Dadizadeh *et al*., 2013; Khodam *et al.*, 2021)

The amount of chlorophyll a reaches the lowest value in this region between 2017 and 2019. Other researchers showed that with the establishment of a high-pressure system over the desert areas of the Emirates, northern Yemen, and part of the Rubab al-Khali desert and the formation of south and southeast currents, significant amounts of dust were transferred to the Persian Gulf and the Oman Sea. This phenomenon caused the creation of dust storms and very fine solid particles suspended in the air and decrease in the incoming light radiation to the water surface and a noticeable decrease in SST. Finally, chlorophyll a level also decreased due to the reduction of photosynthesis (Khodam *et al*., 2021).

In global research, climate change in the ocean (satellite-based estimates over two decades) showed that linear trends in annual primary production between 1998 and 2018 were significantly different on regional scales. In low and middle latitudes, the trend in primary production is generally weak and negative. In polar and coastal regions stronger and positive trends in primary production were observed. Annual trends in global primary production showed an increase between 1998 and 2003, relatively stable trends between 2003 and 2011, and a subsequent decline in primary production until 2015, which indicates a relatively early decline in primary production in the Indian Ocean (Kulk *et al.*, 2020). Because the northern area of the Persian Gulf is considered low latitude, the initial production trend since 2013 has been generally weak and negative. The concentration of chlorophyll a and primary production is similar to the coastal waters of the Arvand River and Rapami region (Ghaemi *et al.,* 2021).

Canonical Correlation Analysis (CCA) of commercial fisheries, environmental and biological factors showed that primary production has a positive relationship with chlorophyll a and negative relationship with air temperature, water temperature, and commercial fisheries. The inverse correlation between SST and chlorophyll a concentration is consistent with the results of the study conducted by Nurdin (Nurdin *et al.*, 2013). The average amount of commercial fish caught in the base years is 17652 kg per year. The annual comparison of the commercial catch predicted under all three scenarios using the LARS-WG model showed that the commercial catch in scenario 2.6 reaches 17530 to 17361 kg from the beginning of the forecast period, which means that it decreases by about 169 kg in catch per year. In the same comparison, the annual fish catch under scenario 4.5 will reach from 17539 to 17354 kg, that is, about 185 kg per year, and under scenario 8.5, it will reach from 17509 to 17123 kg, which means that about 386 kg of catch reduction is expected per year. According to FAO (2018), the maximum catch potential reduction in the exclusive economic zones of the world is predicted to be between 2.8 and 5.3 percent by 2050 according to the RCP2.6 greenhouse gas emission scenario and between 7 to 12.1% based on RCP8.5 greenhouse gas emission scenario(Barange *et al.,* 2018). Although this average is not considered large on a global scale, its effects are much greater on a regional scale. In the coastal waters of the northern Persian Gulf, according to the RCP2.6, RCP4.5, and RCP8.5 greenhouse gas emission scenarios, the maximum reduction potential is 0.69-1.65, 0.64-1.69, and 0.81-2.90, respectively.

Model predictions in marine areas certainly play an important role in the interaction between climate change in the ecosystem and the responses of fisheries managers and planners to minimize threats. It is important to note that these projections only reflect changes in the capacity of the seas to produce fish and do not take into account the management decisions that may or may not be made in response to them. It is concluded that interactions between ecosystem changes and management responses are critical to minimizing threats and maximizing opportunities from climate change (Barange *et al.*, 2018).

The correlation of commercial species with primary production shows that the demersal species *Pomadasys kaakan, Glaucostegus granulatus, Otolithes ruber, Atule mate, Trichiurus lepturus*, and the large pelagic fish species *Planiliza subviridis, Auxis thazard, Scomberoides commersonnianus, Tenualosa ilisha,* *Thunnus tonggol*, *Coryphaena hippurus*, and small pelagic fish species *Liza klunzingeriand* and jellyfish group express a positive relationship. Undoubtedly, the different behavior of different species of aquatic animals concerning primary production indicates that their life cycle and food chains are different. Definitely, due to the positive relationship between their abundance and nutrition with primary production, they are more vulnerable to climate change than other fish species (Fig. 8).

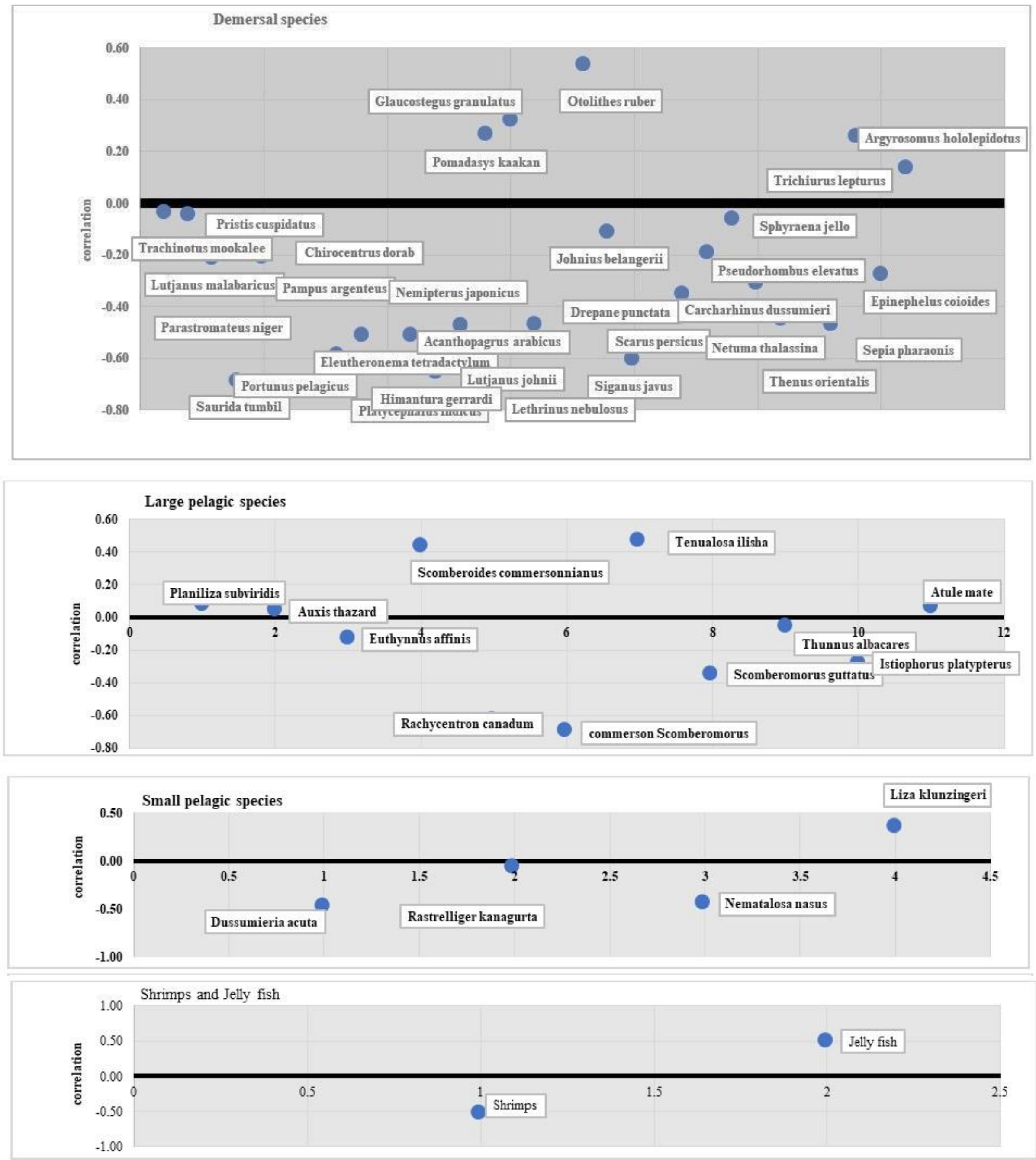


Figure 8: Correlation of commercial fish species with primary production.

Some fish species in the coastal waters are more vulnerable to climate change than other fish species due to the positive relationship between their abundance of nutrition and primary production (Table 2).

Table 2: Fish species more vulnerable to climate change in coastal waters of the northern Persian Gulf

Species	Scientific names
Small pelagic	*Liza klunzingeri*
	Planiliza subviridis
	Auxis thazard
	Scomberoides commersonnianus
Large pelagic	*Atule mate*
	Tenualosa ilisha
	Thunnus tonggol
	Coryphaena hippurus
	Pomadasys kaakan
Demersal	*Glaucostegus granulatus*
	Otolithes ruber
	Trichiurus lepturus

The MTI and fisheries strategies to deal with the predicted climate changes under all three scenarios have shown that there has been a decreasing trend during the predicted years. That is, as the amount of primary production decreases, the level of MTI will increase. The correlation between the total catch and the MTI also shows that if the MTI level increases, the total amount of the catch will increase at the same time (direct relationship), that is, in the study area, the predicted value of the MTI and the total amount of catch will increase. Therefore, to reach a sustainable fishery, it is necessary that if the catch increases, we will reduce the predicted marine trophic level quickly, and if the increase in the catch cannot compensate for the decrease in the marine trophic level, the total catch in the studied area will decrease (Pauly *et al.*, 1998).

The general conclusion in this context is that climate change affects fisheries production through its effects on primary production, food web interactions, life history, and distribution of fish species. Changes in primary production follow changes in the physical and chemical environment (Sarmiento *et al.*, 2004), while changes in the food web are also influenced by the availability of primary production (Brander, 2007). There is much empirical evidence for the effects of climate change on marine ecosystems and their evolving species (Jochum *et al.,* 2012; Rall *et al.*, 2012). The impact of climate change causes a change in the distribution of fish, which is one of the most common ecological reactions in marine and coastal species (Sumaila *et al.*, 2011).

Conclusions

Forecasting climate effects on total fisheries production is challenging, especially at the ecosystem scale. Species identities, habitat associations, physiological responses, life histories, and interspecific interactions vary among systems and are rarely well understood. The details for this prediction are a result of observing the emergence of predictions for several species at the ecosystem scale. At a smaller scale, the displacement of commercial target species leads to the introduction of invasive species, removal of target species, new trophic interactions, and changes in habitats, among other things, causing many uncertain consequences for fisheries and their dependent livelihoods. A wide range of species distribution changes can have significant impacts on fishermen, fishing communities, the fisheries economic sector, and fisheries management institutions. Adaptation and transformative action in fisheries can occur at the individual (fisherman), collective (community), and institutional (government) levels, and awareness of potential disharmony is important.

Future research needs to address the linkages between policy solutions and the social-ecological systems. Further research could look at the implications of more drastic changes, and what policies can counteract the resulting detrimental states. Although not explored here, the resilience of the ecological domain is also key to shifts in species distributions. It is also necessary to investigate the entire Persian Gulf region and the Oman Sea in additional research to investigate the effect of the climate change process more comprehensively. Fisheries managers can make more effective decisions from this forecast.

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Conflicts of interest
The authors declare no conflict of interest for this study.

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