Spatial and temporal variations in stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of the gammarids and the common carp in the southern coast of the Caspian Sea

Rumsliga och tidsmässiga variationer i stabila isotopsignaturer ($\delta^{13}C$ and $\delta^{15}N$) av gammarider och karp längs den södra kusten av Kaspiska havet

Nazila Hajizadeh Koupayeh

Swedish University of Agricultural Sciences, SLU
Faculty of Natural Resources and Agricultural Sciences/Department of Aquatic Resources/
Institute of Coastal Research
Independent project in biology, 60 credits
2020

Spatial and temporal variations in stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of the gammarids and the common carp in the southern coast of the Caspian Sea

Rumsliga och tidsmässiga variationer i stabila isotopsignaturer (13C och 15N) av gammarider och karp längs den södra kusten av Kaspiska havet

Nazila Hajizadeh Koupayeh Swedish University of Agricultural Sciences, Department of Aquatic Resources, Institute of Coastal Research

Supervisor: Rasoul Ghorbani, Gorgan University of Agricultural Sciences and Natural resources, Department of Fisheries and Environment

Assistant Supervisor: Rahmat Naddafi, SLU, Department of Aquatic Resources

Examiner: Andreas Bryhn, SLU, Department of Aquatic Resources

Credits: [60] credits

Level: Second cycle, A2E

Course title: Master thesis in Biology, A2E

Course code: EX0900

Course coordinating department: Department of Aquatic Sciences and Assessment

Editor: Swedish University of Agricultural Sciences, SLU

Publisher: Faculty of Natural Resources and Agricultural Sciences/Department of Aquatic Resources/Institute of Coastal Research

Year of publication: 2020

Place of publication: Öregrund

Illustration:

Keywords: Carbon stable isotope, Caspian Sea, *Cyprinus carpio*, food webs, *Gammarus aequicauda*, nitrogen stable isotope, *Pontogammarus maeoticus*

Abstract

Despite the growing body of research on the use of stable isotope analysis in food web studies, spatial and temporal variations in stable isotope ratios of coastal communities are still poorly understood. The aim of my study is to explore how carbon and nitrogen stable isotopes of selected benthic communities vary spatially and temporally across the south of Caspian Sea. I followed carbon stable isotope ratios ($\delta^{13}C$) and nitrogen stable isotope ratios ($\delta^{15}N$) of gammarids and common carp (*Cyprinus carpio*) in four sites (Anzali, Noshahr, Sari, and Gomishan) from the southwest to the southeast of the Caspian Sea during May-November 2019 to address the following questions: 1) how do carbon and nitrogen stable isotope ratios of gammarids and carps change along the coastline in different months? 2) How do trophic levels of gammarids and carps vary spatially and temporally? My results revealed that there are spatial and temporal variations in stable isotope signatures of the gammarids and carps in the southern coast of the Caspian Sea, although month or site effects were not significant in some cases. Trophic position of gammarids and common carps varied among sites but was independent of month. On average, the amphipod *Pontogammarus maeoticus* in Noshahr presented the highest and the amphipod *Gammarus aequicauda* in Gomishan showed the lowest nitrogen stable isotope ratios among sites. Enrichment of $\delta^{13}C$ in gammarids was the highest in Gomishan, followed by Sari, Noshahr and Anzali. There was an increasing trend in carbon stable isotope ratios of *P. maeoticus* from May to November in Sari, Noshahr and Anzali sites. Among sites, Gammarids in Anzali had the highest C:N ratios. There was a decreasing trend in C:N ratios of *P. maeoticus* from June-November in Anzali, Noshahr, and Sari sites. Carps in Noshahr enriched more with $\delta^{15}N$ than those in other sites. Carps in Gomishan had the highest value of $\delta^{13}C$ among all sites. Gammarids displayed the lowest trophic level in Gomishan among sites and a similar trophic level between Anzali and Sari. Gammarids in Noshahr showed significantly higher trophic level than those in Sari and Anzali. Among sites, *C. carpio* exhibited the highest trophic level in Noshahr. Allochthonous and autochthonous organic matters, sewage discharge, aquaculture wastes, agricultural fertilizers, Chl a concentrations, food quality, amount of lipids, and high degree of trophic variation were discussed as potential reasons for the observed spatial and temporal variation in stable isotope signatures of the gammarids and the carps. Results from this study indicated that low quality allochthonous organic matter originated from the rivers that flow into the Caspian Sea playing an important role in food web and ecosystem function of Caspian Sea. This study notifies water managers to set up proper and timely measures to mediate anthropogenic stressors and rehabilitation of fish spawning habitats.

Keywords: Carbon stable isotope, Caspian Sea, *Cyprinus carpio*, food webs, *Gammarus aequicauda*, nitrogen stable isotope, *Pontogammarus maeoticus*

Populärvetenskaplig sammanfattning

Trots den växande forskningen om användning av stabil isotopanalys i näringsvävsstudier är rumsliga och tidsmässiga variationer i stabila isotopförhållanden i kustsamhällen fortfarande inte väl förstådda. Syftet med min studie är att utforska hur stabila kol- och kväveisotoper från utvalda bentiska samhällen varierar rumsligt och tillfälligt över södra Kaspiska havet. Jag följde kolstabila isotopförhållanden ($\delta^{13}C$) och kvävestabil isotopförhållanden ($\delta^{15}N$) hos gammarider och karp (*Cyprnius carpio*) på fyra platser (Anzali, Noshahr, Sari och Gomishan) från sydväst till sydost i Kaspiska havet under maj-november 2019 för att adressera följande frågor: 1) hur förändras kol- och kvävestabila isotopförhållanden mellan gammarider och karpfiskar längs kustområdena under olika månader? 2) Hur varierar trofiska nivåer av gammarider och karp rumsligt och tillfälligt. Mina resultat avslöjade att det finns rumsliga och tidsmässiga variationer i stabila isotopsignaturer av gammarider och karp längs den södra kusten av Kaspiska havet, även om månads- eller platseffekter inte var betydande i vissa fall. Den trofiska positionen för gammarider och karpfiskar varierade mellan platser men var oberoende av månad. I genomsnitt visade amfipoden *Pontogammarus maeoticus* i Noshahr de högsta och amfipoden *Gammarus aequicauda* i Gomishan visade de lägsta kvävestabila isotopförhållandena bland platserna. Anrikning av $\delta^{13}C$ i gammarider var den högsta i Gomishan, följt av Sari, Noshahr och Anzali. Det var en ökande trend i kolstabila isotopförhållanden mellan *P. maeoticus* från maj till november i Sari, Noshahr och Anzali-platserna. Bland platserna hade gammarider i Anzali de högsta C:N-förhållandena. Det var en minskande trend i C:N-förhållandena av *P. maeoticus* från juni-november på Anzali, Noshahr och Sari-platser. Karp i Noshahr berikades mer med $\delta^{15}N$ än på andra platser. Karp i Gomishan hade det högsta värdet på $\delta^{13}C$ bland alla platser. Gammarider visade den lägsta trofiska nivån i Gomishan bland platserna och en liknande trofisk nivå mellan Anzali och Sari. Gammarider i Noshahr visade signifikant högre trofisk nivå än i Sari och Anzali. Bland platserna uppvisade *C. carpio* den högsta trofiska nivån i Noshahr. Alloktont och autoktont organiskt material, avloppsvatten, akvakulturavfall, jordbruksgödselmedel, Chl a-koncentrationer, näringsvävens kvalitet, mängd lipider och hög grad av trofisk variation diskuterades som potentiella anledningar för den observerade rumsliga och temporära variationen i stabila isotopsignaturer av gammariderna och karpen. Resultaten från denna studie indikerade att alloktont organiskt material med låg kvalitet härstammar från floderna som flödar in i Kaspiska havet och spelar en viktig roll i näringsvävensbanan och ekosystemfunktionen i Kaspiska havet. Denna studie råder vattenförvaltare att inrätta lämpliga och snabba åtgärder för att förmedla antropogena stressfaktorer och rehabilitering av fiskarnas livsmiljöer.

Nyckelord: Kolstabil isotop, Kaspiska havet, *Cyprinus carpio*, näringsväven, *Gammarus aequicauda*, kvävestabil isotop, *Pontogammarus maeoticus*

Table of contents

Contents

List of tables

List of figures

Abbreviations

SLU	Swedish University of Agricultural Sciences
C	Carbon
N	Nitrogen
$\delta^{13}C$	Carbon stable isotope ratio
$\delta^{15}N$	Nitrogen stable isotope ratio
TL	Trophic level
P. maeoticus	*Pontogammarus maeoticus*
G. aequicauda	*Gammarus aequicauda*
C. carpio	*Cyprinus carpio*
Chl a	Chlorophyll a

1. Introduction

The Caspian Sea is the world's largest inland water body with a volume of 80,000 km3, a surface area of 3.8×105 km2, and a catchment area of 3.5 million km2. The main river is the River Volga which discharges into the northern basin and contributes to 82% of the water inflow. In the southern Caspian Sea, Sefidrud and Anzali Wetland provide the main inflow and discharge 4,037 million m3 / year and 2 million m3 / year into the southwestern basin, respectively (Bagheri et al. 2010). The Caspian Sea has been threatened by different anthropogenic pressures such as eutrophication (Dumont 1998), invasive (Kideys et al. 2008, Roohi et al. 2008), oil pollution, untreated sewage, industrial and radioactive wastes, and agricultural activities (Leroy et al. 2020). In this context, the coastal area should be especially important because it is not the only interface zone that receives a large amount of nutrients and particulate inputs but also among the most productive waters that provides goods and service for the society (Costanza et al. 1997). Anthropogenic organic matter and nutrient input can be assimilated by primary producers or directly consumed by consumers, thereby entering food webs (Vizzini et al. 2005, Martínez-Durazo et al. 2019). Understanding trophic interactions and energy flow in the coastal area can provide valuable insights into ecosystem management and conservation (Stachowicz et al. 2007, di Lascio et al. 2013).

Stable isotope analysis provides a powerful tool to evaluate resource dynamics and trophic relationships in aquatic ecosystems (Peterson and Fry 1987, Michener and Schell 1994, Vander Zanden et al. 2016). The ratio of heavy to light carbon isotopes (^{13}C:^{12}C or $\delta^{13}C$) is utilized to explore the origin and pathways of organic matter in food web whereas the ratio of heavy to light nitrogen isotopes (^{15}N:^{14}N or $\delta^{15}N$) is used to quantify trophic position of a consumer (Peterson and Fry 1987, McCutchan Jr et al. 2003). $\delta^{15}N$ and $\delta^{13}C$ are typically enriched by 3-4% and 0-1 % at each trophic level, respectively (DeNiro and Epstein 1981, Minagawa and Wada 1984). Indeed, stable isotopic composition of food sources and trophic fractionation during the feeding process can determine isotopic signatures of the consumers.

Consumers drive their energy (carbon and nitrogen) from either autochthonous sources produced in their habitats or from allochthonous sources imported from terrestrial environments in the aquatic systems. These food sources have distinct stable isotope signatures and can result in specific isotopic signatures in the consumer. The southern Caspian Sea has a long coastline and many large rivers transport terrestrial and organic materials into different part of its coastal zone (Leroy et al. 2018). Thus there is expected to be a spatial variation in stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of the consumers in the southern coast of the Caspian Sea. Furthermore, the amount of transported materials to the coastal area by rivers as well as food quantity and quality may also change at the seasonal scale. Such changes can be reflected in temporal variation in isotopic signatures of consumers. Despite the growing body of research on the use of stable isotope analysis in food web studies (Costanzo et al. 2001, Post 2002, Oeding et al. 2020), spatial and temporal variations in stable isotope ratios of coastal communities are still poorly understood (Schaal et al. 2016). In coastal areas, benthic fauna play an important role in trophic coupling between estuarine benthic and pelagic food webs (Martinetto et al. 2006).

The aim of my thesis is to explore how carbon and nitrogen stable isotopes of selected benthic communities vary spatially and temporally across the south of Caspian Sea. I followed $\delta^{13}C$ and $\delta^{15}N$ of gammarids, an important food resource for the commercial fish species (e.g. sturgeon) (Moiceiev and Filatova 1985), and common carp (*Cyprinus carpio*), a commercially valuable benthic species in the southern coast of the Caspian Sea (Vazirzadeh and Yelghi 2015), in four sites (Anzali, Noshahr, Sari, and Gomishan) from the southwest to the south east of the Caspian Sea during May-November 2019 to address the following questions: 1) how do carbon and nitrogen stable isotope ratios of gammarids and common carp change along the coastal areas in different months? 2) How do trophic levels of gammarids and carp vary spatially and temporally? I hypothesized 1) stable isotope signatures of these two benthic consumers (gammarids and common carp) vary at spatial and temporal scales. The southwestern and the southern coast of Caspian Sea have a subtropical humid climate whereas the eastern coast is situated at a desert climatic zone and is typically warmer (Leroy et al. 2018). Because higher temperature may increase $\delta^{13}C$ enrichment in consumers (Power et al. 2003), organisms in the southeast of the Caspian Sea (Gomishan) would be assumed to have higher $\delta^{13}C$ in their tissue. 2) The trophic position of consumers changes spatially and temporally as a function of their food resources. In addition, I compared trophic position of gammarids and common carp with the trophic positions of other consumers in Gomishan to evaluate the trophic situation of my studied organisms.

2. Material and methods

2.1. Study area

The study was conducted in four sites along the southern coast of the Caspian Sea: Anzali in the southwest, Noshahr and Sari in the south, and Gomishan in the southeast of Caspian Sea during 2019 (Figure 1). The Anzali site is a beach with fine sandy substrate, next to the Anzali wetland complex which is fed by eleven rivers and discharged into the Caspian Sea through five canals (Naderi et al. 2017). A main river adjacent to this site is Sefidrud River, Iran's second longest river after the Karun. In 1962, a dam was built across this river to control flood and irrigation to increase rice production and hydroelectric power production. This river has been contaminated by industrial and hospital effluents without treatment, agricultural fertilizers, and domestic sewage.

The Noshahr site is a steep beach with a rocky substrate and is located in the vicinity of two estuaries, the Chalus River estuary and the Sardabrud River estuary. Water from the Sardabrud River is used to supply drinking water and to support agricultural and aquaculture usage. The Chalus River is a major river of central−northern Iran and passes through the city of Chalus. This river is polluted with sewage discharge and urban wastewater. One dam was constructed across the Chalus River for providing drinking water. There are several seaside villas close to the Noshahr sampling site.

The Sari site is a deep beach with fine sandy substrate, in the vicinity of the Tajan River. A dam was built across the Tajan River to supply water for agricultural and industrial use, mediate flooding, and hydroelectric power production in 1997. The Tajan River has been mostly affected by agricultural activities.
The Gomishan site is a shallow beach with silt/mud substrate. This site is located 20 km from the Gorganrud River estuary, which is mostly surrounded by reeds. A total of 14 dams have been built on the Gorgan River for flood control and water supply in agricultural activities.

Environmental parameters for three regions in the southern coast of the Caspian Sea (southwest, south, and southeast) were obtained from the Iran meteorological organization.

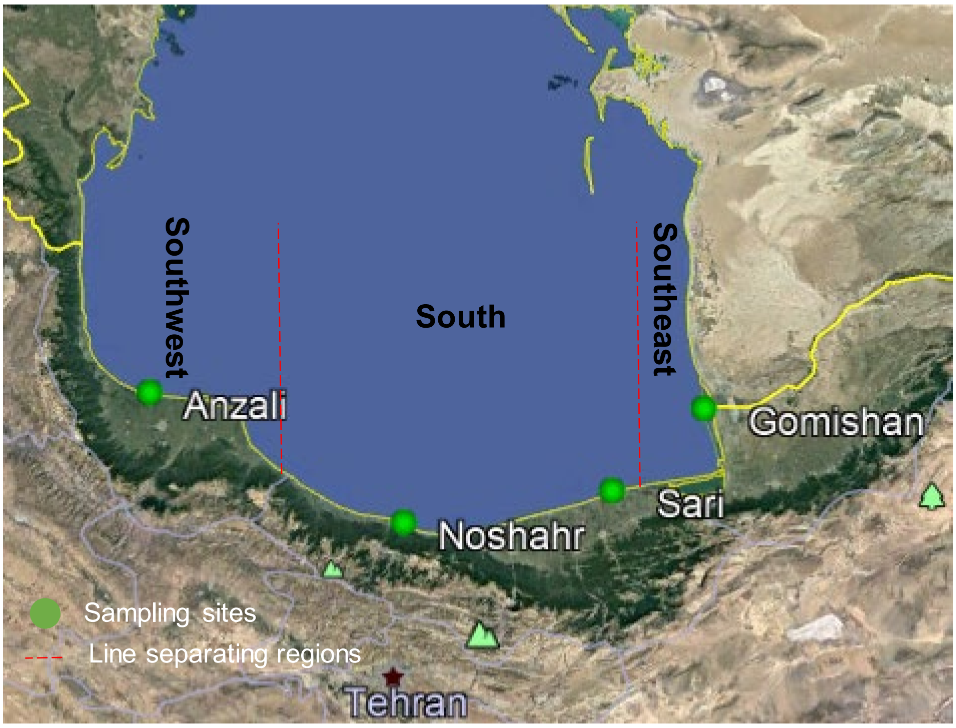


Figure 1. Map of the southern coast of the Caspian Sea. Red dashed lines separate the coast into southwest, south, and southeast regions. Sampling sites are marked with green dots.

2.2. Study organisms

2.2.1. Gammarids

The amphipod species in Anzali, Noshahr, and Sari sites was *Pontogammarus maeoticus*. This species is eurythermic and euryhaline, can tolerate a wide range of temperature and salinity, and is the most abundant and widely distributed species in the southern coast of Caspian Sea (Mirzajani 2003). The amphipod species in Gomishan was *Gammarus aequicauda*, which is limited to the southeast of the Caspian Sea (Stock et al. 1998). Mirzajani (2003) has documented that the fecundity of *P. maeoticus* is the highest in March, and the lowest by the end of summer and in the winter (Mirzajani 2003).

2.2.2. Common carp

C. carpio is a commercially valuable species and a widespread species in the southeast Caspian Sea. During the last three decades, carp populations have declined severely (Vazirzadeh and Yelghi 2015). *C. carpio* is an omnivorous species and can eat aquatic plants but prefers to feed on benthic invertebrates. Wild carp exhibit mostly multiple spawning strategies in the southeastern coasts of the Caspian Sea (Vazirzadeh et al. 2014) and spawn at least over 6 months of the year with two peaks in autumn and spring (Vazirzadeh and Yelghi 2015).

2.3. Sampling, sample processing, and laboratory analyses

At each site, benthic macroinvertebrates samples were collected monthly using an Ekman grab from the depth of 1 m, and sieved on a 500 µm mesh-size on the shore from May to November 2019. The benthic macroinvertebrates were dominated by *P. maeoticus* in the Anzali, Noshahr, and Sari sites. In Goimshan, benthic macroinvertebrates included nereis, chironomids, and bivalves. So, during November and October 2019, the gammarids (*G. aequicauda*) were hand-collected from the aquatic vegetation. Carps were collected bimonthly from the local catches of commercial fishery during May-November 2019. In the Gomishan site, mullets (*Mugilidae*) were also collected for the comparison with carp in May 2019. *Cerastoderma glaucum* (as baseline organism, see below) was also sampled from each site in early and end of summer (June and September) 2019.

In the laboratory, sampled macroinvertebrates were put in bottles containing filtered sea water and left for 24 h to allow them to void their guts. White dorsal muscle tissue of carp was dissected from the upper part of lateral line and was rinsed in a 10% solution of HCL to remove inorganic carbonates and then in distilled water (Bilby et al. 1996). Benthic macroinvertebrates and the dorsal muscle tissue of carps were then frozen until further analyses.

The frozen samples were freeze-dried for 48 hours. After that, 10 to 15 individuals of gammarids from each site at each month were pooled, grounded into a powder and stored in small glass vials for later isotope analysis. Furthermore, dorsal muscle tissue from three individual carps from each site at each month was grounded and placed in three glass vials. From each site and sampling occasion, one individual of *C. glaucum* (only tissue) was grounded and stored in glass vials. In the Gomishan site, 1-3 individuals of each macroinvertebrates species (chironomids, nereis, zebra mussel (only tissue)) from each sampling occasion were pooled, grounded and stored in different labeled glass vails. From this site, three samples of periphytic algae and seagrass were also collected, dried, grounded, and placed in glass vials.

The aim for analysing these samples was to compare isotope signatures and trophic levels of different food web components in the Gomishan. All glass vials were labelled and stored over silica gel desiccant.

From each of the macroinvertebrates vials, three specific amount of samples were put into three tin capsules for stable isotope analyses (Friberg et al. 2009, Premke et al. 2010). For fish species, one sample per vial was transferred to tin capsules (i.e., 3 measurements per site at each month). Nitrogen and carbon contents as well as nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotopic composition were analysed using an Isotope Ratio Mass Spectrometer interfaced with an Elemental Analyser (EA-IRMS).

2.4. Data analyses

Stable isotope ratios are calculated as deviation from standards following the formula:

$$\delta^{15}N \text{ or } \delta^{13}C = ((Rsample - Rstandard)/Rstandard) \times 1000$$

where Rsample and Rstandard is the heavy-to-light isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) of the samples and standards, respectively (Fry 2006). The international standard for nitrogen is atmospheric N^2 and for carbon is a marine limestone called Peedee Belemnite (Peterson and Fry 1987, Fry 2006).

The trophic level (TL) of consumers was calculated according the following equation (Post 2002):

$$TL_i = [(\delta^{15}N_i .\ \delta^{15}N_{PC})/3.4] + 2$$

where TL_i refers to the trophic level of species, $\delta^{15}N_i$ represents the $\delta^{15}N$ of species i, $\delta^{15}N_{pc}$ represents the $\delta^{15}N$ of primary consumers, and 2 is the trophic level of the baseline species used in this study. A commonly used trophic enrichment factor of 3.4 was used to express the mean trophic enrichment of nitrogen between consumers and their prey (Post 2002).

Isotopic signatures of consumers depend on the isotope signatures of primary producer which vary considerably at spatial and temporal scales (Vander Zanden et al. 1999). Thus, trophic position should be standardized according to a common isotopic baseline to deal with such temporal and spatial variation (Post 2002). This would allow comparisons in trophic positions between different systems and different species (Post 2002). Because of highly variable isotopic composition of producers, isotopic signatures of primary consumers that feed on primary producers are used as baseline to calculate trophic position of the sampled consumers (Vander Zanden et al. 1999, Vander Zanden and Rasmussen 2001, Post 2002, Sherwood and Rose 2005). The baseline organism used in the south of Caspian Sea is *C. glaucum* which (as a primary consumer) is assigned a trophic position of 2 (Mirzajani et al. 2016).

2.5. Statistical analyses

Environmental parameters, nitrogen and carbon stable isotope ratios, and trophic level values were tested for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk's test). When these assumptions were met after log-transformation, a two-way ANOVA that considered region/site and season/month as fixed factors, was used to test the temporal and spatial variations in environmental parameters and isotope values. But when the assumptions about homogeneity of variance and normality were failed, a nonparametric Kruskal–Wallis test was used to test the differences among regions/sites and seasons/months. For pairwise multiple comparisons of means, two-way ANOVA analyses were followed by Tukey's test and Kruskal–Wallis testes were followed by post hoc Dunn's test. All statistical tests were performed with software package SPSS 26.0. Statistical significance was accepted at the $P < 0.05$ level.

3. Results

3.1. Spatial variations in environmental parameters

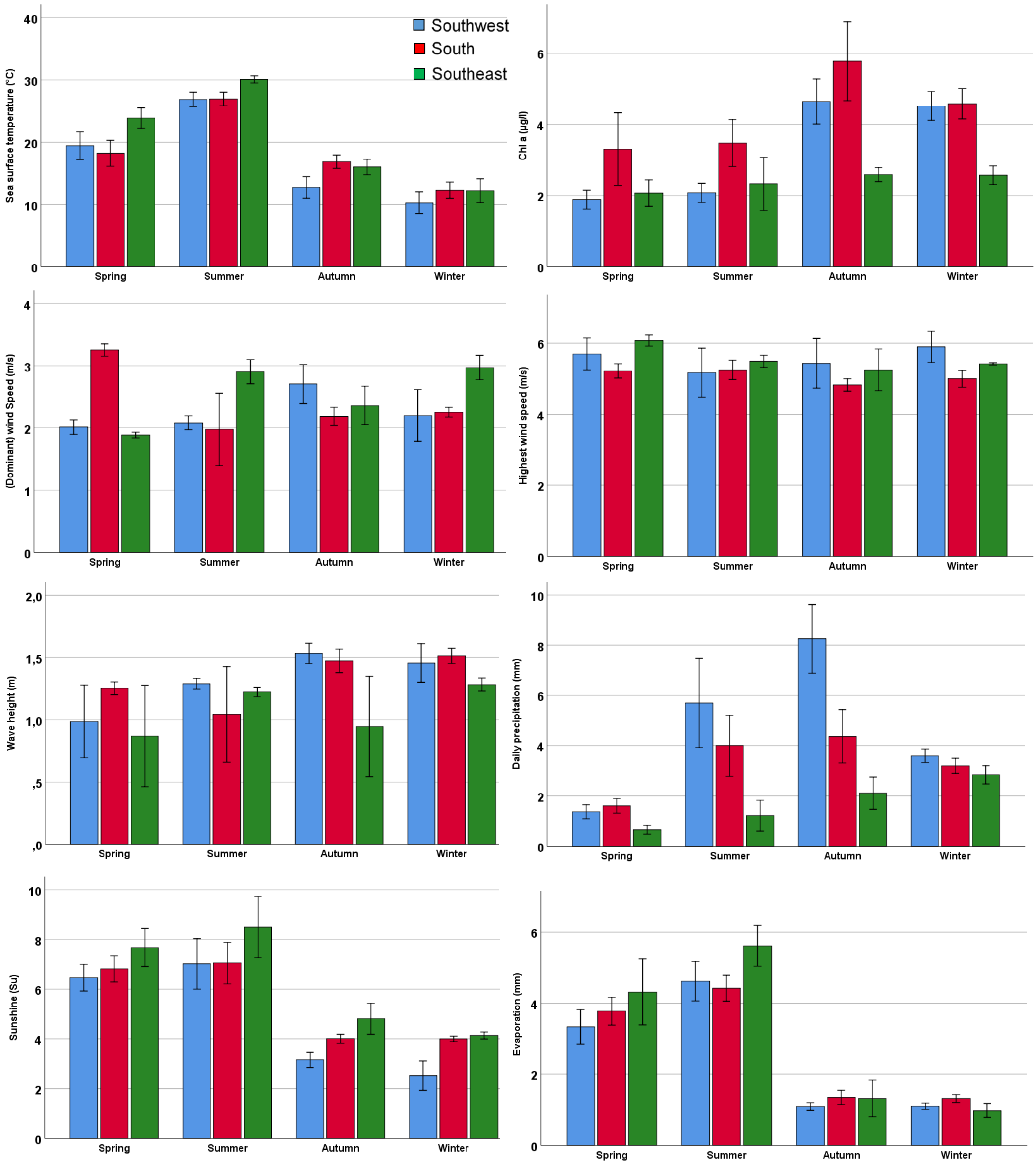
There were no significant differences between regions in minimum air temperature, maximum air temperature, sea surface temperature, evaporation, sunshine, dominant wind speed, (Kruskal–Wallis test, all $P > 0.05$, Figure 2), highest wind speed, maximum daily wind acceleration, and wave height (two-way ANOVA, all $P > 0.05$, Figure 2).

Daily precipitation varied significantly among regions (Kruskal–Wallis test, $P < 0.05$, Figure 2). Dunn’s test revealed that daily precipitation was significantly higher in the southwest than the southeast ($p < 0.05$). The region had a significant effect on the minimum daily wind acceleration (two-way ANOVA, $P < 0.05$, Figure 2), which was higher in the southwest than the southern Caspian Sea (Tukey’s test, $P < 0.05$, Figure 2). Air pressure was significantly different in different regions (Kruskal–Wallis test, $P < 0.05$, Figure 2). The southwest had higher air pressure than both the south and southeast regions (Dunn’s test, $P < 0.05$, Figure 2).

The effect of region on the chlorophyll a (Chl a) concentrations was significant (two-way ANOVA, $P < 0.05$). However, Tukey’s test revealed that only the south region and the southeast region had significantly different Chl a concentrations, which was higher in the former than the latter (Tukey’s test, $P < 0.05$, Figure 2).

3.2. Temporal variations in environmental parameters

Season had a significant effect on minimum air temperature, maximum air temperature, sea surface temperature, evaporation, sunshine, air pressure (Kruskal–Wallis test, all $P < 0.05$), highest wind speed, maximum daily wind acceleration, minimum daily wind acceleration, Chl a concentrations (two-way ANOVA, all $P < 0.05$, Figure 2). Season had no significant effect on the highest wind speed, wave height (two-way ANOVA, P > 0.05, Figure 2), and dominant wind speed (Kruskal–Wallis test, P > 0.05, Figure 2).



Continued

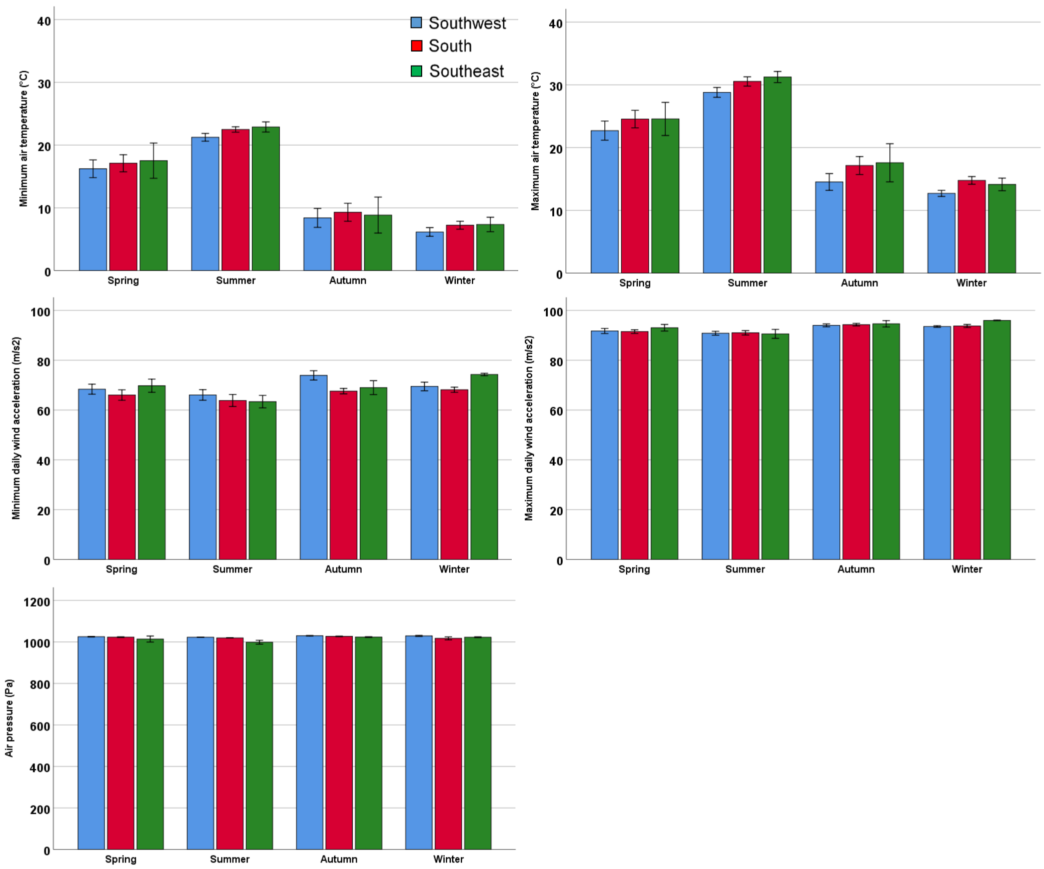


Figure 2. Mean (±SE) environmental parameters of southwest, south, and southeast of Caspian Sea during 2015-2019.

Minimum air temperature, maximum air temperature, evaporation, sunshine, sea surface temperature (Dunn's test, all P < 0.05), maximum daily wind acceleration, were higher in spring and summer than autumn and winter (Tukey's test, all P < 0.05, Figure 2). For these parameters, there were no significant differences between spring and summer as well as between autumn and winter (Dunn's test or Tukey's test, all P > 0.05, Figure 2). Minimum daily wind acceleration was higher in summer than autumn and winter (Tukey's test, P < 0.05, Figure 2). Air pressure was higher in summer than autumn (Dunn's test, P < 0.05, Figure 2). The daily precipitation was the lowest in spring among seasons (Dunn's test, P < 0.05, Figure 2). Chl a concentrations were significantly higher in autumn than in spring and summer (Tukey's test, P < 0.05, Figure 2). No significant differences in Chl a concentrations were found between spring and summer or between autumn and spring (Tukey's test, P < 0.05, Figure 2).

3.3. Spatial and temporal variations in isotope values of consumers

3.3.1. Gammarids

Although site had a significant effect on nitrogen stable isotope ratios of gammarids (Kruskal–Wallis test, n= 69, $P < 0.001$, Figure 3A), no significant effect of month was observed (Kruskal–Wallis test, n= 69, $P > 0.05$, Figure 3A). According to pairwise comparisons of sites, only Anzali and Sari exhibited similar $\delta^{15}N$ signatures in *P. maeoticus* (Dunn's test, $P > 0.05$, Figure 3A). On average, amphipod *P. maeoticus* in Noshahr presented the highest (Dunn's test, all $P < 0.05$) and amphipod *G. aequicauda* showed the lowest (Dunn's test, all $P < 0.01$) nitrogen stable isotope ratios among sites (Figure 3A).

Carbon stable isotope ratios of gammarids varied significantly among sites (two-way ANOVA, $F_{3,59} = 57.4$, $p < 0.001$) and months (two-way ANOVA, $F_{6,59} = 9.0$, $P <0.001$) (Figure 3B). Pairwise comparisons revealed that $\delta^{13}C$ values of this amphipod in different sites are different from each other (Figure 3B, Tukey's test, all $P < 0.05$). Enrichment in $\delta^{13}C$ of gammarids was the highest in Gomishan, followed by Sari, Noshahr and Anzali (Tukey's test, all $P < 0.05$, Figure 3B).

Carbon stable isotope ratios of *P. maeoticus* were not significantly different between May, June, Jul, and Aug (Tukey's test, all $P > 0.05$) as well as between Sep, Oct, and Nov (Tukey's test, all $P > 0.05$, Figure 3B). However, $\delta^{13}C$ values of gammarids in May-Aug were significantly differed from those in Sep-Nov (Tukey's test, all $P < 0.001$, Figure 3B). There was an increasing trend in carbon stable isotope ratios of *P. maeoticus* from May to November in Sari, Noshahr and Anzali sites (Figure 3B). In Gomishan, *G. aequicauda* was more enriched in $\delta^{13}C$ in October than November (Tukey's test, $P < 0.001$, Figure 3B).

C:N ratios of gammarids were also changed spatially and temporally (Kruskal–Wallis test, n= 69, both $P < 0.001$, Fig 3C). Among sites, Gammarids in Anzali had the highest C:N ratios (Dunn's test, all $P < 0.05$) whereas there were no significant differences in C:N ratios of gammarids between other sites (Dunn's test, all $P > 0.05$). There was a decreasing trend in C:N ratios of *P. maeoticus* from June-November in Anzali, Noshahr, and Sari sites (Figure 3C). C:N ratios of gammarids in November differed from those in other months (Dunn's test, all $P < 0.05$) with the exception of September and October (Dunn's test, both $P > 0.05$). Gammarids in October had a lower C:N ratio than those in May-August (Dunn's test, all $P < 0.05$). C:N ratios of gammarids in September were significantly different from those in May-July (Dunn's test, all $P < 0.05$). There were no significant differences in C:N ratios of gammarids between, May, June, July, and August (Dunn's test, all $P > 0.05$).

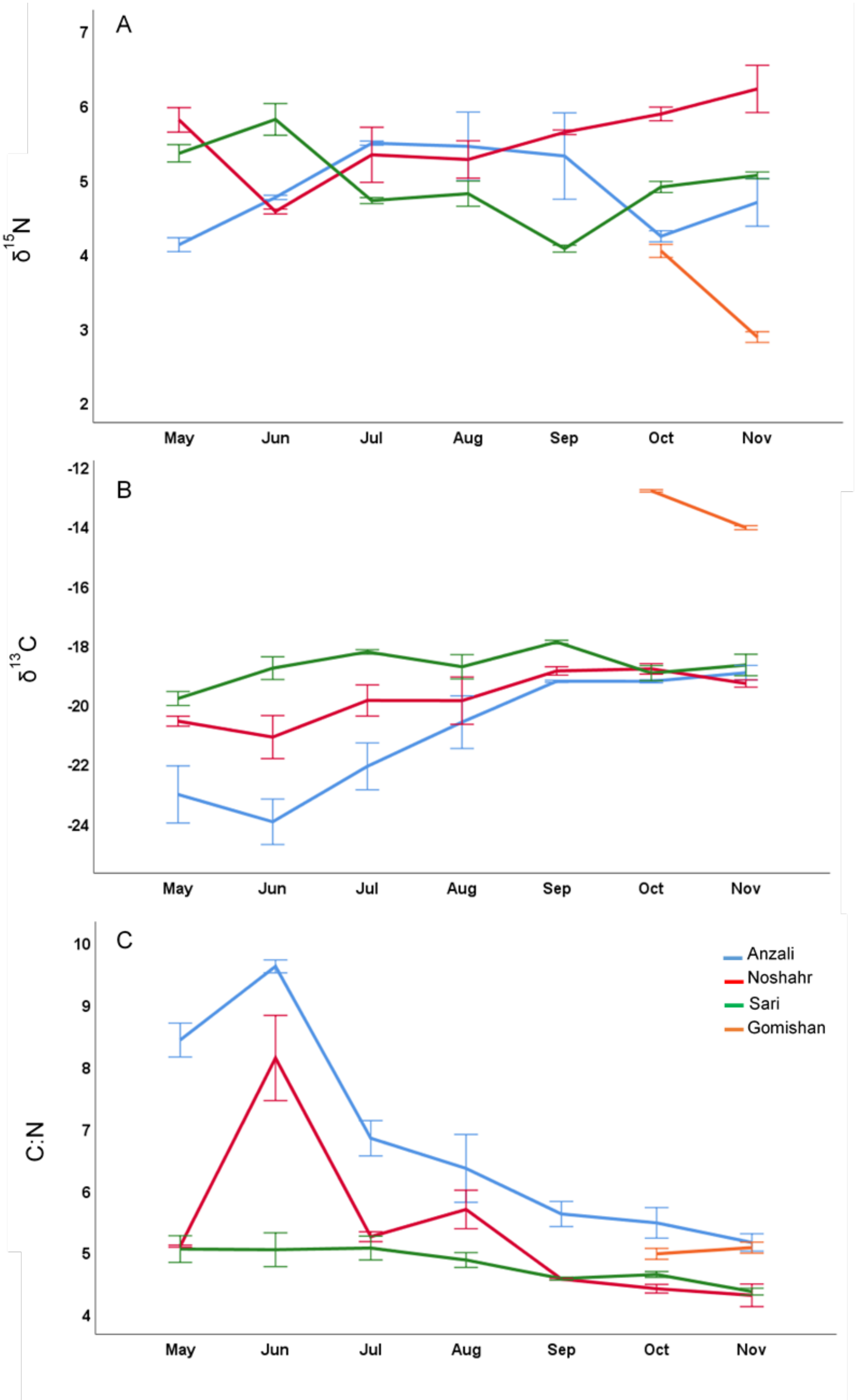


Figure 3. Seasonal trends in mean (±SE) stable nitrogen and carbon isotope ratios and C:N ratios in gammarids samples from Anzali, Noshahr, Sari, and Gomishan stations.

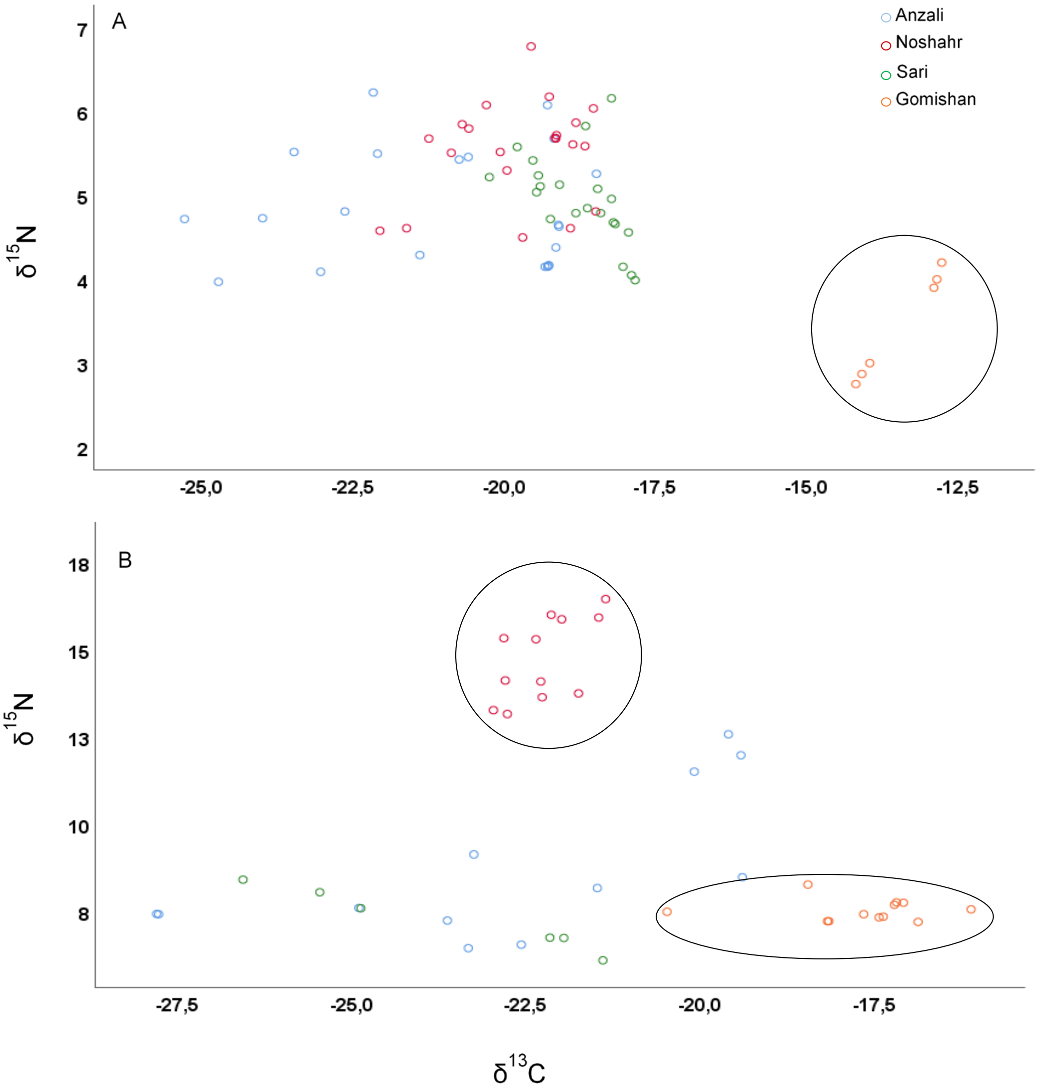


Figure 4. Distribution of carbon and nitrogen stable isotope ratios among gammarids (A) and cyprinus carpio (B) collected from different stations in south of the Caspian Sea. Circles in upper graph (A) and lower graph (B), enclose gammarids and carps having distinct isotope signatures, respectively.

3.3.2. *Cyprinus carpio*

$\delta^{15}N$ values of common carp did not change significantly in different months (Kruskal–Wallis test, n= 42, $P > 0.05$). Site had a significant effect on the $\delta^{15}N$ of common carp (Kruskal–Wallis test, n= 42, $P < 0.05$, Figure 5A). Carps in Noshahr were enriched more with $\delta^{15}N$ than those in Anzali (Dunn's test, $P < 0.01$), Sari (Dunn's test, $P < 0.001$), and Gomishan (Dunn's test, $P < 0.001$) (Figure 5A). There were no significant differences in $\delta^{15}N$ values among carps in the Anzali, Sari, and Gomishan sites (Dunn's test, all $P > 0.05$).

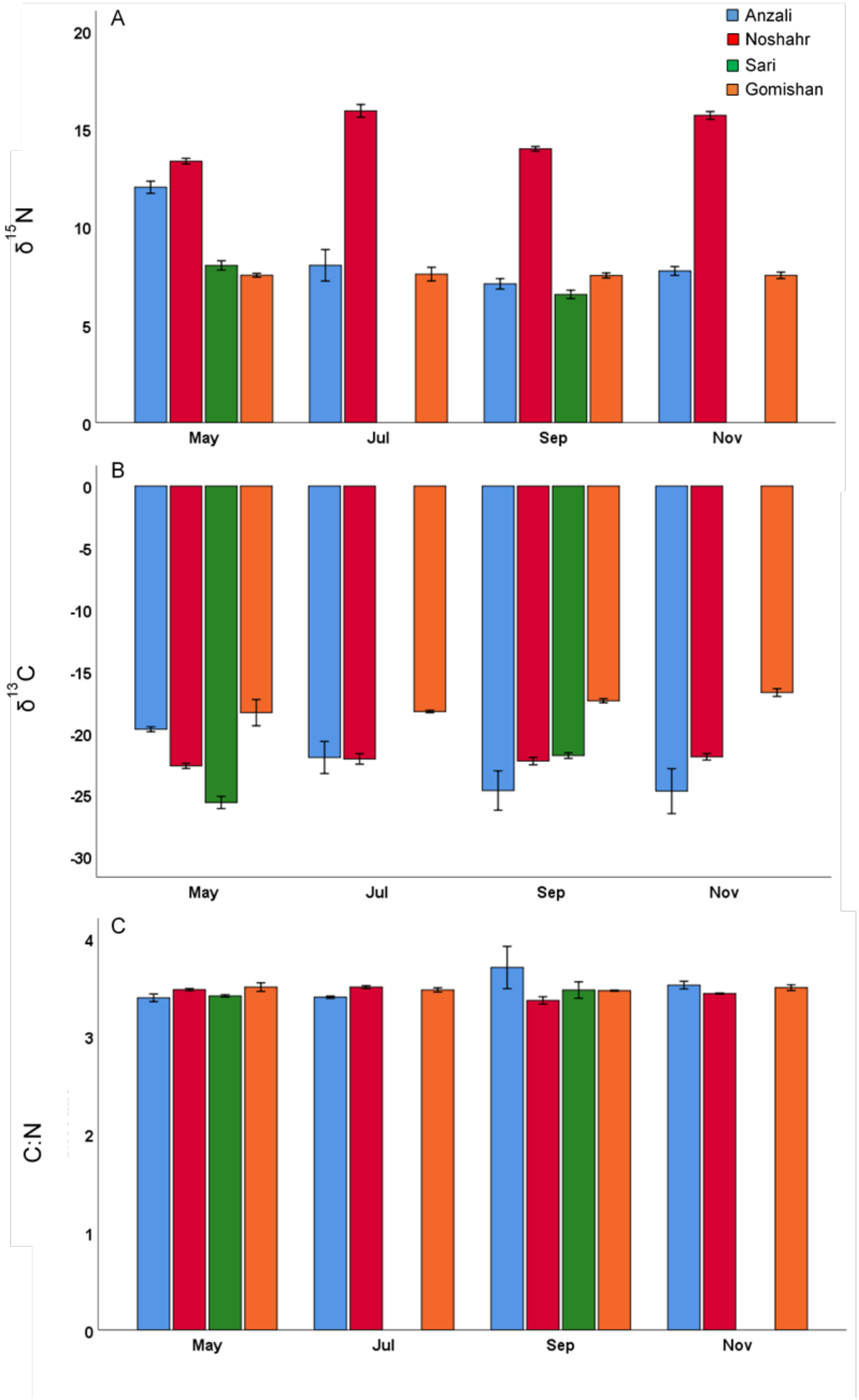


Figure 5. Seasonal trends in mean (±SE) nitrogen and carbon stable isotope ratios and C:N ratios in gammarids samples from Anzali, Noshahr, Sari, and Gomishan sites.

$\delta^{13}C$ values of carp did not differ among months (two-way ANOVA, $P > 0.05$, $F_{3,35} = 0.1$). But enrichment in $\delta^{13}C$ varied among sites (two-way ANOVA, $P < 0.001$, $F_{3,35} = 19.3$) (Figure 5B). Carps in Gomishan had the highest value of $\delta^{13}C$ among all sites. There were no significant differences in $\delta^{13}C$ values among carps in the Anzali, Noshahr, and Sari sites.

C:N ratios in carps were similar among sites (two-way ANOVA, $P > 0.05$, $F_{3,35} = 0.6$) and months (two-way ANOVA, $P > 0.05$, $F_{3,35} = 0.5$) (Figure 5C). Two distinct groups of *C. carpio* (Noshahr and Gomishan) could be differentiated in accordance with their isotope signatures (Figure 4B).

3.4. Isotope signatures of baseline organism and trophic levels

There were no significant differences in stable nitrogen isotope ratios of baseline organism *C. glaucum* between June and September (Two-way ANOVA, $F_{1,19} = 1.2$ for $\delta^{15}N$ and $F_{1,19} = 1.5$ for $\delta^{13}C$, $p > 0.05$). There was an increasing trend in $\delta^{15}N$ and $\delta^{13}C$ values of baseline organism from the southwest to the southeast (Anzali to Gomishan), although only $\delta^{13}C$ values varied significantly among sites (Two-way ANOVA, $F_{3,19} = 0.9$, $p > 0.05$ for $\delta^{15}N$ and $F_{3,19} = 91.9$ for $\delta^{13}C$, $p < 0.001$, Table 1). Month had no significant effect on trophic level of *P. maeoticus* (Kruskal–Wallis test, n= 69, $P > 0.05$) and *C. carpio* (Kruskal–Wallis test, n= 42, $P > 0.05$).

Trophic levels of Gammarids and *C. carpio* varied among sites (Kruskal–Wallis test, both $P < 0.001$, Table 1). Gammarids displayed the lowest trophic level in Gomishan (Dunn's test, all $P < 0.05$) and a similar trophic level between Anzali and Sari (Dunn's test, $P > 0.05$). Gammarids in Noshahr showed significantly higher trophic level than those in Sari (Dunn's test, $P < 0.05$) and Anzali, although the difference between Noshahr and Anzali was not significant (Dunn's test, $P > 0.05$). Among sites *C. carpio* exhibited the highest trophic level in Noshahr (Dunn's test, $P < 0.05$, Table 1). Carps in Anzali, Sari, and Gomishan represented similar trophic levels (Dunn's test, $P > 0.05$) (Table 1).

Table 1. Mean (±SD) stable nitrogen and carbon isotope ratios and trophic level in consumers collected from Anzali, Noshahr, Sari, and Gomishan sites.

Site	Species	$\delta^{15}N$	$\delta^{13}C$	Trophic level*
Anzali	*Pontogammarus maeoticus*	4.86 ± 0.70	-21.02 ± 2.15	2.04 ± 0.21
	Cyprinus carpio	8.73 ± 2.14	-22.76 ± 2.98	3.18 ± 0.63
	Cerastoderma glaucum	4.73 ± 0.83	-21.29 ± 0.66	
Noshahr	*Pontogammarus maeoticus*	5.52 ± 0.60	-19.78 ± 1.06	2.17 ± 0.18
	Cyprinus carpio	14.75 ± 1.19	-22.24 ± 0.54	4.89 ± 0.35
	Cerastoderma glaucum	4,93 ± 0.57	-20.86 ± 0.61	
Sari	*Pontogammarus maeoticus*	4.96 ± 0.54	-18.74 ± 0.70	1.97 ± 0.16
	Cyprinus carpio	7.29 ± 0.89	-23.73 ± 2.16	2.66 ± 0.26
	Cerastoderma glaucum	5.06 ± 0.79	-18.14 ± 0.70	
Gomishan	Chironomid	6.30 ± 0.20	-12.85 ± 0.15	2.28 ± 0.06
	Nereis	7.55 ± 0.23	-14.30 ± 2.18	2.65 ± 0.07
	Gammarus aequicauda	3.45 ± 0.65	-13.45 ± 0.68	1.44 ± 0.19
	Cyprinus carpio	7.54 ± 0.31	-17.68 ± 1.09	2.64 ± 0.09
	Zebra mussel	6.41 ± 0.14	-13.16 ± 0.88	2.31 ± 0.04
	Mullet	6.41 ± 0.16	-18.68 ± 0.12	2.31 ± 0.05
	Cerastoderma glaucum	5.39 ± 0.70	-13.94 ± 1.33	

* Trophic level based on primary consumer (*Cerastoderma glaucum*)

3.5. Food web components in Gomishan

Distribution of carbon and nitrogen stable isotope ratios among samples collected from the Gomishan station revealed that there are some overlaps in trophic niche space between different groups (Figure 6). Different food web components were differentiated in different groups by their isotopic signatures. Seagrass had the lowest $\delta^{15}N$ value whereas carp represented the highest $\delta^{15}N$ value indicating that they are in higher trophic levels than other species (Figure 6). Among primary consumers, nereis was at higher trophic level than zebra mussel and chironomids. *C. glaucum* and algae exhibited similar isotopic signatures (Figure 6). *G. aequicauda* had even lower $\delta^{15}N$ value than *C. glaucum* (Figure 6).

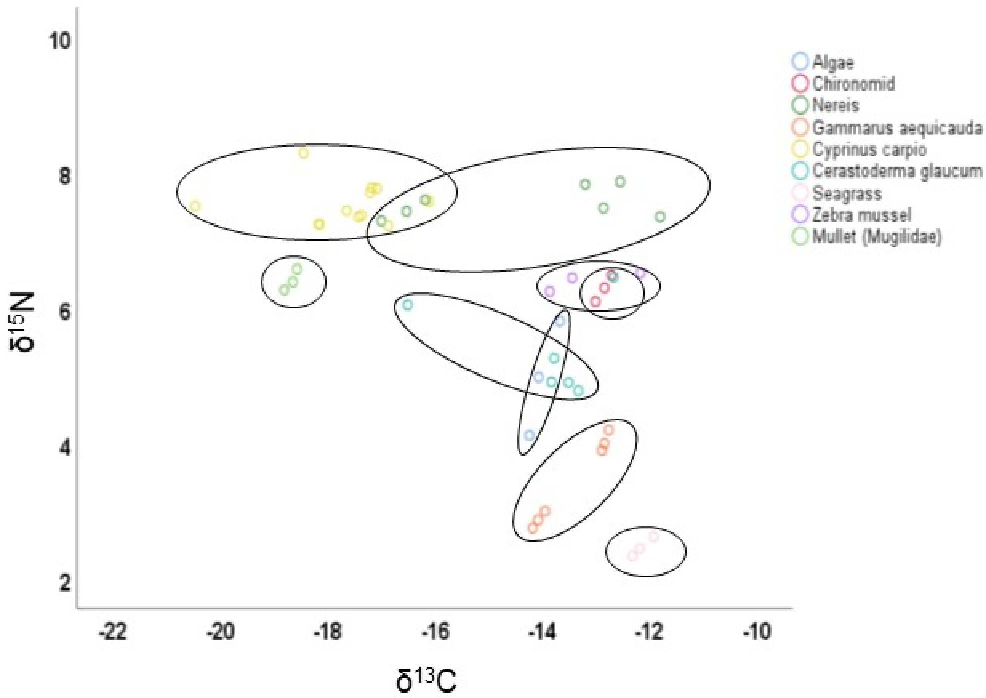


Figure 6. Distribution of carbon and nitrogen stable isotope ratios among samples collected from Gomishan station. Circles enclose different species.

4. Discussion

In this thesis, I showed that the southwest, south, and southeast of Caspian Sea are different with regards to some environmental parameters. My results revealed that there are spatial and temporal variations in stable isotope signatures of the gammarids and carp in the southern coast of the Caspian Sea, although month or site effects were not significant in some cases (supporting partly hypothesis 1). From the southwest to southeast (Anzali to Gomishan), $\delta^{13}C$ values of the baseline organism (*C. glaucum*) increased. All organisms in Gomishan including *C. glaucum*, *G. aequicauda*, nereis, zebra mussel, *C. carpio*, chironomids, and mullets were enriched in $\delta^{13}C$. In freshwater systems, zooplankton (*Daphnia magna*) growing at 26.5°C had higher carbon stable isotope ratio than those that grew at 12.5°C (Power et al. 2003, see also Gu et al. 2006). However, there were no significant differences in sea surface temperature and air temperature between different regions. High δ13C enrichment in consumers of Gomishan can be explained by potentially low amount of terrestrial-derived organic matters in this site. Such organic matters have high depleted $\delta^{13}C$ (range: -30‰ to -23‰, average: -28‰; (Peterson and Fry 1987, Fry and Sherr 1989) and can lead to a high depletion in water isotopic signal. Given the distance between the Gomishan site and Gorganrud estuary, the lack of direct sea current from the estuary to Gomishan, and the vegetation type around Gorganrud (mostly reeds), the input of terrestrial material to Gomishan from Gorganrud River is seemed to be low compared to other sampling sites. In addition, the presence of several dams across Gorganrud River is probably reduced the amount of suspended materials transported by the rivers to Caspian Sea. Apparently, organic matter in Gomishan has more autochthonous origin and is derived from primary producers (algae, seagrass detritus). This will ultimately lead to enrichment in ^{13}C in all organisms living in the Gomishan site.

Gammarids in Gomishan represented the lowest $\delta^{15}N$ among sites. In the Gomishan, amphipod species were different from the amphipod species in other sites (*P. maeoticus*) and samples were collected only for two months. However, the month effect on gammarids $\delta^{13}C$ values was not significant and it does not seem reasonable to see such a large interspecific differences in $\delta^{13}C$ values (see Figure 3B). Nitrogen and carbon signatures of *G. aequicauda* was close to sea grass in Gomishan (see Figure 6) indicating that this amphipod consume mostly seagrass detritus and then has a lower $\delta^{15}N$ value than *C. glaucum* which feed on phytoplankton. Similarly Remy et al. (2017) have found that *G. aequicauda* is the most important dead seagrass (*Posidonia oceanica*) consumer with up to 50% of dead leaves contribution and has a lower turnover rate for C than amphipods feeding on a high quality animal food (Remy et al. 2017).

Both Amphipod *P. maeoticus* and *C. carpio* were more enriched in $\delta^{15}N$ in Noshahr than at other sites. Sewage discharge, urban wastewater, and aquaculture wastes enter into the Noshahr site by Sardabrud River and Chalus River. Urban effluents, agricultural fertilizers, and aquaculture activities can lead to organic enrichment with consequences for benthic communities (Pearson and Rosenberg 1978, McClelland and Valiela 1998, Peterson 1999, Costanzo et al. 2001, Costanzo et al. 2003). It is especially important to note that houses around the Noshahr site can directly discharge their domestic wastes into small streams in the coastal region. Hence, it is assumed that the $\delta 15$N values of gammarids and carp in Noshahr are affected by the organic matters input from Sardabrud River and Chalus River as well as personal houses. Similarly, Costanzo et al. (2001) found that sea grasses in the open sea and in sites in the vicinity of urban centres, were enriched in $\delta^{15}N$ in Moreton Bay (Australia), which was ascribed to sewage effluents (Costanzo et al. 2001). It has also been shown that nitrogen stable isotope ratios in ammonium from wastewater varies from 16% to 25% while it is about 5.4% in ammonium from rainwater (Dillon and Chanton 2008). In the western basin of Lake Superior, organisms inhabiting areas close to large human population were more enriched in $\delta^{15}N$ than those living in surrounding areas (Harvey and Kitchell 2000).

Unlike Gammarids and carps in Gomishan, those inhabited Noshar, Sari and Anzali had depleted $\delta^{13}C$ signatures. Both the Sardabrud River and the Chalus River pass through forest and possibly transfer some terrestrial materials to their estuaries which can enter the Noshahr site as a result of sea currents. Similarly, Sari site is affected by terrestrial materials which are transported with Tajan River to this area. The Sefidrud River and canals which discharge the Anzali wetland, transfer a large amount of allochthonous materials to the coastal areas in the southwest of the Caspian Sea (Nezami and Khodaparast 1996). Thus, high depletion of $\delta^{13}C$ in gammarids and carps in the Noshahr, Sari, and Anzali sites is presumably due to entering of high allochthonous organic matter to these sites by the rivers. Apparently, the amount of allochthonous materials in the Azanli is higher than other sites (probably because of dense vegetation in Anzali wetland) which is reflected in the lowest values of $\delta^{13}C$ in organisms at Anzali.

In the current study, there was a seasonal variation in $\delta^{13}C$ values of gammarids among sites. The gammarids were more enriched in $\delta^{13}C$ in autumn (September-November) than other months. This is probably due to higher Chl a concentrations (high food availability) in autumn than spring and summer in the south of the

Caspian Sea (see Figure 1) because carbon stable isotope ratios is a function of primary productivity in the ecosystem (Gu et al. 1996).

Both site and month had significant effects on C:N ratio of gammarids. The highest C:N ratios of gammarids in Anzali can be explained by more allochthonous sources of carbon in this site (low food quality) compared to other sites. Food with autochthonous sources is energy efficient, more unstable and is preferred by consumers and would lead to low C:N ratio in the consumers (Thorp and Delong 2002, Oeding et al. 2020). In general, the C:N ratio is indicative of food quality and a high C:N ratio can be interpreted as low nutritional, poor food source (Burns and Ryder 2001). This low food quality may finally result in slow growth rate in consumer in higher trophic levels. Amphipods are one of the prey items in *C. carpio* diet in the southern of Caspian Sea (Ghorbani unpublished data). Carp feeding on high C:N ratio gammarids in Anzali may show lower growth rate than carps feeding on low C:N ratio gammarids in other area. But this is an area of research that needs further investigation.

At the Anzali, Noshahr, and Sari sites, C:N ratios of gammarids were higher in June-August than September-November. From June to August, the stored water behind the dam on the rivers is released for irrigation propose in agriculture. Thus a large amount of stored allochthones materials may be released into the southern coastal waters of the Caspian Sea through the connected rivers which in turn may increase the C:N ratio of primary producers and herbivores. Allochthonous input from riparian vegetation have much higher C:N ratios than autochthonous food sources (Elser et al. 2000, Britton et al. 2007). Another reason can be related to fecundity of gammarids. The C:N ratio is used as a proxy for total lipid contents (Schultz et al. 2012). For example, high C:N ratio in soft tissues of Mangrove Oysters (*Crassostrea corteziensis*) was indicative of high amount of lipids in the soft tissue related to reproductive activity (Torres-Rojas et al. 2014) . Thus, the amount of lipids in gammarids was probably high during spring-summer when the fecundity was high, which in turn led to a high C:N ratio during this period.

The trophic position of gammarids and common carps varied among sites (partly supporting hypothesis 2) but were independent of month (partly rejecting hypothesis 2). There were no piscivores in my samples and all consumers fed at the base of the food web, with trophic levels ranging from 1.44 in *G. aequicauda* to 4.89 in *C. carpio*. The lowest trophic level of *G. aequicauda* is presumably because of their feeding on detritus and dead seagrass (Remy et al. 2014). Likewise, Vanderklift and Ponsard (2003) found a $\delta^{15}N$ fractionation of 0.53‰ in detritivores compared to 2.98‰ in herbivores. The highest trophic level of *C. carpio* in Noshahr is probably due to their feeding on local prey (e.g., *P. maeoticus*) with a high nitrogen stable isotope ratio. However, common carp is an omnivorous species and may also consume different prey with variable $\delta^{15}N$ signatures. A high degree of trophic variation was found in juvenile common carp (Weber and Brown 2013) and a piscivory trophic mode was reported by *C. carpio* as small as 11.2 cm (Britton et al. 2007). Mean trophic level of carps in other sites was not significantly different and was around 3. In Gomishan, common carp occupied a higher trophic level than mullet. The $\delta^{15}N$ values of common carp in relation to $\delta^{15}N$ values of benthic invertebrate in Gomishan (see Figure 6) indicate that carp consumes most of them. In another study, it has been shown that *C. carpio* in the southern coast of the Caspian Sea feed mainly on amphipods, chironomids, nereis and bivalves (Ghorbani, unpublished data). Since mullets consume microscopic algae and the minute animals associated with the algae (Brian 2017), their nitrogen stable isotope ratios are likely smaller than common carp.

In conclusion, this study demonstrated that carbon and nitrogen stable isotope ratios of gammarids and cyprinids change among the coastal areas of Caspian Sea. It also revealed that the trophic position of gammarids and common carps varied spatially but not temporally. In addition, the current study highlights the importance of carbon and nitrogen stable isotope ratios to understand trophic interactions and to trace the energy flow in coastal ecosystems. Results from this study suggested that low quality allochthonous organic matters originated from the rivers that flow into the Caspian Sea, playing an important role in the food web and ecosystem function of the Caspian Sea. Moreover, incorporation of the sewage-derived material, aquaculture waste, agricultural runoff, contaminant inputs into food sources in the coastal area can provide a stressful environment for the fauna and threaten the Caspian Sea ecosystem. This study also notifies water managers to set up proper and timely measures to mediate anthropogenic stressors and rehabilitation of fish spawning habitats.

5. References

Bagheri, S., M. Mashhor, M. Makaremi, A. Mirzajani, H. Babaei, H. Negarestan, and W. Wan-Maznah. 2010. Distribution and composition of phytoplankton in the southwestern Caspian Sea during 2001–2002, a comparison with previous surveys. World Journal Fish and Marine Sciences 2:416-426.

Bilby, R. E., B. R. Fransen, and P. A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences 53:164-173.

Brian, W. C. 2017. Review of the freshwater mullets of Iran (Family Mugilidae). Iranian Journal of Ichthyology 4:75-130.

Britton, J. R., R. Boar, J. Grey, J. Foster, J. Lugonzo, and D. Harper. 2007. From introduction to fishery dominance: the initial impacts of the invasive carp Cyprinus carpio in Lake Naivasha, Kenya, 1999 to 2006. Journal of Fish biology 71:239-257.

Burns, A., and D. S. Ryder. 2001. Potential for biofilms as biological indicators in Australian riverine systems. Ecological Management & Restoration 2:53-64.

Costanza, R., R. d'Arge, R. De Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'neill, and J. Paruelo. 1997. The value of the world's ecosystem services and natural capital. nature 387:253-260.

Costanzo, S., M. O'donohue, W. Dennison, N. Loneragan, and M. Thomas. 2001. A new approach for detecting and mapping sewage impacts. Marine Pollution Bulletin 42:149-156.

Costanzo, S. D., M. J. O'Donohue, and W. C. Dennison. 2003. Assessing the seasonal influence of sewage and agricultural nutrient inputs in a subtropical river estuary. Estuaries 26:857-865.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et cosmochimica acta 45:341-351.

di Lascio, A., L. Rossi, P. Carlino, E. Calizza, D. Rossi, and M. L. Costantini. 2013. Stable isotope variation in macroinvertebrates indicates anthropogenic disturbance along an urban stretch of the river Tiber (Rome, Italy). Ecological indicators 28:107-114.

Dillon, K. S., and J. P. Chanton. 2008. Nitrogen stable isotopes of macrophytes assess stormwater nitrogen inputs to an urbanized estuary. Estuaries and coasts 31:360-370.

Dumont, H. 1998. The Caspian Lake: history, biota, structure, and function. Limnology and Oceanography 43:44-52.

Elser, J. J., W. F. Fagan, R. F. Denno, D. R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S. S. Kilham, E. McCauley, and K. L. Schulz. 2000.

Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578-580.
Friberg, N., J. B. Dybkjaer, J. S. Olafsson, G. M. Gislason, S. E. Larsen, and T. L. Lauridsen. 2009. Relationships between structure and function in streams contrasting in temperature. Freshwater Biology 54:2051-2068.
Fry, B. 2006. Stable isotope ecology. Springer.
Fry, B., and E. B. Sherr. 1989. δ 13 C measurements as indicators of carbon flow in marine and freshwater ecosystems. Pages 196-229 Stable isotopes in ecological research. Springer.
Gu, B., A. D. Chapman, and C. L. Schelske. 2006. Factors controlling seasonal variations in stable isotope composition of particulate organic matter in a softwater eutrophic lake. Limnology and Oceanography 51:2837-2848.
Gu, B., C. L. Schelske, and M. Brenner. 1996. Relationship between sediment and plankton isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and primary productivity in Florida lakes. Canadian Journal of Fisheries and Aquatic Sciences 53:875-883.
Harvey, C. J., and J. F. Kitchell. 2000. A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web. Canadian Journal of Fisheries and Aquatic Sciences 57:1395-1403.
Kideys, A. E., A. Roohi, E. Eker-Develi, F. Mélin, and D. Beare. 2008. Increased chlorophyll levels in the southern Caspian Sea following an invasion of jellyfish. International Journal of Ecology 2008.
Leroy, S. A., F. Chalie, F. P. Wesselingh, M. S. Sanjani, H. A. Lahijani, J. Athersuch, U. Struck, G. Plunkett, P. J. Reimer, and P. Habibi. 2018. Multi-proxy indicators in a Pontocaspian system: a depth transect of surface sediment in the SE Caspian Sea. Geologica Belgica 21:143-165.
Leroy, S. A., H. A. Lahijani, J.-F. Crétaux, N. V. Aladin, and I. S. Plotnikov. 2020. Past and current changes in the largest lake of the world: The Caspian Sea. Pages 65-107. Large Asian Lakes in a Changing World. Springer.
Martinetto, P., M. Teichberg, and I. Valiela. 2006. Coupling of estuarine benthic and pelagic food webs to land-derived nitrogen sources in Waquoit Bay, Massachusetts, USA. Marine Ecology Progress Series 307:37-48.
Martínez-Durazo, A., J. García-Hernández, F. Páez-Osuna, M. F. Soto-Jiménez, and M. E. Jara-Marini. 2019. The influence of anthropogenic organic matter and nutrient inputs on the food web structure in a coastal lagoon receiving agriculture and shrimp farming effluents. Science of The Total Environment 664:635-646.
McClelland, J. W., and I. Valiela. 1998. Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries. Marine Ecology Progress Series 168:259-271.
McCutchan Jr, J. H., W. M. Lewis Jr, C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378-390.
Michener, R.H., and D.M. Schell. 1994. Stable isotope ratios as tracers in marine and aquatic food web. In: Lajtha, K., Michener, R.H. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publication, pp. 138-157.
Minagawa, M., and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et cosmochimica acta 48:1135-1140.

Mirzajani, A., A. Hamidian, and M. Karami. 2016. Metal bioaccumulation in representative organisms from different trophic levels of the Caspian Sea. Iranian Journal of Fisheries Sciences 15:1027-1043.

Mirzajani, A. R. 2003. A study on the population biology of *Pontogammarus maeoticus* (Sowinsky, 1894) in Bandar Anzali, southwest Caspian Sea. Zoology in the Middle East 30:61-68.

Moiceiev, P., and Z. Filatova. 1985. Kaspiiskogo Moria: Fauna and bialogiscaya produksia. Moscow, Russia: Nauka press.

Naderi, S., A. Mirzajani, and E. Hadipour. 2017. Distribution of and threats to the Eurasian Otter (Lutra lutra) in the Anzali Wetland, Iran. IUCN/SCC Otter Specialist Group Bulletin 34:84-94.

Nezami, B. S., and S. Khodaparast. 1996. Survey on organic matter accumulation in the Anzali Lagoon sediments. Iranian Scientific Fisheries Journal 5:1-10.

Oeding, S., K. H. Taffs, A. Reichelt-Brushett, and J. M. Oakes. 2020. Carbon and nitrogen stable isotope analyses indicate the influence of land use on allochthonous versus autochthonous trophic pathways for a freshwater Atyid shrimp. Hydrobiologia:1-16.

Pearson, T., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and marine biology annual review 16.

Peterson, B. J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. Acta oecologica 20:479-487.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual review of ecology and systematics 18:293-320.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703-718.

Power, M., K. Guiguer, and D. Barton. 2003. Effects of temperature on isotopic enrichment in Daphnia magna: implications for aquatic food-web studies. Rapid Communications in Mass Spectrometry 17:1619-1625.

Premke, K., J. Karlsson, K. Steger, C. Gudasz, E. von Wachenfeldt, and L. J. Tranvik. 2010. Stable isotope analysis of benthic fauna and their food sources in boreal lakes. Journal of the North American Benthological Society 29:1339-1348.

Remy, F., F. Darchambeau, A. Melchior, and G. Lepoint. 2017. Impact of food type on respiration, fractionation and turnover of carbon and nitrogen stable isotopes in the marine amphipod *Gammarus aequicauda* (Martynov, 1931). Journal of Experimental Marine Biology and Ecology 486:358-367.

Roohi, A., Z. Yasin, A. E. Kideys, A. T. S. Hwai, A. G. Khanari, and E. Eker-Develi. 2008. Impact of a new invasive ctenophore (Mnemiopsis leidyi) on the zooplankton community of the Southern Caspian Sea. Marine Ecology 29:421-434.

Schaal, G., C. Nerot, J. Grall, T. Chouvelon, A. Lorrain, J.-M. Mortillaro, N. Savoye, A. Brind'Amour, Y.-M. Paulet, and H. Le Bris. 2016. Stable isotope ratios in bentho-demersal biota along a depth gradient in the Bay of Biscay: A multitrophic study. Estuarine, Coastal and Shelf Science 179:201-206.

Schultz, S., B. Vallant, and M. J. Kainz. 2012. Preferential feeding on high quality diets decreases methyl mercury of farm-raised common carp (Cyprinus carpio L.). Aquaculture 338:105-110.

Sherwood, G. D., and G. A. Rose. 2005. Stable isotope analysis of some representative fish and invertebrates of the Newfoundland and Labrador continental shelf food web. Estuarine, Coastal and Shelf Science 63:537-549.

Stachowicz, J. J., J. F. Bruno, and J. E. Duffy. 2007. Understanding the effects of marine biodiversity on communities and ecosystems. Annu. Rev. Ecol. Evol. Syst. 38:739-766.

Stock, J., A. Mirzajani, R. Vonk, S. Naderi, and B. Kiabi. 1998. Limnic and brackish water Amphipoda (Crustacea) from Iran. Beaufortia 48:173-234.

Thorp, J. H., and M. D. Delong. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. Oikos 96:543-550.

Torres-Rojas, Y. E., F. P. Osuna, M. B. Tiznado, J. C. Carpizo, and S. A. García. 2014. Seasonal and spatial variation of carbon and nitrogen stable isotopes in mangrove oysters (Crassostrea corteziensis) from the northwest coast of Mexico. Journal of Shellfish Research 33:425-432.

Vander Zanden, H. B., D. X. Soto, G. J. Bowen, and K. A. Hobson. 2016. Expanding the isotopic toolbox: applications of hydrogen and oxygen stable isotope ratios to food web studies. Frontiers in Ecology and Evolution 4:20.

Vander Zanden, M. J., J. M. Casselman, and J. B. Rasmussen. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401:464-467.

Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnology and oceanography 46:2061-2066.

Vanderklift, M. A., and S. Ponsard. 2003. Sources of variation in consumer-diet δ 15 N enrichment: a meta-analysis. Oecologia 136:169-182.

Vazirzadeh, A., B. Mojazi Amiri, and A. Fostier. 2014. Ovarian development and related changes in steroid hormones in female wild common carp (C yprinus carpio carpio), from the south-eastern C aspian S ea. Journal of animal physiology and animal nutrition 98:1060-1067.

Vazirzadeh, A., and S. Yelghi. 2015. Long-term changes in the biological parameters of wild carp (Cyprinus carpio carpio) from the south-eastern Caspian Sea. Iranian Journal of Science and Technology (Sciences) 39:391-397.

Weber, M. J., and M. L. Brown. 2013. Spatiotemporal variation of juvenile common carp foraging patterns as inferred from stable isotope analysis. Transactions of the American Fisheries Society 142:1179-1191.

Vizzini, S., B. Savona, M. Caruso, A. Savona, and A. Mazzola. 2005. Analysis of stable carbon and nitrogen isotopes as a tool for assessing the environmental impact of aquaculture: a case study from the western Mediterranean. Aquaculture International 13:157-165.

Acknowledgements

I would like to thank my supervisor Rasoul Ghorbani for providing the opportunity to work on Caspian Sea and his great help during my filed and laboratory works as well as his valuable advices during the running of this project. Many thanks for all your supports! In addition, I would like to acknowledge my co-supervisor Rahmat Naddafi for his consistent support, guidance, encouragement and patience throughout this study. I would like to express my sincere thanks to Andreas Bryhn for his dedicated support and guidance as a director of studies and for his valuable feedback on the thesis as an examiner. Many thanks to the students in Department of Fisheries and Environment at Gorgan University of Agricultural Sciences and Natural Resources, particularly Atefeh Abbasi, without whom I would not have been able to complete this research. Finally, I would like to thank my family for supporting me during the compilation of this thesis.