

Research Article

Dietary habits change of Lessepsian migrants' fish from the Red Sea to the Eastern Mediterranean Sea

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Abstract

As impacts on the Mediterranean Sea are expected to grow in the future, especially with climate change, habitat degradation, and displacement of native species by non-indigenous species (NIS), the investigation of significant alterations to trophic levels in this diverse marine habitat is important. Analysis of stable isotopes from targeted consumers has previously been shown to reliably reflect that of primary producers, thus enabling us to describe and highlight potential shifts in the food web of a particular ecosystem. In this study, we used $\delta^{13}\text{C}$ values of essential amino acids (AA) in order to examine the dietary composition of established non-native, Lessepsian fish migrants in the Eastern Mediterranean Sea compared to that of the same fish species from their original population in the Gulf of Aqaba, Red Sea. Our data show that a clear variance in carbon isotopic signatures exists in food sources consumed by the same species between the different environments, with the exception of the classic herbivore, *Siganus rivulatus* (Forsskål & Niebuhr, 1775), whose very similar isotopic patterns reflect the algal source they predominantly consume in both locations. With the results of this research, we propose that Lessepsian fishes with the ability to maintain their nutritional patterns, though not necessarily that of their original food source, will acclimatize better in their new habitat. Consequences of flourishing Lessepsian fish populations include a further tropicalization of the Eastern Mediterranean Sea and the likely restructuring of local food webs.



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Introduction

The Mediterranean Sea is a semi-enclosed basin comprising a complex marine ecosystem. (Bethoux et al. 1999). Currently considered among the most impacted of the regional seas, the Mediterranean basin has become susceptible to changes in its biodiversity through various anthropogenic pressures such as climate change, overfishing, habitat degradation, and non-indigenous species (NIS) introductions (Galil 2007; Golani and Bogorodsky 2010). With the opening of the Suez Canal

in 1869, many hundreds of marine species (including over 100 fish species) entered the Mediterranean Sea from the Red Sea (Galil 2007). These invading species, called Lessepsian migrants, arrive through ship ballast water or directly via the Suez Canal (attached to ships, swimming, larval transport) (Padilla and Williams 2004; Zenetos et al. 2005; Bianchi 2007; Galil 2007).

Concomitant with an increase in water temperature over the past decades (Bethoux and Gentili 1999), this massive migration of species from an Indo-Pacific, Indian Ocean, or Red Sea origin over the past 150 years has contributed to a “tropicalization” of the Eastern Mediterranean Sea in particular (Bianchi 2007). The successful establishment of migrating species in a new environment often demands adaptations to core behaviors, essential for the production of new generations or recruitment, leading to a substantial population size and a steady period of reproduction. Given that Lessepsian fish species often constitute over 80% of the commercial catch in the Eastern Mediterranean as of today (Edelist et al. 2013; Öztürk 2021), the ability of these NIS to adapt to different food sources, habitats, and other inorganic environmental factors show the high possibility of their success and a further tropicalization in the Mediterranean Basin as a whole (Golani and Ben-Tuvia 1985; Arndt et al. 2018).

Previous conventional approaches for tracking animal movements have largely relied on extrinsic markers and on stomach content analysis (Rindorf and Lewy 2004; McMahan and Newsome 2019). However, the use of contemporary methods such as molecular DNA profiles and the measurement of naturally occurring stable isotopes in animal tissues offer more reliable and distinct analyses for determining which adaptations have enabled migratory species to thrive in a new environment (Fanelli et al. 2015). Amino acid compound-specific stable isotope analysis (AA-CSIA) has shown to be a powerful tool for examining diet and habitat use, providing key information regarding the isotopic signature of primary producers at the base of a food web (McMahon et al. 2010, 2016).

In this study, we compare the isotopic signature of five essential amino acid (AA) $\delta^{13}\text{C}$ values of two key Lessepsian species *Sargocentron rubrum* (Forsskål, 1775), and *Siganus rivulatus* (Forsskål & Niebuhr, 1775) that entered the Mediterranean a few years after the opening of Suez Canal (Haas and Steinitz 1947) and two relatively “young” immigrants Red Sea goatfish - *Parupeneus forsskali* (Fourmanoir & Guézé, 1976) and Devil firefish *Pterois miles* (Bennett, 1828) (Bariche et al. 2013; Sonin et al. 2013) to baseline isotopic compositions presented by the same species in their native habitat (the Indo-Pacific waters). *P. forsskali* was chosen as a species that outcompeted the native fish species from the same family. *P. miles* was chosen as a species that in the last two decades introduced to many new places all over the world (e.g., the Caribbean and South Atlantic Ocean as in Brazil), therefore six specimens were also collected in Dominica Caribbean. Comparing trophic position indicators of successful invasive species in their native and invaded range can provide insights into behavioral shifts, such as dietary habits, and help us understand how adaptations to environmental differences may shape food web structure and contribute to current ecosystem functioning in the Eastern Mediterranean Sea. The aim of this research was to study the dietary habits change of “old” and “new” Lessepsian migrants' fish from the Red Sea to the Eastern Mediterranean Sea and the establishment of those species.

Material and methods

Fish sampling

Fish species, and green algae, were used in the study. Collections were carefully performed with every effort to minimize unnecessary damage. A portion of the

fish was collected using fish square-shaped hand nets 40/40 cm (aquarium nets) in order to reduce environmental damage by retrieving only the specific fish of interest and eliminating any unwanted bycatch or unintended morbidity. Fish sampling act performed mainly at scuba night dives. Samples from the IUI (The Inter-University Institute for Marine Sciences) Eilat Gulf of Aqaba, and the Israeli Mediterranean Sea shore were collected under the approval provided by the Israel Nature and Park Authority (permit No. 42771), and some fish samples from their collection of confiscated illegal catch. All collected samples were immediately frozen and transferred to the lab for measurement and dissection. At the lab, fish were measured for total length (TL) and weight. A muscle sample was removed and lyophilized for later compound-specific stable isotope analysis.

Compound-specific stable isotope analysis

Approximately 3 mg of lyophilized fish muscle was acid hydrolyzed in 1 ml of 6 nmol Hydrochloric Acid (HCl) at 150 °C for 75 min (Cowie and Hedges 1992) under the nitrogen atmosphere inside a 4 ml glass vial with PTFE cap. Samples were cooled to room temperature (25 °C) and then HCl was evaporated under a gentle stream of nitrogen. Samples were neutralized twice with 1 ml ultra-pure water and evaporation with a gentle stream of nitrogen. We used EZfaast amino acid analysis kit with a slight modification of replacing reagent 6 with dichloromethane as a solvent. For carbon analysis, we injected 1.5 µl in split mode (1:15) at 250 °C. Helium was used as a carrier gas at a constant flow of 1.5 ml/min. The amino acids were separated on a Zebron ZB-50 column (30 m, 0.25 mm, and 0.25 µm) in Thermo Scientific Trace 1300 GC. The gas chromatography (GC) condition was set to optimize peak separation for the desired amino acids as follows: Initial temperature 110 °C ramped to 240 °C at 8 °C per min and then ramped to 320 °C at 20 °C per min and held for 2.5 min. The separated amino acids were split on MicroChannel Device into two direction flows, one toward Thermo Scientific ISQ quadruple for amino acid identification and the second toward Thermo Scientific Delta V advantage for C and N isotope analysis. The ISQ condition was set to transfer line 310 °C, ion source 240 °C, and scan range from 43 to 450 m/z mass range. To define the isotopic ratio of carbon and nitrogen the separated amino acids were combusted in a Thermo scientific GC isolink II at 1000 °C for CO₂ and N₂. Before entering Delta V for the N₂ analysis the sample went through a liquid nitrogen cold trap to freeze down all other gases. From each sample, duplicates were injected for carbon and triplicates for nitrogen.

Data analysis and corrections

Stable isotope ratios were expressed in standard δ notation where the standard for Carbon was relative to Vienna PeeDee Belemnite (VPDB) and for Nitrogen atmospheric N₂ (Air). Separated amino acids were purchased from Sigma Aldrich and analyzed at the Geological Survey of Israel Elemental analyzer isotope ratio mass spectrometry. To extend the Nitrogen isotopic range, two certified amino acids (Alanine +43.25‰ and Valine +30.19‰) were purchased from Arndt Schimmelmann, Biogeochemical Laboratories, Indiana University. We used a standard that contains seven amino acids with the known isotopic ratio (Alanine, Valine, Leucine, Isoleucine, Methionine, Glutamic acid, and Phenylalanine) with an isotopic range for Nitrogen of -6.69‰ to +43.25‰. Since Nitrogen is

not added in the process of derivatization, corrections for nitrogen addition were not required. To account for the carbon that is incorporated during the derivatization process we had to find the correction factor for each amino acid using the equation: $n_{cd} \delta^{13}C_{cd} = n_c \delta^{13}C_c + n_d \delta^{13}C_{d_{corr}}$ where n is the number of moles of carbon, C_c the compound of interest (AA), C_{cd} the derivatized compound, and $C_{d_{corr}}$ the empirically determined correction factor (Docherty et al. 2001). The standard amino acid was used to set $C_{d_{corr}}$ for later calculation of the isotopic ratio of our sample. The standard of amino acids was injected three times after the combustion reactor oxidation for carbon and three more times for nitrogen, to allow for drift correction, the standard was injected again three times for carbon and nitrogen after a maximum of 18 injections. Since AAs differ in the presence of heteroatoms and functional groups, which may lead to different combustion efficiencies and therefore differences in drift, an average of the standard injection from the beginning and the end of the sequence was used. For each sequence of nitrogen, a correction factor was applied based on the linear regression equation of the ratio between the known AA Isotopic ratio and the acquired result for the sequence. The trophic position was calculated from the equation $TP_{Glu/Phe} = ((\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta) / TDF_{AA}) + 1$ where $\beta = -0.36$ and $TDF_{AA} = 4.54$ (Martinez et al. 2020).

Statistical analysis

Statistical analyses were performed using PRIMER-e software. For the carbon principal component analysis (PCA), the analysis of similarities (1-way ANOSIM) was based on the Euclidean distance resemblance matrix.

Results

Isoleucine, methionine, and phenylalanine Amino acid $\delta^{13}C$ isotopic ratio analysis was performed on four representative fish species, *P. forsskali*, *S. rubrum*, *P. miles*, *S. rivulatus* and on green algae (*Ulva* sp.) from two locations – the Israeli coast of the Mediterranean Sea and the Gulf of Aqaba, in the Red Sea. Statistics show a clear and significant separation exists between the Red and Mediterranean Sea sampling groups, *S. rivulatus* and green algae (*Ulva* sp.) showed no difference between the Red and the Mediterranean Sea (see also Suppl. material 1: tables S1–S4).

The highest difference in stable isotope signatures was found between *P. forsskali* individuals caught in the Eastern Mediterranean Sea (Med. $n = 9$, Red. $n = 4$) basin and those originating in the Red Sea ($R = 0.979$, $p = 0.001$), as seen in the clear separation of groups in Figure 1. Similarly, *S. rubrum* individuals (Med. $n = 7$, Red. $n = 7$) also showed a significant difference ($R = 0.848$, $p = 0.001$) between the two locations (Figure 2).

Representing one of the more recent NIS arrivals to the Mediterranean Sea, PCA of samples collected from *P. miles* (Figure 3) show differences in isotopic makeup between the locations (Med. $n = 6$, Red. $n = 6$, Carib. $n = 6$), though with some overlap ($R = 0.487$, $p = 0.001$).

Lastly, very little isotopic change ($R = 0.053$, $p = 0.144$) was observed in $\delta^{13}C$ of *S. rivulatus* populations from the Red Sea compared with those sampled from the Eastern Mediterranean sites (Med. $n = 15$, Red. $n = 13$) (Figure 4), suggesting close similarity in carbon source. Furthermore, within the PCA of the two *Ulva* sp. groups, we see a dominant representation of AA isotope signatures. ($R = 0.168$, $p = 0.073$) (Figure 5), also collected from both locations.

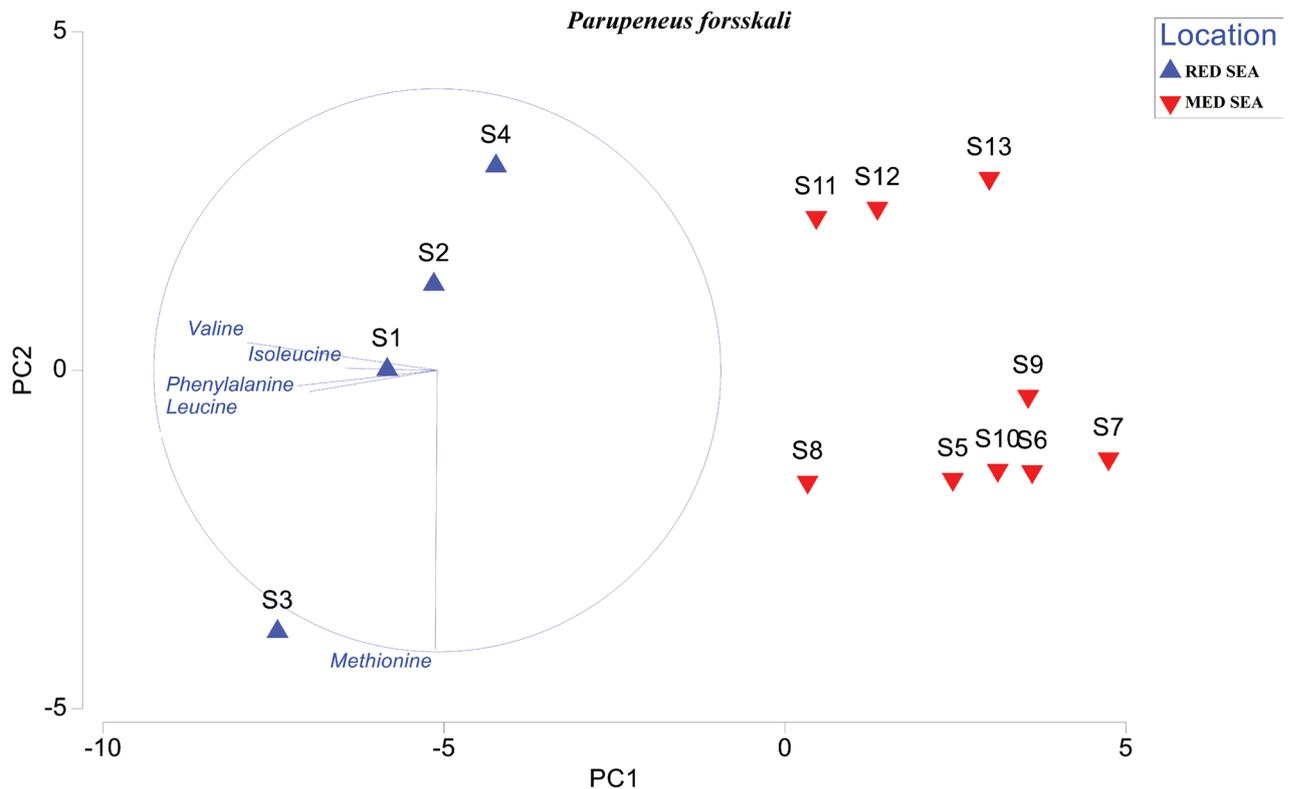


Figure 1. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of *Parupeneus forsskali* from the Red Sea and the Mediterranean Sea indicates a significant difference in the isotopic signature (ONE-WAY ANOSIM $R = 0.979$ $p = 0.001$). Eigenvalues (%) variation PC1 = 73.4 PC2 = 19.7

Discussion

The Eastern Mediterranean Sea and the northernmost tropical Red Sea host a variety of differences between their environments, which include varying temperatures, salinity, habitat structure, as well as bio-community abundance and diversity. Despite these differences, which are themselves on a course of rapid change, a growing number of non-native fish species have exploited local resources and niches to establish successful populations in the Levant basin (Coll et al. 2010) and see also primary results. In the Suppl. material 1: table S1. In assessing any change in trophic level position driven by NIS, analysis of $\delta^{13}\text{C}$ values of essential AAs from targeted consumers (here, sampled fishes) reliably reflect that of primary producers, thus enabling us to describe and highlight potential shifts in the food web of a particular ecosystem.

When foragers from the same species exploit resources across numerous locations, a different isotopic signature is expected. Isotopic analysis in our study indeed revealed significantly different food sources among the following Lessepsian migrants and their original Red Sea population - the squirrelfish, *S. rubrum*; the common Red Sea goatfish, *P. forsskali*; and the lionfish *P. miles*.

Our data support observations reported by Golani et al. (1983), describing *S. rubrum* as a “no preference” bottom feeder, with stomach contents mainly (90%) consisting of Eastern Mediterranean crustacean species, despite abundant access to Lessepsian invertebrates of Red Sea origin. This species has also been observed to exploit under-used niches in deeper water, thus opportunistically capitalizing on new habitats and feeding grounds (Golani and Ben-Tuvia 1985; Farrag et al. 2018). Similarly, though as a relatively new immigrant to the Eastern

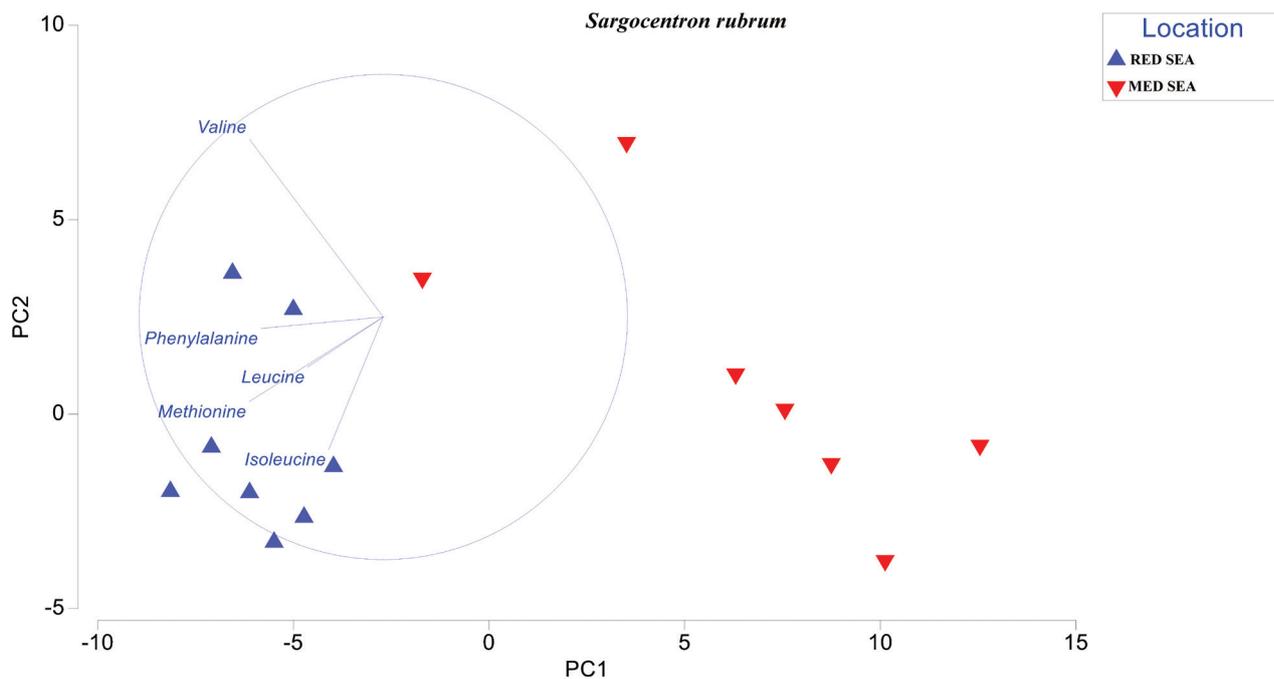


Figure 2. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of *Sargocentron rubrum* from the Red Sea and the Mediterranean Sea indicates a significant difference in the isotopic signature (ONE WAY ANOSIM $R = 0.848$ $p = 0.001$). Eigenvalues (%) variation PC1 = 72.5 PC2 = 24.9.

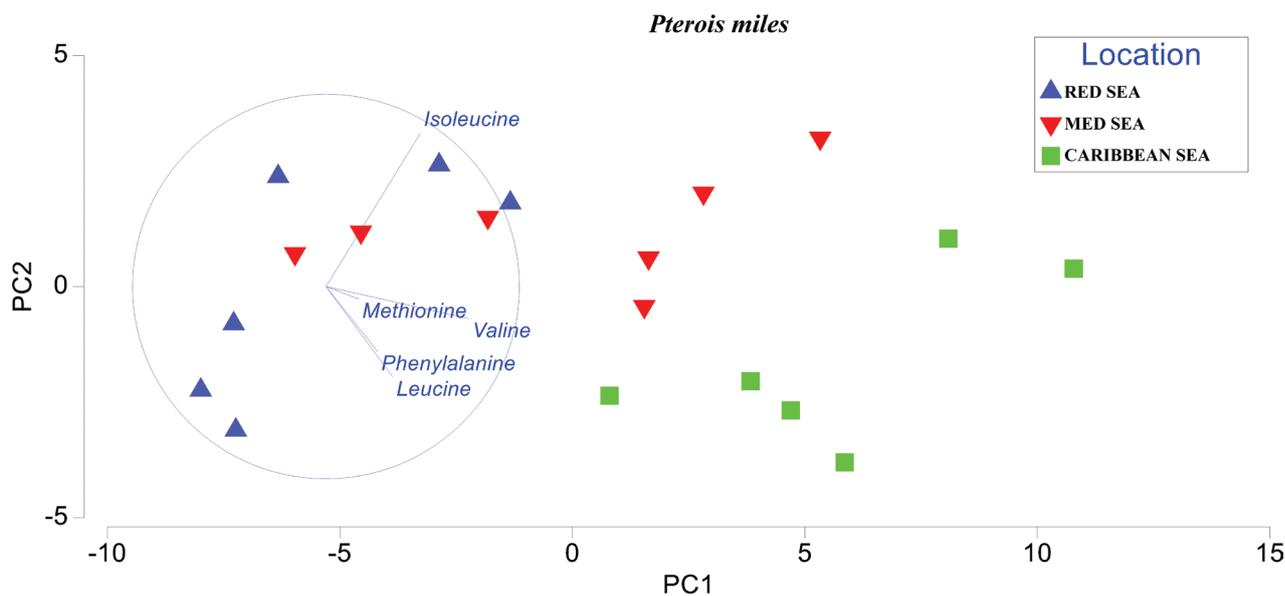


Figure 3. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of *Pterois miles* from the Red Sea and the Mediterranean Sea indicates a significant difference in the isotopic signature (ONE WAY ANOSIM $R = 0.487$ $p = 0.001$). *Pterois miles* from the Mediterranean Sea and the Caribbean ($R = 0.378$ $p = 0.01$). *Pterois miles* (Bennett, 1828) from the Red Sea and the Caribbean ($R = 0.904$ $p = 0.002$). Eigenvalues (%) variation PC1 = 77.6 PC2 = 11.2.

Mediterranean basin, reports on *P. forsskali* describe this species as a strong competitor for resources and space and shown to exhibit habitat flexibility in their newly invaded region (Arndt et al. 2018; Evagelopoulos et al. 2020).

Lastly, the swift and successful invasions of *P. miles* in diverse areas of the globe have been largely attributed to their generalist diet, along with rapid growth rates and high fecundity. Male and female *P. miles* are capable of reproducing year-

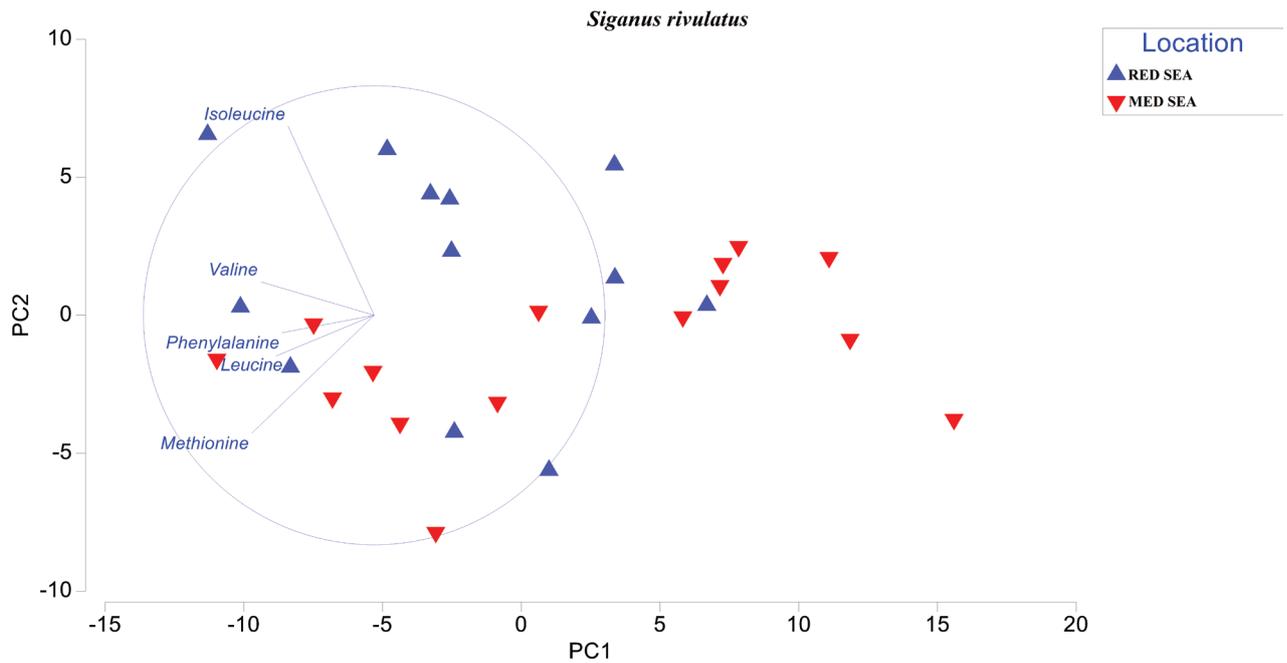


Figure 4. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of *Siganus rivulatus* from the Red Sea and the Mediterranean Sea indicates a significant difference in the isotopic signature (ONE WAY ANOSIM $R = 0.053$ $p = 0.144$). Eigenvalues (%) variation PC1 = 72.4 PC2 = 17.4.

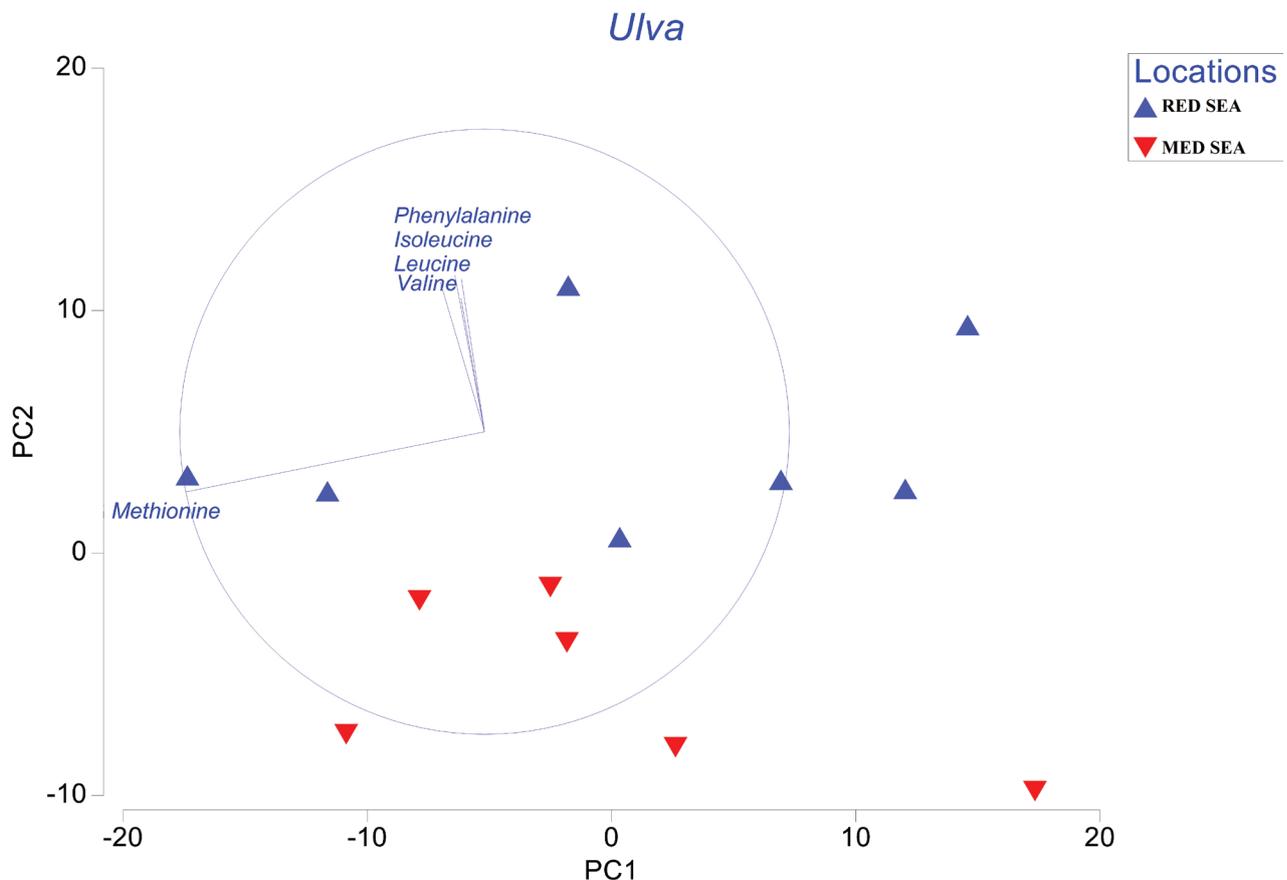


Figure 5. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of green algae *Ulva* sp. from the Red Sea and the Mediterranean Sea indicates a significant difference in the isotopic signature (ONE WAY ANOSIM $R = 0.168$ $p = 0.073$). Eigenvalues (%) variation PC1 = 86.3 PC2 = 8.2.

round and the ovaries of mature females may contain up to 40,000 oocytes, with larger females containing even more oocytes (Gardner et al. 2015). Their growing presence in the Mediterranean indicates that lionfish are thriving and could potentially become a serious threat in the region, particularly in relation to the trophic disruption of local herbivores (Layman et al. 2014; Kleitou et al. 2021).

With the exception of the classic herbivore, *S. rivulatus*, whose very similar isotopic patterns can be attributed to the green algae they predominantly consume in both locations, it appears that successful Lessepsian species that manage to adopt a wider trophic niche breadth, through more “generalist” feeding habits (e.g., feed on a wide variety of food types), become better long-term colonizers (Golani 1998).

Diet selectivity of rabbitfish *S. rivulatus* has been well documented in the Red Sea, due to resource competition among the large population of herbivorous fishes (Lundberg and Golani 1995; Bariche 2006). With their immigration to the Mediterranean Sea close to a century ago, the rabbitfish enjoyed little niche competition from local herbivores, as well as increased access to their preferred food source, the green algae *Ulva* sp., albeit seasonally (Golani 1998; Pickholtz et al. 2018). Our findings show that these classic herbivores do not need to (drastically) change their nutritional habits, thus preserving the same trophic levels between their original habitat and invaded habitat. This possibly alleviates challenges in their ability to establish and maintain a thriving population as compared to many other species which do require a change in their feeding habits in order to survive and thrive.

Whether opportunistic foraging characteristics or relatively similar food sources, in the case of *S. rivulatus*, have contributed to the successful adaptation of certain Lessepsian migrants to the Eastern Mediterranean environment, we maintain that species who can effectively adapt their diet to a new habitat will survive and thus be able to establish a large and stable population there. Although declines in native species abundance and some extirpations have already been reported throughout the Eastern Mediterranean Sea. Native genotypes may be lost, food webs may be altered, and significant economic losses may occur (Kalogirou et al. 2012; Katsanevakis et al. 2014), many established Lessepsian fishes (among them, three are included in our study) have often replaced local species of the same trophic position. In addition, Mediterranean fisheries have also adapted to exploit a number of commercially valuable Lessepsian species, possibly off-setting direct impacts of competitive exclusion in these isotopic niches (Svanbäck and Bolnick 2007).

Nevertheless, the successful establishment of Lessepsian species, particularly in terms of competition and niche partitioning with native and other non-indigenous fish, will likely contribute to further tropicalization and homogenization of the Eastern Mediterranean Sea, and eventually the general Mediterranean basin. While the breadth of these environmental and economic impacts remains unclear, further research should be devoted to understanding the complex and dynamic invasion ecology of the Mediterranean basin in order to monitor these changes. We do not have the ability to prevent the entry of more species through the Suez Canal. Optimal management of fishing may help to mitigate and reduce the ecological and economic impact caused by those migrating species.

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Authors' Contribution

The authors' contributions to the manuscript: Rami Tsadok – research conceptualization, sample design, methodology, investigation, and data collection, data analysis and interpretation, writing - original draft, writing - review & editing. Eli Shemesh - research conceptualization, methodology, and data collection. Stephane Martinez - data analysis, writing review. Debra Ramon - writing original draft, review & editing. Anat Tsemel - data analysis and interpretation. Ziv Zemah-Shamir - writing original draft, review & editing. Itai Kolski - review & editing. Dan Tcheronov - research conceptualization, methodology, writing - original draft, writing - review & editing.

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Supplementary material 1

Primary results

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Data type: xlsx

Explanation note: **table S1**. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of *Parupeneus forsskali* from different sources. **table S2**. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of *Sargocentron rubrum* from different sources. **table S3**. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of *Pterois miles* from different sources. **table S4**. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of *Siganus rivulatus* from different sources. **table S5**. PCA of the $\delta^{13}\text{C}$ of five essential AAs (valine, leucine, isoleucine, methionine, and phenylalanine) of *Ulva* sp. from different sources.

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