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Original article

Comparative Proximate Chemical Composition of Two Invasive Fish Species *Siganus rivulatus* and *Siganus luridus* and the Native Species *Sciaena umbra* off the Western Coast of Tobruk, Eastern Mediterranean Sea

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Abstract
The Mediterranean Sea is increasingly invaded by biological invasions due primarily to the Suez Canal, shipping, and climate change. The present study investigates the proximate chemical composition of two invasive species (*Siganus rivulatus* and *Siganus luridus*) and the native *Sciaena umbra* along the western coast of Tobruk, Libya. Fifteen samples were analyzed for moisture, protein, lipid, ash, and carbohydrate content. The results showed significant interspecific variability, with *S. umbra* with the highest protein (17.67%) and ash (1.11%) and the lowest carbohydrate (2.72%), which are reflective of its carnivorous/omnivorous feeding. *S. rivulatus* and S. *luridus*, on the other hand, had higher carbohydrate (5.14–5.54%) and comparatively higher fat, which are reflective of their herbivorous feeding. Discriminant function analysis (DFA) correctly assigned 93.3% of species as native or invasive. These findings highlight the invasive species' trophic impacts and provide baseline data for biodiversity conservation and fisheries management in the eastern Mediterranean.
Keywords. Proximate Composition, Invasive Species, *Siganus rivulatus, Siganus luridus*, *Sciaena umbra*, Tobruk, Mediterranean Sea.

Introduction

The Mediterranean Sea is one of the most invaded seas globally, with the Suez Canal, high sea traffic, and the aggravating effect of climate change being significant contributors. To date, there have been more than 800 documented Indo-Pacific non-indigenous species, some with severe effects on biodiversity, ecosystem processes, and the natural resource base [1–5].

Among these invaders, *Siganus rivulatus* and *Siganus luridus* have been successful in forming established populations in the eastern Mediterranean, the Libyan coastline being no exception, because of their high ecological fitness and competitive advantage over the resident herbivorous populations. *S. rivulatus* has particularly been found to outcompete the resident *Sarpa salpa* at algal grazing, while *S. luridus* occurs at lower densities and tends to inhabit deeper habitats [6,7]. Its high habitat plasticity, allowing it to live on rocky shores, harbors, and seagrass meadows, is another measure of its ecological success [3]. Their strong grazing pressure was found to reduce canopy-forming algae, change the structure of the habitat, and thereby restrict resource availability to native fauna [6,8].

Nutritionally, both *S. luridus* and *S. rivulatus* are relatively high in protein and lipid content, the latter with a tendency for higher lipid content. Potassium and phosphorus prevail in their elemental composition, while different fatty acid contents have been described. *S. luridus* has with greater linoleic and linolenic acid content, while the DHA to EPA ratio is much greater in *S. rivulatus* [9]. These biochemical characteristics meet not just their energy requirements and ecological abundance but also indicate their potential as economically significant resources for human intake and potential targets for small-scale fisheries [10,11].

Conversely, data on the proximate chemical composition of the native, *Sciaena umbra*, are extremely limited. Investigations conducted were prone to deal with single biochemical constituents, e.g., glycosaminoglycans from skin and bone, and there are no general data yet available for their nutritional composition [12,13]. This ignorance hinders full comprehension of its ecological role, nutritional value, and the potential effects of competition with aliens on native food chains.

This lacuna is to be filled by the present study, a comparative proximate chemical composition of *S. rivulatus, S. luridus*, and *S. umbra* captured from the western shoreline of Tobruk, Libya. The present study, through detailed analysis of moisture, protein, lipid, ash, and carbohydrates, aims to evaluate both the nutritional value as well as the ecological function of invasive and native species. The findings should provide valuable baseline data to preserve biodiversity and manage fisheries sustainably in the eastern Mediterranean.

Methods

Sample Collection and Preparation

Five samples of the indigenous species *Sciaena umbra* were collected in October and November 2024 from the locality of Al-Qardbah, west of Tobruk. The fish were speargun-netted at 5 to 15 m depth. Furthermore, ten specimens of invasive fishes (five of *Siganus rivulatus* and five of *Siganus luridus*) were collected from

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Ain Al-Ghazala locality, west coast of Tobruk (Figure 1) using gillnets at 1–2 m depth off the shore. The samples were directly taken on ice to the laboratory for preserving their biochemical integrity
The fish samples were washed, gutted, and homogenized to be ready to the same quality before undergoing chemical analysis upon receipt.

Chemical Analysis of Samples

Lipids

Extraction of lipids through Soxhlet was carried out with a combination of organic solvents, hexane and petroleum ether. The sample was dried before extraction, and extraction was carried out within a period of 4–6 hours. Extraction was followed by removal of solvent, and excess extract was dried to determine lipid content. Lipid percentage on a dry weight basis was calculated.

Protein

Protein was estimated by the Kjeldahl method. The samples were digested in sulfuric acid with copper sulfate catalyst. The percentage of protein was calculated from total nitrogen by multiplying by 6.25.

Carbohydrates

Estimation of carbohydrates was done by difference as 100% minus the total percentage of moisture, protein, lipid, and ash**.**

Ash

Dry weight was weighed and burnt in a muffle furnace for 4–6 hours at 550–600°C to constant weight. Ash content was calculated based on the before and after weights upon burning.

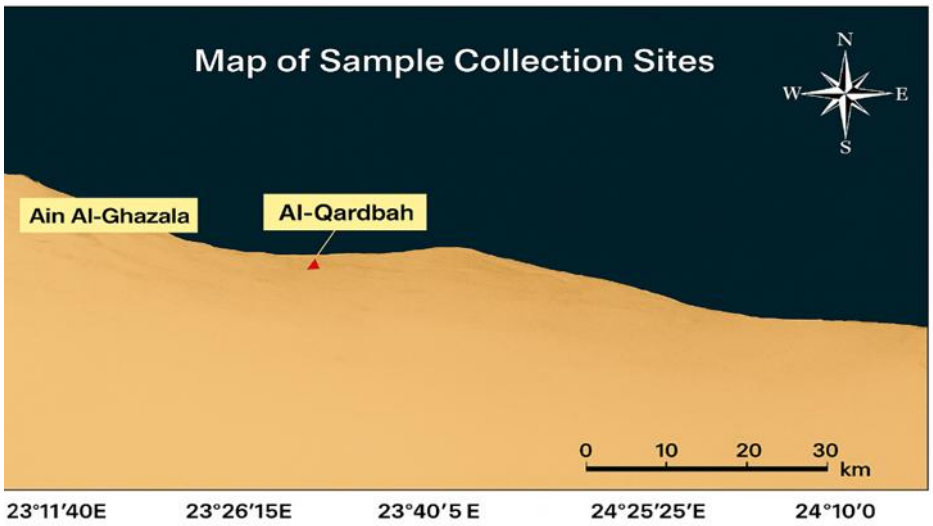

Figure 1. Map of Sample Collection Sites

Statistical Analysis

All statistical tests were conducted using IBM SPSS Statistics software (Version 23). The following procedures were utilized:

- Descriptive statistics including mean, standard deviation, minimum, and maximum.
- Kolmogorov–Smirnov test to verify normality of data distribution.
- Levene's test to verify homogeneity of variances.
- Independent t-test with post hoc Bonferroni correction.
- Discriminant Function Analysis (DFA) to verify differences between species.
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Results and Discussion

The result of the current study (Tables 1–7; Figures 2–4) revealed clear interspecific differences in the proximate composition of the investigated three species: the native *Sciaena umbra* and the alien *Siganus rivulatus* and *Siganus luridus*. S. *umbra* contained the maximum amount of protein (17.67%) and ash (1.11%), but the minimum amount of carbohydrate (2.72%), characteristic of its carnivorous/omnivorous diet and greater reliance on protein- and mineral-rich foodstuffs. On the other hand, the invasive species contained slightly reduced protein (~16.6%) and ash (~0.96%) but increased carbohydrates (5.14–5.54%) and slightly increased lipids, consistent with their herbivorous dietary nature [15–25]. Lipid content was low in all species (1.14–1.65%), as one would expect from wild-caught fish. Moisture content was the most

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frequent constituent with low variability, as one would expect in fresh fish muscle. Similar trends have also been suggested from the Northeastern Mediterranean, where *S. luridus* contained higher lipid content than *S. rivulatus*, and the two species contained nearly identical moisture content [25].

Table 1. Descriptive Statistics of Proximate Composition

Component	Minimum	Maximum	Mean	SD
Moisture (%)	74.36	78.33	76.32	1.12
Ash (%)	0.83	1.13	0.96	0.11
Protein (%)	14.58	18.33	16.62	1.10
Fat (%)	0.83	2.18	1.44	0.37
Carbohydrates (%)	2.51	6.17	4.47	1.38

Finally, these findings suggest that proximate composition is determined by habitat, diet, and species identity. Protein, lipid, and carbohydrate levels are excellent indicators of nutritional quality and ecological fitness [17–22]. Besides, seasonal variation, reproductive stage, and growth phase can also induce variability in protein and lipid levels [23,24]. The current study collectively illustrates that the proximate composition is not merely a quantitative measure of nutritional quality but also a stable biochemical marker to distinguish between native and introduced fish species in the Mediterranean.

Table 2. Normality Test (Kolmogorov–Smirnov)

Component	Statistic	df	Sig.
Moisture (%)	0.113	15	0.200*
Ash (%)	0.227	15	0.060
Protein (%)	0.142	15	0.200*
Fat (%)	0.179	15	0.200*
Carbohydrates (%)	0.208	15	0.081

**Value 0.200 represents the lower bound of true significance.*

Table 3. Descriptive Statistics by Species

Component	Species	Mean	SD
Moisture (%)	*S. umbra*	76.77	0.82
	S. rivulatus	76.77	1.08
	S. luridus	75.42	1.00
Ash (%)	*S. umbra*	1.11	0.01
	S. rivulatus	0.91	0.05
	S. luridus	0.86	0.03
Protein (%)	*S. umbra*	17.67	0.62
	S. rivulatus	15.65	0.70
	S. luridus	16.53	0.89
Fat (%)	*S. umbra*	1.14	0.01
	S. rivulatus	1.52	0.46
	S. luridus	1.65	0.32
Carbohydrates (%)	*S. umbra*	2.72	0.16
	S. rivulatus	5.14	0.75
	S. luridus	5.54	0.51

Levene's test ascertained that all five proximate constituents satisfied the requirement of homogeneity of variances, warranting additional ANOVA analyses (Table 4). The ANOVA test revealed considerable interspecific variation in ash ($F = 81.64$, $p < 0.001$), proteins ($F = 9.24$, $p = 0.004$), and carbohydrates ($F = 40.96$, $p < 0.001$), whereas variation in moisture ($p = 0.075$) and lipid ($p = 0.067$) was not significant. Post hoc Bonferroni comparisons revealed that Sciaena umbra differed significantly from both alien species (*Siganus rivulatus* and *S. luridus*) in ash and carbohydrate contents, and from *S. rivulatus* in proteins (Table 5).

These results exhibit unequivocal evidence of interspecific variation in proximate composition, firmly supporting diet composition and ecological mode variation. Native *S. umbra* contains the highest proportion of protein and ash content, aligning with its carnivorous/omnivorous diet and greater mineral reserves. The

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invasive species, on the other hand, contained significantly higher carbohydrate content, characteristic of their herbivorous diet, while lipid and moisture contents were more or less similar for all species.
Combining these findings with previous reports that indicated that protein, ash, and carbohydrate composition are more likely to vary between fish species, whereas moisture and lipid content is better conserved and less affected by interspecific variability [19,26], establishes the utility of proximate composition as a viable biochemical discriminant to discern native and alien fish species in the Mediterranean.

Table 4. Levene's Test for Equal Variances

Component	p-value	Interpretation
Moisture (%)	0.940	Equal variances (ANOVA valid)
Ash (%)	0.075	Equal variances (α = 0.05)
Protein (%)	0.710	Equal variances
Fat (%)	0.122	Equal variances
Carbohydrates (%)	0.136	Equal variances

Discriminant function analysis (DFA) is a robust multivariate statistical method for fish species classification based on their biochemical makeup. Two discriminant functions were established in the present study to distinguish the indigenous Sciaena umbra from non-native species *Siganus rivulatus* and *S. luridus*. Function 1, depending mainly on ash and carbohydrate levels, accounted for 99.2% of the variance and successfully discriminated the invasive from the native species. Function 2, depending on protein and water, explained 0.8% of the variance and outlined minor biochemical differences between the two invasives (Table 6). The model's overall accuracy of classification was 93.3%, and the classification was perfect for *S. umbra* and *S. rivulatus,* and 80% similarity in *S. luridus*, showing some biochemical resemblance between the two species (Table 7).
These findings are in line with previous research that demonstrated DFA and other multivariate techniques, such as linear discriminant analysis (LDA) and partial least squares discriminant analysis (PLS-DA), could uniformly classify fish species on the basis of biochemical, elemental, or spectral data with high classification accuracy [27–29]. The most discriminatory variables are usually indicative of ecological or dietary features, e.g., ash, carbohydrate, and fatty acid content, indicative of species-specific adaptations. Overall high classification rates (80–100%). Overall, DFA provides a robust and stable pattern for distinguishing between species, which displays the nutritional and ecological diversities of exotic and endemic fish species in the study region.

Table 5. ANOVA Results and Post Hoc Comparisons

Component	F	p	Significant Differences (Bonferroni)
Moisture (%)	3.25	0.075	NS
Ash (%)	81.64	<0.001	*S. umbra* > *S. rivulatus*, *S. luridus*
Protein (%)	9.24	0.004	*S. umbra* > *S. rivulatus*
Fat (%)	3.42	0.067	Marginal NS
Carbohydrates (%)	40.96	<0.001	*S. umbra* < *S. rivulatus*, *S. luridus*

Table 6. Discriminant Function Structure Coefficients

Variable	Function 1	Function 2
Moisture (%)	-0.05	0.70*
Ash (%)	-0.42*	0.12
Protein (%)	-0.12	-0.89*
Fat (%)	0.09	-0.07
Carbohydrates (%)	0.30*	0.12

**Largest absolute correlation for each function.*

Table 7. Discriminant Classification Results

Actual Species	Predicted: S. umbra	*S. rivulatus*	*S. luridus*
S. umbra	100%	0%	0%
S. rivulatus	0%	100%	0%

Ecologically, differences in nutrient composition between species are their different trophic roles. The higher ash and lower carbohydrate content of omnivorous/carnivorous *S. umbra* are in agreement with its diet,

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whereas the higher carbohydrate and lower ash content of herbivorous Siganus species are in agreement with its algae diet [28-30].

The trend demonstrates the intimate relationship between diet and chemical composition of the body: protein storage is a companion of carnivory, but carbohydrate storage is characteristic of herbivory. Variation also illustrates the way invasive species disrupt native food web processes by providing new feeding lifestyles and competition with indigenous organisms. The integration of morphological and biochemical data provides greater insight into the species' biology. For instance, length–weight relationships tell us about the pattern of growth, and proximate composition provides the nutritional substrate for growth as well as information regarding the quality and quantity of consumption. Together, these parameters may be employed to quantify invasive plant ecological impacts; for example, decreases in growth rates may be considered as a sign of stress in adapting to new environments, and differences in chemical content show differences in food web functioning [29,30].

It is therefore necessary to carry out a thorough analysis of morphological and biochemical characteristics for the wise management of marine fisheries, particularly in regions where incursions of alien species have been observed. In this way, integrated analysis more clearly sheds light on the delicate interrelations that modulate marine ecosystems and provides evidence-based recommendations towards sustainable use and protection of biodiversity.

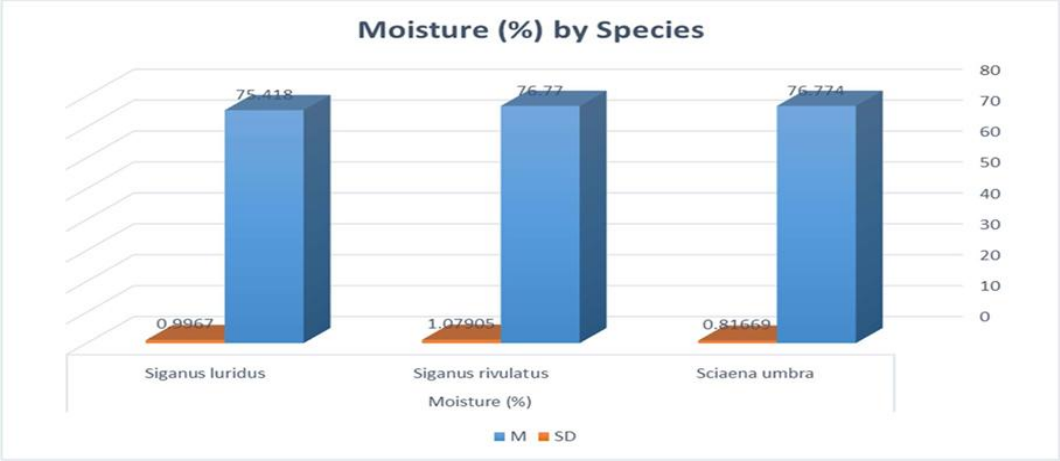

Figure 2. Mean Moisture Content (%) by Fish Species

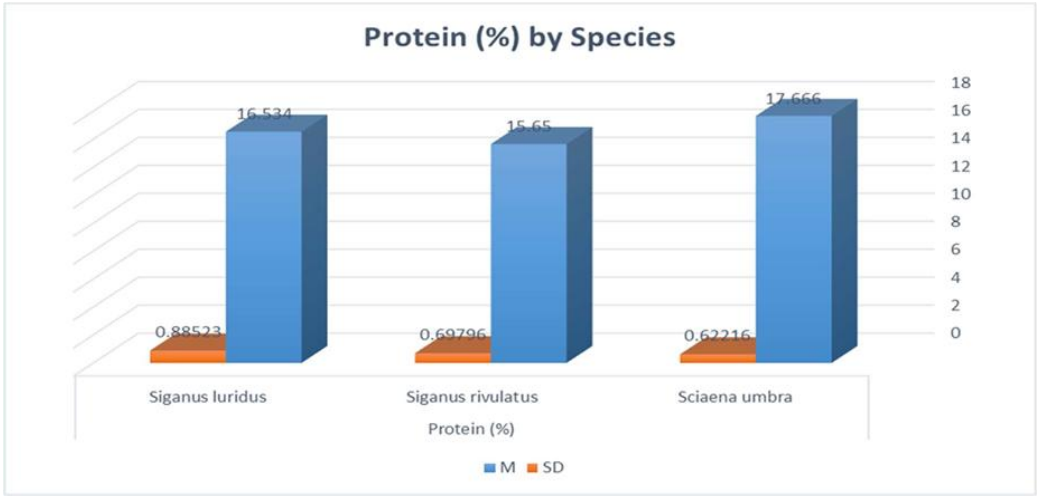

Figure 3. Mean Protein Content (%) by Fish Species

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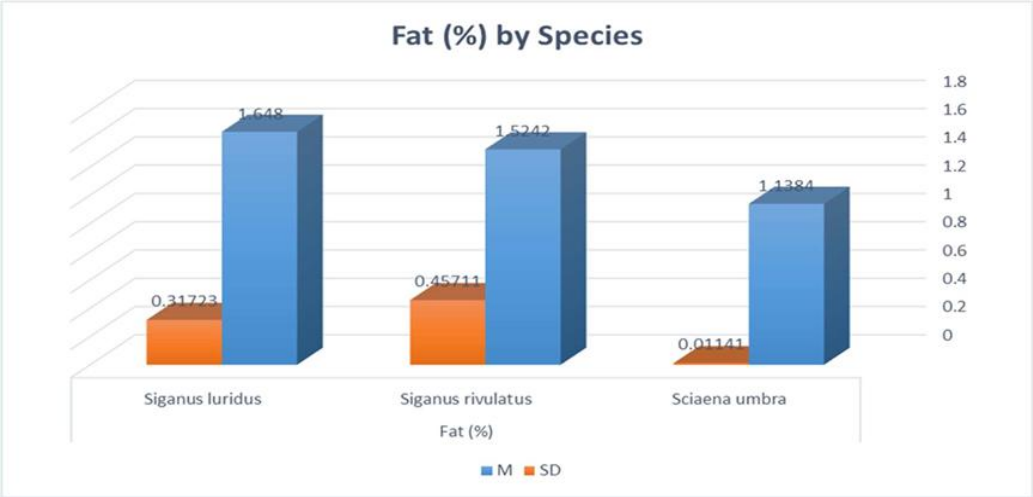

Figure 4. Mean Fat Content (%) by Fish Species

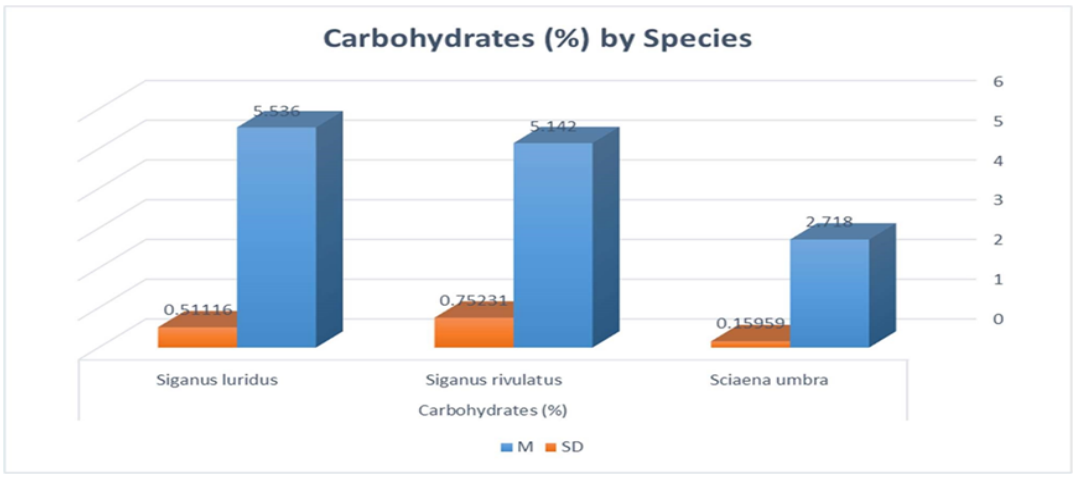

Figure 5. Mean Carbohydrate Content (%) by Fish Species

Conclusion

This study is the first comparative study of the proximate chemical composition of *Sciaena umbra* relative to invasive species on the Libyan coast. The results revealed that *S. umbra* is characterized by higher protein and ash content, while invasive *Siganus species* contain higher carbohydrate content, which reflects differences in their respective dietary niches. Discriminant function analysis (DFA) also demonstrated the utility of proximate composition as a valuable discriminant of species and for monitoring ecological values. Overall, these findings provide ecological threats of invasive species with significant insights and offer scientific bases to inform sustainable fisheries management and biodiversity conservation strategies in the Mediterranean Sea. In addition to highlighting environmental hazards, this study provides a scientific foundation for applied policymaking and management. We recommend integrating proximate composition analysis into routine environmental monitoring and fish certification schemes. Native and exotic species' distinctive biochemical characteristics, as identified using Discriminant Function Analysis (DFA), provide a sturdy and reliable biodiversity monitoring platform. With the use of these indicators, fisheries managers will be in a position to better track the expansion of invasive species, assess their impact on indigenous food webs, and apply targeted management measures, hence promoting the sustainable utilization and conservation of Mediterranean Sea marine biodiversity.

Conflict of interest. Nil

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