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Trophic assessment and isotopic niches of two congeneric pipefishes in a wetland of the Aegean Sea

Sule GURKAN[1], Paula ARAGONESES[2], Deniz INNAL[3], Clara MENDOZA-SEGURA[2], and Miquel PLANAS[2]

[1] Ege University, Faculty of Fisheries, Department of Hydrobiology, 35100 İzmir, Türkiye
[2] Department of Ecology and Marine Resources, Institute of Marine Research (IIM-CSIC) 36208 Vigo, Spain
[3] Mehmet Akif Ersoy University, Science and Art Faculty, Department of Biology, 15100 Burdur, Türkiye

Corresponding authors: Sule GURKAN, sule.gurkan@ege.edu.tr; Miquel PLANAS, mplanas@iim.csic.es

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Abstract

Pipefishes (family: *Syngnathidae*) are representative fishes in many European coastal areas associated with vegetal assemblages and other shallow habitats. The polyandrous pipefishes *Syngnathus abaster* and *Syngnathus acus* co-occur in Metruk Tuzla (Bargilya Tuzla coastal Wetland - BTW, Aegean Sea, Türkiye –a protected but threatened wetland due to anthropogenic disturbances. Both species were sampled to assess the growth and temporal changes on their trophic features using stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and gut content analyses. Both species exhibited positive allometric growth with similar length–weight relationships. The isotopic approach revealed that both species occupied similar trophic niches, as evidenced by comparable $\delta^{13}C$ and $\delta^{15}N$ values (-17.0‰ ± 1.2‰ and 13.2‰ ± 0.6‰, respectively in *S. abaster*; -17.3‰ ± 1.2‰ and 13.3‰ ± 0.7‰ respectively in *S. acus*). The niche area for all sex types (undifferentiated specimens, males and females) in *S. acus* was slightly broader than that in *S. abaster*. Simulation analysis of the trophic position in both species agreed with the isotopic assessment (trophic position = 3.67 ± 0.61 in *S. abaster* and 3.46 ± 0.66 in *S. acus*). Although the trophic positions were consistent with the isotopic assessment, gut content analysis disclosed a notable contribution of copepods, especially *Calanipeda aquaedulcis*, to the dietary regime in both species. Notably, the diet in *S. abaster* was nearly monospecific, whereas cladocerans were crucial in *S. acus*. This study suggests the plausible occurrence of interspecific competition regarding dietary resources. However, we hypothesise that such competition could be mitigated by the occupation of discrete microhabitats in both species. To validate this hypothesis, rigorous investigation and empirical analysis are necessary.

Keywords: Trophic niche; diet; *Syngnathidae*; *Syngnathus abaster; Syngnathus acus*; stable isotope.

Introduction

Wetlands are ecologically vital ecosystems that provide many ecosystem services, including water purification, flood control and habitat for flora and fauna. Wetlands are important targets of conservation or restoration (Cherry, 2011) and one of the most productive ecosystems (Whittaker *et al.*, 1973). Wetlands provide nurseries for fish and other organisms, hosting important fish communities that support small local fishing. Recognising and preserving vital wetland ecosystems is crucial for conserving coastal and marine biodiversity. Studies into the growth patterns, trophic interactions and reproductive strategies have revealed the web of species correlations within wetland ecosystems. This knowledge is essential for effective conservation strategies, especially in the context of threatened wetlands where anthropogenic disturbances pose challenges to the delicate balance of these environments.

In Türkiye a large area of wetlands has deteriorated or been irreversibly lost in the last decades. This decline is due to increased anthropogenic disturbances and will likely accelerate a negative scenario including climate change (Curebal *et al.*, 2015; Murat & Ortaç, 2021; Çolak *et al.*, 2022). Boğaziçi Lagoon, also known as the Bargylia Tuzla coastal wetland (BTW), connects to the heavily damaged Güllük Bay (Aegean Sea) by a submarine strait (Somay-Altaş, 2021). It is one of the last lagoons in Türkiye to survive and a permanent shallow marine lagoon <6-m deep at low tide that includes sea bays and straits and intertidal salt flats (Somay-Altaş, 2021). BTW is a shallow euryhaline area surrounded by several bays and inlets. Salinity during the sampling period fluctuated between 14‰ in spring and 17‰ in summer. The substrate is characterised by silty-sandy and muddy sediments (Demirak *et al.*, 2001). The wetland is a fertile and sensitive oligo-hypersaline area, mainly fed by rainfall, streams and karstic brackish springs. It is one of the most important national wetlands of Türkiye in terms of bird species and biodiversity, covering an important part of the Güllük coastal wetland. Economically important species, such as *Mugil cephalus, Liza aurata, Dicentrarchus labrax, Sparus aurata, Cyprinus carpio, Anguilla anguilla* and *Solea solea*, have been reported in Güllük Delta (Ministry of Agriculture and Rural Affairs Report, 1988; Tokaç *et al.*, 2010; Sağlam *et al.*, 2015; Tosunoğlu *et al.*, 2017). Güllük Delta is inhabited by fish juveniles or noneconomic small fish species that are prey for economical fishes (Tokaç *et al.*, 2010). Güllük Delta is a habitat for noncommercial fish species, such as *Atherina boyeri, Aphanius fasciatus*, *Neogobius fluviatilis, Syngnathus* spp. (this study) and *Chelon* spp. (Sağlam *et al.*, 2015). Some of these species are strongly associated with vegetal assemblages (Altınsaçlı *et al.*, 2015), including *Posidonia oceanica* and *Zostera marina* meadows (Cerim, 2017).

BTW is under threat due to fishing and aquaculture activities, agriculture runoff, domestic waste pollution, port activities and tourism, including a 'Tourism City' project approved by the Ministry of Environment and Urbanisation. Güllük Bay contributes to ~70% of the Turkish aquaculture production, mainly for seabass and seabream (Demirak *et al.*, 2006; Yucel-Gier *et al.*, 2013). These factors put the wetland under threat, because the impact will be far above the carrying capacity of the area, disrupting the ecological balance irreversibly (Yucel-Gier *et al.*, 2013). Therefore, BTW was declared 'Metruk Tuzlası Wetland Protection Area' by the Republic of Türkiye (Ministry of Environment and Forestry, General Directorate of Nature Conservation and National Parks) in 2004 (Anonymous, 2007). The region was taken under the protection area by the National Wetland Commission and considered a wetland candidate for the Ramsar Convention (Tosunoğlu *et al.*, 2017) –an international treaty aimed at conserving wetlands (Davis *et al.*, 1997; Ramsar Convention Secretariat, 2016).

Among the inhabitants of the wetlands, pipefishes (family: *Syngnathidae*) stand out as intriguing subjects for study due to their unique ecological roles and behaviours. Although European pipefishes are classified as *Data Deficient* or *Least Concern* by IUCN (IUCN, 2023), many species are ecologically and biologically vulnerable. However, pipefishes play crucial roles in maintaining the delicate balance of wetland ecosystems. Their presence influences the abundance and distribution of small invertebrates, acting as biotic controllers and influencing the stability and balance of the entire ecosystem. Pipefishes often serve as indicators of wetland and seagrass health, reflecting the overall ecological condition of these fragile habitats (Shokri *et al.*, 2009). Changes in their abundance or behaviour may signal environmental stress or disturbances. In this context, the examination of pipefishes becomes a lens through which we can observe and evaluate the health and resilience of wetlands. As these ecosystems face increasing anthropogenic pressures, examining pipefish ecology provides valuable information for the development of conservation measures for these ecologically significant and imperilled habitats. The study of pipefishes in wetlands goes beyond their individual importance –it contributes to our broader understanding of ecosystem dynamics. The importance of wetlands to pipefishes is multifaceted, encompassing habitat provision, reproductive success, food availability, nursery grounds and ecological balance.

Most native European pipefish species favour shallow marine coastal habitats, although a few exhibit progressive colonisation of freshwater environments. There are two main nearshore representative pipefishes in BTW as follows: the black-striped pipefish *Syngnathus abaster* (Risso, 1827) and the greater pipefish *Syngnathus acus* (Linnaeus, 1758) (Dawson, 1986a). Although both species are categorised as *Least Concern* by IUCN, their population trends are unknown (Smith-Vaniz, 2015; Pollom, 2016). *S. abaster* is a euryhaline fish found among detritus or vegetation, over sand or mud and in marine, brackish and more recently, freshwater environments (Vasileva, 2003; Monteiro & Vieira, 2017; Didenko *et al.*, 2018). *S. acus* inhabits shallow coastal waters, occasionally in brackish waters and estuary mouths. Although usually associated with vegetated substrates, from the intertidal zone to 20 m, it may be found in sandy or muddy areas, particularly in estuarine environments (Dawson, 1986a). However, both species co-occur in Metruk Tuzla –an area subject to significant seasonal fluctuations in the abiotic environment– largely influenced by freshwater inputs. However, historical data for both species are lacking in the studied site and nearby areas.

The euryhaline black-striped pipefish *S. abaster* occupies a wide variety of habitats, with no known threats at a large scale. It is a freshwater and estuarine species occurring in coastal waters and lower reaches of rivers from the Caspian Sea to the Bay of Biscay as well as several rivers in northern Europe and Russia. The species has been progressively colonising pure freshwater ecosystems in Eastern Europe (Vasileva, 2003; Monteiro & Vieira, 2017; Didenko *et al.*, 2018). *S. acus* shows a wider distribution, because it has been identified in waters from the Baltic Sea to the Black Sea and in other southern areas (Morocco, Senegal, Gambia and South Africa).

Studies on the biological or ecological features of *S. abaster* and *S. acus* are limited, especially in *S. acus*. Some have provided information on the morphometrics and meristics (Cakić *et al.*, 2002; Kolangi-Miandare *et al.*, 2013; Vieira *et al.*, 2014; Yildiz *et al.*, 2015; Gürkan & Innal, 2018), whereas others have evaluated the distribution patterns (Dawson, 1986b; Monteiro & Vieira, 2017; Planas *et al.*, 2021), early life history (Silva *et al.*, 2006a), reproduction traits (Silva *et al.*, 2006b; Planas, 2022) or trophic patterns (Franzoi *et al.*, 1993; Vizzini & Mazzola, 2004; Kendrick & Hyndes, 2005; Taşkavak *et al.*, 2010; Gürkan *et al.*, 2021; Planas, 2022) of local populations. Although many European pipefish species are associated with macroalgal assemblages or seagrass habitats and co-occur in the same meadows, the assessment of potential interspecific competition for resources has been poorly investigated. As in most syngnathids, pipefishes commonly forage on small prey, such as crustaceans (Kendrick & Hyndes, 2005; Manning *et al.*, 2019).

S. abaster and *S. acus* are secondary consumers preferring amphipods, copepods, chironomids or cladocerans as well as carideans and isopods, depending on the species, sex, size, developmental stage, region and season (Stefee *et al*., 1989; Taşkavak *et al*., 2010; Didenko *et al*., 2018; Gürkan *et al*., 2021; Planas, 2022).

Based on their distinct morphology, mobility, gape biometrics and foraging skills, we investigated whether the co-occurring pipefishes *S. abaster* and *S. acus* compete for food resources on a microhabitat scale. Notably, the larger mouth width of *S. abaster*, compared with that of *S. acus*, might play an important role in prey selection (Kolangi-Miandare *et al*., 2013; Gürkan & Innal, 2018; Gürkan *et al*., 2021). This study aimed to extend our understanding of syngnathid ecology by describing, for the first time using an isotopic approach, as temporally and spatially vision, the trophic niche characteristics and potential competition for resources in two sympatric pipefishes in the Aegean Sea. Given that BTW is a threatened area, understanding the ecology of pipefishes in the area becomes essential for their conservation. Conservation planning for pipefishes and other syngnathids and habitat protection and rehabilitation will benefit other aquatic species (Shokri *et al*., 2009).

Materials and Methods

Study site and fish collection

The study was performed in Boğaziçi Tuzla lagoon (SW Türkiye (37.190833°N, 27.588889°E) a part of BTW, which is classified as a *Wetland of International Importance*, according to the Ramsar criteria (MEF, 2011). The lagoon is a shallow coastal area (maximum depth: 1.5 m; average depth: 45 cm) in the southern part of BTW (Fig. 1) and separated by the road built on the embankment from Bargil Cove, which is directly connected to Güllük Bay. The water level decreases with summer and fall evaporation, whereas the inward-flowing seawater from these channels prevents from drying up (Altınsaçl *et al*., 2015).

Specimens of the black-striped pipefish *S. abaster* and greater pipefish *S. acus* (Fig. 2) were collected in Metruk Tuzla in spring (April 2018) and early summer (June 2019) –a period corresponding to the breeding season (Tomasini *et al*., 1991; Franzoi *et al*., 1993; Vincent *et al*., 1995; Riccato *et al*., 2003; Planas, 2022) – by using a shore seine net (10-m long; 2-m high; 1.2 × 2-mm mesh size) (Figs. 1, 2) operated several times for 5 min. At the

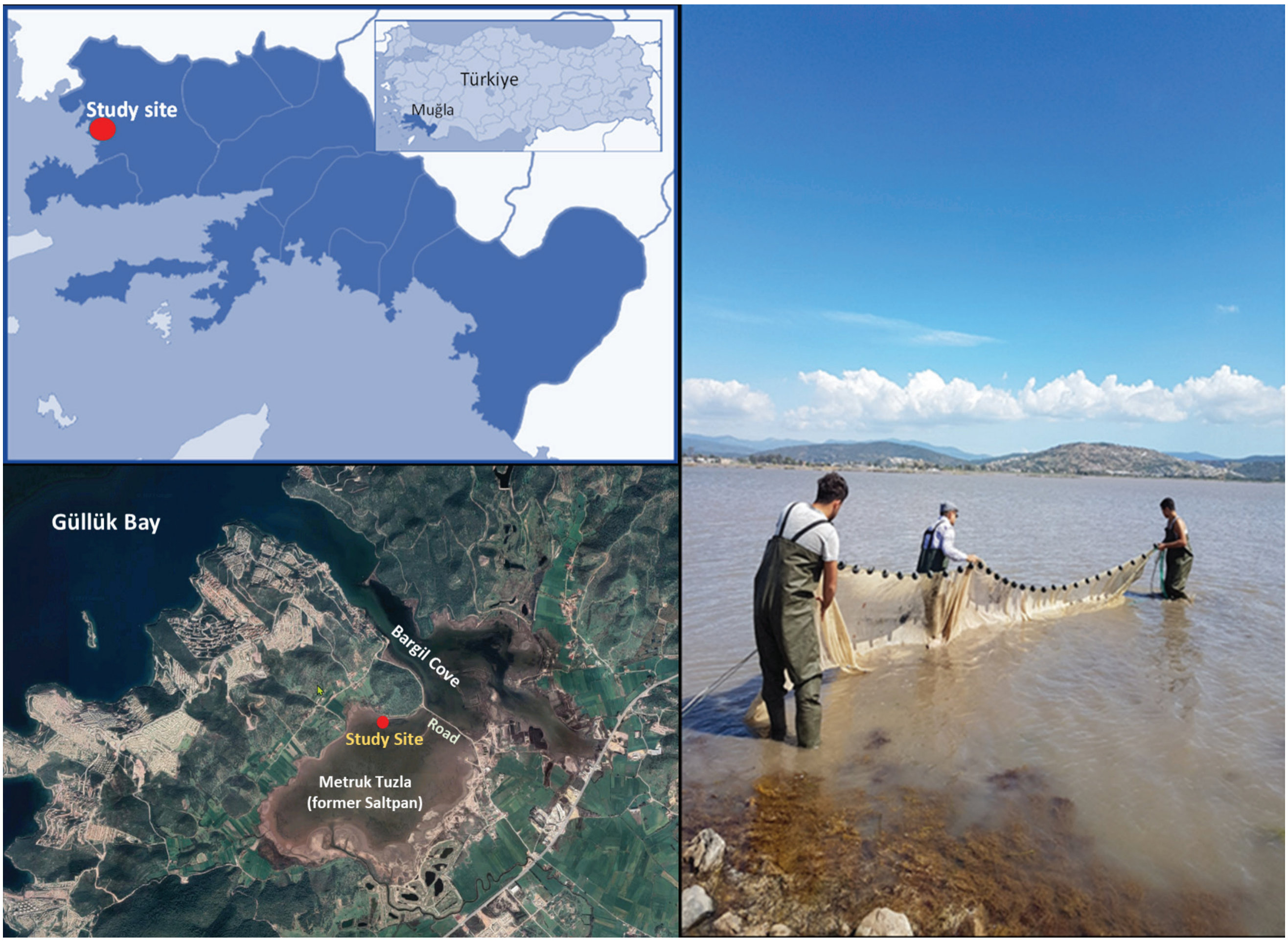

Fig. 1: Study site and shore seine net used for pipefish collection.

Fig. 2: Left: *Syngnathus acus* collected in BTW by the shore seine net. Right: Adults of *Syngnathus abaster* and *S. acus* (females and pregnant males).

end of each sampling operation, the pipefishes were removed from the net, manually sorted, anaesthetised with tricaine methane-sulfonate (MS-222; Sigma-Aldrich, Germany; 1 g mL^{-1}), transferred to plastic containers containing 70% ethanol and transported to the laboratory of the Department of Hydrobiology at Ege University (Türkiye) Because the syngnathids have a relatively undifferentiated gastrointestinal tract, stretched gut lengths of the pipefish specimen was measured (±0.01 mm) between the oesophagus and the intestine using a digital calliper.

Temperature, salinity, pH and oxygen saturation were recorded on the sampling dates (YSI Pro Plus Multiparameter, YSI Inc., Yellow Springs, USA).

Fish sampling and analyses

The collected fishes were analysed for wet weight, standard length (SL), sex and maturity. The reproductive condition was recorded, considering trunk shape in females (i.e., full gonads with hydrated eggs) and pregnancy in males (i.e., brood pouches on the tail carrying fertilised eggs or embryos) (Planas, 2022). Individuals without these characteristics were considered undifferentiated, with most corresponding to preadults or immature fish.

Because pipefishes do not have a fully differentiated tubular gastrointestinal tract, entire guts were dissected out, weighed and preserved in 4% formaldehyde. Food items were identified and quantified (precision of 0.1 mg), according to Demirhindi (1972), under a stereomicroscope to the lowest possible taxonomic level. Fish with empty and full guts were determined. The frequency of occurrence (%F) and numerical presence (%N) of prey items were calculated for each fish species and season as follows (Hyslop, 1980: Liao *et al*., 2001):

$$\%F_i = (n_i / N) * 100,$$

where n_i is the number of individuals with prey item i, and N is the total number of individuals in the sample.

$$\%N_i = (N_i / \Sigma N_i) * 100,$$

where N_i is the number of prey items i in all individuals, and ΣN_i is the sum of all prey items across all individuals.

Fish dorsal fins were removed, rinsed with distilled water, dried in an oven (50°C), sent to the Institute of Marine Research (IIM-CSIC, Spain) and maintained frozen (-20°C) for analyses of stable isotopes (SIA) (δ^{13}C and δ^{15}N), total C and total N.

Stable isotopes

Dried fin samples were homogenised with a pestle and mortar until fine powder, transferred (aliquot of 1-2 mg dry weight) to tin capsules and sent to Servizos de Apoio á Investigación (SAI) of the University of A Coruña (Spain). Fin samples were analysed by continuous-flow isotope ratio mass spectrometry using a FlashEA1112 elemental analyser (Thermo Finnigan, Italy) coupled to DeltaV Advantage (Thermo Scientific) through a ConfloIV interface. Carbon and nitrogen stable isotope abundance was expressed as permil (‰) relative to Vienna Pee Dee Belemnite (VPDB) and Atmospheric Air, according to the following equation:

$$\delta X = (R_{sample} / R_{reference}) - 1,$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio of ^{13}C/^{12}C or ^{15}N/^{14}N. As part of an analytical batch run, a set of international reference materials for δ^{15}N (USGS 40

(-4.52‰), USGS41a (+47.55‰), (IAEA-N-1 (+0.4‰), IAEA-N-2 (+20.3‰) y USGS-25 (-30.4‰) and $\delta^{13}C$ (USGS 40 (-26.39‰), USGS41a (+36.55‰) NBS 22 (−30.031‰) e USGS 24 (-16.049‰)) were analysed. The precision (standard deviation) for the analysis of $\delta^{13}C$ and $\delta^{15}N$ of the laboratory standard (acetanilide) was ± 0.15‰ (1-sigma, n = 10). Standards were run every 10 biological samples. The isotopic analysis procedure fulfilled the requirements of the ISO 9001 standard. The laboratory was submitted to annual intercalibration exercises (Forensic isotope ratio mass spectrometry scheme – FIRMS, LGC Standards, UK).

Due to potential alterations in $\delta^{13}C$ values and, to a lesser extent, $\delta^{15}N$ values, normalisation for lipids is highly recommended in tissues with high lipid content (i.e., C:N > 3.56) (Pinnegar & Polunin, 1999; Sotiropoulos *et al.*, 2004; Sweeting *et al.*, 2006; Post *et al.*, 2007; Logan *et al.*, 2008). In this study, C:N ratios in fin samples of ethanol-preserved specimens ranged from 3.07 to 3.46 (3.07-3.31 in *S. abaster*; 3.11-3.46 in *S. acus*). Studies in syngnathids have demonstrated that preservation in ethanol does not affect $\delta^{15}N$ values in fins (Valladares & Planas, 2012; Planas *et al.*, 2020b). However, $\delta^{13}C$ values might slightly affect the results (Planas *et al.*, 2020b). Consequently, $\delta^{13}C$ data were normalised using the regression equation for dorsal fins in syngnathids (Planas *et al.*, 2020b).

Data treatment

Data and statistical analyses were conducted in R v.3.6.1 (R Core Team, 2020). Data normality and heteroscedasticity were checked with the Shapiro-Wilk normality test and Levene's test, respectively (stats v3.6.2 R package) (R Core Team, 2018).

One- or two-way analyses of variance (ANOVA) (unbalanced design) were applied for mean comparison. When significant, a Tukey HSD post hoc test was applied to check the significance of differences between pairs of group means.

Length-weight relationship (LWR) was estimated as follows (Le Cren, 1951):

$$W = a\mathrm{TL}^{b},$$

where W is the wet weight of fish (g), TL is the total length of fish (cm), a is the intercept of the regression and b is the coefficient expressing the relationship between length and weight. Using the linear regression of the log-transformed equation:

$$\log(W) = \log(a) + b \log(\mathrm{TL}),$$

The parameters a and b were calculated, with a representing the intercept and b the slope of the relationship. The increase in weight was isometric when $b = 3$. When the value of b was other than 3, the weight increase was allometric (positive if $b > 3$ and negative if $b < 3$) (Ricker & Carter, 1958). A positive allometry denotes that fish are heavier, indicating optimum conditions for growth.

The statistical significance of LWR when $P < 0.05$ was analysed with ANOVA. Student's t test was used to verify that the slopes (b) differed significantly from 3.

When comparing both species, we pooled isotopic data from both sampling periods, because the sample size for males and females was not large enough to analyse both periods separately. Stable Isotope Bayesian Ellipses in R (SIBER) v.2.1.4 (Jackson *et al.*, 2011) and nicheRover v.1.1.0 (Lysy *et al.*, 2019) were used to assess differences in the trophic niche features between *S. abaster* and *S. acus*. The niche region was defined as the joint probability density function of the multidimensional niche indicators at a probability alpha of 95%. Uncertainty was accounted for in a Bayesian framework. The analysis provides directional estimates of niche overlap, accounts for species-specific distributions in a multivariate niche space and produces unique and consistent bivariate projections of the multivariate niche region (Swanson *et al.*, 2015).

Total convex hull areas (TA) and core trophic niche breadths were estimated using SIBER while correcting for variable sample sizes (SEAc). Total trophic overlap values for 95% TA were estimated using nicheROVER –a method that is insensitive to sample size and incorporates statistical uncertainty using Bayesian methods (Swanson *et al.*, 2015).

The isotopic characterisation of an adequate baseline (i.e., particulate organic matter, primary producers or secondary producers) is imperative for assessing the trophic position (TP) of a species. Because stable isotope data of organisms in the food web in the study area are lacking –more importantly, a known baseline is lacking– the TP occupied by both species could not be properly analysed. Nevertheless, tentative TP estimates were obtained for *S. abaster* and *S. acus* in Metruk Tuzla based on isotopic data using a hypothetical baseline (Mytilidae *Musculus costulatus* (Risso, 1826); trophic level = 2) from studies in the Cíes Archipelago (NW Spain) (Planas *et al.*, 2020a; Planas, 2022). This approach did not necessarily provide accurate TP estimates but allowed estimation of interspecific differences in TP. TPs in *S. abaster* and *S. acus* were estimated as described by Planas (2022).The analyses were performed using the tRophicPosition v. 0.7.7 package in R (Quezada-Romegialli *et al.*, 2018). For that, we applied experimentally derived trophic discriminant factor values for syngnathids (2.5 for $\delta^{13}C$; 3.9 for $\delta^{15}N$) (Planas *et al.*, 2020a).

Two baselines (spring-summer 2017 and spring-summer 2018) were selected considering isotopic data available in the limited isotopic studies on *S. abaster* and *S. acus* in European populations. Vizzini & Mazola (2004) reported mean $\delta^{13}C$ and $\delta^{15}N$ values of -10.5‰ and 11.9‰ in *S. abaster* from western Mediterranean Stagnone di Marsala (Italy), whereas Planas (2022) provided mean values of -15.4‰ for $\delta^{13}C$ and 11.1‰ for $\delta^{15}N$ in *S. acus* from the Cíes Archipelago. Considering the higher similarity between our data in Metruk Tuzla (-17.0‰ ± 1.2‰ for $\delta^{13}C$ and 13.2‰ ± 0.6‰ for $\delta^{15}N$ in *S. abaster* and -17.3‰ ± 1.2‰ for $\delta^{13}C$ and 13.3‰ ± 0.7‰ for $\delta^{15}N$ in *S. acus*) and those from Planas (2022), we used the isotopic data of the baseline organism (Mytilidae, *M. cos-*

tulatus) in the Cíes Archipelago. Baseline isotopic values are provided in Table S1.

TP analysis was performed using "twoBaselinesFull" model and 20210:40000 iterations (thinning interval: 10; number of chains: 5; and sample size per chain: 2000).

Differences in gut content across species and seasons were assessed by a *t* test and one-way ANOVA.

Significance levels were set at $P < 0.05$, and the results are given as mean ± standard deviation. The graphics were constructed using ggplot2 v3.3.0 in R (Wickham & Chang, 2019).

Bioethics

Animal sampling and manipulation practices were conducted in compliance with all bioethics standards on animal experimentation (Burdur Mehmet Akif Ersoy University, Animal Experiments Local Ethics Committee, Decision number: 1148).

Results

Abiotic data

Temperature and salinity recorded on the sampling dates were 14 and 15°C in spring and 17 and 21°C in summer, respectively. Oxygen saturation and pH levels were 6.8 mg L^{-1} and 8.12 in spring and 6.3 mg L^{-1} and 8.37 in summer, respectively.

Fish populations

A summary of the characteristics of the pipefish collected is provided in Figure S1 and Table S1.

Average TL was 5.9 ± 1.6 cm in *S. abaster* (n = 51; range: 3.3-10.9 cm) and 6.3 ± 1.9 cm in *S. acus* (n = 78; range: 3.1-12.0 cm) (Table 1; Fig. S2). Mean individual weights were 0.115 ± 0.118 g in *S. abaster* (range: 0.015–0.669 g) and 0.124 ± 0.125 g in *S. acus* (range: 0.010–0.610 g).

Table 1. Total length (TL; cm), wet weight (g) and isotope values ($\delta^{13}C$ and $\delta^{15}N$; ‰) in specimens of *Syngnathus abaster* and *Syngnathus acus* collected in Metruk Tuzla (n: sample size; sd: standard deviation; Undif: undifferenciated).

	S. abaster				***S. acus***			
	Pooled	**Undif**	**Male**	**Female**	**Pooled**	**Undif**	**Male**	**Female**
Total length (cm)								
n	51	37	7	7	78	45	9	24
Mean	5.9	5.2	8.8	7.2	6.3	5.0	8.4	7.8
sd	1.6	0.7	1.0	1.5	1.9	0.9	1.9	1.4
Max	10.9	6.6	10.9	10.0	12.0	6.4	12.0	11.6
Min	3.3	3.3	7.7	5.4	3.1	3.1	6.3	6.1
Wet weight (g) (cm)								
n	51	37	7	7	78	45	9	24
Mean	0.115	0.058	0.358	0.168	0.124	0.051	0.272	0.206
sd	0.128	0.023	0.155	0.134	0.125	0.025	0.154	0.130
Max	0.669	0.119	0.669	0.450	0.610	0.096	0.470	0.610
Min	0.015	0.015	0.230	0.069	0.010	0.010	0.098	0.095
$\delta^{13}C$ (‰)								
n	51	37	7	7	75	43	8	24
Mean	−17.0	−17.0	−17.6	−16.6	−17.3	−17.1	−17.6	−17.6
sd	1.2	1.2	1.2	0.9	1.2	1.2	1.6	1.1
Max	−14.4	−14.4	−16.4	−15.4	−14.9	−15.0	−16.3	−16.3
Min	−19.8	−19.8	−19.6	−17.8	−20.5	−22.1	−21.4	−19.0
$\delta^{15}N$ (‰)								
n	51	37	7	7	75	43	8	24
Mean	13.2	13.4	12.8	12.9	13.3	13.4	13.4	13.1
sd	0.6	0.5	0.9	0.6	0.7	0.6	0.6	0.8
Max	14.7	14.7	13.9	13.4	15.2	14.8	14.8	15.2
Min	11.4	12.6	11.4	11.6	11.6	12.2	12.2	11.6

Mean TL in both species did not differ significantly (Kruskal-Wallis test; $X^2_{(1)} = 1.31$; $P = 0.251$) but TL was significantly different across sex groups (undifferentiated < females < males) (Kruskal-Wallis test; $X^2_{(2)} = 1.31$; $P < 0.0001$). The minimum recorded TLs in mature females were 5.4 cm in *S. abaster* and 6.1 cm in *S. acus*, whereas pregnant males had minimum TLs of 7.7 cm in *S. abaster* and 6.3 cm in *S. acus* (Table 1).

Both species showed similar LWRs (Fig. 3). The estimates for coefficient *b* were 3.179 in *S. abaster* and 3.182 in *S. acus*, indicating positive allometric growth in both species (*t* test, $P < 0.0001$) (Table 2).

3.3. Gut content

Only 7.8% of *S. abaster* individuals showed empty guts, whereas the percentage increased to 22.8% in *S. acus*. In *S. abaster*, the dominant prey group was the pseudodiaptomid copepod *Calanipeda aquaedulcis*, both in number (%N; 71.2%) and frequency of occurrence (%F = 42.6%). The contribution of unidentified copepods and fish eggs was important in the diet of the species. In *S. acus*, *C. aquaedulcis* was dominant together with cladocera (pieces) both in %N (47.5% and 45.7%, respectively) and %F (35.3% and 40%, respectively) (Table 3; Fig. S3). The interspecific difference for %N was insignificant (*t* value = -0.019, d.f. = 10, $P > 0.05$).

The results of *t* test analysis showed no significant sex-based differences in prey composition for *S. abaster* or *S. acus* ($P > 0.05$). Regarding seasonal changes in gut contents (Table 3), the findings were most noticeable in summer for *S. acus*, with a numerical increase in the cyclopoid copepod *Halicyclops biscupidatus* (%N = 54.2%). The frequency of cladoceran pieces increased in summer (%F = 35.0%). However, the interseasonal differences in the numerical prey content within each species were insignificant ($P > 0.05$).

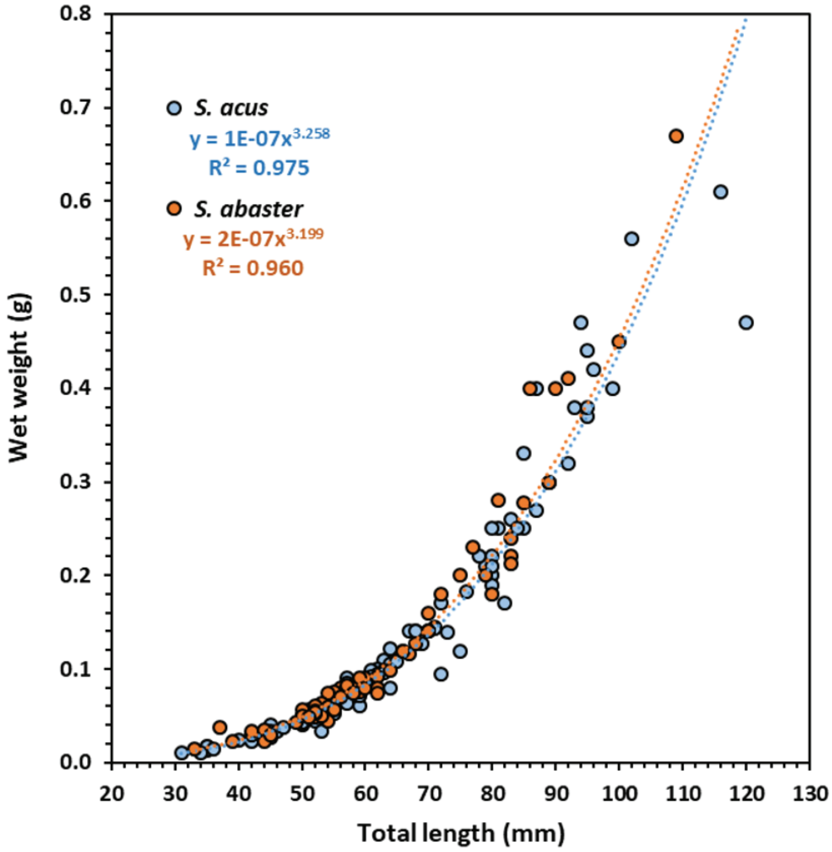

Fig. 3: Relationship between total length (TL) and wet weight (W) ($W = aTL^b$) in the pipefishes *Syngnathus abaster* and *Syngnathus acus* collected in Metruk Tuzla.

Table 2. LWR parameters for *Syngnathus abaster* and *Syngnathus acus* collected in Metruk Tuzla. Equation: log (W) = log a + b log (TL). n: sample size; a: intercept; b: slope; se: standard error; CI: confidence interval (95%).

Species	n	*a*	*b*	se *b*	CI *b*	R²	p	t test p	Growth type
S. abaster	51	0.000294	3.179	0.095	2.989–3.369	0.958	<0.0001	<0.0001	Allometric (+)
S. acus	78	0.000269	3.182	0.057	3.069–3.296	0.976	<0.0001	<0.0001	Allometric (+)

Table 3. Gut contents (pooled, spring and summer samples) in *Syngnathus abaster* and *Syngnathus acus* individuals (%F: frequency percentage; %N: prey number percentage) from Metruk Tuzla. n.d.: not detected.

	S. abaster		***S. acus***		***S. abaster***				***S. acus***			
	Pooled		**Pooled**		**Spring**		**Summer**		**Spring**		**Summer**	
Prey Groups	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**	**%F**	**%N**
Copepoda												
Halicyclops biscupidatus	n.d.	n.d.	1.2	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	54.2
Calanipeda aquaedulcis	42.6	71.8	35.3	47.5	37.0	70.5	47.3	75.5	87.7	44.9	53.6	53.6
Other copepods	37.7	15.5	11.8	4.7	73.0	29.6	18.9	10.2	5.2	4.1	6.2	6.3
Cladocera												
Daphnia longispina	n.d.	n.d.	3.5	0.6	n.d.	n.d.	n.d.	n.d.	0.6	0.5	1.0	0.7
Ceriodaphnia quadrangula	n.d.	n.d.	1.2	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	1.0
Pleopis polyphemoides	n.d.	n.d.	2.3	0.4	n.d.	n.d.	n.d.	n.d.	0.6	0.3	1.0	0.7
Cladocera pieces	n.d.	n.d.	40.0	45.7	n.d.	n.d.	n.d.	n.d.	4.5	49.6	35.0	35.9
Insecta	n.d.	n.d.	1.2	0.2	n.d.	n.d.	n.d.	n.d.	0.6	0.3	n.d.	0.7
Mollusca	n.d.	n.d.	1.2	0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	n.d.
Fish scale	4.9	0.7	1.2	0.2	7.4	0.5	1.3	1.4	0.6	0.3	n.d.	0.7
Unidentified eggs	14.7	12.0	n.d.	n.d.	25.9	11.7	32.4	12.9	n.d.	n.d.	n.d.	n.d.

Isotopic profiles

Isotopic profiles were highly similar in both species (two-way ANOVA, type III; $P > 0.05$) and sex (two-way ANOVA, type III; $P > 0.05$), with mean δ^{13}C and δ^{15}N values of -17.2‰ ± 1.2‰ and 13.3‰ ± 0.7‰, respectively (Table 1; Fig. S4). However, average δ^{15}N was significantly lower in females (13.1‰ ± 0.7‰) than in undifferentiated specimens (13.4‰ ± 0.6‰) (Tukey HSD test; $P_{adj} = 0.036$).

Mean isotopic values were -17.0‰ ± 1.2‰ for δ^{13}C and 13.2‰ ± 0.6‰ for δ^{15}N in *S. abaster* and -17.3‰ ± 1.2‰ for δ^{13}C and 13.3‰ ± 0.7‰ for δ^{15}N in *S. acus* (Table 1).

Spearman's correlations indicated that δ^{15}N values were negatively correlated with TL in both species (*S. abaster*: r(51) = -0.482, $P < 0.001$; *S. acus*: r(78) = -0.458, $P < 0.001$), whereas the correlation between δ^{13}C and TL was statistically insignificant (Fig. S1). The relationship between δ^{15}N and TL was modelled by linear regression. The resulting coefficient b in the models was significant, especially for the greater pipefish ($b = -0.0123$, $P = 0.022$ in *S. abaster*; $b = -0.0125$, $P = 0.007$ in *S. acus*), indicating a small progressive decrease in δ^{15}N with fish growth.

Trophic structure and niche overlap

The average niche area, SEAc, in *S. abaster* (2.06) was slightly smaller than in *S. acus* (2.51) in all sex types (undifferentiated specimens, males and females) (Figs. S4 and S5). The smallest and larger niche areas occurred in females and males, respectively (Table 4).

Isotopic niche overlap analysis with the nicheROVER package revealed high probability (96%) that a specimen of *S. abaster* will be included within the niche of *S. acus* (Fig. 4) The probability decreased to 89% in the reverse condition. Considering inter- and intra-specific niche overlaps concerning the sexual condition (Table 5; Fig. 4), the lower posterior probabilities regarding niche overlap estimates occurred in females and undifferentiated individuals of *S. abaster*, especially compared with males of the same species. Regarding *S. acus*, the probabilities that an individual will be found within estimated trophic niche of the species were high (73%-95%).

Discussion

Our study revealed that *S. acus* and *S. abaster* occupy highly similar trophic niches, foraging partially or almost entirely on similar prey types. Complementary stable iso-

Table 4. Estimated niche areas in *Syngnathus abaster* and *Syngnathus acus* collected in Metruk Tuzla. TA, SEA, SEAb and SEAc (SIBER package): Total area of the convex hull, standard ellipse area and corrected standard ellipse with correction for small sample sizes.

	Syngnathus abaster			***Syngnathus acus***		
Metrics	**Undifferentiated**	**Males**	**Females**	**Undifferentiated**	**Males**	**Females**
TA	6.65	2.19	1.60	8.79	4.11	7.98
SEA	1.66	1.69	1.06	2.11	2.87	2.05
SEAb	1.64	1.97	1.12	2.09	2.59	2.09
SEAc	1.70	2.03	1.28	2.16	3.35	2.14

Table 5. Niche overlap estimates (NicheROVER package) showing posterior probabilities (α = 0.95) that individuals from rows will be found within the niches, indicated by the column header. Results (%) are provided for reproductive stages in *Syngnathus abaster* and *Syngnathus acus* specimens from Metruk Tuzla.

		S. abaster			***S. acus***		
Species	**Sex condition**	**Female**	**Male**	**Undif.**	**Female**	**Male**	**Undif.**
Syngnathus abaster	**Female**	-	51	85	94	88	90
	Male	34	-	58	87	86	71
	Undifferentiated	59	40	-	77	81	95
Syngnathus acus	**Female**	53	70	74	-	87	82
	Male	44	59	65	78	-	76
	Undifferentiated	52	40	86	73	80	-

tope and gut content analyses suggest that both species do not compete entirely for the same resources. Both species foraged upon the copepod *C. aquaedulcis*, which was the primary and nearly exclusive resource for *S. abaster*, whereas cladocerans seemed to play a crucial role in the dietary regime of *S. acus*.

The spatial partitioning of *S. abaster* and *S. acus* is unknown in the Metruk Tuzla lagoon and was not assessed in our study. However, both species are likely to exhibit a spatial or microhabitat partitioning in the area in which *S. acus* would have more easy access to cladocerans present above the phytal bed. We hypothesise that although *S. abaster* feeds on submerged vegetation (Franzoi *et al*., 1993; Vizzini & Mazzola, 2004), *S. acus* would also forage in the water column. These distribution patterns agree with a study in Lake Bafa (Gürkan *et al*., 2021).

The spatial distribution of species along the water column or substrate appears to be a pivotal factor in interspecific competition for food resources. Kehayias & Kourouvakalis (2010) reported that in the surface layer in a coastal area of the eastern Aegean Sea, the most abundant chaetognath species follow the horizontal and vertical distribution of their prey (copepods and cladocerans) and occupy different niches to reduce trophic competition. The study indicated some differences in the vertical distribution of copepods and cladocerans, likely because copepods are better able to tolerate higher temperatures. Differential use of microhabitats in phytal environments has been reported in syngnathid species by Howard & Koehn (1985) and Franzoi *et al.* (1993). In the co-occurring species *S. abaster* and *S. taenionotus* in the Po River Delta (Adriatic Sea), gut content analyses suggested that both species do not compete for food resources, remaining almost completely segregated in different foraging microhabitats during most of their lifespan. The shorter-snouted *S. abaster* fed near the bottom, whereas *S. taenionotus* foraged on prey above algal beds (Franzoi *et al*., 1993).

Most syngnathids forage upon small crustaceans, encompassing different taxa in the diet depending on species, snout and gape morphometrics, seasonal prey availability, size, maturity, etc. (Kendrick & Hyndes, 2005; de Lussanet & Muller, 2007; Manning *et al*., 2019). Consequently, these factors significantly influence foraging and the resulting isotopic profiles in pipefish tissues (Vizini & Mazzola, 2004; Piñeiro-Corbeira *et al*., 2021; Planas, 2022), which in turn will reflect the dietary regime of the consumer (Tieszen *et al*., 1983; Fry, 1988; Hobson *et al*., 1995).

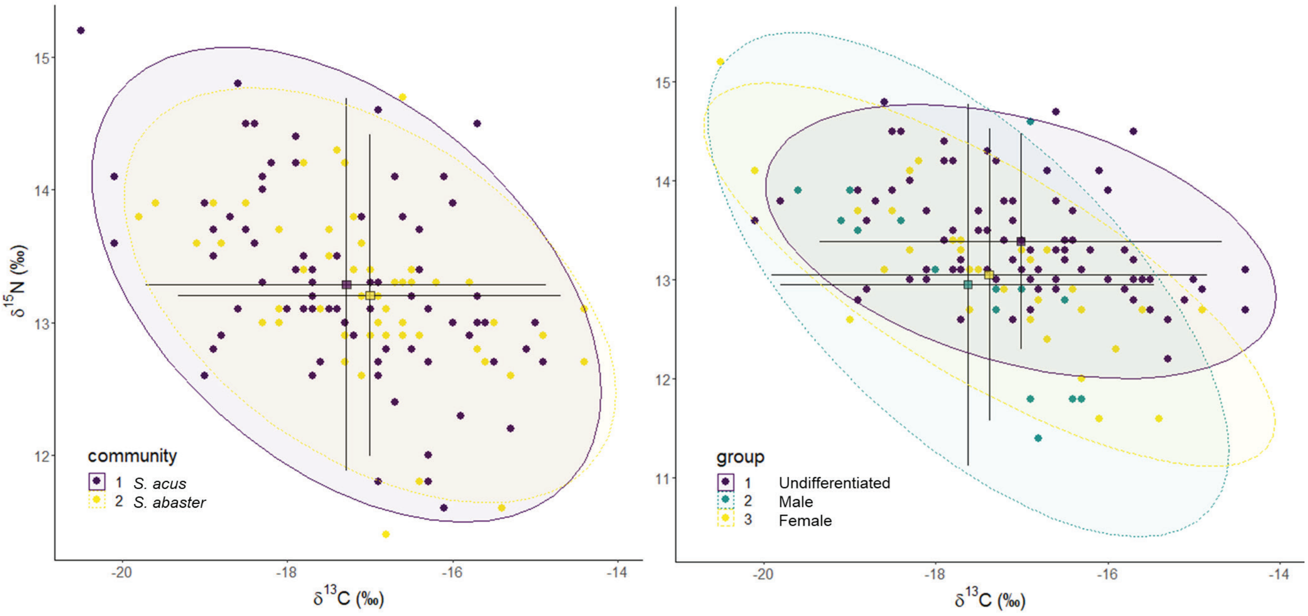

Fig. 4: Stable isotope Bayesian ellipses showing trophic niche widths and overlaps in *Syngnathus abaster* and *Syngnathus acus* specimens collected in Metruk Tuzla. Ellipses with 95% credible intervals for the means are based on standard ellipses corrected for small sample sizes (SEAc; isotopic niche metrics; SIBER package). Centroids and credible intervals are displayed for each ellipse.

Similar to that in Metruk Tuzla, *S. abaster* and *S. acus* from Lake Bafa prefer similar, but not always identical, prey groups (Gürkan *et al.*, 2021). Morphological adaptations in congeneric species may be an important factor in determining the distinction of the ecological niche (Franzoi *et al.*, 1993; Vizzini & Mazzola (2004). Foraging on different prey types in syngnathids varies markedly depending on head and mouth biometrics, type and use of habitat, etc. (Campolmi *et al.*, 1996; Kendrick & Hyndes 2005; de Lussanet & Muller, 2007; Manning *et al.*, 2019; Gurkan and Taskavak 2019). Snout length as a proportion of head length is a widely used morphometric character for species identification in syngnathids, but data on the correlation between mouth size and prey selection are limited (Manning *et al.*, 2019). Similar to *S. taenionotus*, the greater pipefish *S. acus* has a long and terminally cylindrical snout, whereas *S. abaster* has a shorter and conical one (Franzoi *et al.*, 1993). The interspecific differences in feeding habits from Metruk Tuzla specimens would partially rely on morphological differences in gape morphometry, because mouth width was larger in *S. abaster* than in *S. acus*. According to Franzoi *et al.* (1993), these characteristics result in the greater aiming ability of *S. acus*.

This study analysed specimens collected in spring and early summer, when the abundance of copepods, cladocerans and other small prey (e.g., chironomids) increased compared with that in winter (Killi, 2020). The breeding period for *Synganthus* species is from January to July in the Mediterranean Sea, depending on latitude and water temperature (Vincent *et al.* 1995; Takahashi *et al.* 2003; Lyons & Dune 2005). The turbidity of the bottom structure due to the effect of rainfall in spring, the withdrawal of adults to deeper waters due to breeding time and finally the depth limiting effect of the sampling device on the capture of samples resulted in the capture of fewer fish samples in the spring season. Alluvial areas, such as Lake Bafa and Metruk Tuzla, are subjected to significant changes in environmental factors, mainly salinity and temperature, and biotic characteristics. Hence, it was expected that the trophic features in the pipefishes changed accordingly. Such variability would affect prey abundance and distribution, resulting in a seasonal switch in prey preference. This change was reflected in the increased %F for cladocera pieces in the guts of *S. acus* collected in the summer compared with those from early spring. The calanoid copepod *C. aquaedulcis* is a euryhaline, eurythermal and nonspecialised filter feeder (phytodetritophage) that is dominant in confined and relatively oligotrophic Mediterranean wetlands (Boix *et al.*, 2005; Brucet *et al.*, 2008; Lazareva, 2018). The species attains high abundance from spring to autumn, with peaks in summer (Uriarte *et al.*, 2016). In Güllük Bay, copepods and cladocerans are the main zooplankton groups, accounting for 73% and 20% of total zooplankton, respectively (Killi, 2020).

Specimens of *S. abaster* from Stagnone di Marsala (western Mediterranean Sea) mainly fed on Mysidacea and Isopoda (Vizzini & Mazzola, 2004). The dietary regime of *S. acus* from the Cíes Archipelago seemed to prey primarily upon amphipods with varying proportions of carideans, isopods, copepods and mysidaceans (Planas, 2022). However, Gürkan & Taskavak (2019) reported the importance of cladocerans in the diet of the greater pipefish in the Turkish Aegean Sea, whereas Gürkan *et al.* (2021) reported a preference for amphipods and copepods. Studies in *S. abaster* have revealed ontogenetic dietary changes, shifting from harpacticoid copepods to larger prey (Taşkavak *et al.*, 2010). Ontogenic changes in dietary regimes have been reported in *S. acus* and *S. abaster* from Lake Bafa (Türkiye), with an increasing preference for amphipods in larger fishes (Gürkan *et al.*, 2021). The isotopic profiles and the trophic assessment in this study support the occurrence of a slight decrease in the trophic levels of ingested prey. These results suggest a size- or sex-related migration from one habitat to another in the study area and associated changes in prey availability. Such spatial partitioning in separate layers could be an adaptation to reduce intra- and interspecific competition for limited resources, maximising foraging efficiency and providing suitable refuge from predators (Kendrick & Hyndes, 2005).

The depletion observed in $\delta^{13}C$ (-17.0‰ ± 1.2‰) compared with specimens from other regions is noteworthy (Vizzini & Mazzola, 2004; Piñeiro-Corbeira *et al.*, 2021). The finding was supported by gut content data (Gürkan & Taşkavak, 2019; Gürkan *et al.*, 2021; this study), indicating a preference, especially in small pipefishes, for small and less mobile crustaceans (e.g., copepods or cladocerans) with low $\delta^{13}C$ values and positioned at the lower levels of the food web compared with larger crustaceans, such as mysidaceans or amphipods. The $\delta^{13}C$ depletion in those prey would be related to more dependence on sources of terrestrial origin (e.g., insects or detritus) (Deegan *et al.*, 1990; Cole *et al.*, 2020) or less reliance on benthic resources (higher $\delta^{13}C$ values compared with pelagic resources) (Fry & Sherr, 1989).

Estimated TPs (empirical mean ± s.d.) for *S. abaster* and *S. acus* populations in Metruk Tuzla were 3.67 ± 0.61 and 3.46 ± 0.66, respectively. Estimated TP for *S. acus* closely resembled that in Cíes populations (TP = 3.40 ± 0.06) (Planas, 2022). To our knowledge, there are no other references to TP estimates in *S. abaster* and *S. acus*, limiting further comparisons. Nonetheless, our approach suggests that both species are secondary consumers, occupying a similar TP on the food web. This finding aligns with the interspecific similarity revealed by niche structure and overlap assessment.

There is evidence that the size of the studied pipefishes *S. abaster* and *S. acus* is contingent upon the geographical region considered (refer to the review by Gürkan & Innal, 2018). The size of specimens from Metruk Tuzla (5.9 ± 1.6 cm TL in *S. abaster*; 6.3 ± 1.9 cm TL in *S. acus*) was similar to those in nearby Lake Bafa (Türkiye) (Gürkan & Innal, 2018), but notably smaller than that in *S. acus* from the Cíes Archipelago (NW Spain) (34.4 ± 6.8 cm SL) (Planas, 2022). Both species exhibited comparable LWR and positive allometry, aligning with findings in other regions (Veiga *et al.*, 2009; Vieira *et al.*, 2014; Planas, 2022).

Pipefishes are ovoviviparous teleosts with parental care, where the female deposits eggs in a specialised brooding structure of the male, on the male's tails or trunk. The breeding season for most European pipefishes spans from February to September, varying based on region and species (Franzoi *et al.*, 1993; Gürkan *et al.*, 2009; Simal Rodríguez *et al.*, 2021; Planas, 2022). Notably, some species, such as *S. acus*, exhibit temporal migratory events to breeding areas (Planas, 2022).

In Metruk Tuzla, the smaller recorded size in mature males and females was similar in both species (8.4–8.8 cm in males; 7.2–7.8 cm in females). This observation agrees with findings on the Greek coastline, where 50% maturity of males and females of *S. abaster* measured >7 cm in TL (Liousia, 2015). However, the largest mature *S. acus* specimens collected in Metruk Tuzla were notably smaller than those from the Cíes Archipelago (19.1-47.4 cm in ovigerous females; 24.0-49.8 cm in pregnant males), where the largest specimen ever observed for the species was recorded (49.8 cm TL). Thus, at least in the greater pipefish, the size at first maturity appears to be geographically dependent. *S. acus* specimens from Camalti Lagoon (Izmir Bay, Aegean Sea) –recorded by Gurkan *et al.* (2009) in the low-salinity lagoon area near the Gediz River mouth– exhibited larger length values than those in this study. Several factors may contribute to these differences, including genetics, sample size, season, gear selectivity, fish condition, sex, diet, water temperature, habitat structure and other physicochemical parameters (Tesh 1971; Wootton, 1999). Salinity differences can significantly influence fish growth (Boeuf & Payan, 2001) and may explain the variation in fish length, including differences in sexual maturity size. In contrast to *S. acus*, the size ranges for *S. abaster* seem to be relatively conservative and not correlated with the location.

Despite the limited number of males collected, we observed a significant relationship between male size and male brood pouch in both species (Fig. S6). This finding agrees with the hypothesis of length-dependent reproductive fitness proposed by Silva *et al.* (2006b) for *S. abaster*, where larger males are theorised to accommodate more offspring.

The co-occurrence of species, also known as sympatry, may depend on abiotic and/or biotic environmental conditions. Areas of co-occurrence between two species are often believed to emerge in regions where abiotic conditions favour both species, thereby representing an intermediate state between regions where either species might occur alone (allopatry) (Chunco *et al.*, 2012). Hence, under specific environmental conditions, hybridisation may facilitate sympatry, preserving populations in areas where pure species might otherwise be unable to persist. For instance, eutrophication (i.e., turbidity or increased algal cover) may impede species-specific sexual signalling, leading to hybridisation (Seehausen *et al.*, 1977; Sundin *et al.*, 2017). The fishes of the genus *Syngnathus* exhibit sophisticated mating behaviours (Vincent *et al.*, 1995; Silva *et al.*, 2006b), and the absence of pre-mating isolating mechanisms suggests that hybridisation is common in sympatric species of *Syngnathus* (e.g., in southern California). However, hybrids may not be viable in wild populations (Wilson, 2006) or under experimental conditions due to postzygotic reproductive isolation between hybrids and their parental species (Moser & Wilson, 2020). Consistently, there is no evidence of hybridisation between *S. abaster* and *S. acus*.

Both abiotic and biotic interactions in extreme environments may facilitate co-occurrence; however, simple responses to the abiotic environment might not be the primary factors mediating co-occurrence in animals. Sympatry can be fostered by the presence of food resources that minimise competition between ecologically similar species (Chunco *et al.*, 2012). Although some pipefishes are widespread across large marine areas where they co-occur reproductively isolated, others have shorter ranges or inhabit specific sites (Fritzsche, 1980; Garcia *et al.*, 2019). According to the biodiversity related niche differentiation theory (Cazzolla Gatti, 2011), species compete for resources and coexistence is only possible if each uses a different niche of available habitat to reduce interspecific competition. Given that our study was conducted during a limited period of the annual cycle, the presence of seasonal changes, including changes in productivity in both size and overlap of the trophic niches in *S. abaster* and *S. acus*, cannot be ruled out (Lesser *et al.*, 2020).

Syngnathids can play significant roles in the strategic conservation planning of estuarine and wetland ecosystems. This is particularly true when conservation objectives for syngnathids emphasise safeguarding areas with the highest abundance and representation of assemblage variation (Shokri *et al.*, 2009). Our study establishes an important baseline for future assessments, facilitating the evaluation of local extinctions and habitat alterations resulting from changes in the wetland's conservation status. These shifts may arise from the implementation of enhanced protective measures, increased environmental degradation or the impacts of climate change (Monteiro *et al.*, 2023).

Despite the *Least Concern* categorisation by IUCN for *S. abaster* and *S. acus*, the black-striped pipefish is included in Appendix III (Protected species) of the Bern Convention and is considered critically endangered in certain regions (Mayol *et al.*, 2000; Manent Sintes & Abella-Gutiérrez, 2004). To mitigate potential threats, especially in disturbed coastal zones, species not assessed as threatened must be closely monitored (Monteiro *et al.*, 2023). This monitoring aims to ensure that known pressures of exploitation and habitat damage, coupled with ecological shifts due to the progressing threat of climate change, do not escalate to the point of categorising these species as threatened. Given their vulnerability, considering biological and ecological features, such as limited dispersability, reduced home ranges and reproduction with parental care, the protection of areas inhabited by these species must be prioritised.

The lagoon area in BTW is a critical habitat for economically important fish species, including mullet, sea bass, sea bream and common sole, as well as their juvenile stages. It serves as an essential breeding and feeding ground for numerous bird species and is recognised as an important bird area. The Mumcular Dam –constructed to prevent flooding– is a major threat to the lagoon's ecosystem. It has significantly reduced freshwater inflow to the lagoon, leading to increased salinity levels. Insufficient drainage from agricultural lands and pesticide runoff further exacerbate these issues. To mitigate these problems and protect fish populations, actions such as modifying fishing gear, deepening shallow areas and regulating fishing activities, must be taken (Tosunoglu *et al.*, 2017). However, more comprehensive approaches, including improving water quality and restoring freshwater inflow, must address the problems. By implementing these conservation strategies, we can safeguard the ecological integrity of the lagoon and the associated biodiversity.

Conclusions

In this study, we provide insights into the ecology of sympatric pipefishes, focusing on *S. abaster* and *S. acus*. Our observations in Metruk Tuzla highlight that both polyandrous species exhibit similar trophic features, indicating that both species primarily consume prey with similar isotopic signatures in this area. *S. abaster* exhibited monospecific feeding behaviour, primarily targeting copepods. By contrast, *S. acus* exhibited a more diverse diet, incorporating both copepods and cladocerans as primary food sources. This suggests that both species exhibit selective yet flexible carnivorous feeding habits.

Regarding potential interspecific competition for dietary resources, we hypothesise that this phenomenon would be minimised by the occupancy of distinct microhabitats by each species, indicating a form of ecological partitioning. Such partitioning should be further investigated because it contributes to understanding the coexistence of both species in the studied area.

Pipefishes, being euryhaline species, are adaptable to changing environmental conditions. In the study area, anticipated changes, such as increased freshwater inflow, regulated fishing and reduced pollution, could positively impact their populations. Increased freshwater inflow may lead to altered prey availability and improved growth parameters for pipefish species. We emphasise the importance of future investigations. These studies should encompass nearby populations, enabling a comprehensive analysis of genetic structure and local adaptations influenced by varying environmental and physicochemical conditions. Such studies will help understand the resilience or vulnerability of pipefish populations to external pressures. The insights gained will inform conservation strategies for these ecologically significant species, ensuring their protection in the face of environmental challenges.

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Supplementary Data

The following supplementary information is available online for the article:

Fig. S1: Global information on *Syngnathus abaster* and *Syngnathus acus* specimens collected in Metruk Tuzla (Boğaziçi Tuzla Lagoon, Türkiye) in 2019.

Fig. S2: Lengths and weights of *Syngnathus abaster* and *Syngnathus acus* specimens collected in Metruk Tuzla (Boğaziçi Tuzla Lagoon, Türkiye) in 2019.

Fig. S3: Numerical occurrence (%N) of prey items in the guts of *Syngnathus abaster* and *Syngnathus acus* specimens collected in Metruk Tuzla (Boğaziçi Tuzla Lagoon, Türkiye) in 2019.

Fig. S4: Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of *Syngnathus abaster* and *Syngnathus acus* specimens collected in Metruk Tuzla (Boğaziçi Tuzla Lagoon, Türkiye) in 2019.

Fig. S5: Niche plots (95% alpha) (A - $\delta^{15}N$; D - $\delta^{13}C$), density distributions (B) and raw data (C) for each pairwise combination of isotope ($\delta^{13}C$ and $\delta^{15}N$) data in *Syngnathus abaster* and *Syngnathus acus* specimens collected in Metruk Tuzla (Boğaziçi Tuzla Lagoon, Türkiye) in 2019.

Fig. S6: Relationship between total length and pouch length in *Syngnathus abaster* and *Syngnathus acus* males from Metruk Tuzla (Boğaziçi Tuzla Lagoon, Türkiye).

Table S1. $\delta^{13}C$ and $\delta^{15}N$ values used as baseline 1 and baseline 2 (*Musculus costulatus* sampled in the Cíes Archipelago, NW Spain). Data from Planas (2022).

Table S2. General information of *Syngnathus abaster* and *Syngnathus acus* specimens collected in Metruk Tuzla (Boğaziçi Tuzla Lagoon, Türkiye in 2019.