Biol Invasions
https://doi.org/10.1007/s10530-021-02506-7

ORIGINAL PAPER

Trophic ecology of the Atlantic blue crab *Callinectes sapidus* as an invasive non-native species in the Aegean Sea

Herdem Aslan · Michael J. Polito

Received: 16 November 2019 / Accepted: 20 March 2021
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Abstract Invasive non-native crustaceans are a biodiversity and management concern in the Mediterranean Sea. The Atlantic blue crab (*Callinectes sapidus*) was first recorded in the Mediterranean Sea in 1949, but may have arrived as early as in the 1930's. Blue crabs in the Mediterranean Sea are of concern due to their presumed potential for negative consumptive and competitive interactions with native fauna. The aim of this study was to provide a first assessment of the trophic ecology of non-native blue crab in the Northern Aegean Sea using stable carbon ($\delta^{13}C$) and nitrogen isotope ($\delta^{15}N$) analysis. We found limited isotopic niche overlap between blue crabs and seven native species examined at Gökçeada Island in April, June, and August of 2017. In addition, the range of calculated trophic positions of blue crabs at Gökçeada Island (2.0 to 4.4), while broad, is in general agreement with prior studies in both native and non-native ranges. We also observe that trophic position declined and the relative importance of pelagic carbon sources to blue crabs increased from April to August. However, we also found that differing assumptions as to the number and type of food web baselines and trophic discrimination factors led to differing estimates of trophic position in blue crabs at Gökçeada Island by as much as one to two trophic levels. These methodical differences make it challenging to directly compare results within and between studies, and thus limit our ability to assess negative consumptive and competitive interactions of invasive blue crab with native coastal species in the Mediterranean Sea.

Keywords *Callinectes sapidus* · Invasive species · Diet · Mediterranean Sea · Gokceada

Herdem Aslan and Michael J. Polito have contributed equally to this work and share first authorship.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10530-021-02506-7.

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Introduction

Invasive non-native species are considered as one of the key causes of biodiversity changes worldwide (Bax et al. 2003). In the Mediterranean Sea biodiversity changes occur at an unprecedented rate (Zenetos 2009) and it is the most affected area in the world due

Published online: 31 March 2021

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to non-native species (Occhipinti-Ambrogi et al. 2011; Zenetos et al. 2012). By 1950, it is estimated 145 non-native species had been introduced into the Mediterranean Sea, 127 of which have subsequently become established today. The rate of species introductions increased between 1950 and 2012, with 986 introductions reported from the Mediterranean Sea by 2012 (Zenetos et al. 2012). Non-native species are commonly introduced via commercial shipping activities, canals, aquaculture and fisheries, drilling platforms and the aquarium industry (Bax et al. 2003).

The Atlantic blue crab (*Callinectes sapidus* Rathbun, 1896)—hereafter blue crab—is one of 159 invasive non-native Crustacea species currently established in the Mediterranean Sea (Zenetos et al. 2012). Blue crabs are native to the Western Atlantic from Nova Scotia to Uruguay-Argentina (Kennedy and Cronin 2007). They are omnivorous, opportunistic consumers in their native habitats feeding on plants, invertebrates, and vertebrates (Dittel et al. 2006; Seitz et al. 2011). In their native range they are very important consumers in benthic food webs (Baird and Ulanowicz 1989; Dittel et al. 2000), either as a keystone species regulating carbon flows (Baird and Ulanowicz 1989) or by reducing prey abundance and inducing trophic cascades (Silliman and Bertness 2002; O'Connor et al. 2008; Altieri et al. 2012; Mancinelli et al. 2017a).

The blue crab was recorded first recorded in the Eastern Atlantic Ocean in 1901 along the France coast (Bouvier 1901). The first official record in the Mediterranean Sea was 1949 (Giordani Soika 1951), but this species may have arrived as early as in the 1930's (Enzenrob et al. 1997). Since this time there have likely been multiple independent introductions via ballast water and currently blue crab populations have been established in 12 countries along the Mediterranean Sea and the Black Sea (Perdikaris et al. 2016). Blue crab was for the first time reported by Holthuis (1961) from the North Aegean Sea in 1959. Between 1985 and 1995, Enzenrob et al. (1997) reported dense population in 15 lagoon systems along the Mediterranean coast of Turkey. Zaitsev and Özturk (2001) and Yağlıoğlu et al. (2014) first reported this species in the Sea of Marmara and the Turkish coast of the Black Sea in 1967 and 2013, respectively.

This species is a commercially valuable seafood and supports important fisheries in both its native and non-native ranges (Guillory et al. (1998). For example, in the United States of America combined landing of blue crabs were 74,171 metric tons valued at $234.8 million in 2016 (NOAA 2019). Kevrekidis et al. (2013) reported 50–80 metric tons were landed between 2010 and 2011 in northern Greece. Annual landings of blue crabs in Turkey have fluctuated over time with 17–77 metric tons landed in 2008 and 2009, respectively, and 0.6–10.5 metric tons landed between 2015 and 2018 (TUIK 2018). Even so, due to its high establishment rate in the Mediterranean Sea, it ranks among the worst 100 invasive non-native species in this region (Streftaris and Zenetos 2006). Negative impacts of blue crabs include a presumed high degree of competition with native brachyurans, predation on native coastal species, and harvesting difficulties of fish in coastal zones due to the presence of high number of crabs (Perdikaris et al. 2016).

Past studies of blue crabs in Turkey have focused on length–weight relationships, population biology, fishing gear efficiency, genetic differences, seasonal growth rates, settlement and quality of blue crab meat (Atar and Secer 2003; Yerlikaya and Gökoğlu 2004; Gokce et al. 2006; Keskin and Atar 2012; Tureli-Bilen and Yesilyurt 2012; Sumer et al. 2013). However, there is currently little information available on the functional role of this invasive species along the Turkish coast. Even so the European Union's Marine Strategy Framework Directive and Biodiversity Strategy to 2020 list non-native species as one of the most important ecosystem features of concern. These directives propose an integrated assessment of the effects of non-native species in marine ecosystem, following an ecosystem-based approach.

Stable isotope analysis is a widely used method to assess food web and ecosystem structure as the isotopic composition in food sources are mirrored in the tissues of consumer upon consumption (Nielsen et al. 2016). Stable isotope values of carbon ($\delta^{13}C$) in marine food webs can indicate spatial trends in resource use, such as benthic versus pelagic feeding (France 1995). In contrast, stable isotopes values of nitrogen ($\delta^{15}N$) can be used to estimate trophic position because of an approximately 3–5‰ increase in tissue $\delta^{15}N$ values that occurs for each trophic step (Post 2002). Prior studies have used stable isotope to investigate the trophic relationship of native and invasive species in the Mediterranean Sea (e.g. Marić et al. 2016). In addition, recent studies have assessed the trophic ecology and management of blue crab in southern European coastal

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waters (Mancinelli et al. 2013, 2017b, c; Carrozzo et al. 2014), including some that have employed stable isotope analyses in the Adriatic and Ionian Seas (Mancinelli et al 2013, 2016; Carrozzo et al. 2014).

To our knowledge no studies have yet examined the trophic ecology of blue crab in Turkish waters. Therefore, the objective of this study was to use stable isotope analysis to study the trophic ecology of non-native blue crab from Gökçeada Island, Turkey, in the Northern Aegean Sea. Specifically, this research seeks to: (1) quantify the trophic niche (i.e. relative trophic position, basal carbon sources use, and niche size) of non-native blue crabs at Gökçeada Island and assess seasonal variation, (2) compare the trophic niche of non-native blue crabs with native marine species at Gökçeada Island and assess the potential for niche overlap, (3) compare results from Gökçeada Island to past isotopic studies of blue crabs across their native and non-native ranges.

Material and methods

Study area

This study was conducted at Gökçeada Island in the Northern Aegean Sea, a sub-basin in the Mediterranean Sea (Fig. 1). This isolated basin has low water temperatures and is populated by cold water and atypical Atlantic-Mediterranean species (Bianchi et al. 2012). Where the Northern Aegean Sea is influenced by the low-salinity water of the Çanakkale Strait (Dardanelles) input lies a transition area between the eutrophic Black Sea and the highly oligotrophic Mediterranean (Siokou-Frangou et al. 2002). Gökçeada Island (Imbros; 40°09′39″N 25°50′40″E) is Turkey's largest island at 289 km^2 and 95 km of coastline. The wind circulation around Gökçeada is dominated by northeasterly and southwesterly winds (Kocataş and Bilecik 1992).

Sample collection

Invasive blue crab and native marine species from Gökçeada Island were collected during April, June, and August of 2017. Sampling was focused along the south-eastern coast of the island at Gökçeada lagoon (40°09′27.99″N 25°57′50.03″E) and adjacent coastal marine areas (Figure S1). Gökçeada lagoon is hydrologically isolated from the Gökçeada Salt Lake which is approximately 2 km south of the lagoon on the south-eastern coast of the island. This small marine lagoon is connected by a 50 m wide channel to the adjacent coastal marine areas and as such is influenced

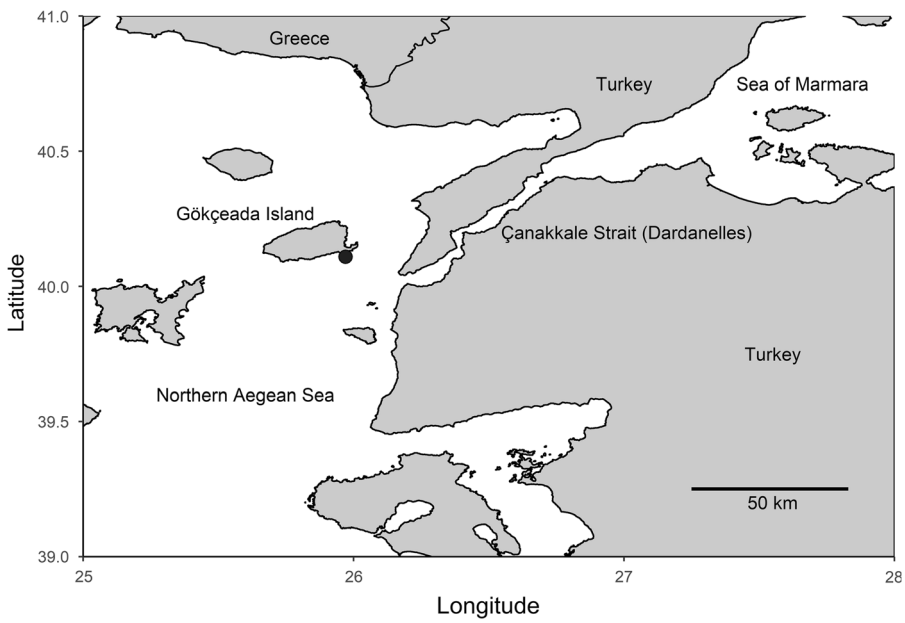


Fig. 1 Gokceada Island, Turkey in the Northern Aegean Sea. Sampling of blue crabs (*Callinectes sapidus*) and native fauna was conducted the along south-eastern coast of Gokceada Island (black point) in coastal marine areas and within a small coastal lagoon

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by the semidiurnal tidal constituents and strong wind-driven water transport present at Gökçeada Island (Alpar et al. 2000). Given this tidal and wind driven water transport the lagoon's size and depth varies daily and seasonally. On average the Gökçeada lagoon is approximately 340 m long and 70 m wide with an area of 0.02 km^2 and a maximum depth of 1.5 m. The lagoon is also connected to a small freshwater stream that reduces in flow during the summer months. In each month water temperature, salinity, dissolved oxygen, pH, TDS (Total Dissolved Solid), and conductivity were measured in the lagoon using a YSI 556 Multiprobe System.

Ten individual blue crab were collected in each month (30 total) from the marine lagoon using deployments of a 90 m static gill net with 28 mm mesh size as well as crab traps. Traps (60 × 60 × 60 cm) were made of vinyl-coated 2 × 2 cm mesh wire with an upper and lower chamber, a 15 cm-wide entrance on four sides, and a conical bait well in the center (Carrozzo et al. 2014). While a comprehensive community assessment of Gökçeada lagoon is lacking, Baltic prawn (*Palaemon adspersus* Rathke, 1837), green shore crab (*Carcinus maenas* Linnaeus, 1758), grooved carpet shell (*Ruditapes decussatus* Linnaeus, 1758), *Gobius* spp, and gilt-head bream (*Sparus auratus* Linnaeus, 1758) have been opportunistically observed and the transient or limited presence of marbled rock crab *(Pachygrapsus marmoratus* Fabricius, 1787), warty crab (*Eriphia verrucosa* Forskål, 1775), Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819), turbinate monodont (*Phorcus turbinatus* Born, 1778), and blotched picarel (*Spicara maena* Linnaeus, 1758) cannot be ruled out (H. Aslan, *pers comms.*). However, the only species other than blue crabs observed and available for sampling in the lagoon during the study period was Baltic prawn and 10 individuals of this species were collected during the month of August using traps. Therefore, we also collected additional native species that may be transient or found at low numbers within Gökçeada lagoon from the adjacent coastal zone of Gökçeada Island. Specifically, in each month nine to ten individual marbled rock crab, warty crab, Mediterranean mussel, turbinate monodont, and blotched picarel were collected using a hand nets and hook and line methods.

Laboratory and stable isotope analyses

The sexes of collected blue crab were identified and the maximum carapace width (CW) was measured as the distance between the two outermost lateral spine tips. Muscle tissue was collected from the claws of blue crabs, warthy crabs, and marbled crabs, the abductor region of Mediterranean mussels, the foot region of turbinate monodont, and along the upper side of lateral line of blotched picarel. Muscle tissues were dried at 60 °C for 24 h in an oven. Approximately 0.575–0.625 mg subsamples of homogenized muscle tissues from each species were weighed into tin capsules and flash-combusted using a Costech ECS4010 elemental analyzer coupled to a ThermoFisher Delta Plus XP or Delta V Advantage continuous-flow stable isotope ratio mass spectrometer. Stable isotope values were normalized using a two-point system with glutamic acid reference material (USGS-40 and USGS-41). Sample precision based on repeated reference material was 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope values were calculated with the following equation and are expressed in standard delta (δ) notation in per mil units (‰):

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The $R_{standard}$ values were based on Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric N_2 (AIR) for $\delta^{15}N$ values. Lipids are depleted in ^{13}C and variable lipid content in muscle tissues can bias $\delta^{13}C$ measurements (Post et al. 2007). Therefore muscle tissue samples with high carbon: nitrogen ratios (C:N > 3.5) were mathematically normalized using the Post et al. (2007) relationship between lipid content, C:N ratio, and aquatic consumer $\delta^{13}C$ values: $\Delta\ \delta^{13}C = -3.32 + 0.99 \times C{:}N$.

Data analysis

The mean carapace width (CW) of sampled blue crabs at Gökçeada Island were compared across sampling months using one-way ANOVA and Tukey–Kramer Multiple-Comparison Tests using JMP (Version 12.1.0). Linear regression was used to test for relationships between CW and muscle tissue $\delta^{13}C$ or $\delta^{15}N$ values. One-way ANOVA and Tukey–Kramer

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Multiple-Comparison Tests were also used to assess differences in blue crabs $\delta^{13}C$ and $\delta^{15}N$ values across months and among species $\delta^{13}C$ and $\delta^{15}N$ values within each sampling month. Data were examined for normality and equal variance and statistical significance was assumed at $p < 0.05$ for all tests.

An isotopic niche approach was used to compare the trophic niche of blue crabs at Gökçeada Island across months and among native and non-native fauna (Newsome et al. 2007). Specifically, using the SIBER package (2.1.3) implemented in program R (R Core Team 2018) the standard ellipse area corrected for sample size was calculated for each group (SEA_c; Jackson et al. 2011). The SEA_c metric is equivalent to a bivariate standard deviation and can be interpreted as a measure of the core isotopic niche area of a population (Jackson et al. 2011). A niche overlap index was calculated for each group as percentage of overall SEA_c isotopic area (i.e., niche space) that overlaps with another group's isotopic area (Jackson et al. 2011). To compare niche size in blue crabs across month and between native and non-native species, a Bayesian approximation of SEA_c with corresponding median and 95% credibility interval values (SEA_b) was calculated (Jackson et al. 2011). Bayesian posterior probabilities ($PP < 0.05$) resulting from pair-wise comparisons of posterior distributions SEA_b were used to reflect significant differences in SEA_b size between groups (Jackson et al. 2011).

We used a Bayesian approach to estimate the trophic position (TP) and the relative importance of pelagic vs. benthic carbon sources (α) to non-native blue crabs from Gökçeada Island using the package tRophicposition 160 (Version 0.7.5) implemented in R (R Core Team 2018). This package employs a Bayesian formulation of the TP equations outlined in Post (2002) coupled with Markov Chain Monte Carlo simulations (Quezada-Romegialli et al. 2018), and has been successfully used to quantify the TP of other invasive brachyurans (e.g. Annabi et al. 2018; Cordone et al. 2020). Specifically, we parameterized this formulation with the $\delta^{13}C$ and $\delta^{15}N$ of blue crabs and representative primary consumers reflecting pelagic and benthic food web baselines (i.e. TP = 2). This pelagic vs. benthic energy pathway approach represents a simplified model of the potential energy pathways in Gökçeada lagoon and the connected coastal areas. When calculating TP and α we used Mediterranean mussels a proxy of pelagic energy pathways and turbinate monodont a proxy of pelagic energy pathways available to blue crabs. We note that these taxa were collected in coastal area adjacent to the lagoon and not in the lagoon itself, which has the potential to impart bias if stable isotope baselines differ spatial with this relatively small area. However, we chose to apply them here given the lack of representative primary consumers available to collect within the lagoon, the lagoon's wind and tidal-driven connectivity with the adjacent coastal zone, the euryhaline status of these two species, the potential for movement in and out of the lagoon by blue crabs, and the use of Mediterranean mussel as a common food web baseline in prior stable isotope studies of blue crabs in the Mediterranean sea (Mancinelli et al. 2013, 2016, 2017a; Carrozzo et al. 2014,).

Six models per sampling month were examined that differed in the number and type of food web baselines and assumed trophic discrimination factors (TDF; i.e. the average change $\delta^{13}C$ and $\delta^{15}N$ values per trophic transfer). Specifically, three variants of food web baseline were examined: a "One Baseline" pelagic baseline model ($TP_{Pelagic}$), a "One Baseline" benthic baseline model ($TP_{Benthic}$), and a 'Two Baselines Full' model that employed both pelagic and benthic baselines ($TP_{Two\text{-}source}$; Quezada-Romegialli et al. 2018). In addition, each of these three model variants were analyzed using two sets of assumed TDFs. First, we incorporated TDFs ($\Delta^{15}N = 3.4 \pm 0.9$; $\Delta^{13}C = 0.4 \pm 1.3$) derived from a review of aquatic consumers by Post (2002) as these values have commonly been used in prior stable isotope studies of blue crabs in the Mediterranean sea (Mancinelli et al. 2013, 2016, 2017a; Carrozzo et al. 2014). Second, we incorporated TDFs ($\Delta^{15}N = 1.6 \pm 0.6$; $\Delta^{13}C = 0.6 \pm 0.7$) derived from a recent controlled feeding that examined blue crab muscle tissue (McCann and Jensen 2018). This allowed us to explicitly assess the degree to which differing baseline and TDF assumptions might influences the resulting estimates of TP and α. Model-checking was performed by calculating Gelman–Rubin diagnostic to assess convergence and by comparing posterior estimates of the mean and standard deviation of TP, $\mu\Delta^{15}N$, and α to their corresponding naïve and time-series Markov Chain Monte Carlo (MCMC) errors to assess precision (Gelman et al., 2014). For all models point estimates of the potential scale reduction factor (R_c) were < 1.01 and below or equal to their corresponding upper

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confidence limits ($R_{95\%}$) indicating model convergence (Table S1 and S2). In addition, both naïve and time-series MCMC errors were less than 5% of their corresponding standard deviations (i.e. standard deviations were > 20 times larger than MCMC errors) indicating model precision (Table S1 and S2).

For each model, the resulting TP and α metrics are presented as median values with 95% credibility intervals. Bayesian posterior probabilities (PP < 0.05) resulting from pair-wise comparisons of posterior distributions of blue crab TP and α were used to reflect significant differences cross months and among models with differing baselines. Finally, to facilitate direct comparison with past studies in the Mediterranean Sea, we also calculated the one and two baseline parametric formations of Post (2002) TP equations and report these metrics mean ± SD averaged across all samples collected between April and August using both the TDF values from Post (2002) and McCann and Jenson (2018).

Results

Environmental conditions

Water temperature and dissolved oxygen content in the coastal lagoon increased during the study period. The lowest values were in April (T = 14.65 °C; DO = 7.92 mg/l), intermediate values in June (T = 21.00 °C; DO = 9.13 mg/l), and the highest values observed in August (T = 27.38 °C; DO = 8.65 mg/l). In contrast, salinity and total dissolved solid decreased from April (S = 34.4‰; TDS = 33.9 g/l), to June (S = 34.06‰; TDS = 33.61 g/l, and August (S = 33.2‰; TDS = 33.1 g/l).

Variation by sampling month

The majority of the blue crabs sampled were males in April (3:7, female: male) and August (4:6) while during June sex ratios were equal (5:5, female: male; Table S3). CW of individual blue crabs varied between 128–154 cm in April, 130–161 cm in June and 130 – 164 cm in August but mean values did not differ among months ($F_{2,29} = 2.35$; $P = 0.1139$; Table S3). Blue crab $\delta^{13}C$ and $\delta^{15}N$ values were not significantly related to CW ($\delta^{13}C$: $R^2 = 0.016$, $P = 0.4991$; $\delta^{15}N$: $R^2 = 0.056$, $P = 0.2080$). In addition, blue crab $\delta^{13}C$ values did not differ among months ($F_{2,29} = 1.43$; $P = 0.2573$). In contrast, blue crab $\delta^{15}N$ values varied significantly among months ($F_{2,29} = 24.50$; $P < 0.0001$). Blue crab $\delta^{15}N$ values in April were significantly higher than those found in June ($P < 0.0001$) and August ($P < 0.0001$) which did not differ from one another ($P = 0.9793$; Table 1).

The core isotopic niche (SEA_c) of blue crabs overlapped substantially (60.7–93.5%) in June and August. In contrast, due to higher $\delta^{15}N$ values in April there was no overlap in the core isotopic niche (SEA_c) of blue crabs relative to June and August (Fig. 2). The core isotopic niche area (SEA_b) of blue crabs did not differ in size across months (all comparisons PP > 0.05), with SEA_b 95% credibility intervals overlapping among all three months (Table 2).

Variation among species

Muscle tissue $\delta^{13}C$ values differed among species from Gökçeada Island within all three sampling months (April: $F_{5,58} = 38.86$; $P < 0.0001$; June: $F_{5,59} = 64.37$; $P < 0.0001$; August: $F_{6,58} = 91.89$; $P < 0.0001$). In April and June, blue crab $\delta^{13}C$ values were significantly higher than all species ($P \leq 0.0001$ to 0.0336) except turbinate monodont ($P = 0.6215$ to 0.7558; Table 1 and S3). In August, blue crab $\delta^{13}C$ values were significantly higher than Mediterranean mussel ($P < 0.0001$), blotched picarel ($P < 0.0001$), and warty crab ($P < 0.0001$), lower than turbinate monodont ($P = 0.0004$), and did not differ from marbled rock crab ($P = 1.0000$) and Baltic prawn ($P = 0.9977$; Table 1 and S3).

Muscle tissue $\delta^{15}N$ values differed among species from Gökçeada Island within all three sampling months (April: $F_{5,58} = 101.47$; $P < 0.0001$; June: $F_{5,59} = 117.34$; $P < 0.0001$; August: $F_{6,58} = 111.55$; $P < 0.0001$). In April, blue crab $\delta^{15}N$ values were significantly higher than Mediterranean mussel ($P < 0.0001$), turbinate monodont ($P < 0.0001$), and marbled rock crab ($P < 0.0001$), and lower than blotched picarel ($P = 0.0490$), and warty crab ($P < 0.0001$; Table 1 and S3). In June, blue crab $\delta^{15}N$ values were significantly higher than turbinate monodont ($P < 0.0001$), lower than blotched picarel ($P < 0.0001$) and warty crab ($P < 0.0001$), and did not differ from Mediterranean mussel ($P = 1.0000$) and marbled rock crab ($P = 0.7055$; Table 1 and S3). In August, blue crab $\delta^{15}N$ values were significantly

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Table 1 The mean ± SD muscle tissue stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of non-native blue crabs (*Callinectes sapidus*) and native species collected at Gokceada Island, Turkey in the Northern Aegean Sea in April, June, and August 2017

Month	Species	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	SEA_b (‰)
April	Blotched picarel (*Spicara maena*)	10	− 20.0 ± 0.2	8.6 ± 0.1	0.7 (0.5–1.3)
	Blue crab (*Callinectes sapidus*)	10	− 14.2 ± 0.5	7.7 ± 0.5	1.3 (0.8–2.3)
	Marbled rock crab (*Pachygrapsus marmoratus*)	10	− 16.3 ± 2.4	5.5 ± 1.2	5.2 (3.3–9.0)
	Warty crab (*Eriphia verrucosa*)	9	− 16.0 ± 1.5	9.4 ± 0.6	2.8 (1.7–5.1)
	Mediterranean mussel (*Mytilus galloprovincialis*)	10	− 20.4 ± 0.4	5.4 ± 0.2	0.9 (0.5–1.5)
	Turbinate monodont (*Phorcus turbinatus*)	10	− 15.1 ± 1.4	4.0 ± 0.7	2.6 (1.7–4.6)
June	Blotched picarel (*Spicara maena*)	10	− 20.0 ± 0.3	8.3 ± 0.5	1.1 (0.7–1.9)
	Blue crab (*Callinectes sapidus*)	10	− 14.3 ± 0.3	6.5 ± 0.5	1.1 (0.7–1.8)
	Marbled rock crab (*Pachygrapsus marmoratus*)	10	− 17.1 ± 2.0	6.9 ± 0.5	3.7 (2.3–6.5)
	Warty crab (*Eriphia verrucosa*)	10	− 16.1 ± 1.2	8.9 ± 1.0	2.5 (1.6–4.2)
	Mediterranean mussel (*Mytilus galloprovincialis*)	10	− 20.3 ± 0.2	6.4 ± 0.2	0.8 (0.5–1.4)
	Turbinate monodont (*Phorcus turbinatus*)	10	− 13.6 ± 1.4	2.6 ± 0.9	3.8 (2.4–6.4)
August	Blotched picarel (*Spicara maena*)	10	− 20.2 ± 0.2	8.7 ± 0.3	0.8 (0.5–1.4)
	Baltic prawn (*Palaemon adspersus*)	10	− 14.1 ± 0.4	6.0 ± 0.6	1.2 (0.8–2.1)
	Blue crab (*Callinectes sapidus*)	10	− 14.5 ± 0.2	6.4 ± 0.4	0.9 (0.6–1.6)
	Marbled rock crab (*Pachygrapsus marmoratus*)	10	− 14.4 ± 1.8	5.5 ± 0.7	3.5 (2.2–6.0)
	Warty crab (*Eriphia verrucosa*)	10	− 16.8 ± 1.2	8.9 ± 1.2	3.7 (2.3–6.5)
	Mediterranean mussel (*Mytilus galloprovincialis*)	10	− 20.1 ± 0.2	7.0 ± 0.3	0.8 (0.5–1.4)
	Turbinate monodont (*Phorcus turbinatus*)	10	− 12.4 ± 1.4	2.8 ± 0.5	2.9 (1.8–5.1)

Isotopic niche size for each species is presented as median (95% credibility intervals) Bayesian approximations of standard ellipse areas (SEA_b)

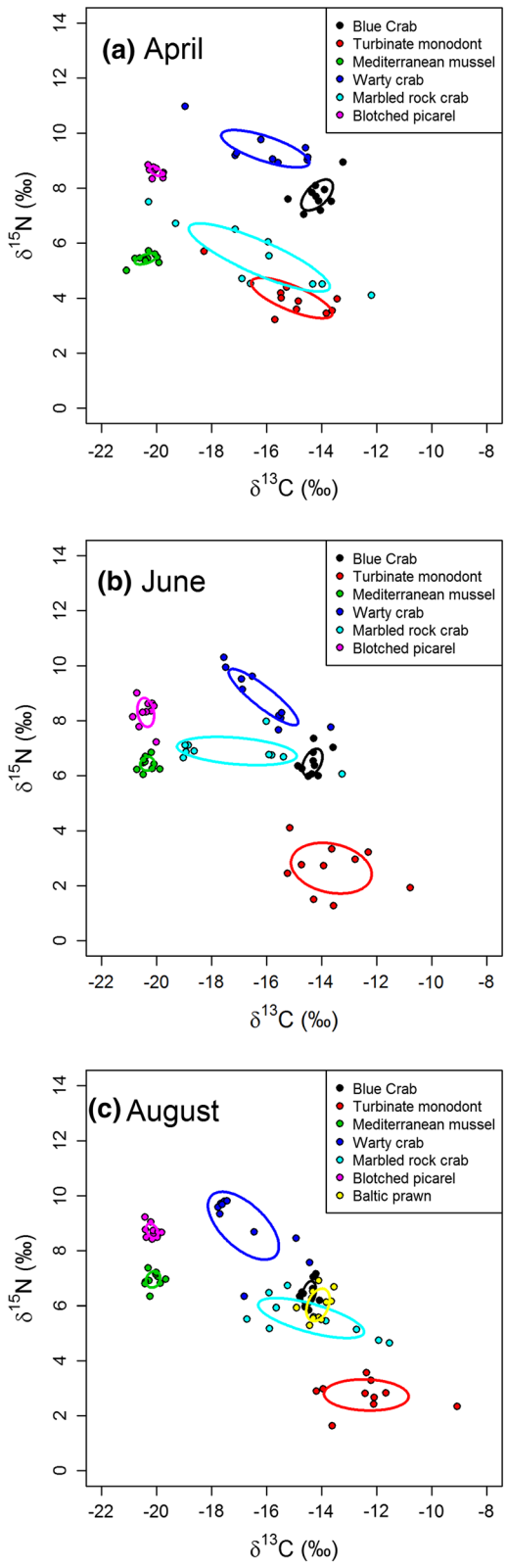
higher than turbinate monodont ($P < 0.0001$) and marbled rock crab ($P = 0.0331$), lower than blotched picarel ($P < 0.0001$) and warty crab ($P < 0.0001$), and did not differ from Mediterranean mussel ($P = 0.5352$) and Baltic prawn ($P = 0.7588$; Table 1 and S3).

The core isotopic niche (SEA_c) of blue crabs from Gökçeada Island did no overlap with any other species isotopic niche in April and June (Fig. 2). In contrast, in August blue crab core isotopic niche overlapped slightly with the core isotopic niches of both marbled rock crab (0.8–8.0%) and Baltic prawn (8.7–18.9%). The core isotopic niche size (SEA_b) of species differed between blue crabs and select native species in all three sampling months (Table 1). In April, blue crabs had smaller isotopic niches relative to both warty crab (PP = 0.0432) and marbled rock crab (PP = 0.0014), but similarly sized isotopic niches relative to other native species (all comparisons PP > 0.05). In April, blue crabs had smaller isotopic niches relative to warty crab (PP = 0.0243), marbled rock crab (PP = 0.0018), and turbinate monodont (PP = 0.0002), but similarly sized isotopic niches relative to all other native species (all comparisons PP > 0.05). In both June and August, blue crabs had smaller isotopic niches relative to warty crab (PP = 0.0243, 0.0009), marbled rock crab (PP = 0.0018, 0.0013), and turbinate monodont (PP = 0.0002, 0.0059), but similarly sized isotopic niches relative to all other native species (all comparisons PP > 0.05).

Trophic position and carbon source

Blue crab TP from Gökçeada Island parameterized with general TDFs from Post (2002) and calculated using a single benthic baseline ($TP_{Benthic}$) were significantly higher than those calculated using a single pelagic baseline ($TP_{Pelagic}$) in each sampling month (April: PP = 0.0010; June: PP < 0.0001; August PP < 0.0001; Table 2). Calculated $TP_{Pelagic}$ using TDFs from Post (2002) for blue crabs in June and August were at the level of an assumed primary

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◀**Fig. 2** The muscle tissue stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of blue crabs (*Callinectes sapidus*) at Gokceada Island, Turkey in the Northern Aegean Sea in **a** April, **b** June, and **c** August 2017. Standard ellipse areas represent the core isotopic niche area of each species

consumer (TP = 2.0). Blue crab $TP_{Two\text{-}source}$ parameterized with TDFs from Post (2002) and calculated using a two baseline (i.e. pelagic and benthic) were similarly higher than $TP_{Pelagic}$ (April: PP = 0.0011; June: PP < 0.0001; August PP < 0.0001; Table 2). While blue crab $TP_{Two\text{-}source}$ and $TP_{Benthic}$ using TDFs from Post (2002) did not differ in April (PP = 0.4760) and June (PP = 0.1610), $TP_{Two\text{-}source}$ was significantly lower than $TP_{Benthic}$ in August (PP = 0.0055). Blue crab $TP_{Benthic}$ and $TP_{Pelagic}$ values using TDFs from Post (2002) from Gökçeada Island did not differ among months (all comparison PP > 0.05). $TP_{Two\text{-}source}$ values using TDFs from Post (2002) were higher in April relative to August (PP = 0.0056; Table 2) but did not differ between April and June (PP = 0.194) or June and August (PP = 0.0581). Blue crab TP estimated parameterized with blue crab muscle tissue specific TDFs from McCann and Jensen (2018) were higher than TP estimates obtained from comparable models parameterized with TDFs from Post (2002; all PP < 0.05), except for $TP_{Pelagic}$ in June (PP = 0.3070) and August (PP = 0.3010; Table 2). Even so differences among TP estimates calculated using TDFs from McCann and Jensen (2018) by month or baseline type followed the same general pattern as differences observed in TP estimates calculated using TDFs from Post (2002; Table 2).

The relative importance of pelagic ($\alpha_{Pelagic}$) vs. benthic carbon sources ($\alpha_{Benthic}$) to blue crabs from Gökçeada Island differed among months (Table 2). While $\alpha_{Benthic}$ was higher than $\alpha_{Pelagic}$ in all months, the relative importance of $\alpha_{Pelagic}$ was significantly higher in August relative to April (PP = 0.0042) and June (PP = 0.0492; Table 2) when parameterized with TDFs from Post (2002). Median estimates of $\alpha_{Pelagic}$ calculated using TDFs from McCann and Jensen (2018) were 6.8 to 9.9 percentage points higher than those calculated using TDFs from Post (2002) but did not differ statistically as 95% credibility intervals overlapped between models with similar structure (PP > 0.05; Table 2). Both sets of TDFs indicated that $\alpha_{Benthic}$ was higher than $\alpha_{Pelagic}$ in all months (Table 2).

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Table 2 The calculated trophic position (TP) and the relative importance of pelagic ($\alpha_{Pelagic}$) and benthic carbon sources ($\alpha_{Benthic}$) to non-native blue crabs (*Callinectes sapidus*) at Gokceada Island, Turkey in the Northern Aegean Sea in April, June, and August 2017

TDF Source	Metric	Blue crab (*Callinectes sapidus*)		
		April	June	August
Post (2002)	$TP_{Pelagic}$	2.7 (2.6–2.8)	2.0 (2.0–2.1)	2.0 (2.0–2.1)
	$TP_{Benthic}$	3.1 (2.9–3.3)	3.1 (2.9–3.4)	3.1 (2.9–3.3)
	$TP_{Two\text{-}sources}$	3.1 (2.9–3.3)	3.0 (2.7–3.2)	2.7 (2.6–2.9)
	$\alpha_{Pelagic}$ (%)	3.9 (0.0–14.1)	15.5 (3.0–27.1)	30.1 (18.9–39.7)
	$\alpha_{Benthic}$(%)	95.9 (85.8–100.0)	84.5 (72.1–98.5)	69.7 (60.3–81.0)
McCann and Jensen (2018)	$TP_{Pelagic}$	3.5 (3.1–4.0)	2.0 (2.0–2.3)	2.0 (2.0–2.2)
	$TP_{Benthic}$	4.4 (3.9–5.2)	3.9 (3.4–3.9)	3.7 (3.2–4.2)
	$TP_{Two\text{-}sources}$	4.3 (3.8–5.0)	3.9 (3.4–4.4)	3.4 (3.0–3.8)
	$\alpha_{Pelagic}$ (%)	10.9 (0.0–26.0)	25.4 (11.3–37.7)	36.9 (27.5–46.3)
	$\alpha_{Benthic}$(%)	89.1 (74.0–100.0)	74.6 (62.3–88.7)	63.1 (53.7–72.5)

Models using a pelagic baseline model ($TP_{Pelagic}$), a benthic baseline ($TP_{Benthic}$), and both pelagic and benthic baselines ($TP_{Two\text{-}source}$) were employed. Models parameterized with trophic discrimination factors (TDF) derived from a review of aquatic consumers by Post (2002; $\Delta^{15}N = 3.4 \pm 0.9$; $\Delta^{13}C = 0.4 \pm 1.3$) or a controlled feeding study that examined blue crab muscle tissue (McCann and Jenson 2018; $\Delta^{15}N = 1.6 \pm 0.6$; $\Delta^{13}C = 0.6 \pm 0.7$). TP and α are presented as median values (95% credibility intervals)

However, when parameterized with TDFs from McCann and Jensen (2018), $\alpha_{Pelagic}$ was higher in August relative to April (PP = 0.0130) but did not differ relative to June (PP = 0.0951; Table 2).

Discussion

This study is the first to examine the trophic ecology of invasive non-native blue crabs in the Northern Aegean Sea using stable isotope analysis. These results indicated that $\delta^{15}N$, but not $\delta^{13}C$, values of blue crabs from Gökçeada Island varied across sampling months leading to a shift in their isotopic niche between April relative to June and August. In addition, only during August did the isotopic niche of blue crabs overlap with those of native species. The calculated TP of blue crabs from Gökçeada Island ranged widely from 2.0 to 4.4. TP estimates were lower in models using a pelagic baseline (2.0 to 3.5) relative to those using a benthic baseline (3.1 to 4.4), or a dual, pelagic / benthic baselines (3.0–4.3) regardless of the TDFs employed. In addition, except for model using solely pelagic baselines, TP estimated were 0.6 to 1.3 lower in models using generalized TDFs (Post 2002) relative to those using blue crab muscle tissue specific TDFs (McCann and Jensen 2018). While these methodological considerations led to uncertainty in the absolute TP of blue crabs from Gökçeada Island, models using different baselines and TDFs generally agreed that relative blue crab TP declined, and the relative importance of pelagic carbon sources increased from April to August.

We used an isotopic niche approach to compare the ecological niches of blue crabs to native fauna at Gökçeada Island. This approach is based on using $\delta^{13}C$ and $\delta^{15}N$ values in bi-variate space as a proxy of the n-dimensional niche space defined by Hutchinson (1957, 1959) to represent an animal's scenopoetic (e.g. benthic vs. pelagic habitat use) and bionomic (e.g. trophic level) niche axes, respectively (Newsome et al. 2007). In this context, isotopic niche areas metrics described the range of dietary resources and habitats used by blue crabs and native fauna, while isotopic niche overlap metrics provided a proxy measure of the potential for competitive overlap between blue crabs and native fauna. The native species examined at Gökçeada Island varied both taxonomically and ecologically. For example, Baltic Prawn marbled crab and warty crab are opportunistic, omnivorous crustaceans (Cannicci et al. 2007; Chartosia et al. 2010), Mediterranean mussel are filter feeding bivalves (Maire et al. 2007), Turbinate monodont are algae grazing gastropods (Sousa et al. 2018), Baltic Prawn

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are an omnivorous species of caridean shrimp (Guerao and Ribera 1996), and Blotched picarel are zooplanktivorus fish (Karachle and Stergiou 2014). The isotopic niche of blue crabs from Gökçeada Island did no overlap with any other species isotopic niche in April and June, and only overlapped slightly with the isotopic niches of marbled rock crab and Baltic prawn in August (Fig. 2).

This low isotopic overlap suggests limited potential for resource competition between blue crabs and native fauna at Gökçeada Island, at least for the species examined here. However, differences in TDF, isotopic turn-over rates, metabolic factors such as growth extrication pathways, and/or spatial variation in stable isotope baselines among taxa has the potential to lead to low isotopic niche overlap even when species share similar diets (Hette-Tronquart et al. 2019). In addition, prior studies indicate the inherent trophic flexibility exhibited by blue crabs, both in their native (Belgrad and Griffen 2016) and non-native ranges (Prado et al. 2020). This suggest that the potential for resource competition between blue crabs and native species, especially ominvours taxa such as marbled rock crab and Baltic prawn, may be higher than suggested based on isotopic niche overlap alone. Therefore, future studies seeking to quantify trophic niche overlap with native species would benefit from integrating stable isotope analyses with other dietary proxies such as gut content and DNA metabarcoding analyses (e.g. Cordone et al. 2020).

Past studies have employed stable isotope analysis to assess the trophic ecology of invasive non-native blue crab in other regions of the Mediterranean Sea region. Mancinelli et al. (2016) focused on blue crabs in the Adriatic Sea, while Carrozzo et al. (2014) and Mancinelli et al. (2013) examined this species in the Ionian Sea. These studies observed mean $\delta^{13}C$ and $\delta^{15}N$ values of blue crabs during the month of June that ranged from -13.7 ± 1.0‰ to -18.8 ± 3.8‰ and 10.6 ± 0.3‰ and 13.0 ± 0.2‰ respectively (Table 3). Moreover, Mancinelli et al. (2017a) examined two locations in the Ionian Sea and three location in the Adriatic Sea and sampled blue crabs in both the winter (January) and summer (July). While Mancinelli et al. (2017a) observed significant spatial and temporal variations in blue crab $\delta^{13}C$ and $\delta^{15}N$ values, no general pattern was evident except that blue crab $\delta^{13}C$ values were higher in the spring relative to the summer (Table 3).

Blue crab $\delta^{13}C$ values in April, June, and August at our study on Gökçeada Island (Aegean Sea) were similar to the lowest $\delta^{13}C$ values observed in past studies in the Adriatic and Ionian Seas (Table 3). However, the mean $\delta^{15}N$ values of blue crabs at Gökçeada Island were lower than any observed in past studies in the region (Table 3). Moreover, calculated TP of blue crabs from Gökçeada Island were generally low relative to the past studies in the Adriatic and Ionian Seas, with differences in calculated TP based on the method employed (Tables 2 and 3). Specifically, when using *M. galloprovincialis* as a single pelagic baseline and TDFs derived from Post (2002), similar to many past studies in the Mediterranean Sea (Table 3), blue crabs from Gökçeada Island had a median $TP_{Pelagic}$ ranging from 2.0 to 2.7 with no overlap in 95% credibility intervals between June and August relative to April (Table 2). In contrast, when using a benthic baseline ($TP_{Benthic}$) or both pelagic and benthic baselines ($TP_{Two\text{-}source}$) blue crabs from Gökçeada Island had a median TP of 2.7 to 3.1 with overlap in 95% credibility intervals across all months (Table 2).

In their native range, the diet composition of blue crabs generally consists of molluscs, arthropods, fishes, polychaetes, and a highly variable percentage of algae, sediment, and detritus (see Belgrad and Griffen 2016 and refences therein). Past studies along the Atlantic coast of the United States have estimated blue crab trophic positions ranging from 2.9 to 3.5 using a combination of food web modeling and stable isotope approaches (Baird and Ulanowicz 1989; Deehr et al. 2014). Similar studies along Northern Gulf of Mexico have estimated TP ranging from 2.1 to 2.9 (Akin and Winemiller 2008; Willson et al. 2009; Llewellyn and La Peyre 2011; Geers et al. 2016). The wide range of blue crab TP values found in their native range agrees with the wide range of TP found in the Mediterranean Sea (Table 3), suggesting a high degree of trophic flexibility in both native and non-native rages.

Our study also highlights the methodological challenges associated with using stable isotopes to quantify the trophic position of blue crabs. Specially, we found that the use of different TDFs and differing assumptions as to the number and type of food web baselines led to differing estimates of the absolute

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Table 3 The muscle tissue stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) and calculated trophic position (TP) of non-native blue crabs (*Callinectes sapidus*) from multiple studies in the Mediterranean Sea

Location (Sea)	Season[a]	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	TP	Baseline	TDF[b]	Source[c]
Mar Piccolo (Ionian)	S	− 23.3 ± 2.3	13.7 ± 2.2	4.2 ± 0.5	Pelagic	a	1
	W	− 17.9 ± 1.6	14.0 ± 0.3	3.9 ± 0.1	Pelagic	a	1
Torre Colimena (Ionian)	S	− 17.0 ± 0.6	10.6 ± 0.3	3.0 ± 0.1	Mixed	a	2,3
	S	–	–	4.2 ± 0.2	Mixed	b	3
	S	− 18.2 ± 2.1	11.6 ± 1.3	3.6 ± 0.3	Pelagic	a	1
	W	− 16.8 ± 1.9	9.2 ± 1.4	2.5 ± 0.5	Pelagic	a	1
Spunderati (Ionian)	S	− 24.6 ± 0.5	14.7 ± 0.6	3.2 ± 0.1	Pelagic	a	1
	W	− 14.3 ± 0.7	13.6 ± 0.4	2.9 ± 0.3	Pelagic	a	1
Acquatina (Adratic)	S	− 13.7 ± 1.0	13.0 ± 0.2	3.7	Mixed	a	2
	S	− 19.5 ± 2.8	10.7 ± 0.6	3.1 ± 0.2	Pelagic	a	1
Alimini Grande (Adriatic)	S	− 22.6 ± 0.9	14.2 ± 1.2	3.2 ± 0.3	Pelagic	a	1
Parilla Lagoon (Adratic)	S	− 18.8 ± 3.8	11.7 ± 2.7	3.7 ± 0.2	Pelagic	a	4
Gökçeada Island (Aegean)	Sp/S	− 14.3 ± 0.4	6.9 ± 0.8	2.2 ± 0.4	Pelagic	c	5
		–	–	3.1 ± 0.1	Benthic	c	5
		–	–	3.0 ± 0.2	Two-source	d	5
		–	–	2.4 ± 0.1	Pelagic	e	5
		–	–	4.5 ± 0.3	Benthic	e	5
		–	–	3.9 ± 0.2	Two-source	f	5

Stable isotope values and TP are presented as mean values (± SD where possible), and standardize to a single decimal place across studies. Values not reported in studies' text or tables were extracted from figures using WebPlotDigitizer (Rohatgi 2019). TP were calculated using either the single or two baseline equation outlined in Post (2002) but studies differed in their application of trophic discrimination factors (TDF). *M. galloprovincialis* was used in all studies with a single pelagic baseline. Mixed baseline studies used a single baseline value averaged across multiple grazers, detritivores, and filter feeding primary consumers. The pelagic, benthic, and two-source (i.e. pelagic and benthic) baselines employed in this study are described in the methods above

[a]S = Summer; W = Winter; Sp = Spring

[b](a) $\Delta^{15}N = 3.4$; (b) $\Delta^{15}N = 1.15 \pm 1.01$; (c) $\Delta^{15}N = 3.4 \pm 0.9$; (d) $\Delta^{15}N = 3.4 \pm 0.9$, $\Delta^{13}C = 0.4 \pm 1.3$; (e) $\Delta^{15}N = 1.6 \pm 0.6$; (f) $\Delta^{15}N = 1.6 \pm 0.6$; $\Delta^{13}C = 0. \pm 07$

[c](1) Mancinelli et al. (2017a), (2) Mancinelli et al. (2013), (3) Carrozzo et al. (2014), (4) Mancinelli et al. (2016), (5) This study

trophic position. Moreover, a review of past studies of blue crabs in the Mediterranean Sea further highlight the potential for methodical differences to make it challenging to directly compare trophic position within and between studies (Table 3). For example, many, but not all, previous studies of blue crab TP in regions of the Mediterranean Sea used generalized TDFs representing an average value across aquatic consumers (e.g. $\Delta^{15}N = 3.4$; Post 2002; Table 3). However, feeding experiments suggest that $\Delta^{15}N$ values in blue crabs are lower than these average value for aquatic consumers (Fantle et al. 1999; Dittel et al. 2000; McCann and Jensen 2018). For example, in our study we used TDFs for claw muscle tissue ($\Delta^{15}N = 1.6$) estimated from adult blue crabs feeding on an experimental diet of hard clam (*Mercenaria mercenaria* Linnaeus, 1758*;* McCann and Jensen 2018). This controlled study reported lower $\Delta^{15}N$ values similar those reported from controlled feeding studies that examined whole body samples of early life stages of this species (Fantle et al. 1999; Dittel et al. 2000). Our analyses indicate that using generalized vs. specific-specific TDFs may results in estimates of TP that differ by as much as a full tropic level or more (Table 2), with generalized TDFs suggesting herbivorous/omnivorous diets and specific-specific TDFs suggesting omnivorous/carnivorous diets in blue crab at Gökçeada Island. Carrozzo et al. (2014) reported similar result when using both generalized ($\Delta^{15}N = 3.4$) and crustacean-specific ($\Delta^{15}N = 1.6$)

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TDFs with baseline value obtained by averaging the $\delta^{15}N$ values of multiple grazers, detritivores, and filter feeding primary consumers as a single mixed baseline in TP calculations (Table 3). While species or crustacean-specific TDFs may be more reflective of isotopic discrimination between blue crabs and their immediate diets, the degree to which these lower TFDs also reflect isotopic discrimination during lower trophic steps in the crab's food web is uncertain. Even so, while these results indicate uncertainty in absolute measures of TP due to the strong influences imparted by TDF choice, our analyses indicate that relative differences (e.g. seasonal) in TP can still be reliably assessed using this approach.

In addition, we found that differing assumptions as to the number and type of food web baselines can lead to large variation in the estimated TP of blue crabs, similar in scale to the influence of differing TDFs. We explored both single baseline (i.e. pelagic or benthic) and two-source (i.e. pelagic and benthic) formulations to estimate the TP of blue crabs on Gökçeada Island. These different approaches resulted in TP estimates for blue crabs at Gökçeada Island that differed by as much as a full trophic level or more (Table 2). Many prior studies of blue crabs in the Mediterranean Sea have commonly assumed a single isotopic baseline representing either pelagic or a mixture of energy pathways (Table 3 and references therein). The pelagic vs. benthic energy pathway approach used in our study represents a simplified model of the potential energy pathways in Gökçeada lagoon and the connected coastal areas. Even so, it highlights how assuming a single vs. multiple energy pathways when calculating the TP can lead to differing results confounding comparison across studies. In addition, isotopic baselines can vary both spatially and temporally (France 1995; Mancinelli et al. 2017a), with mobile aquatic consumers integrating energy pathways across linked food webs along coastal habitat mosaics (Rezek et al. 2020). This, along with logistical constraints, can make it challenging to when seeking to characterize food webs baselines. For example, the two representative primary consumers used in our study reflect well described isotopic differences between pelagic and benthic energy pathways (i.e. lower $\delta^{13}C$ values in pelagic consumers relative to benthic consumers; France 1995). However, we were only able to sample these consumers in the coastal areas adjacent to the lagoon. This sampling artifact has the potential to impart bias if pelagic and/or benthic baselines differ spatially within this relatively small area. The lagoon's connectivity to the adjacent coastal zone, the potential for movement in and out of the lagoon by blue crabs, seasonal variation in the relative influence of different water masses, and the inherent temporal lags between consumer and dietary isotopic values adds additional uncertainty as to the true isotopic baselines experienced by blue crabs during our study. For example, the higher $\delta^{15}N$ values observed in Mediterranean mussels in June and August may reflect difference in fresh vs. marine waters input to the lagoon and coastal zone as stream flow and wind conditions shift in the summer months (Kocataş and Bilecik 1992, H. Aslan pers. comms.; Table 1). Future studies of blue crab trophic ecology at Gökçeada Island would benefit from a more comprehensive assessment of food webs baselines over time and across coastal habitat mosaics.

Uncertainty in the use of TDFs and the challenges in identifying and characterizing representative isotopic baselines are not unique to studies of blue crab, but are inherent to all studies seeking to use stable isotopes to quantify consumer TP. While a full review is outside the scope of this project, we advocate for the use of species, tissue, and diet-specific TDFs values when available and accounting for the presence of multiple energy pathways when calculating TP using stable isotope analysis. Studies using two-source equations and multi-source mixing models leverage the information provided by multiple isotope values (e.g. $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, etc.) to calculate TP while explicitly quantifying the relative use of different energy pathways by consumers (Nelson et al. 2019; Cordone et al. 2020). In addition, recent Bayesian formulation of classical TP equations and stable isotope mixing models allow for including individual variability and propagating sampling error in the modelling approach and provide posterior estimates of parameters and outputs (Quezada-Romegialli et al. 2018; Stock et al. 2018).

Conclusions

We provide a first assessment of the trophic ecology of blue crab as an invasive, non-native species in the Northern Aegean Sea using stable isotope analysis. We found limited isotopic overlap between blue crabs

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and seven native species examined in this study at Gökçeada Island. However, a more comprehensive assessment using a larger number of native taxa is warranted to test for potential competitive interactions. The calculated TP of blue crabs in our study was in general agreement with prior studies of this species. However, methodological differences in TP calculations, such as those that differ in their use of TDFs and/or their assumed number and type of energy pathways, limited our ability estimate absolute TP values and to comparatively assess blue crab trophic ecology across the Mediterranean Sea. This suggests that a meta-analysis of data from prior studies using standardized methods is warranted to better assess variation in the trophic niche of non-native blue crabs across the region. In addition, future stable isotope-based studies using standardized methods across the Mediterranean Sea will allow researchers to better assess the potential for negative consumptive and competitive interactions with native coastal species.

Acknowledgements This research was supported by the postdoctoral research programme (BIDEB 2219) of The Scientific and Technological Research Council of Turkey (TUBITAK). Sample collection and animal use was authorized by the Turkish Ministry of Food, Agriculture and Livestock (T.C. Gida Tarim Hayvancilik Bakanliği) and Canakkale Onsekiz Mart University. We thank to Dr. Onur Gonulal, Bekir Bektas and Esra Barın for assistance with the collection of samples. K. Lamb and T. Mauney provided helpful assistance with stable isotope analysis. We thank Dr. E. Grosholz, Dr. G. Mancinelli, and an anonymous reviewer for their helpful comments and suggestions on an early version of this manuscript.

Data availability Data used in this study is available in Table S3 of the Electronic Supplementary Material.

Declarations

Conflict of interest The authors state no conflicts of interest.

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