

# Interactions of invasive pumpkinseed with a riverine endemic fish indicate trophic overlap but minimal consequences for somatic growth and condition

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**Abstract** – The North American invasive fish pumpkinseed *Lepomis gibbosus* has been associated with some detrimental ecological consequences in some parts of its invasive range. Here, we tested the trophic interactions of pumpkinseed with the endemic stream fish Aegean chub *Squalius fellowesii* ('chub') in a Mediterranean-type water course in SW Anatolia, Turkey, using a bulk stable isotope approach ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ). Seven sampling sites were able to be grouped as chub in pumpkinseed presence ( $n=2$ ) and absence ( $n=3$ ), and pumpkinseed in chub absence ( $n=2$ ). Across these groups, stable isotope metrics revealed that chub trophic (isotopic) niche size was similar in pumpkinseed presence/absence, but pumpkinseed had a larger niche in chub presence. In sites where they are co-habited, their niches showed some convergence, but with no apparent consequences for their growth or body condition, suggesting there were minimal ecological consequences of this sharing of food resources. These results suggest that the trophic consequences of this riverine invasion of pumpkinseed on this endemic chub was minimal.

**Keywords:** Stable isotope analysis / freshwater fish / growth index / diet overlap

## 1 Introduction

Invasive species can cause wide ranging detrimental impacts in their receiving ecosystems, ranging from altered individual behaviours in native species through to alterations in ecosystem functioning (Cucherousset and Olden, 2011). Understanding the disruptions in processes, such predation and competition, caused by invasive species is important in determining the strength and direction of their impacts on native biodiversity (Tran *et al.*, 2015; Britton, 2023). Consequently, an important first step in understanding the extent of the disruption to these processes is determining the extent of the trophic interactions of the invader with native species (Britton, 2018).

Following the introduction of an alien species into a novel ecosystem, theory suggests that if there are some prey resources that are largely unexploited then their exploitation by the introduced species will avoid inter-specific competitive

interactions, increasing their probability of establishment and invasion (Juncos *et al.*, 2015). Conversely, an introduction into an environment where the prey resources are fully exploited and/or are limited could result in competitive interactions increasing, driving a constriction in the size of the trophic niches of all species as they develop dietary specialisms (Tran *et al.*, 2015; Jackson *et al.*, 2016). However, this scenario can also result in each species having larger and convergent trophic niches as all species become more generalized in their resource use to maintain their energetic intake (Svanbäck and Bolnick, 2007). Where the strength of inter-specific competition is high and the alien species establishes an abundant population, some native species may then be displaced from their original trophic niche via competitive exclusion (Bohn *et al.*, 2008). Whilst testing these trophic niche theories for alien freshwater fishes can be challenging due to an absence of information on trophic niche sizes and positions in the pre-invasion period, this can be at least partially overcome where there are control sites available nearby with similar native fish communities present that remain uninvaded (*e.g.* Britton *et al.*, 2010).

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The detrimental impacts of alien species can be elevated when the impacted species are endemic, as their extinction risk rises (De Santis *et al.*, 2021; Trombley *et al.*, 2021). An example of such a species is the Aegean chub, *Squalius fellowesii*, an endemic fish of the Aegean drainages of Anatolia, Turkey, where there are concerns over the consequences of the invasion of the North American centrarchid pumpkinseed *Lepomis gibbosus* (Top *et al.*, 2016a). The Aegean chub is an important socio-economic species due to their consumption by local people, with some concerns on over-exploitation (Balık *et al.*, 2004; Tarkan *et al.*, 2015). Other threats to their populations are habitat loss and degradation (e.g. pollution, channelization, damming), drought and excessive water extraction (Fricke *et al.*, 2007).

The pumpkinseed is a small-bodied, warm-water fish that has been introduced firstly to Europe as an ornamental fish in the 19th century from North America (Copp and Fox, 2007) and is one of the most introduced aquatic species worldwide (Garcia-Berthou, 2007). Its high establishment success is associated with its tolerance to harsh environmental conditions, including high temperatures and hypoxia (Farwell *et al.*, 2007). Studies on invasive pumpkinseed have been conducted on both pond (e.g. Copp *et al.*, 2002; Villeneuve *et al.*, 2005; Fox *et al.*, 2011) and stream dwelling populations (Cucherousset *et al.*, 2009; Fobert *et al.*, 2013; Almeida *et al.*, 2014; Top *et al.*, 2016b; Tarkan *et al.*, 2021). Despite pumpkinseed spreading in Turkey since the 1980s (Tarkan *et al.*, 2015), investigations of stream-dwelling Turkish populations have focused on their habitat use, which suggested minimal evidence that their presence was impacting endemic species (Top *et al.*, 2016b), although high plasticity in pumpkinseed life history traits was noted (Tarkan *et al.*, 2021). There is, however, no information on pumpkinseed-endemic fish trophic interactions in Turkey. Trophic studies on stream-dwelling pumpkinseed from elsewhere in Europe have suggested the species can be highly aggressive in their interactions with native species, such as in Iberia (Almeida *et al.*, 2014), but with their interactions with some species being minimal, such as with brown trout *Salmo trutta* in streams in Southern England (Copp *et al.*, 2017).

The aim here was to thus assess the trophic interactions and consequences of invasive pumpkinseed with endemic Aegean chub ('chub' hereafter) by using isotopic niche as a proxy in a small Mediterranean stream in Turkey. In this stream, the chub is widely distributed and tends to be the most abundant species present, with other native and alien species having very patchy distributions and very low abundance. Where pumpkinseed is present, they also tend to be highly abundant. The trophic interactions, condition and growth rates of chub and pumpkinseed were assessed through samples collected on the stream to represent a design of a natural treatment (sites with co-habiting chub and pumpkinseed) and a control (sites with chub without pumpkinseed and with pumpkinseed without chub). Objectives were to assess the trophic ecology and niche breadth, and growth and condition of chub in pumpkinseed presence and absence, and of pumpkinseed where present, and to discuss the conservation implications.

## 2 Materials and methods

### 2.1 Study area and sites

The study stream was the Sariçay Stream in Muğla-Milas region, south-west Anatolia (Tab. 1; Fig. 1). The stream is approximately 70 km in length and generally comprises of a range of habitat types (e.g. pools and riffles, consisting sand, gravel, mud, and rock as substrate types). Maximum depths rarely exceed 2 m and mean width of the stream is approximately 10 m. A number of alien fishes (pumpkinseed, gibel carp *Carassius gibelio*, common carp *Cyprinus carpio*, eastern mosquitofish *Gambusia holbrooki*, topmouth gudgeon *Pseudorasbora parva*) were present, along with patchily distributed native/endemic fishes (Smyrna chub *Petroleuciscus ninae*, loach *Oxynoemacheilus* sp.), plus the most abundant Aegean chub (Top *et al.*, 2016b). Pumpkinseed was accidentally introduced into the catchment via the Geyik reservoir (GE, Fig. 1) through contaminated stockings of common carp for fisheries enhancement in the early 1990s (Top *et al.*, 2016b). The stream is anthropogenically affected throughout by residential areas (i.e. villages), small-scale factories and sand quarries.

### 2.2 Sampling design and laboratory processing

Sampling was conducted in compliance with ARRIVE guidelines (Percie du Sert *et al.*, 2020) and all methods were carried out in accordance with the relevant guidelines and regulations, with ethical approval also granted by the Animal Experiments Local Ethics Committee of Ankara University (Decision No: 2015-14-157). There were seven field sites used, all of 100 m length (numbered from site 1–upstream to site 7–downstream along the stream) (Fig. 1; Tab. 1). Notably, sampling in reservoirs were conducted in river-type habitats in the reservoirs (i.e. in the stream reaches of the reservoirs). The physical modifications of the basin, which include two large reservoirs and several small ponds formed by disused sandpits, prevented pumpkinseed from occurring throughout the entire stream course. However, they did establish populations in the reservoirs and artificial ponds where chub could not survive, allowing us to observe different species interaction combinations, such as chub-pumpkinseed in both allopatry and sympatry. Accordingly, the study consisted of three treatments; chub in allopatry (Site 1, 4 and 5;  $n=50$ ), pumpkinseed in allopatry (Sites 2 and 3;  $n=40$ ) and both species in sympatry (Sites 6 and 7,  $n=20+20$ ) (Tab. 2). Sampling was conducted in April 2017, with fish captured using a portable electrofishing device without stop nets. In all sites, fishing was continuous within 10-min blocks to enable use of a catch per unit effort (CPUE) metric, which were used as a measure of relative fish abundance, expressed as the number of fish captured per 10 min fishing (Copp *et al.*, 2005). Following capture, fish were euthanized via anaesthetic overdose (2-phenoxyethanol) and transported to the laboratory on an ice water slurry. Water temperature, conductivity, dissolved oxygen, salinity and pH were measured at each sampling site just prior to the fish sampling using a YSI 556 MPS probe. There were only minor differences in most environmental variables across the sampling locations (Tab. 1). The exception was in salinity

**Table 1.** Location, coordinates (Lat., Lon.), sampled species, altitude (Alt., m), temperature (T, °C), pH, dissolved oxygen (DO, mg<sup>-1</sup> and %), conductivity (EC, µS cm<sup>-1</sup>) and salinity (S, ‰) of sampling points measured in April 2017 along the Sarıçay stream.

Site	Coordinates	Sampled species	Alt.	T	pH	DO	DO (%)	EC	S
1	37.431944, 27.874722	Chub	487	12.5	7.6	9.4	101.8	189	0.11
2	37.396944, 27.882500	Pumpkinseed	470	13.8	8.2	11.3	129.5	159	0.08
3	37.328056, 27.818611	Pumpkinseed	61	13.5	8.1	9.3	102.2	340	0.18
4	37.335556, 27.790000	Chub	33	14.7	7.5	2.3	24.2	613	0.34
5	37.353889, 27.740556	Chub	25	15.8	9.4	10.5	126.1	262	0.12
6	37.343611, 27.728611	Chub & Pumpkinseed	24	13.4	8.1	9.5	102.4	435	0.23
7	37.315556, 27.696944	Chub & Pumpkinseed	15	14.0	7.7	2.8	31.6	649	0.34



**Fig. 1.** Location of the sampling sites in the Sarıçay stream.

**Table 2.** Mean TL (mm), TW (g),  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , TP and  $\delta^{13}\text{C}_{\text{corr}}$  ( $\pm 95\%$  CI) of chub and pumpkinseed by treatment (allopatry and sympatry) in the sampling sites in Sarıçay stream.

Site	Species	N	Mean TL	Mean TW	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{15}\text{N}$ (‰)	Mean TP	Mean $\delta^{13}\text{C}_{\text{corr}}$
1	Chub – allopatry	20	116.5 $\pm$ 0.30	19.7 $\pm$ 1.66	-20.63 $\pm$ 0.36	5.65 $\pm$ 0.12	1.67 $\pm$ 0.03	0.56 $\pm$ 0.04
2	Pumpkinseed – allopatry	20	65.2 $\pm$ 0.95	4.7 $\pm$ 2.94	-21.98 $\pm$ 0.27	8.17 $\pm$ 0.15	3.18 $\pm$ 0.04	-1.58 $\pm$ 0.10
3	Pumpkinseed – allopatry	20	64.0 $\pm$ 1.03	3.7 $\pm$ 1.22	-30.41 $\pm$ 0.08	7.70 $\pm$ 0.07	1.72 $\pm$ 0.02	-0.47 $\pm$ 0.01
4	Chub – allopatry	20	149.8 $\pm$ 0.73	48.2 $\pm$ 6.36	-26.62 $\pm$ 0.14	9.06 $\pm$ 0.37	0.42 $\pm$ 0.11	-0.27 $\pm$ 0.13
5	Chub – allopatry	10	131.7 $\pm$ 1.46	34.1 $\pm$ 12.81	-26.70 $\pm$ 0.38	18.06 $\pm$ 0.29	5.19 $\pm$ 0.09	-2.30 $\pm$ 0.10
6	Chub – sympatry	20	136.7 $\pm$ 0.80	33.0 $\pm$ 6.14	-26.44 $\pm$ 0.34	17.92 $\pm$ 0.40	2.34 $\pm$ 0.12	1.40 $\pm$ 0.32
7	Chub – sympatry	20	120.5 $\pm$ 0.40	20.8 $\pm$ 2.26	-25.97 $\pm$ 0.22	14.57 $\pm$ 0.45	1.57 $\pm$ 0.18	1.86 $\pm$ 0.21
6	Pumpkinseed – sympatry	20	69.8 $\pm$ 2.13	8.3 $\pm$ 8.46	-26.80 $\pm$ 0.17	19.00 $\pm$ 0.33	2.66 $\pm$ 0.09	1.05 $\pm$ 0.17
7	Pumpkinseed – sympatry	20	58.8 $\pm$ 1.02	3.7 $\pm$ 2.18	-27.16 $\pm$ 0.22	15.29 $\pm$ 0.60	2.29 $\pm$ 0.18	-0.76 $\pm$ 0.04

and conductivity, which generally increased with distance downstream, plus low dissolved oxygen values were evident at both sites 4 and 7, due to local issues (Tab. 1). Also, samples of fish putative prey resources (submerged macrophytes, zooplankton, macrobenthos) were collected from each sampling site. Macrophytes and macrobenthos were collected with a scoop and a grab from the stream bottom and surface, while zooplankton was collected with a zooplankton net.

In the laboratory, the collected fishes were identified as either chub or pumpkinseed, measured (total length and weight, nearest mm and g, respectively). For ageing, scale samples of each species were taken from the area between lateral line and dorsal fin. All specimens were stored at  $-20\text{ }^\circ\text{C}$ , before defrosting and a sample of dorsal muscle tissue excised from each individual for bulk stable isotope analysis (SIA). As white muscle tissue has lower variability in nitrogen isotopic signature compared to other tissues it does not require acidification to remove inorganic carbonates (Pinnegar and Polunin, 1999). The muscle samples, plus those of the fish putative prey, were then dried in an oven for 24 h at a constant temperature ( $60\text{ }^\circ\text{C}$ ) before being ground to fine powder using an agate pestle and mortar. A 1 mg sample of homogenized tissue from each fish and invertebrate material and 2 mg of plant material, each was weighed accurately (*i.e.*  $0.150 \pm 0.001$ ) and put into  $5 \times 9$  mm tin cups using an ultra-microbalance (Sartorius MSA3.6P-000- DM Cubis Micro Balance). Samples were analysed by an elemental analyser coupled with a continuous flow isotope ratio mass spectrometer at Akdeniz University Food Safety and Agricultural Research Centre. All stable isotope values are reported in the  $\delta$  notation:  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000]$ , where  $R$  is  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  ratios, and the standards are atmospheric nitrogen and PeeDee Belemnite. The secondary standards for stable N isotope USGS-34 Potassium Nitrate and for stable C isotope CH-6 sucrose and total C and N ratio were estimated by using aspartic acid, cyclohexanone-2,4-DNPH, acetanilide and nicotinamide standards. The standard errors of the replicates of all analyses were  $0.03\text{‰}$  for  $\delta^{13}\text{C}$  and  $0.11\text{‰}$  for  $\delta^{15}\text{N}$ . The ageing of the collected scales was through counting true annuli (Vilizzi *et al.*, 2013). Scale impressions of 10 scales for each specimen on acetate strips were read on a micro-projector (magnification:  $\times 48$ ). Two independent readers estimated the ages without knowing prior biological status of the fish and a final evaluation was made when age estimates differed. If the disagreement

continued, the sample was excluded from the dataset. Rejected samples together with regenerated scales, meant that 3.4% of all sampled fish were removed from further age-based analyses. After age estimation, total scale radius and the distances from the scale focus to each annulus of one scale per fish was measured for the back-calculation of length at age (Bagenal and Tesch, 1978).

### 2.3 Data analyses

Somatic growth rates of both fishes were calculated using the Fraser-Lee equation (Francis, 1990):

$$L_t = c + (TL_c - c)(S_t/R),$$

where  $L_t$  is TL when annulus  $t$  was formed,  $TL_c$  is TL at capture,  $S_t$  is the distance from scale focus to the annulus  $t$ ,  $R$  is scale radius, and  $c$  is the intercept on the length axis from the linear relationship of TL *versus* scale radius. A fixed body-scale intercept ( $c = 17.7$  mm for chub  $22.7$  mm for pumpkinseed) was used for each sampled population to reduce bias due to differences in the size distribution. The relative growth of both species across the sampling locations were compared through a relative growth index (GI) following (Hickley and Dexter, 1979). This involved use of the Walford (Walford, 1946) method to obtain a straight line by plotting TL at mean age  $n$  of all specimens against TL at age  $n + 1$ , with GI then calculated as the mean value of the growth in each age class of the species for a certain site relative to TLs at age, using the following formulae:

$$l_n = L_\infty(1 - k^n),$$

where  $L_\infty = l_i/(1-k)$  and  $l_i$  is the intercept,  $l_n$  is the TL at age  $n$  and  $k$  is the slope of the Walford plot (Hickley and Dexter, 1979) and:

$$GI = \sum TL_{oi}/TL_{ri} \times 100,$$

where  $TL_{oi}$  and  $TL_{ri} \times 100$  are the observed and reference mean TLs respectively of the species at age  $i$ . Because of observed high levels of variability in the estimated length at older ages in the reviewed dataset, which suggested some potential issues of ageing accuracy and precision (Beamish and McFarlane, 1983), only comparable ages (*i.e.* the first four age

classes) of the fish from each site were used in calculations. The relative body condition (RC) of Le Cren (Le Cren, 1951) was used to measure variation in body condition using according to  $W/W'$ , where  $W$  is the actual weight of an individual and  $W'$  is the expected weight from the  $WL$  relationship. RC values  $>1$  or  $<1$  show that specimens are in better or worse condition than the other fish at the same TL range. For the proper use of this index, all populations are required to be sampled at the same time of year (Knaepkens *et al.*, 2002) hence, samples were collected from all sampling sites on the same day.

For the fish stable isotope data, with the  $\delta^{13}\text{C}$  values were arithmetically transformed prior to analysis to account for muscle lipid content (Kiljunen *et al.*, 2006). As the fish putative prey SI data revealed significant differences in  $\delta^{13}\text{C}$  between the sites ( $F=16.90$ ,  $df=13$ ,  $P < 0.01$ ) (Supplementary material, Table S1) then, for each site, the fish  $\delta^{13}\text{C}$  data were standardised by their conversion to  $\delta^{13}\text{C}_{\text{corr}}$  according to Olsson *et al.* (2009):

$$\delta^{13}\text{C}_{\text{corr}} = (\delta^{13}\text{C}_i - \delta^{13}\text{C}_{\text{meaninv}})/\text{CR}_{\text{inv}},$$

where  $\delta^{13}\text{C}_{\text{corr}}$  is the corrected carbon isotope ratio of the individual fish,  $\delta^{13}\text{C}_i$  is the uncorrected isotope ratio of that fish,  $\delta^{13}\text{C}_{\text{meaninv}}$  is the mean invertebrate isotope ratio and  $\text{CR}_{\text{inv}}$  is the carbon range of the invertebrate ( $\delta^{13}\text{C}_{\text{max}} - \delta^{13}\text{C}_{\text{min}}$ ) (Olsson *et al.*, 2009). The  $\delta^{15}\text{N}$  were then converted to trophic position (TP) to provide more meaningful ecological metric:

$$\text{TP}_i = ((\delta^{15}\text{N}_i - \delta^{15}\text{N}_{\text{base}})/3.4) + 2,$$

where  $\text{TP}_i$  is the trophic position of the individual fish,  $\delta^{15}\text{N}_i$  is the isotopic ratio of that fish,  $\delta^{15}\text{N}_{\text{base}}$  is the isotopic ratio of primary consumers (*i.e.* baseline invertebrates), 3.4 is the fractionation between trophic levels and 2 is the trophic position of the baseline organism (Post, 2002).

To then test the corrected SI data, the site-level data were grouped into 'experimental', and 'control' stretches, where the experimental group were sites where chub co-habited with pumpkinseed, and the control groups were sites where pumpkinseed and chub were in allopatry. Standard ellipse areas (SEAs) were calculated for each group using the SIBER package in R (Jackson *et al.*, 2011). SEA is a bivariate estimate of the core isotopic niche width considering 40% of the central data points (Jackson *et al.*, 2011, 2012, 2016). Bayesian estimates of SEA (SEAb) were calculated using a Markov chain Monte Carlo simulation (104 iterations per category) to test for differences in sizes of isotopic niches by calculating the probability that the relative posterior distributions of the niche sizes were significantly smaller or larger between the species ( $\alpha=0.05$ ) (Jackson *et al.*, 2011, 2012). Thus, no overlap in the 95% confidence intervals of SEAb indicated a significantly smaller or larger niche size (depending on the direction of the difference between the species). Isotopic niche overlaps (%) between the species were calculated using the SEAc in SIBER (subscript 'c' indicates a small sample size correction was used; Jackson *et al.*, 2012). The extent of niche overlap between the species used pumpkinseed as the comparator species.

A permutational univariate analysis of variance (PERMANOVA) was used to test the significance of differences of

CPUE, standard length, weight, TP and  $\delta^{13}\text{C}_{\text{corr}}$  between treatments using PERMANOVA 1.0.1. + add-in to PRIMER version 6.1.11 (PRIMER-E Ltd., Plymouth, UK, Anderson *et al.*, 2008). This procedure was performed with Type III sums of squares following normalization of the data and was based on a Euclidian distance matrix and 9999 permutations of the residuals under a reduced model. The use of PERMANOVA versus traditional parametric analysis of variance (ANOVA) is advantageous as the stringent assumptions of normality and homoscedasticity in the data are substantially relaxed in PERMANOVA, enabling its use with real-world ecological data sets.

### 3 Results

#### 3.1 Fish sample characteristics

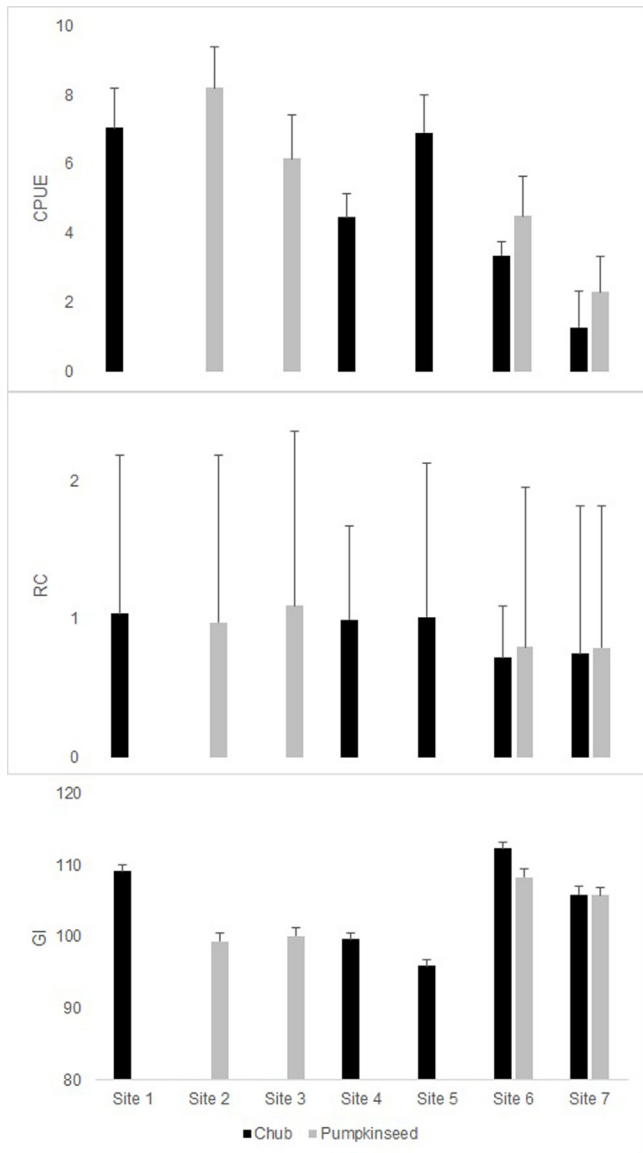
There were 312 chub sampled from the five sites where they were present, varying from 31 individuals at site 7 (pumpkinseed present) to 87 at site 4 (pumpkinseed absence), with fish present between 75 and 216 mm in length and 4.6 to 125.0 g in mass (Tab. 2). Pumpkinseed were collected from 4 sites, with sample sizes varying from 82 at site 7 (chub present) to 406 at site 2 (chub absent); all pumpkinseed were between 23 and 124 mm in length and 0.2–39.2 g in mass (Tab. 2).

Both mean TL and TW of chub were significantly higher in pumpkinseed absence than presence (TL:  $F=3.24$ ,  $df=1$ ,  $P < 0.02$ ; TW:  $F=2.81$ ,  $df=1$ ,  $P < 0.01$ ). This was true for pumpkinseed, where both mean TL and TW were significantly higher in the absence of chub (TL:  $F=19.61$ ,  $df=1$ ,  $P < 0.001$ ; TW:  $F=26.22$ ,  $df=1$ ,  $P < 0.001$ ) (Fig. 2). There were significant differences in relative abundance (as CPUE) for both species, being significantly higher in chub where pumpkinseed were absent compared with where pumpkinseed were present ( $F=6.50$ ,  $df=1$ ,  $P < 0.01$ ). Pumpkinseed were also more significantly abundant in the absence of chub ( $F=22.36$ ,  $df=1$ ,  $P < 0.001$ ) (Fig. 2). The relative condition was significantly higher for chub in pumpkinseed absence ( $F=3.28$ ,  $df=1$ ,  $P < 0.01$ ) and for pumpkinseed in chub absence ( $F=15.40$ ,  $df=1$ ,  $P < 0.0001$ ) (Fig. 2). Growth rates (as  $GI$ ) of both species were higher when sympatry (both species together) (chub:  $F=49.10$ ,  $df=1$ ,  $P < 0.01$ ; pumpkinseed:  $F=21.14$ ,  $df=1$ ,  $P < 0.001$ ) (Fig. 2).

#### 3.2 Stable isotope ecology and metrics

Comparison between treatment groups revealed chub TP and  $\delta^{13}\text{C}_{\text{corr}}$  was significantly higher in pumpkinseed presence than absence (TP:  $F=5.323$ ,  $df=1$ ,  $P < 0.01$ ;  $\delta^{13}\text{C}_{\text{corr}}$ :  $F=24.876$ ,  $df=1$ ,  $P < 0.001$ ) (Tab. 2). Pumpkinseed TP was also highest in chub presence ( $F=25.431$ ,  $df=1$ ,  $P < 0.001$ ) (Tab. 2). In general, the relationship between TL and both TP and  $\delta^{13}\text{C}_{\text{corr}}$  were not significant, with the exception of positive relationship for  $\delta^{13}\text{C}_{\text{corr}}$  in pumpkinseed (Fig. S1).

The chub had similar sized isotopic niches (probability 0.21) between pumpkinseed presence and absence, and with some shift in niche position between the contexts (niche overlap was 45% between pumpkinseed presence/absence) (Tab. 3; Fig. 3). Pumpkinseed in chub presence had a larger isotopic niche (probability 0.23) than in chub absence, and overlapped by 67% (Tab. 3, Fig. 3).



**Fig. 2.** Catch per unit effort (CPUE), relative condition (RC) and growth index (GI) of Aegean chub and pumpkinseed in the sampling sites.

## 4 Discussion

Comparison of the isotopic niche sizes of the two fishes across the different contexts suggested that the consequences of pumpkinseed presence for chub were minor, with similar sized niches in their presence/absence and maintaining some of their isotopic space between pumpkinseed presence/absence (45%). Despite chub sharing their isotopic niches with pumpkinseed where they are co-habited, there were few apparent consequences for their growth or body condition.

Although empirical evidence showed that non-native fishes can have detrimental ecological impact for native fishes and communities as a result of trophic interactions (Cucherousset and Olden, 2011; Britton, 2023), co-occurrence with non-native pumpkinseed in the present study revealed no significant impact on chub growth and relative condition. In

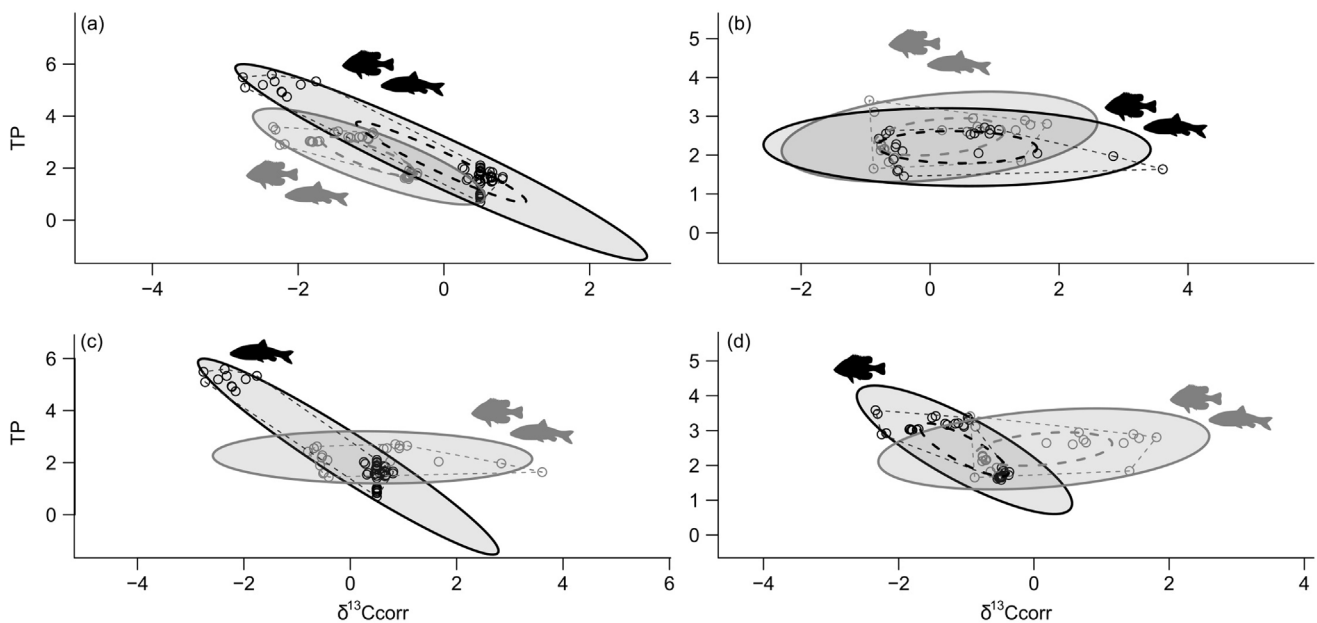
fact, a significant increase in growth but reduced condition was observed in both species in case of co-occurrence. This was supported by the data from a previous study of habitat use of both species in one of the same sampling sites (site 6) in Sarıçay stream that showed a microhabitat partition (Top *et al.*, 2016b). These outputs were also consistent with Stakėnas *et al.* (2013) and Jackson *et al.* (2016) who, despite showing higher habitat and resource partitioning between brown trout and pumpkinseed in a similar stream ecosystem in England, also found little evidence of any ecological impacts arising from these processes. The results are, however, contrary to Copp *et al.* (2017) who used also stable isotope analysis in experimental pond conditions, detecting high niche overlap and reduced niche size in native gudgeon *Gobio gobio* and roach *Rutilus rutilus* in pumpkinseed presence. In a similar experimental study on impact of pumpkinseed on perch *Perca fluviatilis*, no effect on growth was observed in either species, but both species shifted their diet to avoid competition (Fobert *et al.*, 2011). These results support an earlier study in the stream on habitat interactions of pumpkinseed with endemic fishes suggesting little evidence for impact (Top *et al.*, 2016b), meaning there is currently little need for further management actions to mitigate the effects of their ecological interactions.

The stable isotope analysis approach used in this study complements previous trophic studies on this stream that assessed the trophic interactions between non-native topmouth gudgeon, pumpkinseed and native/endemic species using traditional stomach analyses (Karakuş, 2014). Then, significant feeding overlaps were apparent between the two invaders, and between pumpkinseed and native *Oxynoemacheilus* sp. (Karakuş, 2014). However, since this study, the topmouth gudgeon population has declined markedly and *Oxynoemacheilus* sp. was captured in very low abundance, meaning it was not possible to test their isotopic niches in the present study. Whilst other studies have noted that dietary shifts in native fishes might occur after an introduction of non-native species *e.g.* Britton *et al.* (2010), such changes were not clearly apparent in the present study. This might be related to biotic resistance of the stream, evidenced by minimal habitat/food interactions of pumpkinseed with native species or their presence has not been sufficient to exert a long-term shift in stream community structure. Also, the data have the caveat that they were non-replicated field samples that were only sampled once and subject to uncontrolled environmental conditions (*e.g.*, fish abundance, food availability, ecosystem size).

Pumpkinseed are highly invasive in much of Europe, especially in the south *e.g.* Iberian Peninsula, (Godinho *et al.*, 1997) where they mainly invade impounded reaches of large river systems (Mesquita *et al.*, 2006; Almeida *et al.*, 2009). Though not so wide-spread, pumpkinseed has established populations in Turkey (Tarkan *et al.*, 2015) but still there is no report on their invasive populations causing ecological impacts, which could also be due to lack of relevant studies. Future climate change models predicted a country-scale range expansion of pumpkinseed and potential niche overlaps with some common endemic cyprinid species (Emiroğlu *et al.*, 2023), with likely increases in dispersal *via* fluvial networks. Thus, the negligible ecological impact found in the present study should be approached with caution, as pumpkinseed is likely to spread to other systems under future scenarios of climate change where detrimental impacts could be incurred.

**Table 3.** Isotopic niche sizes (as standard ellipse areas, SEAc and SEAb, with 95 % CI) and ellipses overlap for intra and inter-specific status.

Treatment	Species	N	Mean TP ± SE	Mean $\delta^{13}C_{corr} \pm SE$	SEAc	SEAb (95% CI)	% overlap (95% CI)
Allopatry	Chub	50	2.23 (0.22)	-0.04 (0.16)	1.57	1.91 (1.72–2.01)	45.15 (30–59)
Sympatry	Chub	20	2.21 (0.09)	0.42 (0.27)	1.66	1.65 (1.38–1.86)	
Allopatry	Pumpkinseed	40	2.45 (0.12)	-1.03 (0.10)	0.87	0.92 (0.81–1.01)	66.99 (47–86)
Sympatry	Pumpkinseed	20	2.47 (0.11)	0.15 (0.22)	1.50	1.51 (1.25–1.70)	



**Fig. 3.** Isotopic niches (as standard ellipse areas, SEAc) for (a) chub allopatry vs. pumpkinseed allopatry, (b) chub sympatry vs. pumpkinseed sympatry, (c) chub in allopatry vs. sympatry, (d) pumpkinseed allopatry vs. sympatry. TP=trophic position (calculated from  $\delta^{15}N$  data),  $\delta^{13}C_{corr}$ =corrected value of  $\delta^{13}C$ .

However, this may just be suggestive of future impacts, as climate change would also affect the distribution, abundance and growth of native species (e.g. Van der Putten, 2010) and impact of non-native species could be context-dependent (i.e. based on the recipient ecosystem) (Catford *et al.*, 2021).

Detrimental impacts of non-native species on native species have been widely recognized and reported (Gozlan *et al.*, 2010; Britton, 2023), mainly through processes such as increased predation and interspecific competition, but also through habitat modifications and interactions resulting in interference (Gozlan *et al.*, 2010; Cucherousset and Olden, 2011; Almeida *et al.*, 2014). In the literature, there is usually a bias to focus on the cases where high abundances of invaders occur, but this inherently results in high ecological impacts, which potentially overstates the ecological consequences of non-native species (Gozlan, 2008; Jackson *et al.*, 2015). It is therefore highly crucial for prioritizing proper management actions (Britton *et al.*, 2023) to report such cases where impact of non-native fishes is little/relatively benign (e.g. Fobert *et al.*, 2011) or biotic resistance of the native species and ecosystems is considerably high. The example provided here of the

pumpkinseed in the Sarıçay Stream is such an example, where their impacts on the endemic Aegean chub appeared to be minimal, despite their relatively high resource sharing in sites where they co-occur.

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### Supplementary Material

**Table S1.** Mean, min, max  $\delta^{13}C$ ,  $\delta^{15}N$  of macroinvertebrates in the sampling sites in Sarıçay stream.

**Fig. S1.** Relationships of TP and  $\delta^{13}C_{corr}$  versus total length (TL) of Aegean chub (left side) and pumpkinseed (right side).

The Supplementary Material is available at <https://www.kmae-journal.org/10.1051/kmae/2023010/olm>.

## References

- Almeida D, Almodovar A, Nicola GG, Elvira B. 2009. Feeding tactics and body condition of two introduced populations of pumpkinseed *Lepomis gibbosus*: taking advantages of human disturbances? *Ecol Freshw Fish* 18: 15–23.
- Almeida D, Vilizzi L, Copp GH. 2014. Interspecific aggressive behaviour of invasive pumpkinseed *Lepomis gibbosus* in Iberian fresh waters. *PLoS One* 9: e88038.
- Anderson MJ, Gorley RN, Clarke KR. 2008. PERMANOVA+ for primer: guide to software and statistical methods.
- Bagenal TB, Tesch FW. 1978. Age and growth. In: Bagenal TB, Methods for Assessment of Fish Production in Fresh Waters. Oxford, pp. 101–136.
- Balik S, Sarı HM, Ustaoglu MR, İlhan A. 2004. Age and growth characteristics of chub (*Leuciscus cephalus* Linnaeus, 1758) population in Işıklı Lake, Çivril, Denizli, Turkey. *J Fish Aquat Sci* 21: 257–262.
- Beamish R, McFarlane GA. 1983. The forgotten requirement for age validation in fisheries biology. *Trans Am Fish Soc* 112: 735–743.
- Bøhn T, Amundsen PA, Sparrow A. 2008. Competitive exclusion after invasion? *Biol Invasions* 10: 359–368.
- Britton JR. 2018. Empirical predictions of the trophic consequences of non-native freshwater fishes: a synthesis of approaches and invasion impacts. *Turkish J Fish Aquat Sci* 19: 529–539.
- Britton JR, Davies GD, Harrod C. 2010. Trophic interactions and consequent impacts of the invasive fish *Pseudorasbora parva* in a native aquatic food web: a field investigation in the UK. *Biol Invasions* 12: 1533–1542.
- Britton JR. 2023. Contemporary perspectives on the ecological impacts of invasive freshwater fishes. *J Fish Biol* <https://doi.org/10.1111/jfb.15240>
- Britton JR, Lynch AJ, Bardal H, Bradbeer SJ, Coetzee JA, Coughlan NE, Dalu T, Tricarico E, Gallardo B, Lintermans M, Lucy F. 2023. Preventing and controlling non-native species invasions to bend the curve of global freshwater biodiversity loss. *Environ Rev* <https://doi.org/10.1139/er-2022-0103>.
- Catford JA, Wilson JR, Pyšek P, Hulme PE, Duncan RP. 2021. Addressing context dependence in ecology. *Trends Ecol Evol* 37: 158–170.
- Copp GH, Britton JR, Guo Z, Edmonds-Brown VR, Pegg J, Vilizzi L, Davison PI. 2017. Trophic consequences of non-native pumpkinseed *Lepomis gibbosus* for native pond fishes. *Biol Invasions* 19: 25–41.
- Copp GH, Fox MG, Kovac V. 2002. Growth, morphology and life history traits of a coolwater European population of pumpkinseed *Lepomis gibbosus*. *Arch Hydrobiol* 155: 585–614.
- Copp GH, Fox MG. 2007. Freshwater Bioinvaders: Profiles, Distribution, and Threats (ed. Gherardi, F.), Berlin 289–306.
- Copp GH, Wesley KJ, Vilizzi L. 2005. Pathways of ornamental and aquarium fish introductions into urban ponds of Epping Forest (London, England): the human vector. *J Appl Ichthyol* 21: 263–274.
- Cucherousset J, Copp GH, Fox MG, Sterud E, Van Kleef HH, Verreycken H, Zahorska E. 2009. Life-history traits and potential invasiveness of introduced pumpkinseed *Lepomis gibbosus* populations in northwestern Europe. *Biol Invasions* 11: 2171–2180.
- Cucherousset J, Olden JD. 2011. Ecological impacts of nonnative freshwater fishes. *Fisheries* 36: 215–230.
- De Santis V, Quadroni S, Britton RJ, Carosi A, Roberts CG, Lorenzoni M, Crosa G, Zaccara S. 2021. Biological and trophic consequences of genetic introgression between endemic and invasive *Barbus* fishes. *Biol Invasions* 23: 3351–3368.
- Emiroğlu Ö, Aksu S, Başkurt S, Britton JR, Tarkan AS. 2023. Predicting how climate change and globally invasive piscivorous fishes will interact to threaten populations of endemic fishes in a freshwater biodiversity hotspot. *Biol Invasions* <https://doi.org/10.1007/s10530-023-03016-4>.
- Farwell M, Fox MG, Moyes CD, Burness G. 2007. Can hypoxia tolerance explain differences in distribution of two co-occurring north temperature sunfishes? *Environ Biol Fish* 78: 83–90.
- Fobert E, Fox MG, Ridgway M, Copp GH. 2011. Heated competition: How climate change will affect competing non-native pumpkinseed and Eurasian perch in the U.K. *J Fish Biol* 79: 1592–1607.
- Fobert E, Zieba G, Vilizzi L, Godard MJ, Fox MG, Stakenas S, Copp GH. 2013. Predicting non-native fish dispersal under conditions of climate change: case study in England of dispersal and establishment of pumpkinseed *Lepomis gibbosus* in a floodplain pond. *Ecol Freshw Fish* 22: 106–116.
- Fox MG, Villeneuve F, Copp GH. 2011. Seasonal reproductive allocation, local-scale variation and environmental influences on life history traits of introduced pumpkinseed (*Lepomis gibbosus*) in southern England. *Fundam Appl Limnol* 178: 231–243.
- Francis RICC. 1990. Back-calculation of fish length: a critical review. *J Fish Biol* 36: 883–902.
- Fricke R, Bilecenoglu M, Sarı HM. 2007. Annotated checklist of fish and lamprey species of Turkey, including a Red List of threatened and declining species. *Stuttg Beitr Nat kd Biol* 706: 1–169.
- Garcia-Berthou E. 2007. The characteristics of invasive fishes: what has been learned so far? *J Fish Biol* 71: 33–55.
- Godinho F, Ferreira MT, Cortes RV. 1997. The environmental basis of diet variation in pumpkinseed sunfish, *Lepomis gibbosus*, and largemouth bass, *Micropterus salmoides*, along an Iberian river basin. *Environ Biol Fishes* 50: 105–115.
- Gozlan RE. 2008. Introduction of non-native freshwater fish: is it all bad? *Fish Fish* 9: 106–115.
- Gozlan RE, Britton JR, Cowx I, Copp GH. 2010. Current knowledge on non-native freshwater fish introductions. *J Fish Biol* 76: 751–786.
- Hickley P, Dexter FK. 1979. A comparative index of quantifying growth in length of fish. *Aquac Res* 10: 147–151.
- Jackson AL, Inger R, Parnell AC, Bearhop, S. 2011. Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *J Anim Ecol* 80: 595–602.
- Jackson MC, Donohue I, Jackson AL, Britton JR, Harper DM, Grey J. 2012. Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. *PLoS One* 7: e31757.
- Jackson MC, Grey J, Miller K, Britton JR, Donohue I. 2016. Dietary niche constriction when invaders meet natives: evidence from freshwater decapods. *J Anim Ecol* 85: 1098–1107.
- Jackson MC, Ruiz-Navarro A, Britton JR. 2015. Population density modifies the ecological impacts of invasive species. *Oikos* 124: 880–887.
- Juncos R, Milano D, Macchi PJ, Vigliano PH. 2015. Niche segregation facilitates coexistence between native and introduced fishes in a deep Patagonian lake. *Hydrobiologia* 747: 53–67.
- Karakuş U. 2014. Dietary interactions between non-native species *Pseudorasbora parva* (Temminck & Schlegel, 1846) and some other fish species in Sarıçay Stream-Muğla. Msc thesis, Muğla, Turkey.
- Kiljunen M, Grey J, Sinisalo T, Harrod C, Immonen H, Jones RI. 2006. A revised model for lipid-normalizing  $\delta^{13}\text{C}$  values from aquatic organisms, with implications for isotope mixing models. *J Appl Ecol* 43: 1213–1222.



- Knaepkens G, Knapen D, Hanfling B, Verheyen E, Eens M. 2002. Genetic diversity and condition factor: a significant relationship in Flemish but not in German populations of the European bullhead (*Cottus gobio* L.). *Heredity* 89: 280–287.
- Le Cren ED. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *J Anim Ecol* 20: 201–219.
- Mesquita N, Coelho MM, Filomena MM. 2006. Spatial variation in fish assemblages across small Mediterranean drainages: effects of habitat and landscape context. *Environ Biol Fishes* 77: 105–120.
- Olsson K, Stenroth P, Nyström PER, Graneli W. 2009. Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? *Freshw Biol* 54: 1731–1740.
- Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, *et al.* 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol* 18: e3000410.
- Pinnegar JK, Polunin NVC. 1999. Differential fractionation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among fish tissues: implications for the study of trophic interactions. *Funct Ecol* 13: 225–231.
- Post DM. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703–718.
- Stakėnas S, Vilizzi L, Copp GH. 2013. Habitat use, home range, movements and interactions of introduced *Lepomis gibbosus* and native *Salmo trutta* in a small stream of Southern England. *Ecol Freshw Fish* 22: 202–215.
- Svanbäck R, Bolnick DI. 2007. Intraspecific competition drives increased resource use diversity within a natural population. *Proc Royal Soc B* 274: 839–844.
- Tarkan AS, Marr SM, Ekmekci FG. 2015. Non-native and translocated freshwater fishes in Turkey. *FISHMED* 2015.003: 28.
- Tarkan AS, Karakuş U, Top N, Keskin E, Ünal EM, Britton JR. 2021. Invasion of pumpkinseed *Lepomis gibbosus* is facilitated by phenotypic plasticity across its invasion gradient. *Biol Invasions* 23: 3201–3214.
- Top N, Tarkan AS, Akbaş F, Karakuş U. 2016a. Growth and life history traits of Aegean chub, *Squalius fellowesii* (Günther, 1868) in streams of the Muğla Province, Aegean Coast, Turkey. *J Appl Ichthyol* 32: 532–537.
- Top N, Tarkan AS, Vilizzi L, Karakuş U. 2016b. Microhabitat interactions of non-native pumpkinseed *Lepomis gibbosus* in a Mediterranean-type stream suggest no evidence for impact on endemic fishes. *Knowl Manag Aquat Ecosyst* 417: 36.
- Tran TNQ, Jackson MC, Sheath D, Verreycken H, Britton JR. 2015. Patterns of trophic niche divergence between invasive and native fishes in wild communities are predictable from mesocosm studies. *J Anim Ecol* 84: 1071–1080.
- Trombley CA, Schwalb AN, Hardy TB, Cottenie K. 2021. Spatio-temporal analyses show conflicting evidence of the role of an invasive minnow in the decline of an endangered desert fish endemic to the south-western USA. *Freshw Biol* 66: 2158–2169.
- Van der Putten WH, Macel M, Visser ME. 2010. Predicting species distribution and abundance responses to climate change: why it is essential to include biotic interactions across trophic levels. *Philos. Trans. R. Soc. B* 365: 2025–2034.
- Vilizzi L, Copp GH, Britton JR. 2013. Age and growth of European barbel *Barbus barbus* (Cyprinidae) in the small, mesotrophic River Lee and relative to other populations in England. *Knowl Manag Aquat Ecol* 9: 409.
- Villeneuve F, Copp GH, Fox MG, Stakėnas S. 2005. Interpopulation variation in growth and life-history traits of the introduced sunfish, pumpkinseed *Lepomis gibbosus*, in southern England. *J Appl Ichthyol* 21: 275–281.
- Walford LA. 1946. A new graphic method of describing the growth of animals. *Biol Bull* 90: 141–147.

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