

Article

Seasonal and Spatial Variation in the Diet of *Gambusia holbrooki* in Different Water Bodies of Karaburun Peninsula (Western Türkiye)

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Abstract: The Eastern mosquitofish *Gambusia holbrooki* Girard, 1859, has been widely introduced into tropical and temperate countries as a biological agent to control mosquitos, which are associated with diseases such as malaria and yellow fever. However, the species exhibits invasive characteristics by competing with native species for food and habitat use. This study investigates the feeding ecology of *G. holbrooki* populations from three distinct freshwater environments (Lake Iris, Eğlenhoca Reservoir, and Parlak Reservoir) on the Karaburun Peninsula (North-Western Türkiye), a region outside its native range. The primary aim was to evaluate seasonal and spatial variations in the diet of the species. A total of 871 specimens were analysed: 247 from Iris Lake, 318 from Parlak Reservoir, and 306 from Eğlenhoca Reservoir. Low percentages of empty stomachs ($\leq 20\%$) across all populations indicate high feeding intensity. The results reveal that *G. holbrooki* exhibits a generalist feeding strategy, consuming a wide range of food items such as insects, zooplankton, and plant material across all the investigated environments. The relative importance of food groups (determined by the Importance Index, MI%) varied seasonally but not spatially. In all three populations, the most important food source in the spring was dipterans, followed by plants in the summer and fall, and cladocerans in the winter. Plants accounted for the largest percentage of the diet in every population (MI% $> 65\%$). These findings suggest that *G. holbrooki* adapts its diet to seasonal food availability.

Keywords: Cladocera; Diptera; invasive species; feeding ecology; mosquitofish



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1. Introduction

The survival and genetic integrity of native fish species are seriously threatened worldwide by numerous introductions of non-native species as a result of anthropogenic activities [1,2]. Among these, invasive species negatively affect global biodiversity and cause significant economic and ecological damage [3]. The Eastern mosquitofish *Gambusia holbrooki* Girard, 1859, together with the congeneric Western mosquitofish *Gambusia affinis* (Baird & Girard, 1853), have been introduced in more than 50 countries and are now found in all continents except Antarctica [4,5]. These species were introduced in tropical and temperate countries in the early 1900s as biological agents to control mosquitoes and to prevent diseases, including malaria and yellow fever [5]. Despite scientific evidence suggesting that native fish are equally effective, if not even more so, and that the negative effects of introducing non-native fish outweigh any positive effects [6–9], the recent literature

reports that some local authorities in Türkiye continue to promote the introduction of these non-native fish as effective mosquito control agents [10].

The Eastern mosquitofish generally prefers shallow, stagnant, or slow-moving waters, especially along well-vegetated shorelines, and it can potentially interact with other species including various zooplankton, invertebrates, amphibians, and other fish in habitats rich in biodiversity [11]. Its ability to switch prey depending on the varying abundance of prey species in the environment allows it to compete with other species for food and habitat use [12]. Both experimental and observational studies have reported that *G. holbrooki* is sometimes insufficient to control larval mosquitoes, although it is also effective at consuming zooplankton and other invertebrates [13–15]. On the other hand, it is also suggested that mosquitofish have contributed to the spread of mosquitoes by preying on or outcompeting indigenous invertebrate predators of mosquito larvae, and native species might be even more effective at controlling mosquitoes than mosquitofish [11,14,16–18]. Pyke [11] reported evidence of two conflicting statements about the effectiveness of *G. holbrooki* in controlling mosquito larvae: one suggests that the fish is generally effective in this role, while the other argues that *G. holbrooki* is generally ineffective due to the low levels of mosquito larvae in its diet. Given its well-documented invasive traits and its designation as a potential pest [19], yet its widespread introduction as an effective biological control agent for mosquitoes, it is essential to investigate the feeding ecology of *G. holbrooki* in introduced environments to determine which characterization holds greater weight. Furthermore, the omnivorous feeding behaviour of *G. holbrooki* and its diet composition may affect its potential effectiveness as a mosquito control agent in newly colonized habitats [17,20,21].

Diet composition analysis is a widely used direct method for interpreting the ecological role of fish and their potential impact on food webs [22]. Estimating fish feeding patterns across time and space provides critical insights into the feeding ecology of invasive fish, and these findings are valuable for managing their populations effectively [23]. Additionally, seasonal and spatial changes in food supply, such as the increased availability of plant matter during warmer seasons, are known to induce temporal changes in the diet of omnivorous fish with high trophic plasticity [24]. Studies on the seasonal diet shift of *G. holbrooki* have shown that its diet fluctuate with prey availability ranging from insect to zooplankton [25,26]. Therefore, the objectives of this study are (1) to investigate the seasonal diet of three introduced populations of *G. holbrooki* in Western Türkiye and (2) to analyse and compare the feeding strategies of these populations.

2. Materials and Methods

2.1. Study Area

Karaburun is a peninsula in the Aegean Sea (Western Türkiye) located outside the urban pressures of Izmir, one of the country's largest metropolitan cities (Figure 1). The area has a low human population, and agricultural land is scarce due to its predominantly mountainous terrain. Several reservoirs have been created for drinking and utility water purposes.

In this study, *G. holbrooki*'s specimens were collected from three different water bodies on the Karaburun Peninsula (Figure 1): Eğlenhoca and Parlak reservoirs and Lake Iris. The Eğlenhoca Reservoir, built in 2007 and with a surface area covering approximately 0.05% of the territory, is the largest of the agricultural irrigation and utility water reservoirs on the Karaburun Peninsula. Four other reservoirs—Bozköy, Karareis, Salman, and Parlak—were built between 2014 and 2018 for the similar water supplies purposes [27]. Lake Iris, located near the Karareis Reservoir, is the only wetland in the Karaburun Peninsula.

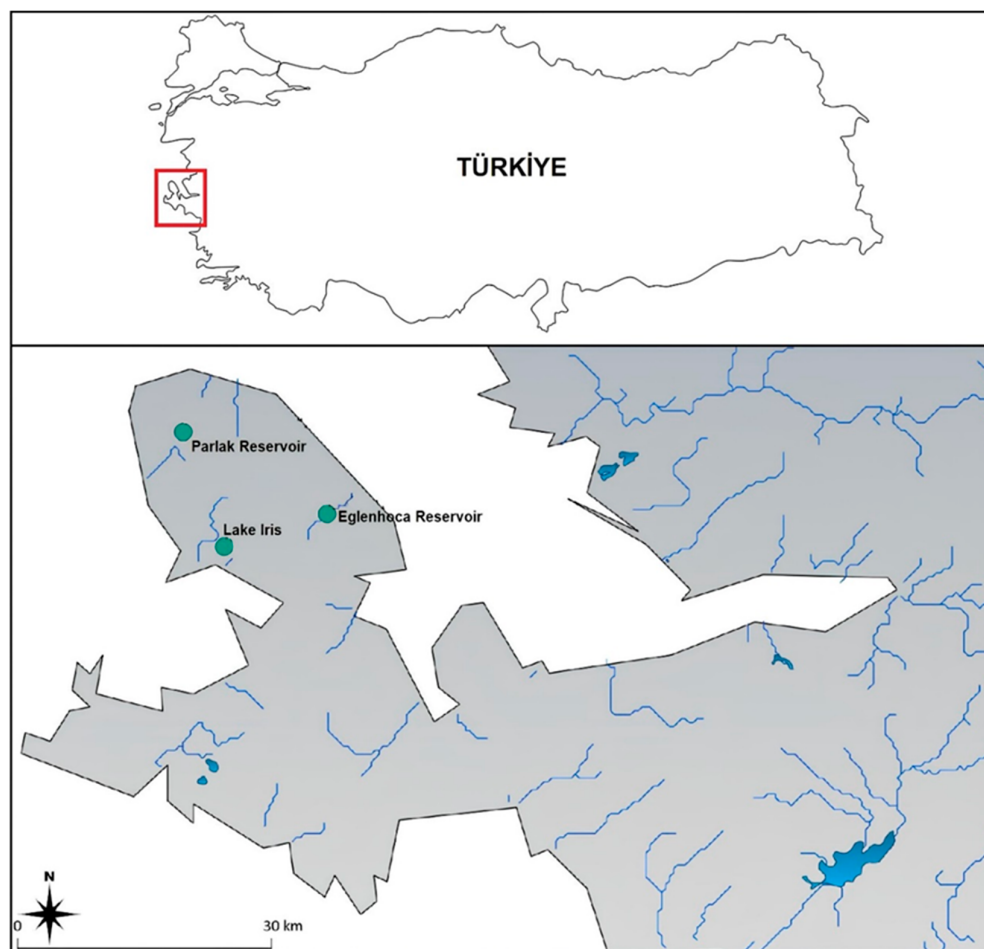


Figure 1. The map of the study area with the sites for *Gambusia holbrooki* specimens captured. The red rectangle indicate the position of the study area within Türkiye. The map was created using the QGIS 3.16 software available from <http://qgis.org> (accessed on 12 October 2024).

2.2. Sampling and Laboratory Procedures

The field surveys of this study were conducted within the scope of the project titled “Karaburun-Ildır Bay Special Environmental Protection Area Terrestrial Biodiversity Research Project” funded by the Republic of Türkiye Ministry of Environment, Urbanisation and Climate Change; General Directorate for the Protection of Natural Assets. Accordingly, the treatment of collected fish specimens was consistent with the Republic of Türkiye’s animal welfare laws and guidelines. Fish surveys were conducted during four seasons between May 2021 and February 2022 by electro-fishing (SAMUS 1000 portable electro-shocker; frequency 50–55 Hz; 20–50 cm fishing depth) along the banks of each water body. Fish were sampled over a period of approximately 15 min at midday from approximately 50 m from the shoreline at a maximum depth of 80 cm at each sampling site. The same sites in each water body were sampled in the seasonal surveys. Notably, no native fish species were observed in these water bodies, suggesting that the feeding ecology of *G. holbrooki* will be a key factor in understanding its role in the investigated environments. All collected fish specimens were ethically euthanized in the field with an overdose of clove oil (100 mg/L) and then fixed in a formaldehyde solution for further analyses. Each sampling site was considered a different statistical population.

In the laboratory, fish were measured to the nearest 0.1 cm for total length (TL), and body mass (W) was determined using an electronic balance with an accuracy of 0.01 g. Each specimen was dissected from the oesophagus to the anus, and the digestive tracts were removed. For diet analyses, gut contents were analysed under a binocular microscope,

and each food item was categorized to the lowest possible taxonomic level using specific identification keys [28–30]. Countable food items, such as insects and zooplankton, were counted, and then all food items were dried at 80 °C (2–4 h) and weighed to the nearest 0.0001 g.

2.3. Diet Analyses

The feeding density of each population was examined by estimating the proportion of empty stomachs. The feeding habits of the populations were measured using two different indices to assess the level of consumption of specific foods, (a) the index of relative importance (IRI) for countable foods, and (b) the modified index of relative importance (MI) for uncountable foods. The two indices were calculated as follows:

$$\text{IRI} = (\text{N}\% + \text{W}\%) \times \text{F}\% \quad (1)$$

and

$$\text{MI}\% = \text{F}\% \times \text{W}\%. \quad (2)$$

Here, F% represents the percentage frequency of occurrence [(number of digestive tracts containing a food item/total number of digestive tracts with food) $\times$ 100], N% represents the percentage number of digestive tracts with a certain food item against the total number of digestive tracts, and W% represents the percentage mass of a certain food item against the mass of all taxa consumed [31].

Shannon index (H') was used to compare dietary diversity between the three populations as follows:

$$H' = -\sum p_i \ln p_i \quad (3)$$

where p_i represents the F% values for each food type. The analysis was performed using the PRIMER v6 software package [32]. The modified Costello's method [33] was used to determine the feeding strategy and interpret the importance of foods in the diet of each population on a graphical basis [34]. According to this method, the prey-specific abundance ($P_i\%$) was plotted against the frequency of occurrence ($F_i\%$). The calculation of prey-specific abundance is as follows:

$$P_i = (\Sigma S_i / \Sigma St_i) \times 100; \quad (4)$$

where P_i is the prey-specific abundance of prey i , S_i is the digestive tract content (volume, weight or number) comprised prey i , and S_{ti} is the total digestive tract content in only those predators with prey i in their digestive tract [34]. This graphical method assists in gaining the ecological insight from gut content data, and helps evaluate important factors of the feeding behaviour of fish, such as feeding strategy (specialized vs. generalist), prey importance (dominant vs. rare), and niche width [35].

A one-way ANOVA was used to compare the overall and seasonal dietary importance (F% and MI%) across the three populations. Before performing the ANOVA, the normality of the data was assessed using the Kolmogorov–Smirnov test, and homogeneity of variances was checked with Levene's test. Given that the data met the assumptions of normality and homogeneity, the parametric ANOVA was conducted using IBM SPSS Statistics 28.0 (IBM Corp., Armonk, NY, USA). A significance level of $p < 0.05$ was set to determine statistical differences.

3. Results

A total of 871 specimens of *G. holbrooki* were collected from the three sampling sites (247 from Iris, 318 from Parlak, and 306 from Eğlenhoca, respectively). Table 1 provides information on the sampling sites and the fish sizes (TL and W). The proportions of

empty stomachs (%) were similar in all three populations (Table 2), and the low values calculated during winter (5.8% for Lake Iris, 6.3% for Parlak Reservoir, and 5.1% Eğlenhoca Reservoir) indicate a high feeding density (Tables 3–5). However, the lowest feeding densities, indicated by the highest proportion of empty stomachs values, were found at Lake Iris in summer (34.4%), Parlak Reservoir in autumn (41.2%), and Eğlenhoca Reservoir in spring (50.8%).

Table 1. The number of individuals (n) analysed per sampling sites with information on size ranges (TL; total length and W; body mass).

No	Site	Coordinate	n	TL, cm (min.-max.)	Mean TL $\pm$ SD	W, g (min.-max.)	Mean W $\pm$ SD
1	Lake Iris	38.49250 N 26.45000 E	247	1.6–4.0	2.43 $\pm$ 0.49	0.05–0.80	0.20 $\pm$ 0.16
2	Parlak Reservoir	38.61306 N 26.40667 E	318	1.6–5.3	3.17 $\pm$ 0.72	0.05–3.80	0.47 $\pm$ 0.47
3	Eğlenhoca Reservoir	38.52656 N 26.55944 E	306	1.8–5.3	3.17 $\pm$ 0.57	0.08–1.72	0.38 $\pm$ 0.28

Table 2. Percentage values of frequency of occurrence (F%), relative importance index (IRI%), and the modified index of relative importance (MI%) for the functional food items, as well as the numbers of individuals (n), proportion of empty stomachs (PES%), and diversity index (H') values of the three *Gambusia holbrooki* populations (Karaburun Peninsula, Türkiye) analysed. (In bold are reported the highest values estimated for each of the indices for each population).

Foods	Lake Iris			Parlak Reservoir			Eğlenhoca Reservoir		
	F%	IRI%	MI%	F%	IRI%	MI%	F%	IRI%	MI%
Diptera	40.20	31.13	16.80	41.11	10.48	17.83	38.31	11.60	9.47
Ephemeroptera	3.02	0.36	0.30	2.37	0.22	0.47	3.63	0.17	0.17
Plecoptera	1.51	0.06	0.04	-	-	-	0.40	<0.01	<0.01
Trichoptera	0.50	0.01	0.01	3.56	0.19	0.39	0.81	<0.01	<0.01
Odonata	-	-	-	0.79	0.01	0.02	2.42	0.17	0.19
Hemiptera	-	-	-	-	-	-	1.21	0.01	0.01
Coleoptera	-	-	-	0.79	0.01	0.03	0.40	<0.01	<0.01
Terrestrial insects	3.02	0.21	0.16	1.98	0.04	0.09	4.03	0.18	0.18
Ostracoda	9.05	1.92	0.63	-	-	-	0.81	0.01	<0.01
Copepoda	12.56	6.20	0.74	6.72	0.14	0.14	8.87	0.32	0.09
Cladocera	31.16	59.25	12.43	51.78	88.87	13.41	39.52	87.46	2.63
Gammaridae	-	-	-	-	-	-	0.40	<0.01	<0.01
Crustacea *	-	-	-	0.40	0.02	0.04	-	-	-
Acaridae	5.03	0.75	0.53	1.98	0.01	0.01	2.42	0.05	0.01
Bivalvia	1.51	0.06	0.05	0.40	0.01	0.02	-	-	-
Pisces	-	-	-	-	-	-	0.40	0.02	0.02
Plant material	62.31	-	68.30	28.46	-	67.57	40.73	-	87.22
PES (%)	19.4			20.4			19.0		
H'	2.48			2.27			2.49		

* unidentified.

Table 3. Seasonal values of frequency of occurrence (F%), relative importance index (IRI%), and modified index of relative importance (MI%) for functional food items along with individual numbers (n), proportion of empty stomachs (PES%), and diversity index (H') values of the *Gambusia holbrooki* population observed in Lake Iris. (In bold are reported the highest values estimated for each of the indices for each population).

Foods	Spring			Summer			Autumn			Winter		
	F%	IRI%	MI%	F%	IRI%	MI%	F%	IRI%	MI%	F%	IRI%	MI%
Diptera	49.06	42.07	46.40	62.50	50.80	28.44	47.37	100.00	15.94	4.08	0.09	0.16
Ephemeroptera	11.32	4.22	6.27	-	-	-	-	-	-	-	-	-
Plecoptera	3.77	0.32	0.43	2.50	0.11	0.13	-	-	-	-	-	-
Trichoptera	1.89	0.16	0.25	-	-	-	-	-	-	-	-	-
Terrestrial insects	9.43	1.89	2.70	-	-	-	-	-	-	2.04	0.02	0.04
Ostracoda	26.42	12.76	8.33	5.00	0.39	0.32	-	-	-	4.08	0.10	0.16
Copepoda	15.09	14.36	2.45	-	-	-	-	-	-	34.69	7.93	11.53
Cladocera	20.75	23.22	3.97	10.00	37.87	20.60	-	-	-	95.92	91.85	88.11
Acaridae	5.66	0.25	0.22	17.50	10.83	12.61	-	-	-	-	-	-
Bivalvia	5.66	0.74	1.08	-	-	-	-	-	-	-	-	-
Plant material	22.64	-	27.90	37.50	-	37.91	61.40	-	84.06	-	-	-
PES (%)	8.6			34.4			25.0			5.8		
H'	3.0			1.97			0.99			1.26		

Table 4. Seasonal values of frequency of occurrence (F%), relative importance index (IRI%), and the modified index of relative importance (MI%) for the functional food items along with individual numbers (n), proportion of empty stomachs (PES%), and diversity index (H') values of *Gambusia holbrooki* population observed in Parlak Reservoir. (In bold are reported the highest values estimated for each of the indices for each population).

Foods	Spring			Summer			Autumn			Winter		
	F%	IRI%	MI%	F%	IRI%	MI%	F%	IRI%	MI%	F%	IRI%	MI%
Diptera	69.70	74.93	75.15	25.35	80.95	1.13	40.00	6.88	5.37	42.86	10.89	27.38
Ephemeroptera	3.03	0.33	0.40	5.63	3.90	0.33	3.33	0.83	1.16	-	-	-
Trichoptera	-	-	-	7.04	7.67	0.82	-	-	-	3.36	0.04	0.10
Odonata	-	-	-	1.41	0.20	0.01	3.33	0.15	0.18	-	-	-
Coleoptera	3.03	0.21	0.26	1.41	0.33	0.04	-	-	-	-	-	-
Terrestrial insects	9.09	1.18	1.37	1.41	0.25	0.02	-	-	-	0.84	0.01	0.02
Copepoda	3.03	0.14	0.02	-	-	-	-	-	-	13.45	0.48	1.11
Cladocera	12.12	22.68	2.38	1.41	6.69	<0.01	36.67	91.52	5.43	96.64	88.54	70.21
Crustacea *	-	-	-	-	-	-	3.33	0.62	0.86	-	-	-
Acaridae	-	-	-	-	-	-	-	-	-	4.20	0.04	0.11
Bivalvia	3.03	0.52	0.65	-	-	-	-	-	-	-	-	-
Plant material	27.27	-	19.78	66.20	-	97.65	33.33	-	87.00	5.04	-	1.07
PES (%)	25.0			26.0			41.2			6.3		
H'	2.05			1.73			1.96			1.69		

* unidentified.

Table 5. Seasonal values of frequency of occurrence (F%), relative importance index (IRI%), and the modified index of relative importance (MI%) for the functional food items along with individual numbers (n), proportion of empty stomachs (PES%), and diversity index (H') values of *Gambusia holbrooki* population observed in Eğlenhoca Reservoir. (In bold are reported the highest values estimated for each of the indices for each population).

Foods	Spring			Summer			Autumn			Winter		
	F%	IRI%	MI%	F%	IRI%	MI%	F%	IRI%	MI%	F%	IRI%	MI%
Diptera	62.07	56.42	84.04	20.37	47.57	0.46	19.44	89.64	2.26	55.91	15.73	39.60
Ephemeroptera	-	-	-	16.67	32.41	1.54	-	-	-	-	-	-
Plecoptera	-	-	-	-	-	-	-	-	-	1.08	0.01	0.02
Trichoptera	-	-	-	-	-	-	-	-	-	2.15	0.04	0.10
Odonata	-	-	-	7.41	7.94	0.99	1.39	0.34	0.01	1.08	0.01	0.03
Hemiptera	10.34	1.32	2.00	-	-	-	-	-	-	-	-	-
Coleoptera	-	-	-	-	-	-	1.39	0.31	0.01	-	-	-
Terrestrial insects	-	-	-	1.85	0.55	0.07	2.78	1.26	0.03	7.53	0.44	1.13
Ostracoda	6.90	0.73	0.95	-	-	-	-	-	-	-	-	-
Copepoda	20.69	40.88	11.15	-	-	-	-	-	-	23.66	1.32	2.90
Cladocera	-	-	-	5.56	6.60	0.02	-	-	-	95.70	82.44	54.97
Gammaridae	3.45	0.65	1.00	-	-	-	-	-	-	-	-	-
Acaridae	-	-	-	1.85	3.97	0.01	4.17	8.45	0.01	2.15	0.01	0.02
Pisces	-	-	-	1.85	0.96	0.16	-	-	-	-	-	-
Plant material	3.45	-	0.86	72.22	-	96.75	76.39	-	97.69	6.45	-	1.22
VI (%)	50.8			5.3			21.7			5.1		
H'	1.82			1.97			1.27			1.96		

Insects (Diptera, Ephemeroptera, Trichoptera, and terrestrial insects), zooplankton (Cladocera and Copepoda), mites, and plant material (macrophytes and filamentous algae) were all common food groups consumed by all three populations, indicating an omnivorous diet (Figure 2). Due to the difficulty of identifying macroinvertebrates or zooplankton that have semi-digested structures, they were classified into the major groups.

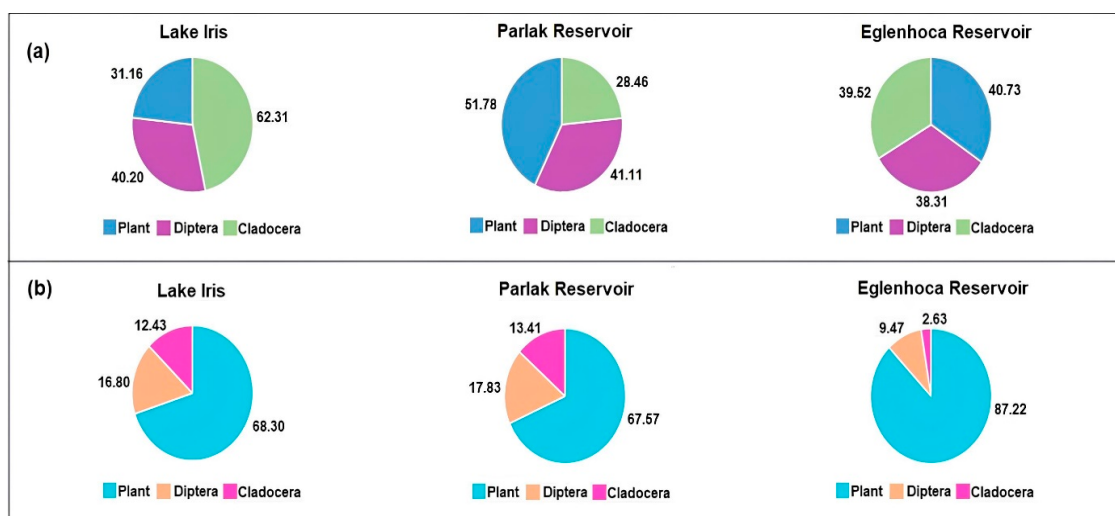


Figure 2. (a) F% and (b) MI% values of the three dominant food types in the diet of *Gambusia holbrooki* populations in water bodies on Karaburun Peninsula, Türkiye.

Diptera, the predominant insect group in the diet across all three populations, was detected in approximately 40% of the examined fish (Table 2). This group included subgroups such as Chironomidae, Ceratopogonidae, Culicidae, Simuliidae, and Tipulidae. Among these, Culicidae, which encompasses mosquitoes, represented 5.6%, 7.1%, and 4.7% in the İris, Parlak, and Eğlenhoca populations, respectively. The values of prey diversity and the H' index were very close among the populations, varying between 2.27 for the Parlak population and 2.49 for the Eğlenhoca population (Table 2). According to the F%, IRI%, and MI% analyses, all three populations fed mainly on plant and zooplankton (Cladocera; see Table 2). Plant material was the main food source in İris and Eğlenhoca lakes by frequency of occurrence (F%), and in all lakes by importance index (MI%). In terms of the relative importance index (IRI%) for countable groups, Cladocera was the most important food item, followed by Diptera (Table 2).

The diet of the three populations showed temporal, but not spatial differences. For certain food groups, including plant material, Cladocera, Copepoda, and Diptera, both F% and MI% values varied seasonally across all water bodies ($p < 0.05$). However, neither F% nor MI% values of each food showed significant spatial differences across the three water bodies ($p > 0.05$). Seasonal differences were observed for Copepoda ($p = 0.008$), Cladocera ($p = 0.001$), and plant material ($p = 0.005$) based on F% values. Specifically, differences were found between summer and winter ($p = 0.003$) and autumn and winter ($p = 0.002$) for Copepoda. For Cladocera, winter differed significantly from the other seasons ($p = 0.003$). In the case of plant material, only spring and winter showed no significant differences ($p > 0.05$), while differences were observed between the other seasons ($p < 0.05$). Additionally, MI% values varied seasonally for Diptera ($p = 0.07$) and plant material ($p = 0.01$).

The F%, MI% and IRI% values of food groups observed in the digestive tract of *G. holbrooki* were analysed seasonally for each population. Food diversity was highest in the spring and summer for the İris population, with Diptera showing the highest F% values during these seasons. The importance index (MI%) for plants increased in summer and autumn, while Cladocera had the highest MI% in winter (Table 3). For the Parlak population, Diptera, Cladocera, and plants were the main food sources year-round. This population consumed Diptera more frequently in spring and summer, with the highest IRI% and MI% values in summer. Plants dominated the diet of the population in summer and autumn, while zooplankton was the primary food consumed in winter (Table 4). For the Eğlenhoca population, food diversity peaked in summer and winter. Dipterans were also present in spring with the highest F%, IRI% and MI% values, similar to the other two populations. While plants had the highest F% and MI% values in summer and autumn, zooplankton was the main food consumed in winter, consistent with the other two populations (Table 5).

According to the graphical representations of the modified Costello's method (Figure 3), the distribution of the food categories in the graphs, generally located in the lower part of the $P_i\%$ axis, indicate that all three analysed populations follow a generalist feeding strategy. Surprisingly, only the İris population deviated from this strategy and specialized in Copepoda. The three main food categories (Cladocera, Diptera, and Plant) exhibited relatively high values on the F_i axis for each graph, suggesting their dominance in prey importance and contribution to niche width within-phenotype for all three populations. As presented in the graphs, the other food categories (except Copepoda in the İris population) are grouped towards the lower-left corner, indicating their rare consumption by the three populations.

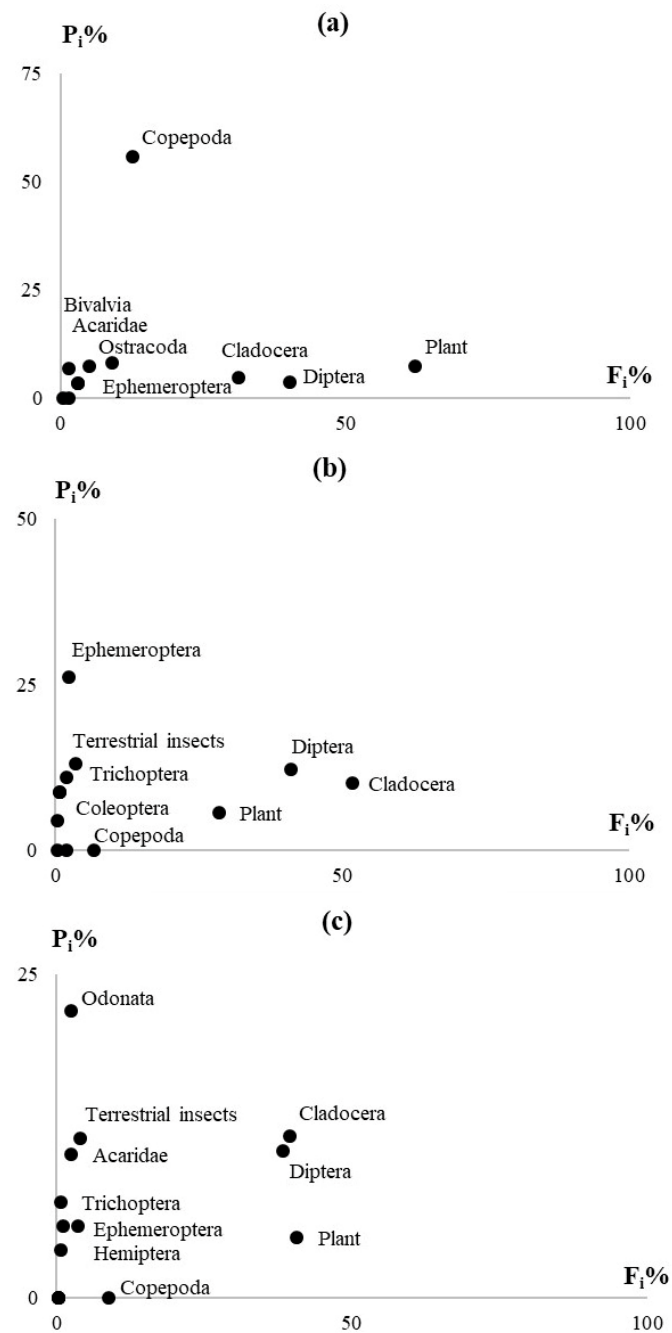


Figure 3. Modified Costello feeding strategy diagrams for three *Gambusia holbrooki* populations: (a) Lake Iris, (b) Parlak Reservoir, and (c) Eğlenhoca Reservoir. Prey-specific abundance ($P_i\%$) plotted against frequency of occurrence ($F_i\%$) of the food items in the diet of each population.

4. Discussion

Previous studies on different fish species have reported significant variations in feeding intensity according to the season: it typically increases in late spring following reproductive activity (to recover the fitness expended during the reproductive activity), peaks in summer and autumn due to the increased food availability, and decreases to its lowest values in winter [36–38]. However, *G. holbrooki* exhibits annual variations in gonad condition and, as a live-bearing species, can release its brood at different times from spring to autumn [39–41]. Despite being generalist feeders, the irregularity in feeding intensity observed in the populations studied may be linked to its reproductive strategy. The high feeding intensity, especially in winter, could be attributed to the species' tendency to not reproduce during

colder seasons. In fact, no gonadal or fry development was observed in the fish samples examined.

In general, *G. holbrooki* primarily fed on plant material and zooplankton in all three lakes. However, when each population was assessed seasonally, the species' diet pattern was determined by the food supply in the environment. Particularly in spring, the importance of Diptera in the diet increased, whereas in summer and autumn, there was a tendency towards plant material. In winter, all three populations predominantly fed on zooplankton. Opportunistic feeders, like *G. holbrooki*, select food that is most abundant in a given area and therefore readily available [42]. Apart from interspecific competition, the duration and quantity of food resources in the environment play a crucial role, especially for generalist and opportunistic fish species. Many dipterans enter diapause in winter conditions, with growth usually resuming with the rise in spring temperatures [43]. Dipterans are typically dominant aquatic insects in freshwater ecosystems, particularly in lakes [44], and it is expected that they are consumed by opportunistic *G. holbrooki* during warm periods. The increase in temperature effects the presence or seasonal growth patterns of algal communities from spring to the end of autumn, and algal accumulation is particularly high during the summer [45,46]. Consequently, it is inevitable that *G. holbrooki* feeds on macrophytes or filamentous algae, which are abundant and easily accessible during this period. Zooplankton communities also undergo seasonal changes in abundance and taxonomic composition, with biomass typically reaching its minimum density in winter [47,48]. However, the scarcity of plants and aquatic insects in cold conditions may attract the fish to zooplankton groups present in the environment, even if their abundance is limited. A similar pattern of seasonal variation in the diet of species living in lentic ecosystems has been observed in several studies [17,49,50]. Therefore, it is not surprising that *G. holbrooki*, a generalist and opportunistic fish, can adapt its diet pattern during seasons of high resource availability.

Dipterans are a dominant insect group in aquatic environments, with the highest number of species and high dominance, serving as the main food source for many fish species [51,52]. However, in the three populations studied, fish fed intensively on this food for only a short period of the year. Pyke [11] reported that *G. holbrooki* reduces its predation on mosquito larvae in the presence of aquatic vegetation. Further, it is questionable whether this periodic predation is sufficient to effectively control mosquitoes. The results of this study seem to validate the reports from previous researchers suggesting that *G. holbrooki* is generally ineffective in controlling mosquito larvae, as they are not commonly found in their diet. Several other studies have also suggested that the effectiveness of *G. holbrooki* in controlling larval mosquitoes may depend on differences between habitats and their food supply [50,53,54]. However, one of the shortcomings of this study is that no specific sampling was carried out to determine the presence, composition, or abundance of macroinvertebrates or dipterans in the environment. Thus, unfortunately, our dataset may not fully support the demonstration and discussion of fish selectivity for invertebrates or dipterans, and it would be premature to make definitive judgments, such as 'fish do not prefer dipterans' or 'are ineffective in mosquito control', based solely on the results of this study, which rely exclusively on the analysis of fish digestive tract contents.

This study avoids classifying and evaluating dipterans at lower taxonomic levels because their semidigested structures make them difficult to identify. For this reason, the calculations for the indices were made directly for the Diptera, rather than for subgroups that can be identified at low rates. Specifically, Chironomidae and Ceratopogonidae larvae were common among the identifiable dipterans while Culicidae larvae were observed only in a limited number of fish. The effectiveness of this fish, which primarily feeds intensively

on dipterans and rarely on Culicidae larvae only in spring, in mosquito control remains thus controversial in the light of these findings.

Among the three wetlands studied, Lake Iris is the only natural lake experiencing significant fluctuations in water level throughout the year. Particularly, certain areas of the lake dry out during the summer and remain dry until the autumn rains. The samples collected in this lake were captured in a small puddle during the summer and autumn seasons. Consequently, the seasonal drying of the lake was reflected in the diet of the fish: the feeding density was low, and food diversity differed from that of the other two populations. During spring, when water levels were high, the fish's diet was more diverse, whereas in the autumn, when water levels were at their lowest, the species fed on only two food items. In Lake Iris, a sensitive ecosystem devoid of native fish species, the negative impact of *G. holbrooki* may be particularly noticeable on the macroinvertebrate or zooplankton composition, which it feeds upon.

The observation of a generalist feeding strategy (Figure 3) in all three populations demonstrating similar feeding patterns (Figure 2) is supported by numerous studies on this invasive species [25,50,54]. Although this feeding strategy minimizes direct competition with native species by enabling the fish to exploit a wide range of available resources, it may also enhance its adaptability to new environments, thereby increasing its invasiveness over time [55]. The positioning of plants, cladocerans and dipterans—key components of the diet of all three *G. holbrooki* populations—on the graphs (Figure 3) and their relatively higher values on the $F_i\%$ axis indicate their significant contribution to the niche width of the fish. In Lake Iris, copepods were represented with significantly higher values than the other three foods, particularly in winter, highlighting the substantial individual contribution of this organism to the niche breadth of the fish species, as evidenced by its position on the feeding strategy plot (Figure 3).

5. Conclusions

In summary, this study shows that the dietary pattern of non-native *G. holbrooki* shows a particularly seasonal variation with little spatial variation in three different water bodies in Western Türkiye. Given the well-documented threats posed by *G. holbrooki* when introduced to new ecosystems, caution should be exercised to avoid further introductions or spread of its range. Vigilant monitoring and promotion of effective methods aiming to eradicate or control existing populations of *G. holbrooki* are thus essential to preserve both biodiversity and the ecological balance of ecosystems where the species may potentially become established.

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