DOI: https://doi.org/10.3897/arphapreprints.e183353

Trophic ecology of the invasive marbled crayfish in ephemeral streams of semi-arid environments

Rotem Mor, Oren Kolodny, Tamar Guy-Haim

Trophic ecology of the invasive marbled crayfish in ephemeral streams of semi-arid environments

Rotem Mor[1], Oren Kolodny[1,*], Tamar Guy-Haim[2,3,*†]

[1] Department of Ecology, Evolution, and Behavior, Institute for Life Sciences, The Hebrew University of Jerusalem, Jerusalem 9190401, Israel
[2] Israel Oceanographic and Limnological Research, Tel Shikmona, P.O.B. 2336, Haifa 3102201, Israel
[3] Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel

[*] These authors supervised this study jointly.
[†] Corresponding author: tamar.guy-haim@ocean.org.il

Abstract

The marbled crayfish (*Procambarus virginalis)* is an invasive, parthenogenetically reproducing species that has spread to freshwater ecosystems in over 24 countries across North America, Europe, Africa, and the Middle East. To date, the trophic ecology of the marbled crayfish has only been studied in European lentic and lotic perennial ecosystems. This study aims to shed light on the trophic ecology of the marbled crayfish in ephemeral streams of semi-arid environments in the Middle East, using $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis. We also conducted a diet regime experiment to test the impact of herbivorous and omnivorous diets on crayfish survival. In agreement with studies elsewhere, our analysis confirmed that *P. virginalis* functions as a flexible omnivore, occupying an intermediate trophic level (trophic position: 2.3–2.9), with the highest values observed during summer. The diet regime experiment showed that juveniles can survive on various diets, with only small differences in survival rates among the three diets that were tested. Surprisingly, the highest survival rate was observed when the crayfish were fed a poor plant-based diet. Adults and sub-adults also showed high survival rates under all tested diets, as well as some extent of cannibalism, which was amplified when fed purely plant-based diets. Cannibalism was primarily directed at small-sized juveniles (0-2 months old). In contrast, cannibalism among adults and sub-adults was very limited, even when reared at high densities.

Keywords: Bayesian mixing models, cannibalism, diet regime experiment, food web, isotopic niche breadth, Kaplan-Meier survival analysis, opportunistic feeding, stable isotope analysis

Introduction

The effects of invasive species are a world-wide concern, threatening biodiversity and altering ecosystem functioning, posing growing ecological and economic challenges (Pyšek et al., 2020; Maciaszek et al., 2022). How they interact with the indigenous species and how they might affect the ecosystem over time are important questions for scientists, policy makers, and the general public. The marbled crayfish, *Procambarus virginalis* (Lyko, 2017) is a freshwater decapod crustacean that has successfully established in numerous sites in Europe, North America, Africa, Asia and, in the last decade, also in Israel (Lipták et al., 2019; Maciaszek et al., 2022; Yanai et al., 2024), spreading via aquaculture and the international pet trade. It is recognized as one of the most invasive crayfish (Lipták et al., 2019) and was therefore included in the European Union's list of Invasive Alien Species (IAS) of Union Concern (European Commission, 2016). The first invasion in Israel was reported in 2018 in social media and was verified in December 2022 by morphological and molecular identification (Yanai et al., 2024).

Invasive crayfish are among the most destructive invasive species. They are generalist omnivores, potentially affecting all trophic levels and altering the food web (Maciaszek et al., 2022). Their foraging and burrowing make them potential ecosystem engineers - they can change sediment properties, increase bioturbation, create thermal refuges and drive other abiotic changes to the ecosystem (Twardochleb et al., 2013; Emery-Butcher et al., 2020; Maciaszek et al., 2022; Rilov et al. 2024). Invasive crayfish were also found to negatively affect biomass and abundances of macrophytes, invertebrates, fish, and amphibians in experimental settings (Twardochleb et al., 2013).

Many Middle Eastern countries are characterized by semi-arid climatic conditions (e.g., Maruani and Amit-Cohen, 2009), where freshwater ecosystems are typically small, fragile, and often seasonal. Understanding the impacts of the marbled crayfish invasion on these systems is crucial. This study aims to examine the trophic interactions of the marbled crayfish in semi-arid environments and assess its potential effects on local ecosystems. Given the potential impact of *P. virginalis* on the ecosystem, it is important to understand its role within local food webs to identify native species at risk. Determining

its trophic position will also inform management strategies to mitigate its effects, prevent further spread, and predict its ecological impacts in current and future invaded systems.

Previous invasions of *P. virginalis* have been studied across a range of aquatic ecosystems differing in habitat type and water depth. Several studies have used stable isotope analysis (SIA) to examine its trophic role and interactions within recipient ecosystem (Lipták et al., 2019; Linzmaier et al., 2020; Veselý et al., 2021; Teesalu et al., 2025). These studies were conducted in permanently flooded systems where fish represent the top predators, and in some cases other invasive crayfish were also present (Lipták et al., 2019; Linzmaier et al., 2020; Veselý et al., 2021). Overall, SIA results indicate that *P. virginalis* occupies a flexible, intermediate trophic position, with diet composition varying among sites. The main food sources included detritus, algae, zoobenthos and macrophytes (Veselý et al., 2021), detritus and algae (Lipták et al., 2019), macrophytes and periphyton (Teesalu et al 2025), or arthropods, snails and mussels (Linzmaier et al., 2020). Experimental evidence further suggests a preference for animal prey when available, including mobile invertebrates and carrion (Linzmaier et al., 2020). These findings suggest that the marbled crayfish can alter food-web structure and energy pathways in invaded ecosystems (Lipták et al., 2019).

Our study sites differ from those previously studied in several aspects: (1) Two of the water bodies studied are ephemeral, occasionally dry, while moisture is found only in underground burrows; (2) There are no indigenous crayfish species in Israel, and the studied sites had no other invasive crayfish prior to the current invasions; (3) None of the sites are inhabited by fish, which may prey on the invasive crayfish of compete with them, and thus control their populations. These differences make it difficult to predict which ecological niche and trophic position *P. virginalis* might occupy. To establish trophic position and interactions of the marbled crayfish, we used two complimentary approaches: (1) Stable isotope analysis of the crayfish and their potential food sources across different sites and seasons, and (2) Diet regime experiments. These approaches enabled us to assess the trophic position, isotopic niche breadth and main food sources of *P. virginalis*, and to test the impact of herbivorous and omnivorous diets on crayfish survival, while examining the extent of intraspecific predation (cannibalism). This

investigation is especially relevant in seasonal freshwater ecosystems, where the ecological succession is slow at the onset of winter (the wet season), and for several weeks after the hydroperiod begins, macrophytes and animal prey are scarce. During this time the crayfish that survived the dry summer are active, and cannibalistic behavior might be intensified (Fox, 1975; Dong and Polis, 1992).

Materials and methods

Study sites

Crayfish for SIA and diet regime experiments were collected from three inland water bodies in Israel, where established populations have been reported: (1) Ein Meshotetim, Siah Stream (32.801°N; 34.974°E), (2) Tzaanan Pool, Guvrin Stream (31.604°N; 34.959°E), and (3) Mekorot Pool, Guvrin Stream (31.613°N; 34.946°E) (Yanai et al., 2024) (Fig. 1). All crayfish specimens were collected between October 2022 and September 2023. All potential food sources were collected in June 2023.

Ein Meshotetim is a contact spring in the Siah Stream, located in Mount Carmel near the city of Haifa, in north-western Israel. The spring feeds an inner pool carved in the rock (ca. 1 x 1 x 1 m), with limited lighting, that intermittently flows through a hatch into an outside rock pool (ca. 1.5 x 1.5 x 1 m), which is occasionally emptied. Tzaanan Pool is located in the Guvrin stream in central-eastern Israel, near Beit Guvrin. It is a seasonal pool, with a maximum depth of 120 cm, which naturally dries out every year for at least 1-2 months (mid-October to mid-December). Approximately 1 km downstream from Tzaanan Pool is Mekorot Pool. The water in this pool originates from surplus groundwater discharged from a nearby drinking water facility. The pool is shallow, with depth ranging from a few centimeters to 40 cm, depending on the daily volume of water released.

Laboratory diet regime experiments were conducted using crayfish from Tzaanan Pool. SIA was performed on individuals collected from Ein Meshotetim, Tzaanan Pool, and Mekorot Pool.

Figure 1. Study sites: (A) Ein Meshotetim, (B) Tzaanan Pool, and (C) Mekorot Pool.

Sampling of crayfish and potential food sources

A total of 101 crayfish and 94 samples of potential food sources were collected manually and using hand nets between October 15, 2022, and September 7, 2023. the collected samples and their sampling dates are provided in Table 1. All crayfish sampled for SIA were frozen at -80 °C until further processing. Sampling was conducted under INPA permit no. 2024/43690 and in accordance with the handling requirements of invertebrates at the Hebrew University of Jerusalem.

Stable Isotope Analysis

Carbon and nitrogen content, and stable isotope ratios (^{13}C:^{12}C, ^{15}N:^{14}N) of the crayfish and potential food sources were measured to estimate the trophic position and main food sources of the crayfish in the three study sites. The crayfish were individually measured for total length (rostrum to telson, in cm) and weighed to the nearest 0.1 mg. Potential food sources were also weighed. Muscle tissue was dissected from the abdominal segment of each crayfish, except 20 juveniles (<2.51 cm, <0.27 g), for which the entire body was used for analysis. In all other species, the whole organism was analyzed.

All samples were oven-dried at 60 °C for 48 hours. Dried samples were homogenized using a mortar and pestle, weighed, and shipped to the Stable Isotope Facility at Cornell University (USA) for SIA. The isotopic composition of organic carbon and nitrogen was determined by the analysis of CO_2 and N_2 continuous-flow produced by combustion on a

Carlo Erba NC2500 connected on-line to a DeltaV isotope ratio mass spectrometer coupled with a ConFlo III interface.

Stable isotope ratios were calculated using the δ-notation - the deviation in per mill (‰) from the international standards: $\delta_{sample} = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 10^3$

where, R represents the $^{15}N:^{14}N$ or $^{13}C:^{12}C$ ratio. Stable isotope data are expressed relative to international standards of Vienna PeeDee belemnite and atmospheric N_2 for carbon and nitrogen, respectively. The analytical precision for the in-house standard was ± 0.04‰ [1σ] for both $\delta^{13}C$ and $\delta^{15}N$. For animal-derived samples with C:N > 3.5, we used a lipid correction of $\delta^{13}C_{corr} = \delta^{13}C - 3.32 + 0.99 \times C:N$ (Post et al., 2007). All graphs shown in this study present the lipid-corrected $\delta^{13}C$ ($\delta^{13}C_{corr}$). Isotopically similar sources were grouped into composite source pools to reduce model complexity and improve parameter identifiability (Phillips et al., 2014).

Trophic niche and contribution of food sources

Bayesian mixing models were applied using R package MixSIAR v. 3.1.12(Stock et al., 2018) to assess the relative contribution of potential food sources to the crayfish diet in each site and season. Each model was run with identical parameters (number of MCMC chains = 3; chain length = 300000; burn in = 200000; thin = 100), and model convergence was determined using Gelman-Rubin and Geweke diagnostic tests (Stock et al., 2018).

The trophic breadth of each crayfish population (n ≥ 20) during summer were assessed by calculating Standard Ellipse Area (SEA) using the R package SIBER v. 2.1.6 (Jackson et al., 2011). Size-corrected SEAs (SEA_C) were calculated for each site during summer, which adjusts for underestimation of ellipse area at small sample sizes and allows for inter-study comparison of ellipse sizes (Jackson et al., 2011).

The trophic enrichment factors used in the models follow Lipták et al. (2019), Ruokonen et al. (2012) and Veselý et al. (2021), and are 3.23‰ ± 0.41 for $\delta^{15}N$ and 0.47‰ ± 1.23 for $\delta^{13}C$ for animal food, and values of 2.4‰ ± 0.42 for $\delta^{15}N$ and 0.40‰ ± 0.28 for $\delta^{13}C$, for plant-based foods and detritus. The trophic position (TP) of the crayfish in the different

sites and seasons was calculated by: $TP_c = \lambda + (\delta^{15}N_c - \delta^{15}N_{base})/\Delta_n$ (Post, 2002), where $\delta^{15}N_c$ is the isotopic N value of the consumer, $\delta^{15}N_{base}$ is the $\delta^{15}N$ mean values of the baseline, for which we use the isotopic N value of the small invertebrates in the ecosystem, which function as a baseline (Jackson et al., 2017). For Tzaanan Pool we used insects and annelids, for Ein Meshotetim insects, gastropods and zooplankton, and for Mekorot Pool insects and zooplankton. λ is the trophic position of the baseline; we used the value 2 following Lipták et al. (2019). Δn is the trophic enrichment factor of $\delta^{15}N$ between trophic levels, which was set to 3.23 following Lipták et al. (2019). $\delta^{15}N_c$ is the isotopic N value of the consumer, $\delta^{15}N_{base}$ is the $\delta^{15}N$ mean values of the baseline, for which we use the isotopic N value of the small invertebrates in the ecosystem, which function as a baseline (Jackson et al., 2017). For Tzaanan Pool we used insects and annelids, for Ein Meshotetim insects, gastropods and zooplankton, and for Mekorot Pool insects and zooplankton. λ is the trophic position of the baseline; we used the value 2 following (Lipták et al., 2019). Δn is the trophic enrichment factor of $\delta^{15}N$ between trophic levels, which was set to 3.23 following Lipták et al. (2019).

Diet regime experiments

Crayfish were fed *ad libitum* under three dietary regimes: (1) a poor vegetarian (herbivorous) diet consisting of lettuce only; (2) a rich vegetarian diet including lettuce, carrots and kohlrabi; and (3) an omnivorous diet, composed of the same vegetables, supplemented with fish (commercially grown redbelly tilapia) and fish food (Dajana Mini Tropical pellets, composed of fish, crustaceans, and plant material, and SISO blood worms, composed of freeze dried blood worms). The crayfish were kept in 48x42x69 cm plastic tanks throughout the experiment. Tanks were filled with 60-70 L water at the start of the experiment. Rostrum to telson lengths were measured and the crayfish were categorized according to their sizes at the beginning of the experiment: (1) 0-0.7 cm; (2) 0.8-1.5 cm; (3) 1.6-3.4 cm; (4) 3.5-5.8 cm.

The first experiment lasted 69 days, from 21 September to 28 November 2023, with 3 tanks for each treatment. Each tank had 37 crayfish from size class 1, 3 from size class 2, 5 from size class 3 and 3 from size class 4. The second experiment lasted 68 days, 3

January to 10 March 2024, with juvenile crayfish reared in the absence of adults. Each tank had 28 0-0.7 cm long crayfish, one tank per diet. Feeding was carried out 1-2 times per week. The tanks were maintained at room temperature (21.8 °C) under 12:12 h light:dark regime. All crayfish were manually surveyed and counted by the same observer to maintain consistency.

Statistical analysis

All statistical analyses were conducted in R (version 4.4.1). Differences in $\delta^{15}N$ and $\delta^{15}C_{corr}$ values among sites and seasons were tested using Kruskal-Wallis rank-sum tests, followed by pairwise comparisons with Dunn's post-hoc tests and Holm's correction for multiple testing. Differences between juveniles and adults within each site and season were evaluated using Wilcoxon rank-sum tests. Relationships between stable isotope values ($\delta^{15}N$, $\delta^{15}C_{corr}$) and crayfish length and weight were assessed using linear regression, with separate models fitted for each site-season combination and for pooled seasonal data. Survival analyses for the dietary regime experiment were performed using Kaplan-Meier survival curves and log-rank tests to compare survival between diet types and size classes. Differences in juvenile growth (length) among diet treatments were tested with one-way ANOVA, followed by Tukey's HSD post-hoc tests. Significance was accepted at $p < 0.05$ for all tests.

OpenAI's ChatGPT (version GPT-5, accessed February 2025) was used in this study for assistance in refining and troubleshooting R code. All code was verified by the authors. The source code was uploaded to GitHub and is openly accessible via: https://github.com/TamarGuy-Haim/marbled-crayfish.

Results

Stable isotopes

Mean $\delta^{15}N$ values (± SD) for *Procambarus virginalis* varied across sites and seasons, ranging from 8.30 ± 1.78 ‰ in Mekorot Pool in the summer to 13.67 ± 1.29 ‰ in Tzaanan Pool in the summer. Mean $\delta^{13}C_{corr}$ values ranged from -31.39 ± 1.74 ‰ in Tzaanan Pool

in the autumn to -23.66 ± 2.14 ‰ in Ein Meshotetim in the summer (Table 1, Fig. 2). In all sites and seasons in which our sample included both adults and juveniles (Mekorot Pool - summer, Tzaanan Pool - summer and autumn), adults consistently showed higher mean $\delta^{15}N$ values than the juveniles. During summer, when 4-14 taxa were collected per site, *P. virginalis* was the most $\delta^{15}N$-enriched species at all three sites.

Table 1. Summary data. All samples collected, including data on sample size, season, site, isotopic values, and C:N ratio.

Taxon	n	Season	Length (mean ± SD) (cm)	Weight (mean ± SD) (g)	$\delta^{15}N$ (mean ± SD) (‰)	$\delta^{13}C$ (mean ± SD) (‰)	$\delta^{13}C_{corr}$ (mean ± SD) (‰)	C:N (mean ± SD)
Mekorot Pool								
P. virginalis (all)	26	Summer	3.49 ± 1.67	0.88 ± 1.14	8.30 ± 1.78	-26.80 ± 1.88	-26.44 ± 1.75	3.65 ± 0.50
P. virginalis (juveniles)	9	Summer	1.73 ± 0.25	0.07 ± 0.03	7.78 ± 2.50	-27.74 ± 2.46	-26.83 ± 2.40	4.28 ± 0.12
P. virginalis (adults)	15	Summer	4.54 ± 1.18	1.45 ± 1.26	8.70 ± 1.24	-26.28 ± 1.41	-26.28 ± 1.41	3.32 ± 0.06
Leaves	2	Summer	NA	NA	0.90 ± 0.05	-30.57 ± 0.01	-30.57 ± 0.01	58.23 ± 18.24
Detritus	5	Summer	NA	NA	8.38 ± 4.68	-18.01 ± 2.10	-18.01 ± 2.10	23.20 ± 2.13
Tadpoles	5	Summer	NA	NA	6.36 ± 0.16	-26.00 ± 0.22	-24.80 ± 0.30	4.57 ± 0.10
Insect larvae	1	Summer	NA	NA	9.92	-39.53	-37.24	5.67
Zooplankton	1	Summer	NA	NA	1.50	-29.96	-27.42	5.92
Tzaanan Pool								
P. virginalis (all)	10	Autumn	3.10 ± 1.66	0.74 ± 0.80	11.56 ± 0.44	-31.80 ± 1.48	-31.39 ± 1.74	3.72 ± 0.49
P. virginalis (all)	23	Summer	5.46 ± 3.20	4.51 ± 6.70	13.67 ± 1.29	-28.87 ± 2.33	-28.53 ± 2.11	3.69 ± 0.56
P. virginalis (all)	9	Winter	5.65 ± 2.1	3.26 ± 4.26	10.83 ± 0.57	-28.25 ± 0.83	-28.25 ± 0.83	3.30 ± 0.05
P. virginalis (adults)	5	Autumn	4.64 ± 0.53	1.44 ± 0.46	11.85 ± 0.37	-32.63 ± 1.29	-32.63 ± 1.29	3.27 ± 0.05
P. virginalis (adults)	15	Summer	7.05 ± 2.62	6.66 ± 7.47	14.16 ± 1.25	-27.94 ± 2.11	-27.94 ± 2.11	3.34 ± 0.07
P. virginalis (adults)	9	Winter	5.65 ± 2.1	3.26 ± 4.26	10.83 ± 0.57	-28.25 ± 0.83	-28.25 ± 0.83	3.30 ± 0.05
P.virginalis (juveniles)	5	Autumn	1.57 ± 0.18	0.05 ± 0.02	11.27 ± 0.282	-30.97 ± 1.25	-30.15 ± 1.14	4.18 ± 0.11
P.virginalis (juveniles)	7	Summer	2.06 ± 0.23	0.11 ± 0.04	12.59 ± 0.70	-30.69 ± 1.77	-29.56 ± 1.83	4.50 ± 0.18
Detritus	5	Summer	NA	NA	4.92 ± 0.34	-9.14 ± 0.44	-9.14 ± 0.44	99.39 ± 8.87

Taxon	n	Season	Length (mean ± SD) (cm)	Weight (mean ± SD) (g)	$\delta^{15}N$ (mean ± SD) (‰)	$\delta^{13}C$ (mean ± SD) (‰)	$\delta^{13}C_{corr}$ (mean ± SD) (‰)	C:N (mean ± SD)
Macrophytes	5	Summer	NA	NA	10.74 ± 0.08	-30.08 ± 0.05	-30.08 ± 0.05	9.64 ± 0.14
Notonectidae	5	Summer	NA	NA	10.74 ± 0.16	-32.44 ± 0.19	-30.75 ± 0.24	5.07 ± 0.07
Periphyton	3	Summer	NA	NA	6.68 ± 0.05	-38.94 ± 0.70	-38.94 ± 0.70	13.17 ± 0.48
Tadpoles	5	Summer	NA	NA	12.13 ± 0.08	-30.76 ± 0.42	-29.17 ± 0.52	4.97 ± 0.12
Annelida	4	Summer	NA	NA	12.06 ± 0.05	-29.62 ± 0.10	-27.18 ± 0.02	5.81 ± 0.10
Ein Meshotetim								
P.virginalis (all)	5	Autumn	6.45 ± 0.33	4.40 ± 0.35	9.77 ± 0.084	-31.28 ± 0.61	-31.28 ± 0.61	3.24 ± 0.05
P.virginalis (all)	20	Summer	5.32 ± 1.31	2.04 ± 1.81	10.72 ± 0.50	-23.66 ± 2.14	-23.66 ± 2.14	3.35 ± 0.06
P.virginalis (all)	8	Winter	6.78 ± 0.97	4.50 ± 1.96	9.547 ± 0.10	-29.21 ± 1.73	-28.01 ± 2.36	4.46 ± 1.81
P.virginalis (adults)	5	Autumn	6.45 ± 0.33	4.40 ± 0.35	9.769 ± 0.08	-31.28 ± 0.61	-31.28 ± 0.61	3.24 ± 0.05
P.virginalis (Adults)	20	Summer	5.32 ± 1.31	2.04 ± 1.81	10.715 ± 0.50	-23.66 ± 2.14	-23.66 ± 2.14	3.35 ± 0.06
P.virginalis (Adults)	8	Winter	6.78 ± 0.96	4.50 ± 1.96	9.55 ± 0.10	-29.21 ± 1.73	-28.01 ± 2.36	4.46 ± 1.81
Chronomus larvae	3	Summer	NA	NA	8.26 ± 0.07	-28.31 ± 0.06	-26.98 ± 0.05	4.70 ± 0.03
Culex larvae	1	Summer	NA	NA	6.80 ± NA	-26.28 ± NA	-24.55 ± NA	5.11 ± NA
Culex pupa	4	Summer	NA	NA	7.41 ± 0.07	-26.40 ± 0.03	-24.64 ± 0.07	5.13 ± 0.05
Ephemeroptera larvae	3	Summer	NA	NA	4.38 ± 0.08	-35.55 ± 0.05	-32.08 ± 0.07	6.86 ± 0.12
Insect larvae	11	Summer	NA	NA	6.76 ± 1.59	-29.41 ± 4.04	-27.30 ± 3.24	5.48 ± 0.91
Zooplankton	3	Summer	NA	NA	6.74 ± 0.03	-26.06 ± 0.10	-23.83 ± 0.13	5.61 ± 0.14
Detritus	5	Summer	NA	NA	5.23 ± 0.19	-20.53 ± 0.32	-20.53 ± 0.32	16.27 ± 0.58
Leaves	2	Summer	NA	NA	0.15 ± 1.52	-30.87 ± 0.01	-30.87 ± 0.01	83.49 ± 8.34
Periphyton	20	Summer	NA	NA	6.31 ± 2.61	-27.78 ± 2.78	-27.78 ± 2.78	6.33 ± 3.21
Melanopsis praemorsa	5	Summer	NA	NA	8.07 ± 0.94	-23.84 ± 0.28	-21.76 ± 0.40	5.46 ± 0.25
Theodoxus	2	Summer	NA	NA	8.68 ± 1.52	-25.18 ± 0.01	-23.36 ± 0.15	5.20 ± 0.15
Physella acuta	4	Summer	NA	NA	7.65 ± 0.04	-29.24 ± 0.20	-27.71 ± 0.32	4.89 ± 0.15
Gastropods	11	Summer	NA	NA	8.03 ± 0.85	-26.05 ± 2.59	-24.21 ± 2.86	5.21 ± 0.33

232

233

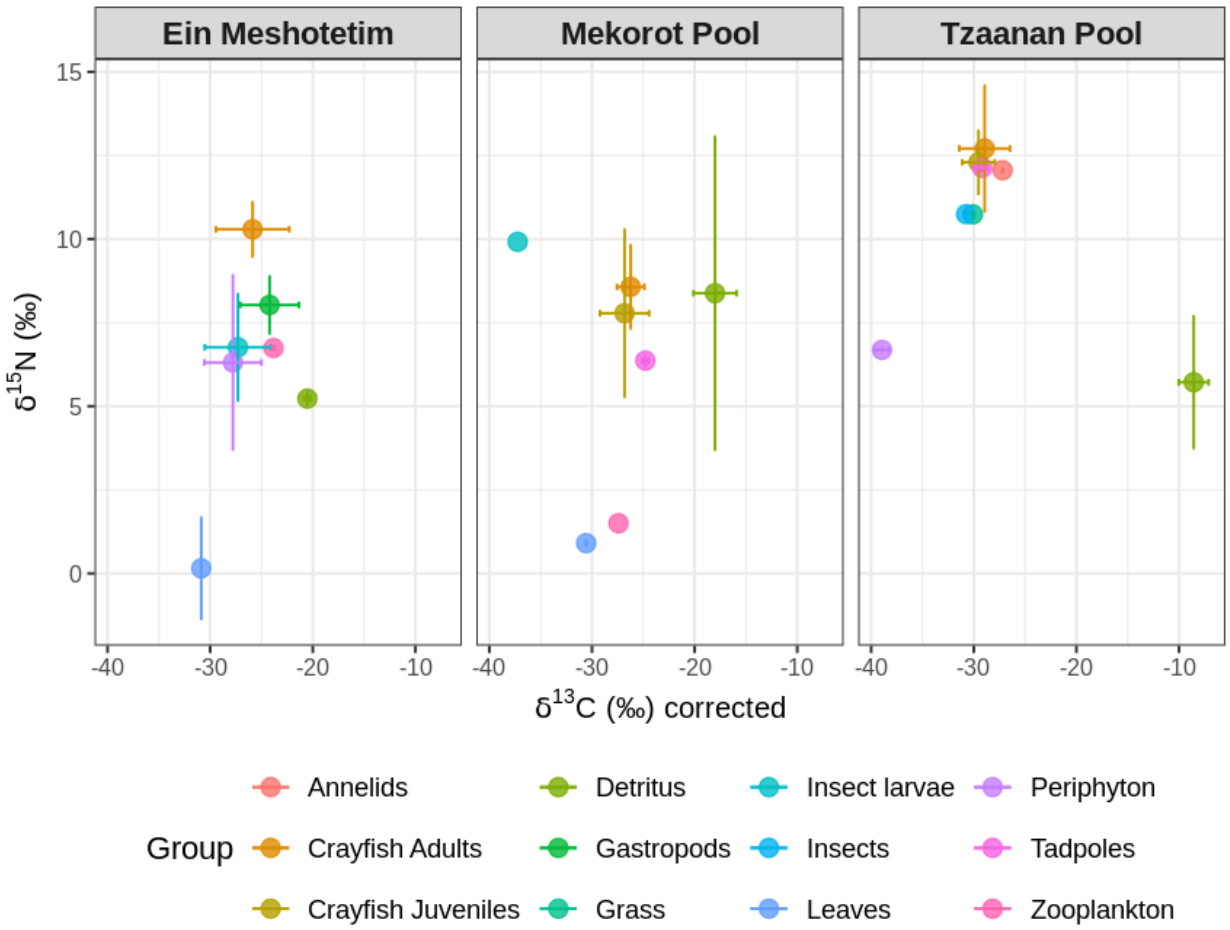


Figure 2. Isotope biplot of $\delta^{13}C$ and $\delta^{15}N$ values of *Procambarus virginalis* and food sources across study sites and seasons.

$\delta^{15}N$ values in crayfish from Tzaanan Pool (12.56 ± 3.17 ‰, n = 42) were significantly higher than in crayfish from Ein Meshotetim (10.29 ± 3.99 ‰, n = 33) and Mekorot Pool (8.30 ± 2.53 ‰, n = 26) (Kruskal-Wallis followed by Dunn's post-hoc test with Holm correction, $\chi^2 = 62.04$, $df = 2$, $p < 0.001$, all pairwise comparisons were significant with $p < 0.005$). $\delta^{13}C_{corr}$ values varied in samples from different sites ($\chi^2 = 24.43$, $df = 2$, $p < 0.001$). Samples from Tzaanan Pool (-29.15 ± 4.97 ‰) had higher $\delta^{13}C_{corr}$ than samples from the other sites ($p < 0.001$), whereas $\delta^{13}C_{corr}$ values in Mekorot Pool (-26.44 ± 1.75 ‰) were not significantly different from those in Ein Meshotetim (-25.87 ± 3.53 ‰) ($p = 0.630$).

We found significantly lower $\delta^{15}N$ in juveniles (length < 2.51 cm, weight < 0.27 g) than in adults in Mekorot Pool (Wilcoxon rank-sum test, p = 0.020). However, these differences were not significant in Tzaanan Pool (p = 0.753). Differences in $\delta^{13}C_{corr}$ between juveniles

and adults were not significant both in Mekorot Pool ($p = 0.916$) and Tzaanan Pool ($p = 0.298$).

$\delta^{15}N$ values were significantly different in different seasons when comparing crayfish samples from the same site. Summer samples had higher values than samples collected in autumn and winter. In Tzaanan pool (Kruskal-Wallis test, $\chi^2 = 30.35$, $df = 2$, $p < 2.56 \times 10^{-7}$), summer samples (average = 13.67 ± 3.08 ‰, n = 23) had higher $\delta^{15}N$ than autumn samples (11.56 ± 0.44 ‰, n = 10) ($p = 4.51 \times 10^{-4}$) and than winter samples (10.83 ± 0.91 ‰, n = 9) ($p = 1.80 \times 10^{-6}$). In Ein Meshotetim (Kruskal-Wallis: $\chi^2 = 15.03$, $df = 2$, $p = 0.0005$), summer samples (10.72 ± 0.50 ‰, n = 20) had significantly higher $\delta^{15}N$ than autumn (9.77 ± 0.08 ‰, n = 5) ($p = 0.005$) and winter samples (9.55 ± 1.00 ‰, n = 8) ($p = 0.006$).

Similar comparison of $\delta^{13}C_{corr}$ values of crayfish samples from the same site in different seasons, showed significant differences. In general, autumn samples had lower values than summer and winter samples. Significant differences were found in Tzaanan Pool (Kruskal-Wallis: $\chi^2 = 13.158$, df = 2, p = 0.001), where summer (average = -28.53 ± 2.40 ‰) had significantly higher values than autumn (-31.39 ± 1.74 ‰) ($p = 0.004$). Winter samples (-28.25 ± 4.97 ‰) also had significantly higher $\delta^{13}C_{corr}$ than autumn samples ($p = 0.0027$). In Ein Meshotetim (Kruskal-Wallis: $\chi^2 = 20.167$, df = 2, $p = 4.177 \times 10^{-5}$), $\delta^{13}C_{corr}$ values were significantly higher in summer (-23.66 ± 5.72 ‰) than autumn (-31.28 ± 0.61 ‰) ($p = 0.00018$) and winter (-28.01 ± 2.36 ‰) ($p = 0.007$). In Mekorot Pool only summer samples were collected.

In Ein Meshotetim and Tzaanan Pool, $\delta^{15}N$ values of the crayfish were positively correlated with $\delta^{13}C_{corr}$ values ($r^2 = 0.512$, $p \leq 0.01$; $r^2 = 0.469$, $p \leq 0.01$, respectively) (Supplementary material, Figs. S1, S2). In both sites, the correlation was highest during summer (Ein Meshotetim: $r^2 = 0.697$, $p < 0.001$; Tzaanan Pool: $r^2 = 0.714$, $p \leq 0.001$). However, a significant negative correlation between $\delta^{15}N$ and $\delta^{13}C_{corr}$ values was found in Mekorot pool ($r^2 = -0.847$, $p < 0.001$) (Supplementary material, Fig. S3). Significant but contrasting trends were observed across sites: $\delta^{15}N$ values varied by season and site, with adults consistently more enriched than juveniles. $\delta^{13}C_{corr}$ values also varied

significantly, and the correlation between $\delta^{15}N$ and $\delta^{13}C_{corr}$ differed in both strength and direction among sites. These patterns likely reflect site-specific differences in resource availability and how ontogenetic shifts in diet interact with local conditions.

Mean $\delta^{15}N$ values of primary producers (leaves, periphyton and macrophytes) varied among sites and taxa, ranging from 0.15 ± 1.52 ‰ for leaves in Ein Meshotetim to 10.74 ± 0.08 ‰ for macrophytes in Tzaanan Pool. Similarly, mean $\delta^{13}C_{corr}$ values showed substantial variation, ranging from -38.94 ± 0.70 ‰ for periphyton in Tzaanan Pool to -27.78 ± 2.78 ‰ for periphyton in Ein Meshotetim. $\delta^{15}N$ values for detritus varied across sites and ranged from 4.92 ± 0.34 ‰ in Tzaanan Pool to 8.38 ± 4.68 ‰ in Mekorot Pool, while mean $\delta^{13}C_{corr}$ values ranged from -20.53 ± 0.32 ‰ in Ein Meshotetim to -9.14 ± 0.44 ‰ in Tzaanan Pool. Among consumers, $\delta^{15}N$ values in Tzaanan Pool (Notonectidae, tadpoles and Annelida) ranged from 10.74 ± 0.16 ‰ for Notonectidae to 12.13 ± 0.08 ‰ for tadpoles. $\delta^{13}C_{corr}$ values ranged from -32.44 ± 0.19 ‰ for Notonectidae to -29.62 ± 0.10 ‰ for Annelida. In Mekorot Pool, consumers included tadpoles, zooplankton and insect larvae with $\delta^{15}N$ values ranged from 1.50 ‰ for zooplankton to 9.92 ‰ for insect larvae, and $\delta^{13}C_{corr}$ values from -37.24 ‰ for insect larvae to -26.00 ± 0.22 ‰ for tadpoles. In Ein Meshotetim, consumers (insect larvae, zooplankton and gastropods) exhibited, $\delta^{15}N$ values between 6.74 ± 0.03 ‰ for zooplankton and 8.03 ± 0.85 ‰ for gastropods. $\delta^{13}C_{corr}$ values ranged from -29.41 ± 4.04 ‰ for insect larvae to -24.21 ± 2.86 ‰ for gastropods.

Estimation of trophic position

The highest trophic position of *P. virginalis* was found in Ein Meshotetim during summer (TP = 2.93 ± 0.16). In all sites, trophic position values were highest in the summer. In every site and season where both adults and juveniles were sampled, the juveniles consistently showed lower trophic position than the adults: 2.64 ± 0.78 versus 2.89 ± 0.39 in Mekorot Pool during summer (Wilcoxon rank-sum test, $p = 0.021$), 1.98 ± 0.09 versus 2.16 ± 0.12 in Tzaanan Pool during autumn ($p = 0.037$) and 2.46 ± 0.22 versus 2.93 ± 0.39 in Tzaanan Pool during summer ($p = 0.004$) (Fig. 3).

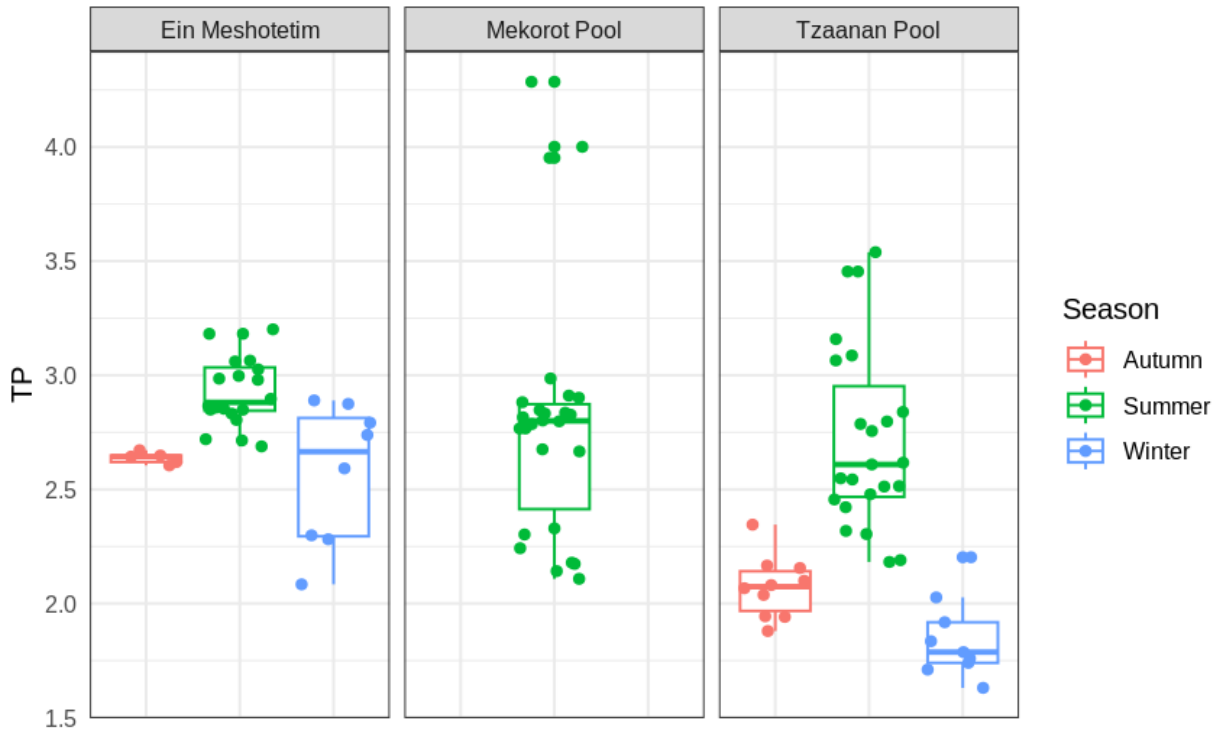


Figure 3. The trophic position (TP) of *Procambarus virginalis* across study sites and seasons, estimated using invertebrate $\delta^{15}N$ as a baseline and calculated following Post (2002).

Ontogenetic effect

The correlations between crayfish weight and $\delta^{13}C_{corr}$ and between weight and $\delta^{15}N$ (Fig. 4) showed diverse trends across sites and seasons. In Tzaanan Pool in the autumn, a positive correlation between crayfish weight and $\delta^{15}N$ values was observed ($r^2 = 0.52$, $p = 0.018$). Samples from Ein Meshotetim in the summer and winter exhibited significant negative relationships between weight and $\delta^{15}N$ values ($r^2 = 0.52$, $p = 3.0 \times 10^{-4}$; $r^2 = 0.65$, $p=0.016$; respectively). Significant negative relationships between weight and $\delta^{13}C_{corr}$ values were found in samples from Tzaanan Pool in the autumn ($r^2 = 0.53$, $p = 0.016$) and Ein Meshotetim in the summer ($r^2 = 0.83$, $p = 2.7 \times 10^{-8}$). In Ein Meshotetim in the autumn $\delta^{13}C_{corr}$ was negatively correlated with length ($r^2 = 0.92$, $p = 0.009$), but not with weight. In all other cases where a significant correlation was found between $\delta^{15}N$ or $\delta^{13}C_{corr}$ and weight, a corresponding correlation was also found with length. When pooling samples across all seasons, negative correlations were observed in Ein Meshotetim between $\delta^{15}N$ and weight ($r^2 = 0.68$, $p = 3.8 \times 10^{-9}$), $\delta^{15}N$ and length ($r^2 = 0.69$, $p = 2.8 \times 10^{-9}$), $\delta^{13}C_{corr}$ and

weight (r^2 = 0.49, p = 6.6x10^{-6}), and $\delta^{13}C_{corr}$ and length (r^2 = 0.44, p = 2.9x10^{-5}). No significant correlations were found in other sites when data from all seasons were combined. The contrasting ontogenetic trends across sites may reflect site-specific differences in resource use or limited robustness of the isotopic signals.

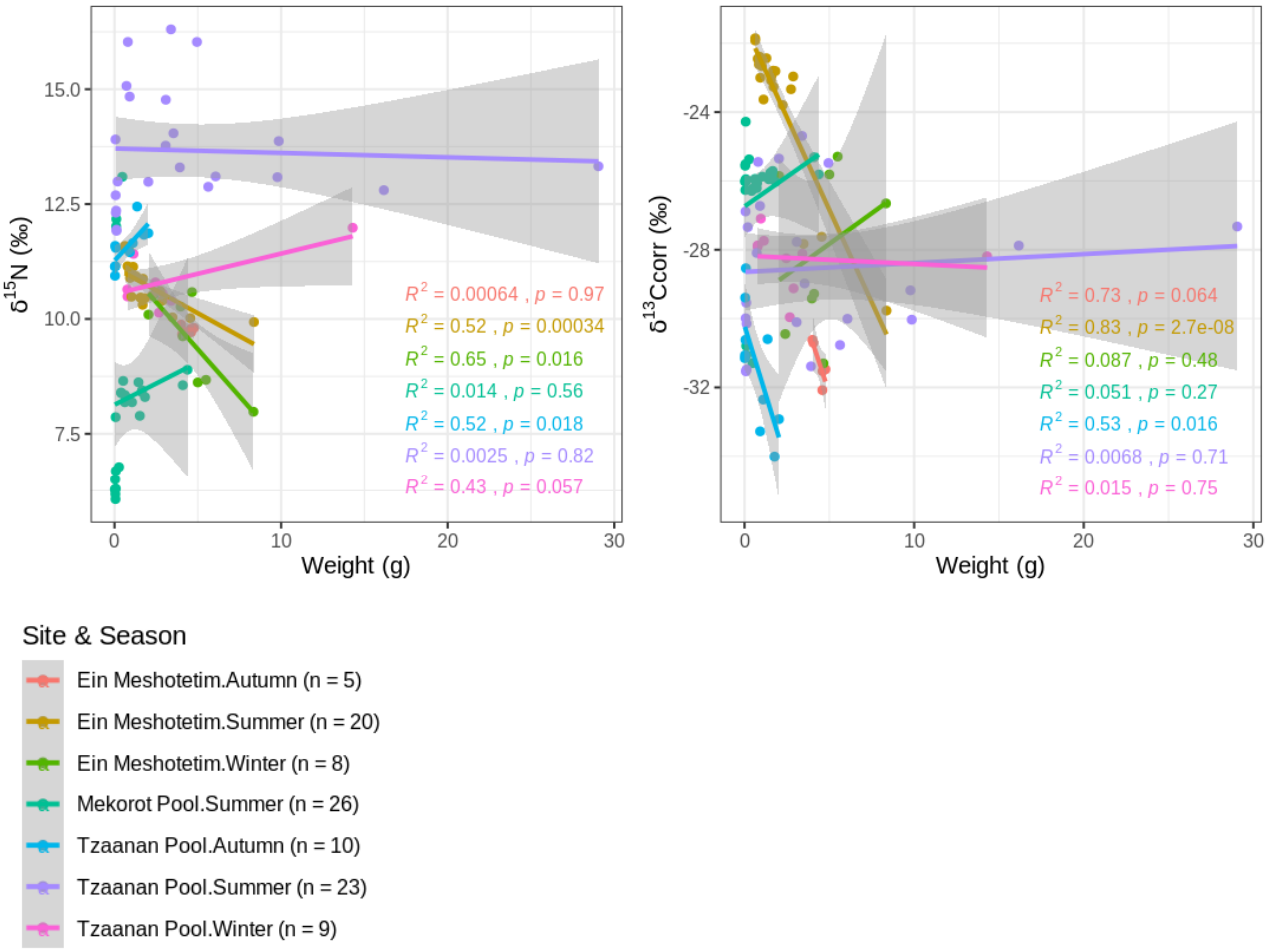
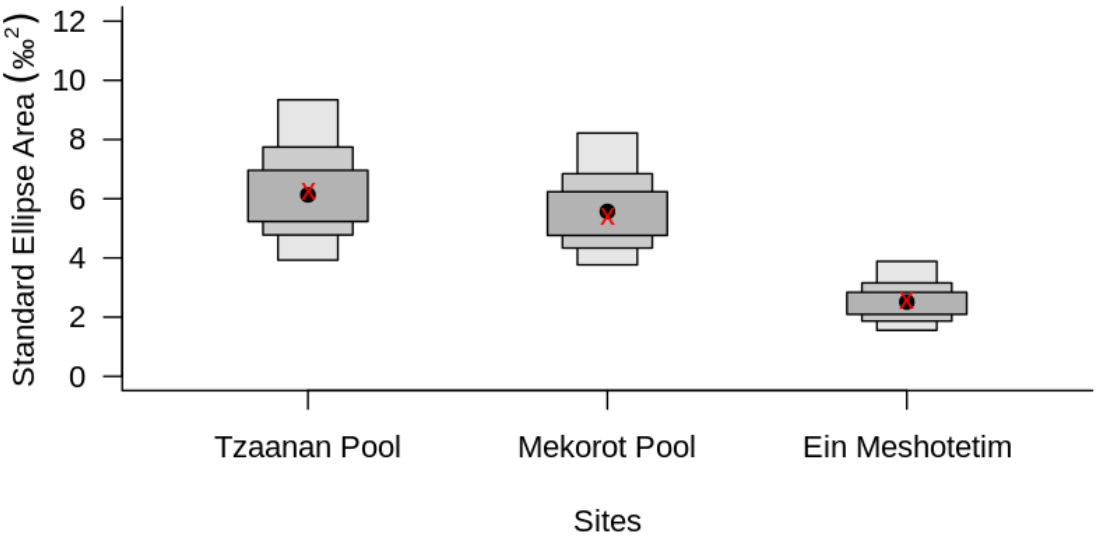


Figure 4. Ontogenetic effects. Linear regression analysis between weight (g) and $\delta^{15}N$ values, and weight and $\delta^{13}C_{corr}$ values, for *P. virginalis* collected in different sites and seasons.

Isotopic niche breadth

The isotopic niche breadth of the crayfish was calculated using the size-corrected standard ellipse area (SEA_C) based exclusively on summer specimens, as summer was the only season sampled consistently across all three study sites (n ≥ 20 per site). The

largest isotopic niche breadth was observed in Tzaanan Pool (SEA_C=6.09), followed by Mekorot Pool (SEA_C=5.55), with the smallest niche breadth in Ein Meshotetim (SEA_C=2.50) (Fig. 5).



.

Figure 5. Trophic niche breadth of the marbled crayfish *Procambarus virginalis* in the three study sites in the summer (n>20 per site) estimated by size-corrected standard ellipse area (SEA_C) boxplots (showing 95, 75 and 50% credibility intervals). Black circles represent means; red x symbols represent the maximum likelihood estimates of SEA_C.

Bayesian mixing models

To estimate the dietary composition of *Procambarus virginalis*, we applied Bayesian mixing models using $\delta^{13}C$ and $\delta^{15}N$ from summer samples, when potential food sources were also collected. The results revealed variable and site-specific dietary patterns (Fig. 6). In Ein Meshotetim, the crayfish diet was dominated by zooplankton (posterior median = 46.6% ± 14.6%) and gastropods (34.9% ± 9.1%). In Tzaanan Pool, macrophytes (36.3% ± 14.8%) and tadpoles (34.8% ± 20.1%) were the main contributors. In Mekorot Pool, tadpoles (50.5% ± 11.3%) and leaf litter (27.0% ± 4.8%) were the primary sources. These findings indicate an omnivorous diet in *P. virginalis*, with spatial variability reflecting local resource availability. It is important to note that Bayesian analyses provide probabilistic estimates rather than definitive conclusions. The results reflect the posterior medians, but a wide range of possible values is also plausible, as indicated by the broad posterior

distributions. Therefore, the uncertainty in these estimates must be considered. The outcomes are highly sensitive to the assumptions made during model setup, such as the food sources and trophic enrichment factors selected, and the results should be interpreted with caution, recognizing that different assumptions could lead to varying conclusions.

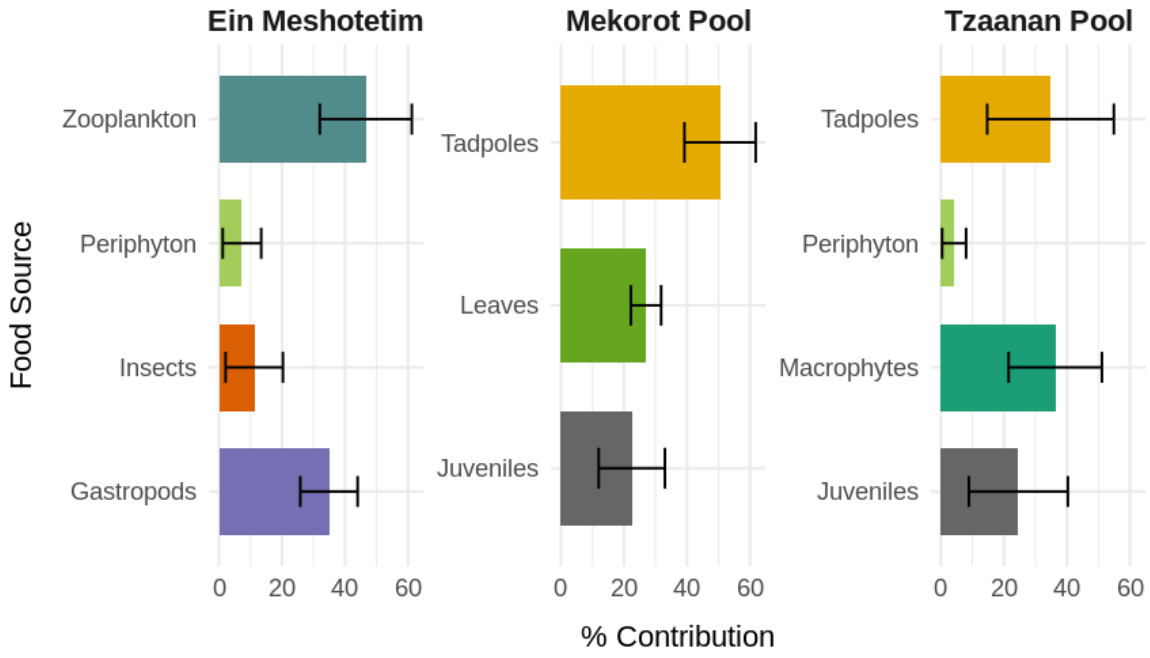


Figure 6. Estimated relative contributions (%) of food sources used by *Procambarus virgnialis* in the study sites, based on Bayesian mixing models. Bars represent mean contributions and error bars represent mean ± SD.

Diet regime experiment

To examine the effect of dietary regime on survival in *Procambarus virginalis*, we conducted a controlled feeding experiment. The raw data is openly accessible via https://doi.org/10.5281/zenodo.17998016. Kaplan–Meier curves, illustrating survival patterns per diet type and size class, are presented in Fig. 7. Juveniles of the smallest size class (length 0-0.7 cm) reared without adults exhibited high survival rates (>75%) across all diets, with no significant differences between dietary treatments (log-rank test: $X^2 = 0.1$, $p = 1$). Survival of juveniles appeared to be largely unaffected by diet.

In contrast to survival, juvenile growth - measured by body length - was significantly affected by diet type (ANOVA test: $p < 0.001$). Crayfish fed a rich vegetarian diet were significantly larger (average length = 1.78 cm, n = 21) than those fed a poor vegetarian diet (average length = 1.52 cm, n = 27, Tukey test: $p = 0.00033$). Crayfish in the omnivorous diet group were smaller (average length = 1.62 cm, n = 21) from the rich vegetarian group (Tukey test: $p = 0.050$). These results suggest that although diet composition had little effect on survival, it did influence juvenile growth rates, with a nutritionally richer plant-based diet supporting enhanced growth.

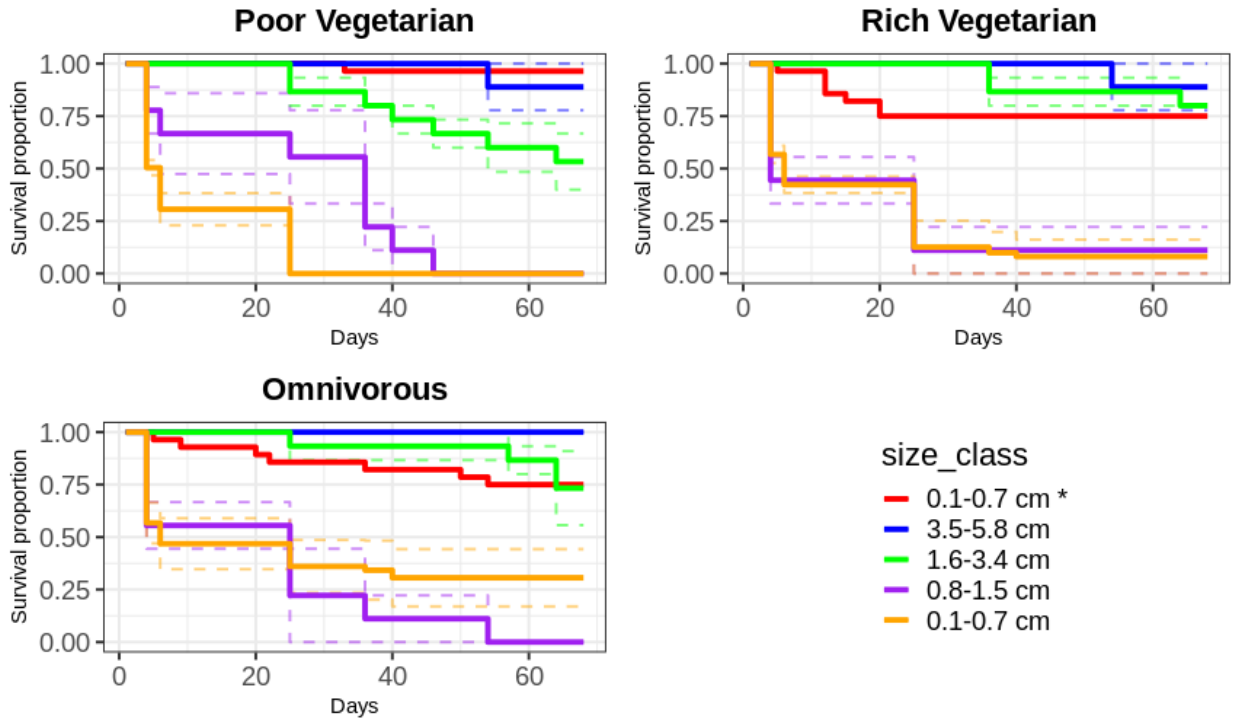


Figure 7. Survival proportion of the marbled crayfish per diet, represented by Kaplan-Meier plots. Lines represent different size class. Dashed lines represent standard error. The red line marked by an asterisk represents an experiment with juveniles reared in the absence of adults.

Survival of the smallest crayfish size class in the presence of adults dropped below 30% in all three diets. The poor vegetarian diet showed significant survival differences between size classes (log-rank test: $X^2 = 30.6$, $p = 4 \times 10^{-6}$) as well as the omnivorous diet (log-rank test: $X^2 = 10.2$, $p = 0.04$), which might mean that more juveniles were preyed upon than adults in those diets. When examining survival differences by size class across diets,

a significant effect was found in juveniles (log-rank test: $X^2 = 21.8$, $p = 2 \times 10^{-5}$, table 2). That is, in diets where adults lacked animal-based food, we observed higher mortality among juveniles (0-0.7 cm). This is likely driven by increased cannibalistic predation on this size class. However, no significant differences in survival were observed for the other size classes across diets.

Table 2: Log-Rank tests for the effects of diet and size class on marbled crayfish survival.

By diet:

	Poor vegetarian diet	Rich vegetarian diet	Omnivorous diet
Chi-sq	30.6	8.5	10.2
p	4.0×10^{-6}	0.07	0.04

By size class:

	0-0.7 cm, juveniles only	3.6-5.8 cm	1.6-3.5 cm	0.8-1.5 cm	0-0.7 cm
Chi-sq	0.1	0.8	0.8	1.7	21.8
p	1.0	0.7	0.7	0.4	2.0×10^{-5}

Discussion

This study is the first to assess the trophic position of the invasive crayfish *Procambarus virginalis* in the Levant, along with its trophic interactions and potential ecosystem impacts. Understanding the trophic dynamics of invasive species can be an important tool in predicting their ecological consequences following invasion (Gallien and Carboni 2017; Jackson et al., 2017). Our findings provide insights regarding the trophic role of *P. virginalis* at the Levantine invasion sites. Moreover, these insights may also be relevant to other invaded water bodies characterized by periodical drying and lacking indigenous crayfish species and fish that might provide top-down control of crayfish populations. The highest $\delta^{15}N$ values recorded in Tzaanan Pool and Ein Meshotetim were from *P. virginalis*, while in Mekorot Pool, *P. virginalis* had the second highest values among those recorded in this study, suggesting that *P. virginalis* could be one of the top predators at the invasion sites in Israel. Although the $\delta^{15}N$ values of *P. virginalis* and the calculated trophic positions

were high compared to other taxa at all sites, they varied significantly across locations. These findings indicate that *P. virginalis* feeds on different trophic levels at each site, highlighting its high trophic plasticity and establishment potential across diverse environments. Interestingly, $\delta^{15}N$ values of juvenile crayfish differed from those of adults in Mekorot Pool, but not in Tzaanan Pool. A possible explanation might be that Mekorot Pool is a poor habitat, where adults may resort to cannibalism in the absence of abundant, nutritious food sources, leading to a separation in trophic levels between juveniles and adults. In contrast, Tzaanan Pool offers a greater diversity and abundance of food resources, which are likely shared by *P. virginalis* across all size classes, resulting in similar $\delta^{15}N$ values throughout the crayfish ontogeny.

Ontogenetic trophic shifts are common among aquatic species (Mittelbach et al., 1988; Nakazawa 2015). Here we found a significant negative correlation between weight and $\delta^{15}N$ in *P. virginalis* collected in Ein Meshotetim. This may suggest that crayfish on this site do not consume as much animalic food in later stages of their life. An opposite trend was found in crayfish from Tzaanan Pool. This contrasting ontogenetic shift between the two sites may reflect differences in resource availability. Tzaanan Pool is a relatively resource-rich habitat, where a wide variety of potential prey items exist. In such conditions, it is plausible that as *P. virginalis* individuals grow larger, their predatory capacity increases and they incorporate more animal-derived food into their diet, leading to the positive ontogenetic trend observed. Ein Meshotetim, on the other hand, is a resource-poor habitat, with limited options beyond periphyton on the pool walls. Under such constraints, larger individuals may not have access to more protein-rich prey, resulting in the negative correlation between weight and $\delta^{15}N$ detected there. This negative ontogenetic effect is in accordance with other studies showing that juvenile crayfish feed predominantly on zooplankton and invertebrates, whereas adult crayfish consume primarily large amounts of detritus and macrophytes (Stenroth et al., 2006). Nevertheless, it should be noted that these correlations are not particularly strong and may be influenced by a few outliers, so the observed trends should be interpreted with caution.

Biota in ephemeral streams generally exhibit seasonal variation in $\delta^{15}N$ values (Jardine and Cunjak, 2009; Jardine et al., 2014). Accordingly, we detected seasonal differences in the crayfish $\delta^{15}N$ values, with higher values in the summer. Tzaanan Pool experiences a dry period from mid-October to mid-December, and in some years an even longer period, potentially leading to a dietary shift throughout the year. Elevated $\delta^{15}N$ values during summer likely reflect a combination of increased consumption of animal prey (Hellmann et al., 2013), seasonal enrichment of baseline nitrogen pools driven by enhanced microbial denitrification (Lehmann et al., 2004) and intensified internal nitrogen recycling associated with reduced hydrological connectivity (Olin et al., 2013). The mean TP calculated for *P. virginalis* in the invasion sites in Israel was consistent with values reported for several European sites, ranging from 1.82 ± 0.31 (Lipták et al., 2019) to 3.20 (Linzmaier et al., 2020).

The isotopic niche breadth of the crayfish was different across sites. In Ein Meshotetim, the niche breadth was narrower than the other sites, with a SEAc of less than a half than those of Tzaanan Pool and Mekorot Pool. This is consistent with the limited food sources available in the inner pool, which include few gastropods, sparse zooplankton, few occasional fallen leaves, and periphyton, thereby possibly restricting the isotopic diversity in this habitat compared to both other sites. The isotopic niche breadth in Mekorot Pool and Tzaanan Pool is similar, which may be attributed to their proximity and potential connectivity. Mekorot Pool is located approximately one kilometer downstream from Tzaanan Pool. Although the stream is not perennial, crayfish movement between the two pools is plausible during the rainy season (November-March). Furthermore, there may be unidentified invasion sites along the stream between the two pools that could facilitate this connectivity. Nevertheless, when comparing isotopic niche breadth across studies, the SEA_C of Ein Meshotetim falls within the range observed in previous studies (0.09–3.41; Linzmaier et al., 2020; Veselý et al., 2021; Teesalu et al., 2025), while Tzaanan Pool and Mekorot Pool exhibit notably higher SEA_C values, indicating a particularly diverse diet in these sites. The Bayesian mixing model results suggest that *P. virginalis* are omnivorous, consistent with other studies Linzmaier et al., 2020; Veselý et al., 2021; Teesalu et al., 2025). Of the potential food sources included in our models, 1-2 were

primary producers and 2-3 were consumers. In two of the sites, one of the vegetal food sources accounted for 27-36% of the crayfish's diet, representing one of the two primary food sources, while the other primary source was animal-based. In the third site, both primary food sources were animal-based. These findings indicate that *P. virginalis* exhibit omnivorous and flexible feeding behavior in the invasion sites in the Middle East. However, the primary food sources still appear to be predominantly animal-based.

The diet regime experiment, however, suggests a slightly different interpretation. Our results indicate that the survivability of juveniles in the absence of adults was high (>75%) and unaffected by diet across all treatments, indicating that juvenile crayfish do not require animal-based food to survive. In contrast, juvenile survival in the presence of adults was consistently low (<31%) across all diets, pointing to substantial predation pressure. Except in juveniles, non-significant differences in survival were observed across diets and size classes. These findings highlight the possibility that additional factors may be at play, alongside those that we outlined following the isotopic analysis. If survival rates remain largely unaffected by diet over the course of two months, it raises the question of why *P. virginalis* predominantly consume animal-based food sources, as shown in the SIA and the mixing model. It is possible that diet does not directly influence survivability, but instead provides other benefits, such as improved health, growth, or physical condition (Das et al., 2024).

It is important to emphasize the differences, advantages, and limitations of the two approaches. The diet regime experiments do not represent natural prey composition or the integrated dietary patterns over time, as they reflect controlled and short-term conditions. In contrast, stable isotope analysis (SIA) provides an integrated view of assimilated diets but relies on trophic enrichment factors and prey item choices, which may introduce bias and uncertainty.

One of the key factors we believe contributed to the successful establishment of marbled crayfish populations in the ephemeral Beit Guvrin stream, where Tzaanan and Mekorot pools are located, is the absence of natural predators. Previous studies conducted in habitats with fish have shown higher $\delta^{15}N$ values in fish compared with crayfish,

suggesting that they may prey on the crayfish (Lipták et al., 2019; Linzmaier et al., 2020; Veselý et al., 2021). The dry period of the ephemeral stream prevents fish from establishing there and releases the crayfish from potential top-down control. However, the dry season also presents a major challenge for the crayfish. Remarkably, *P. virginalis* is capable of surviving for several months without water by taking refuge in underground burrows within the mud (Dorn and Volin, 2009; Maciaszek et al., 2022). Although many individuals perish during this period, as evidenced by the numerous dead specimens that we observed, it appears that a sufficient number survive to sustain the population.

A primary concern regarding the invasion of *P. virginalis* is its potential impact on amphibian populations. As noted previously, ecological succession is slow at the onset of winter (the wet season), leading to a period during which the pool begins to fill. During this time, crayfish that survived the dry summer are fully active, while other aquatic organisms are still scarce, with many in early developmental stages (e.g., eggs, tadpoles). For example, Israel is the southernmost range limit of the distribution of the banded newt (*Triturus vittatus*), and its population in Israel is critically endangered (Gafny, 2004). The newts typically begin their aquatic phase and reproduction shortly after the pools are filled (Degani et al., 2013). Thus, the paucity of potential prey in the pool at this phase may lead the crayfish to exert excessive predation pressure on the eggs and tadpoles of the newts. This may lead to extirpation of the newt population at this site in the near future.

Numerous eradication strategies have been implemented worldwide to control invasive crayfish, with mixed success. Temporary drainage has proven effective in several habitats in Switzerland, where invasive *P. leniusculus* and *P. clarkii* were present (Krieg et al., 2020). However, in ponds in Britain and Poland this approach resulted only in population suppression rather than eradication (Stebbing et al., 2014). In the case of the ephemeral Beit Guvrin stream, this method may be effective, depending on the timing of drainage and the length of time to which it will extend the dry period that the crayfish must face. Drainage has sometimes been combined with soil treatment using calcium hydroxide, yielding partial success but posing substantial risks to non-target organisms (Krieg et al., 2020). Biocides have also been proposed, yet no crayfish-specific compounds currently exist, and trials using organic treatments (e.g., livestock manure) have failed to achieve

eradication (Stebbing et al., 2014; Krieg et al., 2020). Other approaches, including trapping, hand removal, electricity, and excavation of streambeds during dry periods, have been shown to suppress crayfish populations but have not resulted in complete eradication (Stebbing et al., 2014; Krieg et al., 2020; Caruana et al., 2024).

Biological control through the introduction of predatory fish has been explored as another eradication strategy (Stebbing et al., 2014; Krieg et al., 2020). Many fish species, including native species found in Israel such as *Clarias gariepinus* (Tyser and Douthwaite, 2014), are known to prey on crayfish and could potentially help suppress populations. Like the previous approaches, this approach poses additional challenges, such as the risk of predation on non-target species, potential environmental impacts, and the possibility of predatory fish migrating away from the target area (Stebbing et al., 2014). In Ein Meshotetim, which is an almost completely closed system, this method might be effective. The use of pathogens has also been suggested, but no successful large-scale applications exist, and the ecological risks of pathogen release remain substantial (Simberloff and Stiling, 1996; Messing and Wright, 2006).

Our study raises some questions that will, for now, remain open. Are the invaded ecosystems still undergoing adjustment or reorganization in response to the disturbance caused by the invasion, or have they reached a new stable state? *P. virginalis* was first recorded in Tzaanan Pool in 2018, and in Ein Meshotetim in 2022 (Yanai et al., 2024). The period since then is unlikely to have been sufficient for the systems to reach a new balance, and further ecological changes are likely to occur. Such changes may include shifts in trophic interactions, changes in the abundance and composition of local species and changes to the abiotic components of the ecosystem: *P. virginalis* activity, such as burrowing and sediment disturbance, may directly modify the physical characteristics of the habitat.

Predicting exactly how ecological systems at invasion sites will change in the future, and which species may be harmed or potentially benefit, remains challenging. With the influence of regional climate change, 2025 was one of the driest years on record in the Beit Guvrin area, with Tzaanan Pool drying out just weeks after refilling, rather than

retaining water until the end of the fall, as observed in previous years since the establishment of the crayfish population. This, combined with the manual collection of crayfish from their burrows, raises the possibility that the marbled crayfish population may have been eradicated from this site. Future monitoring could confirm or refute this hypothesis.

Acknowledgements

This study was supported by the Israel Science Foundation (ISF) grants no. 1655/21 to T.G.-H. and no. 1826/20, 1788/25 to O.K., and the Minerva Center for the Study of Population Fragmentation.

We thank Khristina Ermak from the Guy-Haim Lab for her assistance in preparing samples for SIA, and the Kolodny family, Noa Truskanov, Zohar Yanai, Erga Peter and Ofer Friedman for their help in field sampling, and to the members of the Kolodny Lab for their constructive feedback throughout this work.

Author contributions

Conceptualization: Oren Kolodny, Tamar Guy-Haim; Data curation: Rotem Mor, Tamar Guy-Haim; Funding acquisition: Oren Kolodny, Tamar Guy-Haim; Methodology: Tamar Guy-Haim; Project Administration: Oren Kolodny, Tamar Guy-Haim; Software: Tamar Guy-Haim, Rotem Mor; Supervision: Oren Kolodny, Tamar Guy-Haim; Validation: Oren Kolodny, Tamar Guy-Haim; Visualizations: Rotem Mor; Writing – original draft: Rotem Mor, Oren Kolodny, Tamar Guy-Haim; Writing – review and editing: Rotem Mor, Oren Kolodny, Tamar Guy-Haim.

References

Caruana, A., Camilleri, B., Farrugia, L., and Jones, J. P. G. (2024). Mechanical excavation of wetland habitat failed to eradicate invasive American red swamp crayfish (*Procambarus clarkii*) in Malta. *Ecological Solutions and Evidence*, *5*(2). https://doi.org/10.1002/2688-8319.12325

587 Das, K., Roy, K., Mráz, J., Buřič, M. and Kouba, A. (2024). Considerations for protein
588 and amino acids in standardized reference diet for parthenogenetic marbled
589 crayfish *Procambarus virginalis* model organism. *Scientific Reports*, *14*(1), p.16395.

590 Degani, G., Pearlson, O.R.E.N. and Goldberg, T.A.L.I., 2013. Impermanent Breeding
591 Site Selection—Fitness of Gonadal Cycle and Larval Growth of *Triturus vittatus*
592 vittatus (Urodela) from the Southern Limit of Its Distribution. *Amer. Open. Anim.*
593 *J*, *1*, pp.16-30.

594 Dong, Q., and Polis, G. A. (1992). The dynamics of cannibalistic populations: A foraging
595 perspective. In B. J. Crespi and M. A. Elgar (Eds.), *Cannibalism: Ecology and*
596 *Evolution among Diverse Taxa* (pp. 13–37).

597 Dorn, N. J., and Volin, J. C. (2009). Resistance of crayfish (*Procambarus* spp.)
598 populations to wetland drying depends on species and substrate. *Journal of the*
599 *North American Benthological Society*, *28*(4), 766–777. https://doi.org/10.1899/08-
600 151.1

601 Emery-Butcher, H. E., Beatty, S. J., and Robson, B. J. (2020). The impacts of invasive
602 ecosystem engineers in freshwaters: A review. In *Freshwater Biology* (Vol. 65,
603 Issue 5, pp. 999–1015). Blackwell Publishing Ltd. https://doi.org/10.1111/fwb.13479

604 European Commission. (2016). *Commission Implementing Regulation (EU) 2016/1141*.

605 Fox, L. R. (1975). Cannibalism in Natural Populations. *Annual Review of Ecology,*
606 *Evolution, and Systematics* , *6*, 87–106.

607 Gafny, S. (2004). *Threatened amphibians of Israel*. *Endangered Species in Israel: Red*
608 *List of Threatened Animals. Vertebrate (eds Dolev A, Perevolotsky A)*, pp.55-68

609 Gallien, L. and Carboni, M. (2017_. The community ecology of invasive species: where
610 are we and what's next?. *Ecography*, *40*(2), 335-352.

611 Hellmann, C., Wissel, B., and Winkelmann, C. (2013). Omnivores as seasonally
612 important predators in a stream food web. *Freshwater Science*, *32*(2), 548-562.

613 Jackson, A. L., Inger, R., Parnell, A. C., and Bearhop, S. (2011). Comparing isotopic
614 niche widths among and within communities: SIBER - Stable Isotope Bayesian
615 Ellipses in R. *Journal of Animal Ecology*, *80*(3), 595–602.
616 https://doi.org/10.1111/j.1365-2656.2011.01806.x

617 Jackson, M. C., Evangelista, C., Zhao, T., Lecerf, A., Britton, J. R., and Cucherousset,
618 J. (2017). Between-lake variation in the trophic ecology of an invasive crayfish.
619 *Freshwater Biology*, *62*(9), 1501–1510. https://doi.org/10.1111/fwb.12957

620 Jardine, T., and Cunjak, R. A. (2009). Detecting marine nutrient and organic matter
621 inputs into multiple trophic levels in streams of Atlantic Canada and France.
622 *Challenges for diadromous fishes in a dynamic global environment*: proceedings of
623 the international symposium "Challenges for Diadromous Fishes in a Dynamic
624 Global Environment" held in Halifax, Nova Scotia, Canada, June 18-21, 2007.

Jardine, T. D., Hadwen, W. L., Hamilton, S. K., Hladyz, S., Mitrovic, S. M., Kidd, K. A., Tsoi, W. Y., Spears, M., Westhorpe, D. P., Fry, V. M., Sheldon, F., and Bunn, S. E. (2014). Understanding and overcoming baseline isotopic variability in running waters. *River Research and Applications*, *30*(2), 155–165. https://doi.org/10.1002/rra.2630

Krieg, R., King, A., and Zenker, A. (2020). Measures to Control Invasive Crayfish Species in Switzerland: A Success Story? *Frontiers in Environmental Science*, *8*. https://doi.org/10.3389/fenvs.2020.609129

Lehmann, M. F., Bernasconi, S. M., McKenzie, J. A., Barbieri, A., Simona, M., and Veronesi, M. (2004). Seasonal variation of the δC and δN of particulate and dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling in a eutrophic lake. *Limnology and Oceanography*, *49*(2), 415-429.

Linzmaier, S. M., Musseau, C., Matern, S., and Jeschke, J. M. (2020). Trophic ecology of invasive marbled and spiny-cheek crayfish populations. *Biological Invasions*, *22*(11), 3339–3356. https://doi.org/10.1007/s10530-020-02328-z

Lipták, B., Veselý, L., Ercoli, F., Bláha, M., Buřič, M., Ruokonen, T. J., and Kouba, A. (2019). Trophic role of marbled crayfish in a lentic freshwater ecosystem. *Aquatic Invasions*, *14*(2), 299–309. https://doi.org/10.3391/ai.2019.14.2.09

Maciaszek, R., Jabłońska, A., Prati, S., Wróblewski, P., Gruszczyńska, J., and Świderek, W. (2022). Marbled crayfish Procambarus virginalis invades a nature reserve: how to stop further introductions? *European Zoological Journal*, *89*(1), 888–901. https://doi.org/10.1080/24750263.2022.2095046

Maruani, T., and Amit-Cohen, I. (2009). The effectiveness of the protection of riparian landscapes in Israel. *Land Use Policy*, *26*(4), 911–918. https://doi.org/10.1016/j.landusepol.2008.11.002

Messing, R.H. and Wright, M.G. (2006). Biological control of invasive species: solution or pollution?. *Frontiers in Ecology and the Environment*, *4*(3), pp.132-140.

Mittelbach, G.G., Osenberg, C.W. and Leibold, M.A. (1988). Trophic relations and ontogenetic niche shifts in aquatic ecosystems. In *Size-structured populations: ecology and evolution* (pp. 219-235). Berlin, Heidelberg: Springer Berlin Heidelberg.

Nakazawa, T. (2015). Ontogenetic niche shifts matter in community ecology: a review and future perspectives. *Population Ecology*, *57*(2), 347-354.

Olin, J. A., Hussey, N. E., Rush, S. A., Poulakis, G. R., Simpfendorfer, C. A., Heupel, M. R., and Fisk, A. T. (2013). Seasonal variability in stable isotopes of estuarine consumers under different freshwater flow regimes. *Marine Ecology Progress Series*, *487*, 55-69.

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., ... and Semmens, B. X. (2014). Best practices for use of stable isotope mixing models in

food-web studies. *Canadian Journal of Zoology*, 92(10), 823–835. https://doi.org/10.1139/cjz-2014-0127

Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, *83*(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., and Montaña, C. G. (2007). Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, *152*(1), 179–189. https://doi.org/10.1007/s00442-006-0630-x

Pyšek, P., Hulme, P. E., Simberloff, D., Bacher, S., Blackburn, T. M., Carlton, J. T., Dawson, W., Essl, F., Foxcroft, L. C., Genovesi, P., Jeschke, J. M., Kühn, I., Liebhold, A. M., Mandrak, N. E., Meyerson, L. A., Pauchard, A., Pergl, J., Roy, H. E., Seebens, H., … Richardson, D. M. (2020). Scientists' warning on invasive alien species. *Biological Reviews*, *95*(6), 1511–1534. https://doi.org/10.1111/brv.12627

Rilov, G., Canning-Clode, J., and Guy-Haim, T. (2024). Ecological impacts of invasive ecosystem engineers: A global perspective across terrestrial and aquatic systems. In *Functional Ecology* (Vol. 38, Issue 1, pp. 37–51). British Ecological Society. https://doi.org/10.1111/1365-2435.14406

Ruokonen, T. J., Kiljunen, M., Karjalainen, J., and Hämäläinen, H. (2012). Invasive crayfish increase habitat connectivity: a case study in a large boreal lake. *Knowledge and Management of Aquatic Ecosystems*, *407*. https://doi.org/10.1051/kmae/2013034

Stebbing, P., Longshaw, M., and Scott, A. (2014). Review of methods for the management of non-indigenous crayfish, with particular reference to Great Britain. In *Ethology Ecology and Evolution* (Vol. 26, Issues 2–3, pp. 204–231). Universita di Firenze. https://doi.org/10.1080/03949370.2014.908326

Stenroth, P., Holmqvist, N., Nyström, P., Berglund, O., Larsson, P. and Granéli, W. (2006). Stable isotopes as an indicator of diet in omnivorous crayfish (Pacifastacus leniusculus): the influence of tissue, sample treatment, and season. *Canadian Journal of Fisheries and Aquatic Sciences*, *63*(4), pp.821-831. https://doi.org/10.1139/f05-265

Stock, B. C., Jackson, A. L., Ward, E. J., Parnell, A. C., Phillips, D. L., and Semmens, B. X. (2018). Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ*, *2018*(6). https://doi.org/10.7717/peerj.5096

Teesalu, P., Muuga, J.-M., Hurt, M., Kaldre, K., Nõges, T., and Ercoli, F. (2025). Effects of temperature on marbled crayfish (Procambarus virginalis, Lyko 2017) invasion ecology. *Hydrobiologia*. https://doi.org/10.1007/s10750-025-05828-y

Twardochleb, L. A., Olden, J. D., and Larson, E. R. (2013). A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*, *32*(4), 1367–1382. https://doi.org/10.1899/12-203.1

Tyser, A. B., and Douthwaite, R. J. (2014). Predation on invasive redclaw crayfish Cherax quadricarinatus by native fishes in the Kafue River, Zambia. *African Journal of Aquatic Science*, *39*(4), 473–477. https://doi.org/10.2989/16085914.2014.980774

Veselý, L., Ruokonen, T. J., Weiperth, A., Kubec, J., Szajbert, B., Guo, W., Ercoli, F., Bláha, M., Buřič, M., Hämäläinen, H., and Kouba, A. (2021). Trophic niches of three sympatric invasive crayfish of EU concern. *Hydrobiologia*, *848*(3), 727–737. https://doi.org/10.1007/s10750-020-04479-5

Yanai, Z., Guy-Haim, T., Kolodny, O., Levitt-Barmats, Y., Mazal, A., Morov, A. R., Sagi, A., Truskanov, N., and Milstein, D. (2024). An overview of recent introductions of non-native crayfish (Crustacea, Decapoda) into inland water systems in Israel. *BioInvasions Records*, *13*(1), 195–208. https://doi.org/10.3391/bir.2024.13.1.17

Supplementary figures

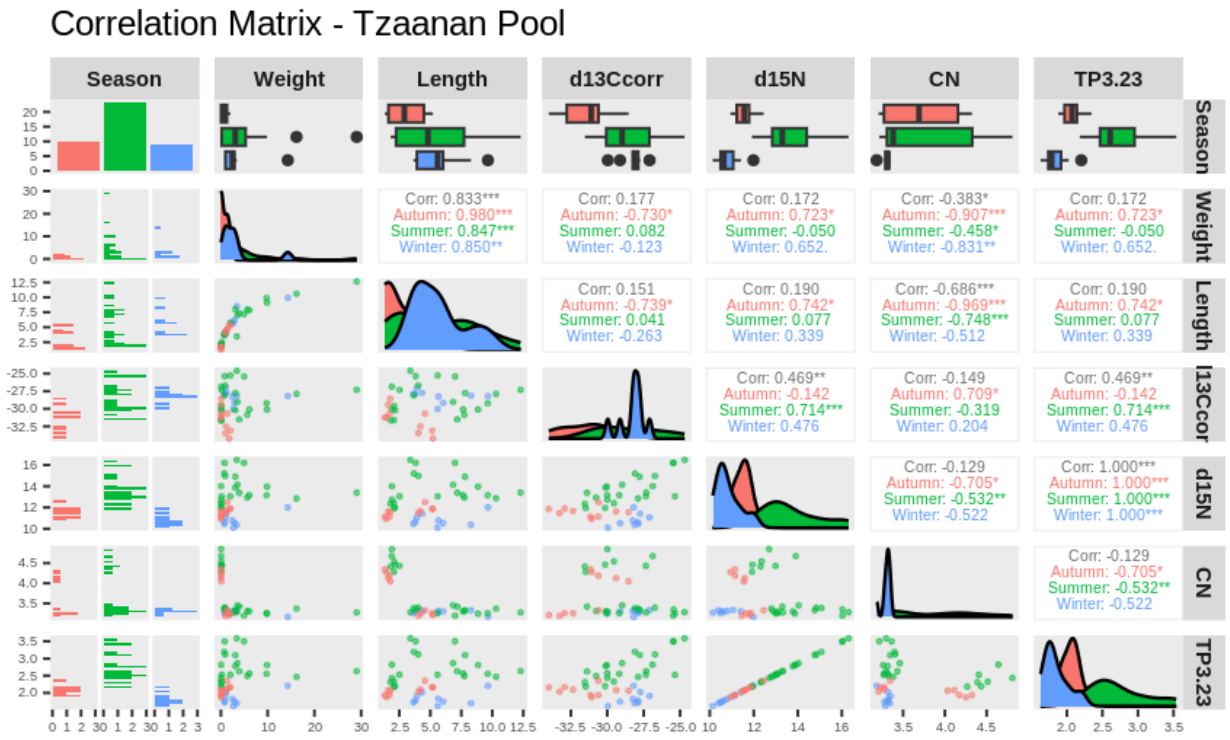


Figure S1. Correlation matrix of isotopic values and other traits for crayfish sampled in Tzaanan Pool across seasons. Distributions are shown on the diagonal, scatter plots bellow, and Pearson correlation coefficients above.

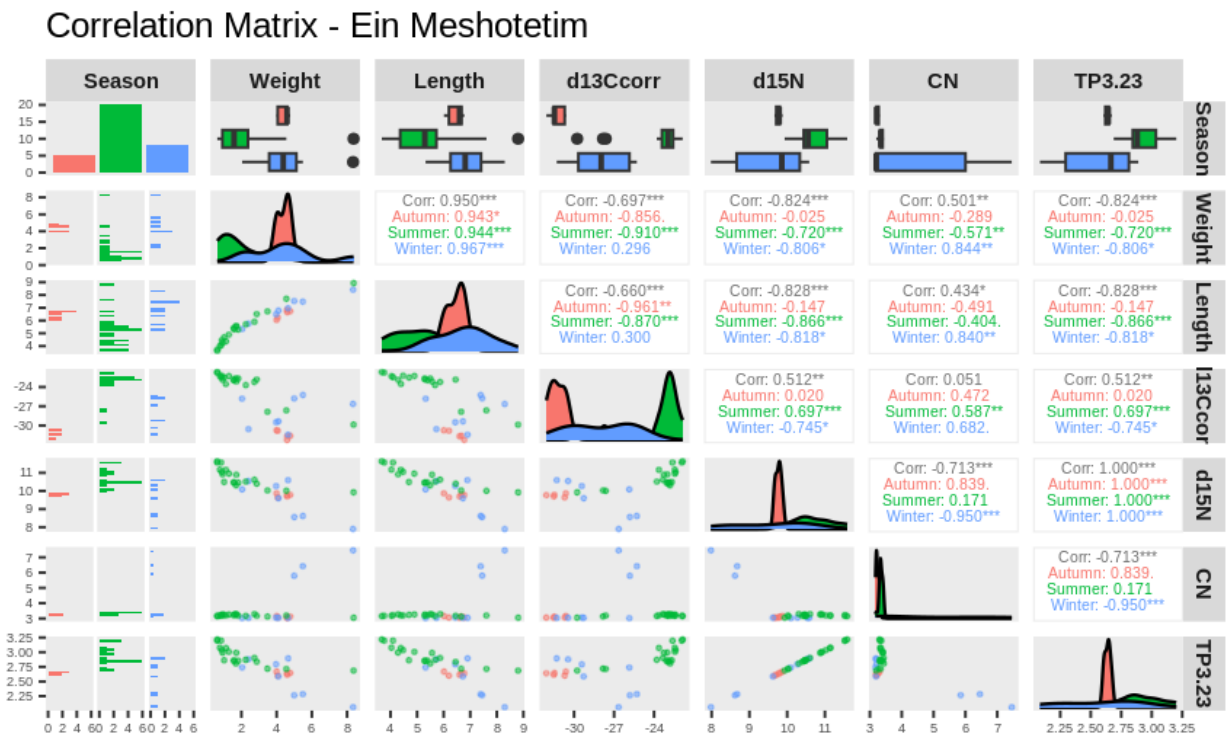


Figure S2. Correlation matrix of isotopic values and other traits for crayfish sampled in Ein Meshotetim across seasons. Distributions are shown on the diagonal, scatter plots bellow, and Pearson correlation coefficients above.

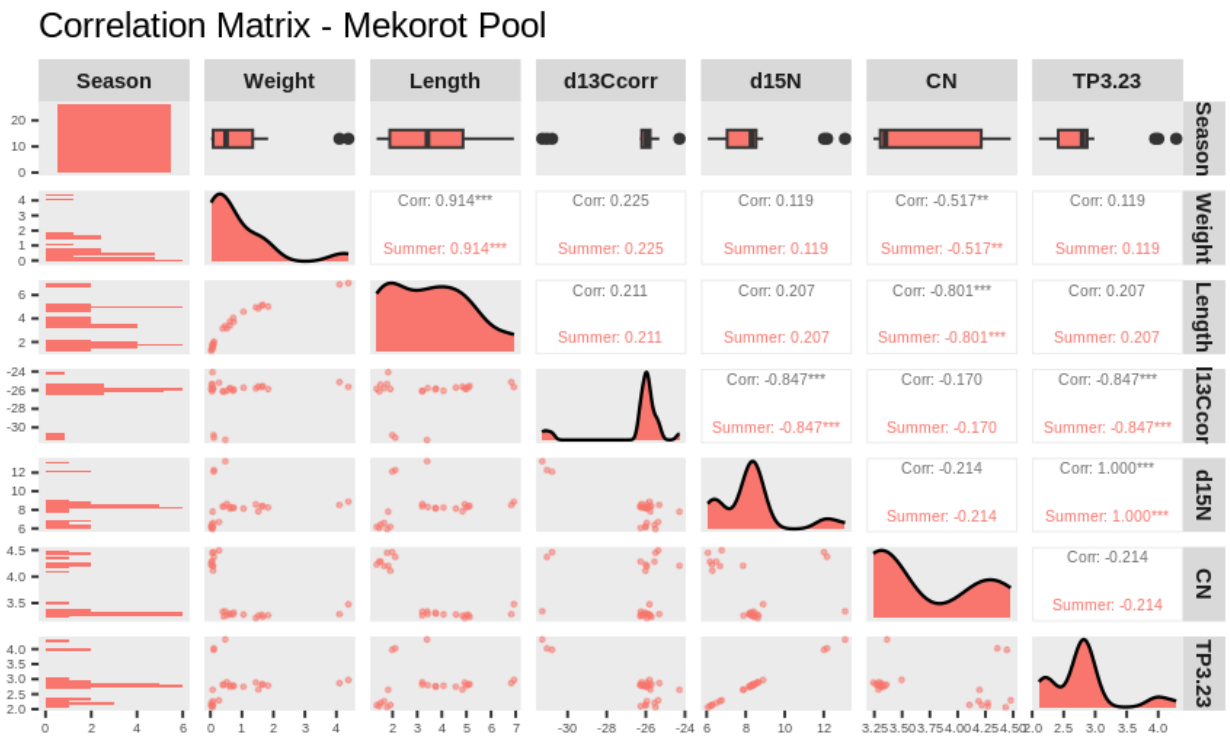


Figure S3. Correlation matrix of isotopic values and other traits for crayfish sampled in Mekorot Pool. Distributions are shown on the diagonal, scatter plots bellow, and Pearson correlation coefficients above.

Figure S4. High crayfish densities observed at Ein Meshotetim on October 15th, 2022, when no population control measures were applied. The photograph illustrates the extent of crowding that occurred in the absence of top-down regulation.