

## Article

# Feeding of the Amphipod *Gammarus aequicauda* in the Presence of the Planktonic Cladoceran *Moina salina* and the Benthic Chironomid Larvae *Baetendipes noctivagus*

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**Abstract:** The energy balance approach helps to understand the limits within which the ‘predator–prey’ relationship can form, but cannot explain the formation of trophic links in natural multispecies systems. A time balance approach to the study of feeding has been developed to complement it. In the hypersaline Lake Moynaki (Crimea) which has a simple food web, there is one omnivorous species, *Gammarus aequicauda*, and two abundant prey, planktonic *Moina salina* and benthic *Baetendipes noctivagus*. The size of *M. salina* ranges from 1.0 to 1.2 mm, and the size of *B. noctivagus* larvae from 9.0 to 10.0 mm. In experiments, elements of time balance were evaluated during feeding of *G. aequicauda* in the presence of both prey species, i.e., the duration of time spent searching before capture, handling prey, and resting between attempts to eat a chironomid larva. In all experiments, *G. aequicauda* consumed both kinds of prey. The maximum predation rate of *G. aequicauda* on the smaller sized *M. salina* was limited by searching and handling time, but on the larger *B. noctivagus* it was limited by digestion time. Feeding solely on chironomid larvae may well satisfy the energy needs of *G. aequicauda*. However, the food ration of *G. aequicauda* may be 50% higher if it feeds in the presence of both prey species than if feeding only on chironomid larvae. The presence of chironomid larvae more than halved the consumption of *M. salina*, compared to when cladocerans were the only prey.

**Keywords:** *Gammarus aequicauda*; *Moina salina*; *Baetendipes noctivagus*; prey–predator relations; hypersaline lake

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## 1. Introduction

Trophic relationships are an important factor determining the functioning of ecosystems [1–3]. Ivlev (1955) clearly established complexity of the interactions affecting prey selectivity and diet size [4]. Even though several diverse studies have been devoted to the formation and dynamics of trophic relationships in aquatic ecosystems [5–8], their main patterns are not completely understood. The energy–balance approach helps to describe the limits within which the ‘predator–prey’ relationship can form [5,9,10], but because averages are used, understanding of the mechanisms for the formation of trophic links in natural multispecies systems has remained poorly understood. To date, several works have shown that the use of several types of prey by a predator can stabilize the system of trophic relationships, while allowing the predator to receive a larger diet [11–15]. V.S. Ivlev pointed out that, to analyze feeding, it is important to take into account time as one of the resources that determine the size of the diet [8,16,17], as well as the ratio of energy spent on searching for and consuming prey by a predator with the energy obtained by consuming this prey [16]. At present, the importance of these factors is recognized and their roles are being studied experimentally [18–22].

Starting with the work of [8,16,17,23], a time balance approach to the study of feeding has been developed [18,20,22,24–28]. The essence of this approach is that it takes a certain amount of time to search, capture, eat and digest one prey, which can be described by a simple equation [22,29]:

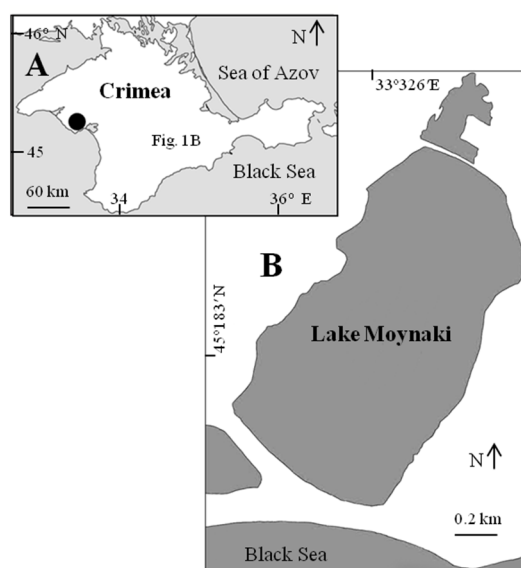
$$C = W/T = W/(T_1 + T_2 + T_3), \quad (1)$$

where  $C$  is the rate of energy intake of a prey item,  $W$  is the mass of one prey,  $T$  is the total time ( $T$ ) spent on various stages of the search and consumption of prey,  $T_1$  is the average duration of the search for the prey before it is captured, and  $T_2$  is handling time spent on attacking, processing, and eating the prey,  $T_3$  is the time spent on digesting the prey.

Each component of the time balance depends on the characteristics of the predator and prey [8,19,30,31]. For example, if the prey is much smaller than the predator, the time spent on eating it will be very small, and this can often be neglected in such cases. If a predator's gut can accommodate many items of prey, then the duration of digestion can be neglected in the equation. Predator consumption rate can be classified into two types [18,32]: handling-limited predation and digestion-limited predation. One predator, as in our case, can demonstrate the first type for smallsize prey, and the second type for large-size prey [21,29].

Ecosystems are typically characterized by the presence of a large number of species and the complexity of trophic relationships in them, which can be complex to investigate. Using the method of stable isotopes allows the establishment and quantification of the available trophic links [33–36], but not the mechanisms that cause their formation and existence. Hypersaline water bodies are characterized by low species diversity and the relative simplicity of trophic networks [6,37]. Therefore, to interpret the formation of trophic relationships in hypersaline water bodies, studies should be carried out on the inhabitants of these water bodies rather than in ecosystems with higher biodiversity.

In Lake Moynaki (Figure 1), one of many similar hypersaline lakes in Crimea, omnivorous *Gammarus aequicauda* (Martynov, 1931) is practically the only facultative predator in the lake; the main basis of its diet is two prey species, one of planktonic cladocerans *Moina salina* (Daday, 1888) and the other of benthic chironomid larvae *Baeotendipes noctivagus* (Kieffer, 1911) [21,29].



**Figure 1.** Map of the hypersaline Lake Moynaki (A) —the Crimean scale, (B) —the local scale).

Our previous studies have shown that *G. aequicauda* consumes both planktonic and benthic animals, suppressing the development of the prey populations to varying de-

grees [21,22]. In previous studies, the feeding of *G. aequicauda* in the presence of only one species of prey (*M. salina* [21] or *B. noctivagus* [29]) was monitored separately, and the balance of time for each case was determined. However, in nature, a predator generally exists together with several types of prey. Consequently, additional data are needed to understand the natural patterns. Previous experiments on the feeding of *G. aequicauda* with two types of prey at once (brine shrimp and chironomid larvae) established that the order of eating different prey also affected the diet size [38].

The objectives of our study were to: 1. measure the difference in the time budget when *G. aequicauda* feeds in the presence of two prey species (*M. salina* and *B. noctivagus*), which are significantly different in size and ecology, 2. compare the obtained characteristics with those when feeding on one type of prey, 3. test the hypotheses that (a) feeding on two types of prey at the same time, greatly differing in size, can significantly increase the total diet of the predator, (b) the presence of a larger prey reduces the pressure of the predator on a smaller prey species.

## 2. Materials and Methods

All animals for experiments were caught in the small hypersaline lake Moynaki (45°10'37" N, 33°18'50" E) (Figure 1), which is described in several articles [21,22,29,39]. The animal diversity is not great in the lake, with only 7–8 invertebrate species [39,40]. The cladocera *M. salina* and the amphipod *G. aequicauda* are the most abundant species in the lake plankton, while the larvae of the chironomid *B. noctivagus* dominate in the benthos. In the lake, abundance of these species ranged in summertime of 2018–2020, for *G. aequicauda*, from 300 to 65,000 ind./m<sup>3</sup>, for *B. noctivagus*, from 120 to 3700 ind./m<sup>2</sup>, and for *M. salina*, from 3500 to 420,000 ind./m<sup>3</sup> [21,29,39,40].

All experiments were conducted during July–September 2021 in the laboratory. Up to 3–4 h passed between the capture of animals in the lake and their placement in experimental vessels. Prior to the start of the experiments, the *G. aequicauda* were kept in vessels without food for a day (24 h). The initial numbers of prey in the experiments were 50 *M. salina* and 1 *B. noctivagus*, combined with 1 *G. aequicauda*. The experiments were carried out in vessels with a diameter of 8 cm and a volume of 500 mL under aeration conditions with a compressor. The compressor was not connected immediately, but only after *G. aequicauda* started eating a chironomid larva, to reduce disturbance to *G. aequicauda* as much as possible. Half of the vessels were completely without sediments and half had a 0.8–1.0 cm layer of sediments (silty sand from the lake). For each experimental trial, there were 30 replicates, which were conducted in different days. The salinity in the vessels did not differ from that in the lake and ranged from 53 to 59 g/L, and the water temperature was between 23 and 26 °C in all experiments. The experiments started in the morning, from 7 am to 10 am, to provide the maximum natural light period for the experiments, which lasted not less 8 h. The size of *M. salina* ranged from 1 to 1.2 mm, and their wet mass was from 0.075 to 0.13 mg, averaging 0.1 mg; the size of a *B. noctivagus* larva was from 9 to 10 mm, and their average wet weight was 4.3 mg, and the size of *G. aequicauda* ranged from 9 to 10 mm, with a wet mass averaging 23.7 mg.

The duration of time spent searching until capture, to eat (handling time), and the rest between different attempts to eat larva were evaluated with a stopwatch [29]. The *M. salina* individuals remaining immediately after the capture of the *B. noctivagus* chironomid by a *G. aequicauda*, but before it started eating the chironomid larva, were transferred with some water into another container, taking care to minimally disturb the *G. aequicauda*. This was simple, as *M. salina* were always concentrated on the surface or in the subsurface layer. It was observed that *G. aequicauda* were not distracted from eating the chironomid larva. *M. salina* were then counted in a Bogorov chamber [21,40] and carefully transferred back to the 0.5 L container, where the *G. aequicauda* was eating the chironomid larva. After the complete consumption of the chironomid larva, *M. salina* were again removed and counted. Subsequently, *M. salina* were also counted after 4 and 8 h.

Average values, as well as standard deviations (SD), coefficients of variation (CV), correlation (R), and determination ( $R^2$ ) coefficients, were calculated in the MS Excel 2007 program. Before the evaluation of the significance of average value differences by Student's  $t$ -test, normality tests [41] were applied. For correlation coefficients, the confidence level ( $p$ ) was determined [42].

### 3. Results

#### 3.1. General Ethological Observations

There was spatial separation between the prey: *M. salina* was concentrated mainly near the surface, with a smaller portion in the middle layer whereas chironomid larvae were always in the near-bottom water layer. *G. aequicauda* used almost all the space in the vessel. Therefore, the strategy of *G. aequicauda* could be different, aimed at searching for and capturing chironomid larvae, i.e., being at the bottom most of the time, or searching for and consuming *M. salina* with an ascent to the upper layers of the water. Different individuals of *G. aequicauda* showed slightly different swimming behavior. Some of them moved mainly in the near-bottom layer of water, while others spent more time in the middle and upper layers. However, not a single individual of *G. aequicauda* spent all of its time at the bottom or in the upper water column. In the process of feeding, the first prey of *G. aequicauda* could be either a chironomid larva or *M. salina*.

#### 3.2. Search and Capture of a Chironomid Larva

In the vessels without bottom sediments, the values of the time to the capture of the chironomid larva by *G. aequicauda* fell clearly into two clusters. In one group, the value varied from 0.2 to 5.0 min (average 1.9 min, CV = 0.77), and in the other, the this varied from 10.5 to 38 min (average 17.5 min, CV = 0.50) (Table A1). The ranges of change in these two clusters did not intersect, and differences were statistically significant ( $p = 0.01$ ). Seventy-two percent of *G. aequicauda* were in the first group. In vessels with sediments, *G. aequicauda* were also divided into two groups according to the time before capture. Thirty-five percent of all subjects were in the fast group (average 1.2 min, CV = 1.05), and 65% were in the other group (average 12.5 min, CV = 0.37). Differences are statistically significant ( $p = 0.01$ ). When chironomid larvae were in vessels with sediments, if they were not captured by *G. aequicauda* in the first 4–5 min, they managed to build a protective cover around themselves. Differences for both groups' values in vessels with and without sediments did not statistically differ significantly, and nor did for average group values.

#### 3.3. Eating a Chironomid Larva

Eating an entire larva (handling time) could be carried out either in one go or in several approaches with interruptions. Both in vessels with sediments and vessels without, about 48% of *Gammarus* ate a whole larva at one approach with a mean consumption time of 49 min in vessels without sediments and 59 min (CV = 0.14) in vessels with them (CV = 0.24) (Table A1). The differences were not statistically significant. Approximately 52% of *G. aequicauda* ate the larva in 1–6 visits (mean = 2) in all variants of the experiments (Table A1). The total duration of eating minus the time for breaks averaged 60 min in vessels with and without sediments (CV = 0.29). In the process of eating the larvae, *G. aequicauda* took breaks; the total duration of breaks in vessels without sediments averaged 63 min (CV = 0.66), i.e., significantly ( $p = 0.001$ ) 70% more than in vessels without sediments (average 38 min, CV = 0.70).

#### 3.4. *Moina salina* Consumption by *Gammarus aequicauda*

Before capturing a chironomid larva, *G. aequicauda* ate a number of *M. salina* in 52% of cases in vessels without sediments and in 76% of cases in vessels with sediments (Table A1), and the differences were statistically significant ( $p = 0.05$ ). Before the capture of a

larva, *G. aequicauda* ate on average 2.3 ind. of *M. salina* in vessels without sediments and 4.1 ind. in a vessel with them, but differences were not statistically significant. Thus, the rate of eating *M. salina* by *G. aequicauda* may be regarded as the same in both variants of the experiment, on average 0.4 ind./min (Table A1). *G. aequicauda*, which had eaten a chironomid larva in one run, began to consume *M. salina* at an average rate of 0.28 ind./min in vessels without sediments, and at a significantly ( $p = 0.05$ ) lower speed of 0.11 ind./min in vessels with sediments (differences were significant,  $p = 0.05$ ). *G. aequicauda* that took breaks in eating a chironomid larva consumed *M. salina* during these breaks. In vessels without sediments, during rest periods, *G. aequicauda* ate an average of 8.3 ind. of *M. salina* in total, and in vessels with sediments, 2.9 ind. of *M. salina*. The *M. salina* consumption rate was practically the same in both cases, at 0.13 ind./min. Thus, during the first 4 h of the experiment, an average of 23 ind. of *M. salina* were eaten in vessels with and without sediments (Table A1). Between hours four and eight of the experiment, *G. aequicauda* ate *M. salina* at a similar rate in both variants of the experiment, 0.06 and 0.05 ind./min, respectively, in vessels without sediments and with them. We note that the maximum consumption rate of *M. salina* by *G. aequicauda* decreased from morning (beginning of the experiment) to the end of the day (end of the experiment) from 0.4 to 0.05–0.06 ind./min (Table A1). Using the data for the period before the capture of chironomid larvae, we assessed the dependence of the rate of eating *M. salina* by *G. aequicauda* on the average concentration of *M. salina* contained in vessels with and without sediments; the relationship was significantly positive ( $R = 0.42$ ,  $p = 0.05$ ). However, as the coefficient of determination (0.17), showed, less than 20% of the variability in the rate of *M. salina* consumption by *G. aequicauda* can be explained by differences in the average concentration of *M. salina*.

#### 4. Discussion

The data do not contradict the previously found results of *G. aequicauda* feeding on *M. salina* [21] and *B. noctivagus* larvae [29] separately. However, in the situation of two prey types at the same time, the quantitative characteristics of feeding behavior changed. For example, when the prey was only chironomid larvae, the time interval before the capture of the prey was shorter than in this study. In particular, in vessels without bottom sediments, it was less than a minute [29], while in the new experiments it was almost five minutes on average. This could be attributed to distribution of attention between the two prey types. The duration of consumption of the chironomid larvae by *G. aequicauda* in all cases of this study was similar to the previous study [29]. It should be noted that the duration of eating of a particular type of prey varied little, and was mainly determined by the capabilities of the predator and the characteristics of the prey [18–22,27]. In general, the authors conclude that the presence of *M. salina* has relatively little effect on the feeding behavior of *G. aequicauda* when consuming chironomid larvae, as previously shown for another prey type, planktonic brine shrimp [22]. Only the duration of the search before capture significantly changed and increased. For *M. salina*, the situation was different. When *G. aequicauda* were not distracted by chironomid larvae, they ate an average of 48 *M. salina* in 4 h (Table A1). In experiments, where there were both *M. salina* and chironomid larvae, *G. aequicauda* ate an average of only 22 individuals of *M. salina* during this time. The presence of chironomid larvae more than halved the consumption of *M. salina*. This was also noted previously in the case of *G. aequicauda* feeding on both brine shrimp and *B. noctivagus* [38]. It is possible to draw the general conclusion from these two results that, when a predator feeds on two prey types, a smaller prey can benefit, i.e., population suppression by a predator at a slower rate.

*G. aequicauda* changed feeding behavior when there were two types of prey (Table A1), in comparison with having only *M. salina* [21]. There were interruptions in the capture of *M. salina*, which were associated with eating a chironomid larva. The rate of *M. salina* consumption by *G. aequicauda*, as shown earlier [21], varied widely, increasing with an increase in the concentration of *M. salina*, from 0.04 to 9.8 ind./hour. The lowest

rate was observed at *M. salina* concentrations of about 20–50 ind./L. The results obtained in this current study were on average higher, and the short-term *M. salina* consumption rates ranged from 3 to 24 ind./hour. The authors cannot unequivocally explain the differences between the new and previous results. Most likely, these can be explained by the following methodological differences between the previous [21] and current experiments, and some other unaccounted for factors. The results obtained earlier probably characterize average daily consumption rates, while the maximum values in the new experiments refer to the morning hours. Our new results may suggest the presence of a diurnal rhythm in the feeding of *G. aequicauda*, with a maximum rate of prey consumption in the morning. The results of the new experiments showed that when calculating the rates of *M. salina* consumption by *G. aequicauda*, the rates obtained for intervals of 4 and 8 h were lower than for certain shorter periods (Table A1). Taking all this into account, it can be assumed that the average daily rates of *M. salina* consumption by *G. aequicauda* can be 3–5 times less than the average morning rates. Diurnal behavioral and feeding rhythms are present in various types of crustaceans, including this species [43–45]. Other reasons for the differences in values are also possible, which may somehow be related to the state of *G. aequicauda*, its high food excitation in the presence of chironomid larvae, and some other unaccounted-for environmental factors. Temperature and salinity in our experiment had lower variability, so it was unlikely that these were the reasons. In our experiments, there was only a slight dependence of the *M. salina* consumption rate on their concentration. Most likely, this was also associated with the higher food excitation of *G. aequicauda* at a relatively low *M. salina* concentration. At low food concentrations, animals often reduce the intensity of feeding, because energy expenditure on searching for and consuming one piece of prey can be greater than the amount of energy received when eating it [16,17,20,21]. The presence of chironomid larvae removes this restriction when feeding on small *M. salina*. Other hypotheses also can be put forward to explain this. However, only one thing can be stated: The processes of trophic relationships are very complex, and we are only beginning to get closer to understanding their complexity and the relationship between causes and effects.

If the gut volume of a predator and the volume of prey are close, we cannot neglect  $T_3$ , the time necessary to digest prey. Given Equation (1), it becomes clear why, after eating one chironomid larva, *G. aequicauda* does not attempt to capture the next larva until 20–120 min have passed [22,29]. For the second larva there is simply no room in the gut. *M. salina* are about 40 times smaller in mass, and *G. aequicauda* can consume these almost continuously, without a break for digestion. So  $T_3$  can be excluded from Equation (1). The rate of *M. salina* consumption by *G. aequicauda* would be inversely proportional to  $(T_1 + T_2)$  and, if  $T_1 \geq T_2$ , directly proportional to the prey concentration [21]. The rate of *B. noctivagus* larvae consumption by *G. aequicauda* is constant in a wide range of *B. noctivagus* concentrations due to  $T_3$  limiting consumption rate [29]. Based on this, the ratio of the components of the time balance during nutrition is determined largely by prey size, which must be taken into account when modeling trophic relationships.

The next question to be discussed is: does the presence of two alternative prey give the predator any energetic benefit over a single prey source? Using already published [29] and new data (Table A1), it can be concluded that the presence of *M. salina* only slightly affected the total chironomid larvae consumption by *G. aequicauda*. If only chironomid larvae are present, the diet of *G. aequicauda* for a long period would consist of only chironomid larvae and would be equal, as may be calculated by Equation (1). In the presence of two prey kinds, *G. aequicauda* would also consume *M. salina* in proportion to their number, additionally to chironomids. For example (Table A1), *G. aequicauda* consumed an average of 22 individuals of *M. salina* in 4 h. Taking into account the difference in body weight of the chironomid larva and *M. salina*, this gives at least a 50% (in mass) increase in the diet of the *G. aequicauda* during this time. In the case that the concentration of chironomid larvae would be significantly lower than that in the experiment, then the role of *M. salina* in the total diet of *G. aequicauda* may be even greater. All of this leads

to an understanding of the ecological role of the predator's use of alternative prey that differs greatly in size. At given concentrations of two prey, the consumption of a larger prey may be energetically more profitable because it allows, on average, the consumption of a large amount of biomass per unit of time. However, with other combinations of prey for *G. aequicauda* (*Artemia* and chironomid larvae), it appeared that the consumption of smaller prey turned out to be more profitable for the predator, but in that case the prey differed in mass much less than in the experiment presented here [22].

*G. aequicauda* feeding on *Artemia* and chironomid larvae is digestion-limited predation [22], but feeding on *M. salina* it is handling-limited predation [21]. Based on this, we assume the following general rule: to obtain some benefit from consuming two alternative prey, a predator needs to have one prey species for digestion-limited predation and another prey species for handling-limited predation. The dependence of the consumption of biomass/energy by the predator on the size of the prey is not linear; it has a more complex form. However, size is also not the only characteristic of the prey that affects the rate of its consumption by the predator [8].

Feeding on chironomid larvae, when there are enough of them, may well satisfy the energy needs of *G. aequicauda* [29]. Specialization in feeding only on them would probably be beneficial for *G. aequicauda* in a constant environment. However, in reality, chironomid larvae pupate and then fly out of the lake en masse and, after that, there are practically no large enough larvae in the lake for a certain period of time. If *G. aequicauda* had targeted and eaten only chironomid larvae, they would starve after the pupation event. In hypersaline water bodies such as Lake Moynaki, there are often periods of near-bottom oxygen deficiency or even anoxic events, and benthic animals, including chironomid larvae, may die en masse. In this situation, *G. aequicauda* would have to switch to planktonic prey, such as *M. salina*. *G. aequicauda* can successfully exist in lakes by feeding only on small planktonic animals [21]. It should be noted that hypersaline waters, like other extreme habitats, are characterized by steep animal population fluctuations [5,40,46,47]. Predators in hypersaline waters, therefore, can sustainably exist only with a wide range of prey of different sizes. *G. aequicauda* is one of the most halotolerant amphipod species [21]. In general, *Gammarus* spp. are omnivorous and, in the absence of a preferable resource, they also can feed on microbial biomass and plant leaves [31,40,48,49]. The size spectrum of its prey is very wide, from small harpacticoids (<0.5 mm) to *Artemia* and chironomid larvae (>10 mm) [21,29]. This is one of the features that allow the species to successfully exist in a rapidly changing environment, from brackish to hypersaline water bodies [21,50]. The predators use alternative prey, plants or detritus to compensate for the sharp fluctuation of single prey species populations; this annual stabilization pattern is common in highly fluctuating environments [51,52].

A wide range of *G. aequicauda* prey also contributes to the stabilization of food webs and the ecosystem as a whole. Small species, like *M. salina*, if they are the only prey, can quickly be reduced by *G. aequicauda* in water bodies [21,22,39]. The presence of large prey can also significantly reduce the pressure of the predator on them, as shown in this study and earlier [38]. Thus, if the predator feeds on different types of prey, it reduces the likelihood of excessive prey predation and loss of smaller prey from the community [38]. General ecosystem resilience also follows this [46,53,54].

The results of these new experiments with and without bottom sediment have shown that sediment can alter the characteristics of feeding behavior of *G. aequicauda* to some extent (Table A1). For example, the probabilities of a chironomid larva and a cladoceran being the first prey were the same in vessels without soil. In vessels with soil, the probability of *Moina* becoming the first prey was significantly higher than that of a chironomid larva. A similar phenomenon was noted by the authors earlier, during other experiments with *G. aequicauda* [29,38]. In the presence of bottom sediments, a chironomid larva, for example, may make the tube around its body, or deeper, bury into the sediment, using that as a defense mechanism [29,38,55]. An increase in habitat complexity (sediments, vegetation, etc.) supports stability of coexistence of prey and predator

species; it may modify feeding behavior and decrease predator success [38,39,55–57]. However, we do not have enough data here to accurately discuss this.

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## Appendix A

**Table A1.** Elements of *Gammarus aequicauda* feeding behavior in the presence of two types of prey, a cladoceran *Moina salina* and a chironomid larva *Baeotendipes noctivagus* (there were 30 replicates of each treatment).

Feeding Behavior Parameters of <i>G. aequicauda</i>	Without Bottom Sediments			With Bottom Sediments		
	Average	Range	CV	Average	Range	CV
<b>Feeding on Chironomid Larva</b>						
Time to capture chironomid larvae, min	4.9	0.2–17.5	1.177	8.7	0.2–15.3	0.766
% of eating the entire larva (handling time) in one time	48.3	–	–	47.8	–	–
Handling time to completely consume one prey, min	49.2	29.5–74.0	0.240	58.8	44.1–70.1	0.140
The number of approaches spent on the complete consumption of one chironomid larva	2.3	1.0–6.0	0.675	2.0	1.0–5.0	0.571
The duration of the time of complete eating in several visits, min	59.6	34.5–112.8	0.379	60.3	52.8–76.3	0.139
The total duration of rest during the period of eating, min	63.3	7.7–168.7	0.657	38.1	2.7–80.8	0.700
<b>Feeding on <i>Moina salina</i></b>						
% cases when <i>Moina</i> was captured before catching a chironomid larva	52.0	–	–	76.0	–	–
The number of <i>Moina</i> eaten before the capture of a chironomid larva, ind.	2.3	0–8.0	1.105	4.1	0–10.0	0.738
The rate of <i>Moina</i> consumption before capturing a chironomid larva, ind./min	0.4	0–1.6	0.765	0.4	0–0.9	0.679



Time spent searching for and handling one <i>Moina</i> , min	2.5	0.63	0.780	2.5	1.1	0.683
The number of <i>Moina</i> consumed after fully eating a chironomid larva before the 4th hour of an experiment, ind.	13.5	9.0–20.0	0.271	19.5	13.0–24.0	0.175
The rate of <i>Moina</i> consumption in the period after larva eating up to 4 experiment hours, ind./min	0.3	0.13–0.41	0.348	0.11	0.07–0.14	0.191
The number of <i>Moina</i> consumed during breaks when eating a chironomid larva, ind.	8.3	4.0–16.0	0.382	2.9	0–8.0	1.116
The rate of <i>Moina</i> consumption during these breaks, ind./min.	0.13	0.04–0.35	0.593	0.08 (0.14 *)	0.01–0.4 *	1.152 *
Average time spent searching for and handling one <i>Moina</i> , during these breaks, min	7.7	2.9–25.0	0.601	12.5 (7.1 *)	100 (2.5 *)	1.202
The number of <i>Moina</i> eaten in the first 4 h of an experiment, ind.	19.7	9.0–32.0	0.347	25.3	13.0–33.0	0.213
The <i>Moina</i> consumption rate by <i>G. aequicauda</i> in the first 4 h of the experiment, ind./min	0.08	0.04–0.13	0.361	0.11	0.05–0.14	0.252
Time spent searching for and handling one <i>Moina</i> in the first 4 h of an experiment, min	12.5	7.7–25.0	0.360	9.1	7.1–20.0	0.254
The number of <i>Moina</i> eaten between 4 and 8 h of an experiment, ind.	15.2	10.0–27.0	0.267	12.4	8.0–19.0	0.225
The <i>Moina</i> consumption rate between 4 and 8 h of experiment, ind./min	0.06	0.04–0.11	0.267	0.05	0.03–0.08	0.226
Time spent searching for and handling one <i>Moina</i> between 4 and 8 h of an experiment, min	16.7	9.1–25.0	0.268	20.0	12.5–33.3	0.231
The total number of <i>Moina</i> eaten in 8 h, ind.	34.9	23.0–44.0	0.160	37.7	23.0–45.0	0.128
Average <i>Moina</i> consumption rate in the whole experiment, ind./min	0.07	0.05–0.09	0.167	0.08	0.05–0.1	0.131

\*—excluding that experiment where *Gammarus* did not consume *Moina* at that time.

## References

1. Poisot, T.; Mouquet, N.; Gravel, D. Trophic complementarity drives the biodiversity–ecosystem functioning relationship in food webs. *Ecol. Lett.* **2013**, *16*, 853–861.
2. Barnes, A.D.; Jochum, M.; Lefcheck, J.S.; Eisenhauer, N.; Scherber, C.; O'Connor, M.I.; de Ruiter, P.; Brose, U. Energy flux: The link between multitrophic biodiversity and ecosystem functioning. *Trends Ecol. Evol.* **2018**, *33*, 186–197.
3. Dahlin, K.M.; Zarnetske, P.L.; Read, Q.D.; Twardochleb, L.A.; Kamoske, A.G.; Cheruvilil, K.S.; Soranno, P.A. Linking terrestrial and aquatic biodiversity to ecosystem function across scales, trophic levels, and realms. *Front. Environ. Sci.* **2021**, *9*, 692401.
4. Ivlev, V.S. *Experimental Ecology of the Feeding of Fishes*; Yale University Press: New Haven, CT, USA, 1961; 302p.

5. Hildrew, A.G.; Raffaelli, D.G.; Edmonds-Brown, R. *Body Size: The Structure and Function of Aquatic Ecosystems*; Cambridge University Press: Cambridge, UK, 2007; 356p.
6. Hammer, U.T. *Saline Lake Ecosystems of the World*; Junk Publishers: Dordrecht, Netherlands, 1986; 616p.
7. Flood, P.J.; Duran, A.; Barton, M.; Mercado-Molina, A.E.; Trexler, J.C. Invasion impacts on functions and services of aquatic ecosystems. *Hydrobiologia* **2020**, *847*, 1571–1586.
8. Kwak, I.S.; Park, Y.S. Food chains and food webs in aquatic ecosystems. *Appl. Sci.* **2020**, *10*, 5012.
9. Winberg, G.G. Diversity and unity of life phenomena and quantitative methods in biology. *Zh. Obshch. Biol.* **1981**, *13*, 5–19 (in Russian)
10. Alimov, A.F.; Bogatov, V.V.; Golubkov, S.M. *Production Hydrobiology*; Nauka: Saint-Petersburg, Russia, 2013; 343p. (in Russian)
11. Kalinkat, G.; Rall, B.C.; Vucic-Pestic, O.; Brose, U. The allometry of prey preferences. *PLoS ONE* **2011**, *6*, e25937.
12. Egilmez, H.I.; Morozov, A.Y. Tri-trophic plankton models revised: Importance of space, food web structure and functional response parametrisation. *Math. Model. Nat. Phenom.* **2016**, *11*, 16–33.
13. Schneider, F.D.; Brose, U.; Rall, B.C.; Guill, C. Animal diversity and ecosystem functioning in dynamic food webs. *Nat. Commun.* **2016**, *7*, 12718.
14. Nordberg, E.J.; Schwarzkopf, L. Predation risk is a function of alternative prey availability rather than predator abundance in a tropical savanna woodland ecosystem. *Sci. Rep.* **2019**, *9*, 7718.
15. Saha, N.; Kundu, M.; Saha, G.K.; Aditya, G. Alternative prey influence the predation of mosquito larvae by three water bug species (Heteroptera: Nepidae). *Limnol. Rev.* **2020**, *20*, 173–184.
16. Ivlev, V.S. Hunting time and the path traveled by the predator in relation to the abundance of prey populations. *Zool. Zhurn.* **1944**, *23*, 139–145 (in Russian)
17. Ivlev, V.S. On the utilization of food by planktophage fishes. *Bull. Math. Biophys.* **1960**, *22*, 371–389.
18. Jeschke, J.M.; Kopp, M.; Tollrian, R. Predator functional responses: Discriminating between handling and digesting prey. *Ecol. Monogr.* **2002**, *72*, 95–112.
19. Van Gils, J.A.; Piersma, T. Digestively constrained predators evade the cost of interference competition. *J. Anim. Ecol.* **2004**, *73*, 386–398.
20. Tiselius, P.; Saiz, E.; Kiørboe, T. Sensory capabilities and food capture of two small copepods, *Paracalanus parvus* and *Pseudocalanus* sp. *Limnol. Oceanogr.* **2013**, *58*, 1657–1666.
21. Shadrin, N.; Yakovenko, V.; Anufrieva, E. *Gammarus aequicauda* and *Moina salina* in the Crimean saline waters: New experimental and field data on their trophic relation. *Aquac. Res.* **2020**, *51*, 3091–3099.
22. Shadrin, N.; Yakovenko, V.; Anufrieva, E. Behavior of *Gammarus aequicauda* (Crustacea, Amphipoda) during predation on *Artemia* (Crustacea, Anostraca): New experimental results. *Int. Rev. Hydrobiol.* **2020**, *105*, 143–150.
23. Rashevsky, N. Some remarks on the mathematical theory of nutrition of fishes. *Bull. Math. Biophys.* **1959**, *21*, 161–183.
24. MacArthur, R.H.; Pianka, E.R. On optimal use of a patchy environment. *Am. Nat.* **1966**, *100*, 603–609.
25. Ostrovskaya, N.A. Mathematical model of food consumption by planktonic animals. *Biol. Morya* **1975**, *33*, 22–27 (in Russian)
26. Charnov, E.L. Optimal foraging, the marginal value theorem. *Theor. Popul. Biol.* **1976**, *9*, 129–136.
27. Shadrin, N.V. Some theoretical aspects of copepod nutrition. In *Ecology of Marine Organisms*; Naukova Dumka: Kyiv, Ukraine, 1981; pp. 38–44 (in Russian)
28. Vilenkin, B.Ya.; Berezkina, E.V. Experimental estimation of the time spent on food consumption in the process of feeding *Puntius arulius* (Pisces, Cyprinidae). *Zool. Zhurn.* **1989**, *68*, 94–102 (in Russian)
29. Shadrin, N.; Yakovenko, V.; Anufrieva, E. Can *Gammarus aequicauda* (Amphipoda) suppress a population of *Baeotendipes noctivagus* (Chironomidae) in a hypersaline lake? A case of Lake Moynaki (Crimea). *Aquac. Res.* **2021**, *52*, 1705–1714.
30. Souna, F.; Lakmeche, A.; Djilali, S. The effect of the defensive strategy taken by the prey on predator—Prey interaction. *J. Appl. Math. Comput.* **2020**, *64*, 665–690.
31. Cozzoli, F.; Shokri, M.; Boulamail, S.; Marrocco, V.; Vignes, F.; Basset, A. The size dependency of foraging behaviour: An empirical test performed on aquatic amphipods. *Oecologia* **2022**, *199*, 377–386.
32. Sentis, A.; Hemptinne, J.L.; Brodeur, J. Parsing handling time into its components: Implications for responses to a temperature gradient. *Ecology* **2013**, *94*, 1675–1680.
33. Michener, R.H.; Kaufman, L. Stable isotope ratios as tracers in marine food webs: An update. In *Stable Isotopes in Ecology and Environmental Science*; Michener, R., Lajtha, K., Eds.; Blackwell Publishing: Oxford, UK; pp. 238–282.
34. Vander Zanden, H.B.; Soto, D.X.; Bowen, G.J.; Hobson, K.A. Expanding the isotopic toolbox: Applications of hydrogen and oxygen stable isotope ratios to food web studies. *Front. Ecol. Evol.* **2016**, *4*, 20.
35. McCormack, S.A.; Trebilco, R.; Melbourne-Thomas, J.; Blanchard, J.L.; Fulton, E.A.; Constable, A. Using stable isotope data to advance marine food web modelling. *Rev. Fish Biol. Fish.* **2019**, *29*, 277–296.
36. Saccò, M.; Humphreys, W.F.; Stevens, N.; Jones, M.R.; Takulis, F.; Thomas, E.; Blyth, A.J. Subterranean carbon flows from source to stygofauna: A case study on the atyid shrimp *Stygiocaris stylifera* (Holthuis, 1960) from Barrow Island (WA). *Isot. Environ. Health Stud.* **2022**, *58*, 247–257.
37. Saccò, M.; White, N.E.; Harrod, C.; Salazar, G.; Aguilar, P.; Cubillos, C.F.; Meredith, K.; Baxter, B.K.; Oren, A.; Anufrieva, E.; et al. Salt to conserve: A review on the ecology and preservation of hypersaline ecosystems. *Biol. Rev.* **2021**, *96*, 2828–2850.
38. Shadrin, N.; Yakovenko, V.; Anufrieva, E. Feeding behavior of *Gammarus aequicauda* in the presence of two prey species of *Artemia* sp. and *Baeotendipes noctivagus*. *J. Exp. Zool. A: Ecol. Integr. Physiol.* **2022**, *337*, 768–775.

39. Shadrin, N.; Yakovenko, V.; Anufrieva, E. Suppression of *Artemia* spp. (Crustacea, Anostraca) populations by predators in the Crimean hypersaline lakes: A review of the evidence. *Int. Rev. Hydrobiol.* **2019**, *104*, 5–13.
40. Shadrin, N.V.; Yakovenko, V.A.; Anufrieva, E.V. Appearance of a new species of Cladocera (Anomopoda, Chydoridae, Bosminidae) in the hypersaline Moynaki Lake, Crimea. *Biol. Bull.* **2021**, *48*, 934–937.
41. Thode, H.C. *Testing for Normality*; Marcel Dekker Inc.: New York, NY, USA, 2002; 368p.
42. Müller, P.H.; Neuman, P.; Storm, R. *Tafeln Der Mathematischen Statistik*; VEB Fachbuchverlag: Leipzig, Germany, 1979; 280p.
43. Morillo-Velarde, P.S.; Lloret, J.; Marín, A.; Sánchez-Vázquez, F.J. Effects of cadmium on locomotor activity rhythms of the amphipod *Gammarus aequicauda*. *Arch. Environ. Contam. Toxicol.* **2011**, *60*, 444–451.
44. de Azevedo Carvalho, D.; Collins, P.A.; De Bonis, C.J. The diel feeding rhythm of the freshwater crab *Trichodactylus borellianus* (Decapoda: Brachyura) in mesocosm and natural conditions. *Mar. Freshw. Behav. Physiol.* **2013**, *46*, 89–104.
45. Santos, A.D.; López-Olmeda, J.F.; Sánchez-Vázquez, F.J.; Fortes-Silva, R. Synchronization to light and mealtime of the circadian rhythms of self-feeding behavior and locomotor activity of white shrimps (*Litopenaeus vannamei*). *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2016**, *199*, 54–61.
46. Hemraj, D.A.; Hossain, M.A.; Ye, Q.; Qin, J.G.; Leterme, S.C. Plankton bioindicators of environmental conditions in coastal lagoons. *Estuar. Coast. Shelf Sci.* **2017**, *184*, 102–114.
47. Anufrieva, E.; Kolesnikova, E.; Revkova, T.; Shadrin, N. Spatio-temporal variability of zooplankton and zoobenthos as the elements of integrated zoocenosis in a marine lake (Crimea, Black Sea): What is a general pattern? *J. Sea Res.* **2022**, *185*, 102231.
48. Kelly, D.W.; Dick, J.T.; Montgomery, W.I. The functional role of *Gammarus* (Crustacea, Amphipoda): Shredders, predators, or both? *Hydrobiologia* **2002**, *485*, 199–203.
49. Georgievová, B.; Zhai, M.; Bojková, J.; Šorfová, V.; Syrovátka, V.; Polášková, V.; Schenková, J.; Horsák, M. Does predation by the omnivorous *Gammarus fossarum* affect small-scale distribution of macroinvertebrates? A case study from a calcareous spring fen. *Int. Rev. Hydrobiol.* **2020**, *105*, 162–170.
50. Evans, E.W.; Stevenson, A.T.; Richards, D.R. Essential versus alternative foods of insect predators: Benefits of a mixed diet. *Oecologia* **1999**, *121*, 107–112.
51. Harwood, J.D.; Obrycki, J.J. *The Role of Alternative Prey in Sustaining Predator Populations*. Second International Symposium on Biological Control of Arthropods: Davos, Switzerland, 2005; pp. 453–462.
52. Remonti, L.; Balestrieri, A.; Raubenheimer, D.; Saino, N. Functional implications of omnivory for dietary nutrient balance. *Oikos* **2016**, *125*, 1233–1240.
53. Layman, C.A.; Quattrochi, J.P.; Peyer, C.M.; Allgeier, J.E. Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecol. Lett.* **2007**, *10*, 937–944.
54. Breaux, N.; Lebreton, B.; Palmer, T.A.; Guillou, G.; Pollack, J.B. Ecosystem resilience following salinity change in a hypersaline estuary. *Estuar. Coast. Shelf Sci.* **2019**, *225*, 106258.
55. Hölker, F.; Stief, P. Adaptive behaviour of chironomid larvae (*Chironomus riparius*) in response to chemical stimuli from predators and resource density. *Behav. Ecol. Sociobiol.* **2005**, *58*, 256–263.
56. González, M.J.; Burkart, G.A. Effects of food type, habitat, and fish predation on the relative abundance of two amphipod species, *Gammarus fasciatus* and *Echinogammarus ischnus*. *J. Great Lakes Res.* **2004**, *30*, 100–113.
57. Médoc, V.; Albert, H.; Spataro, T. Functional response comparisons among freshwater amphipods: Ratio-dependence and higher predation for *Gammarus pulex* compared to the non-natives *Dikerogammarus villosus* and *Echinogammarus berilloni*. *Biol. Invasions.* **2015**, *17*, 3625–3637.