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Assessing Local Distribution of Alien Ponto-Caspian Mysids in Lithuanian Waters, the Baltic Sea Basin: Do Sampling Method and Time Matter?

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Abstract: Alien Ponto-Caspian mysids *Paramysis lacustris*, *Limnomysis benedeni* and *Hemimysis anomala* were introduced into Lithuanian waters from Ukraine's water reservoirs in the early 1960s. Their expansion from the primary introduction site in the Kaunas Reservoir, located on the Nemunas River, proceeded through secondary introductions and natural dispersal. The two species, *P. lacustris* and *L. benedeni*, are currently quite widespread in Lithuanian waters, whereas *H. anomala* has been observed exclusively in the Kaunas Reservoir until recently. Here, we present data from the most recent comprehensive survey of Ponto Caspian mysids and analyze the impact of sampling method on the likelihood of species detection. The results clearly indicate that the detection of larger-sized, more mobile species with good swimming abilities, such as *P. lacustris*, requires, in addition to conventional macroinvertebrate sampling, the use of devices designed to capture active nektobenthic animals. For this purpose, an epibenthic dredge or sledge is recommended. In contrast, the detection probability of the smaller-sized *L. benedeni* was not affected by the sampling method. The recent detection of the bloody-red mysid *H. anomala* near the Nemunas Delta suggests it may now be well-established in the area. However, due to its nocturnal lifestyle, the effective detection of this mysid requires dusk or nighttime sampling using equipment appropriate for capturing nektobenthic fauna.

Keywords: Ponto-Caspian; mysid; inland waters; alien; sampling method; nocturnality; crypsis

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1. Introduction

Ponto-Caspian mysids first invaded the Baltic Sea basin due to deliberate translocation from Ukraine's water reservoirs [1,2]. The three species, *Paramysis lacustris* (Czerniavsky, 1882), *Limnomysis benedeni* Czerniavsky, 1882, and *Hemimysis anomala* G.O. Sars, 1907, had been introduced into the Kaunas Reservoir located on the Nemunas River in 1960–1961 [1,3]. The establishment of populations of *P. lacustris* and *L. benedeni* (Figure 1) was documented in the year following their introduction [4]. Later, these species expanded across Lithuanian inland waters due to natural dispersal and secondary deliberate translocations into numerous lakes in an attempt to improve fish food bases [5]. The survival of the last mysid *H. anomala* in the Kaunas Reservoir was confirmed in 1967 by its detection at 18–20 m depth. Later, the bloody-red mysid *H. anomala* was only recorded from the Kaunas Reservoir and the Nemunas bed up to 50 km above the dam [1,6].

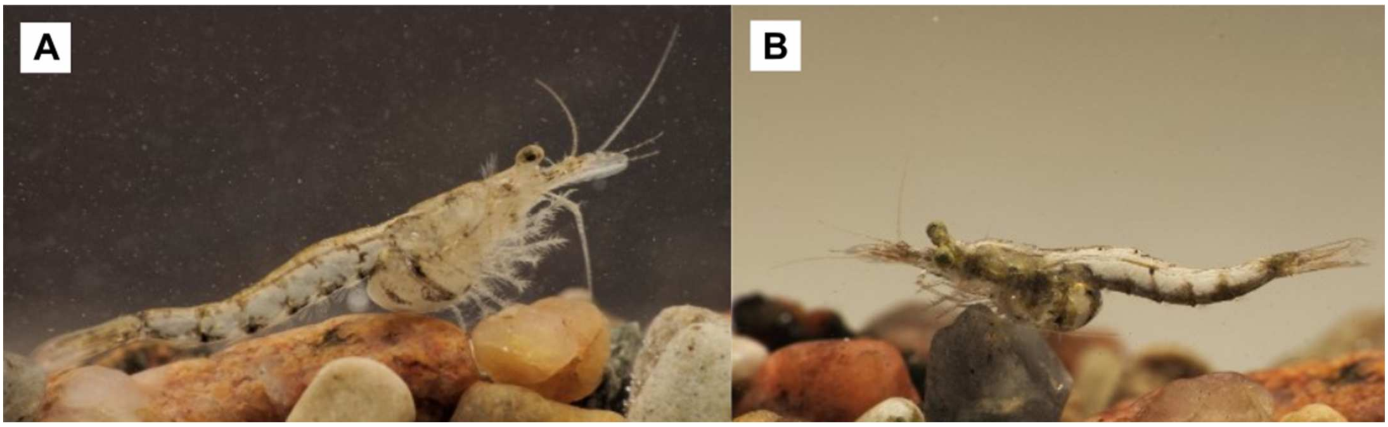

Figure 1. Ponto-Caspian mysids *Paramysis lacustris* (**A**) and *Limnomysis benedeni* (**B**). Photographs by D. Copilaș-Ciocianu.

Recently, *P. lacustris* has been the most widespread Ponto-Caspian mysid species in Lithuanian waters occurring in rivers, lakes, water reservoirs, and the Curonian Lagoon. Another mysid *L. benedeni* has narrower local distribution, mainly inhabiting large rivers, the lagoon, and a few lakes. Finally, the last species, *H. anomala*, has only been observed since its introduction in the Kaunas Reservoir [3,7]. However, this species is widely distributed in the Baltic Sea, and based on molecular investigation, it was concluded that these invaders of the sea are descendants of the population from the Kaunas Reservoir [8].

In contrast to local distribution, globally, the bloody-red mysid *H. anomala* is the most widespread Ponto-Caspian mysid species. This species, which originates from the Ponto-Caspian region including the freshwater and brackish waters of the Black Sea, the Sea of Azov, the eastern Caspian Sea and their basins, is currently widely distributed throughout the European mainland and the Baltic Sea and has invaded the United Kingdom and even North America, including the Laurentian Great Lakes and surrounding lakes and rivers [8–11]. The mysid *L. benedeni* is also widely distributed in European inland waters [9,12], whereas *P. lacustris* has the narrowest documented invasive distribution. This species is known mainly from the Baltic States, where it was deliberately introduced [13], and its presence has been reported in Belarus [14], the Middle Danube [15,16], and Germany [17].

The contrasting patterns of global and local distribution of the three mysis species deliberately introduced into Lithuanian waters may be linked to differences in their dispersal potential. On the other hand, detectability may vary among mysid species due to differences in their biological traits, behavioral patterns, and environmental preferences. As a result, distribution data for certain species may be incomplete due to limitations in sampling methods. In shallow waters, macroinvertebrates are typically collected using a dip net; however, this method may be inadequate for detecting mobile mysid species capable of evading the sampling gear. Therefore, the application of reliable sampling technique to detect mysids, as well as other invasive species, is of primary importance, especially during the early stages of an invasion, when control and eradication measures may still be feasible. Robust sampling methods are critical for monitoring the distribution of alien species, particularly at potential invasion gateways. The purpose of this study was to present the results of the most recent and comprehensive survey of Ponto-Caspian mysid species in the Nemunas River basin. This survey employed two sampling devices: a dip net and an epibenthic dredge. The use of different devices revealed variation in mysid catchability. Consequently, a secondary objective of this study was to conduct a retrospective analysis to test the impact of the sampling method on the probability of detecting mysid species. This study also analyzed the most recent data on the occurrence of *H. anomala* in Lithuanian inland waters.

2. Materials and Methods

The survey of macroinvertebrate assemblages in the Nemunas River basin was undertaken in the late season of 2015. The material was collected using the two methods. Firstly, macroinvertebrates were collected using a standard dip net (25 × 25 cm opening, 0.5 mm mesh size) with a 10-min sampling effort (5-min kick sampling of stony/pebbly bottoms, and 5-min sweep sampling of submerged vegetation) [18]. Secondly, nektobenthic macroinvertebrates were collected using a modified 60-cm-wide epibenthic dredge with a 0.5-mm mesh net; the dredge was slowly pulled (~10 m min^{-1}) for a ~20-m distance. Both types of samples were collected in the wadable nearshore zone, i.e., at 0.3–1.5 m depth. From the collected material, only information on Ponto-Caspian mysids will be presented here.

In October 2012, a dense culture of *H. anomala* was discovered in a 'monk' outlet of a pond located in the Kintai fish farm [19]. The farm is set on the bank of the Minija River (adjacent to the Nemunas Delta) and is fed by its waters. The detection of the species within the fish farm suggested that *H. anomala* may also be present in nearby natural environments. Thus, in 2015 and 2016, we took samples at nighttime using epibenthic dredging in the Minija River. The results of these samplings, along with those from dip net sampling in the Šyša River in 2018, a tributary of the Nemunas River where *H. anomala* was detected, are presented in this study.

To analyze the impact of sampling method, i.e., dip net vs. epibenthic dredging, on the probability of detecting the aforementioned species, data from 2015 were combined with data from 2007–2008 at study sites where both methods were applied simultaneously (see Table S1). To test the effects of the sampling method and relative abundance on the detection probability (based on presence–absence data) of the mysid species *P. lacustris* and *L. benedeni*, logistic regression and classification tree analysis [20,21] were performed in the R 4.1.2 environment [22]. To compare the efficiency of different sampling methods between mysid species, medians and means were calculated, and the Wilcoxon matched-pairs test was applied to assess the difference between the means.

3. Results

Combined sampling using a dip net and a dredge revealed the presence of two mysid species, *P. lacustris* and *L. benedeni*, in the Nemunas River and several of its tributaries. In general, *L. benedeni* dominated numerically in the lower Nemunas, while *P. lacustris* was more prevalent in the middle reaches of the river and was almost the only mysid species found upstream the Kaunas Reservoir (Figure 2 and Table S1). However, this mysid survey also indicated that *L. benedeni* likely inhabits the Nemunas River upstream of the Kaunas Reservoir, as the species was detected at a study site above the town of Alytus. A new finding on its distribution is that *L. benedeni* is well-established in the middle reaches of the Šešupė River (Figure 2).

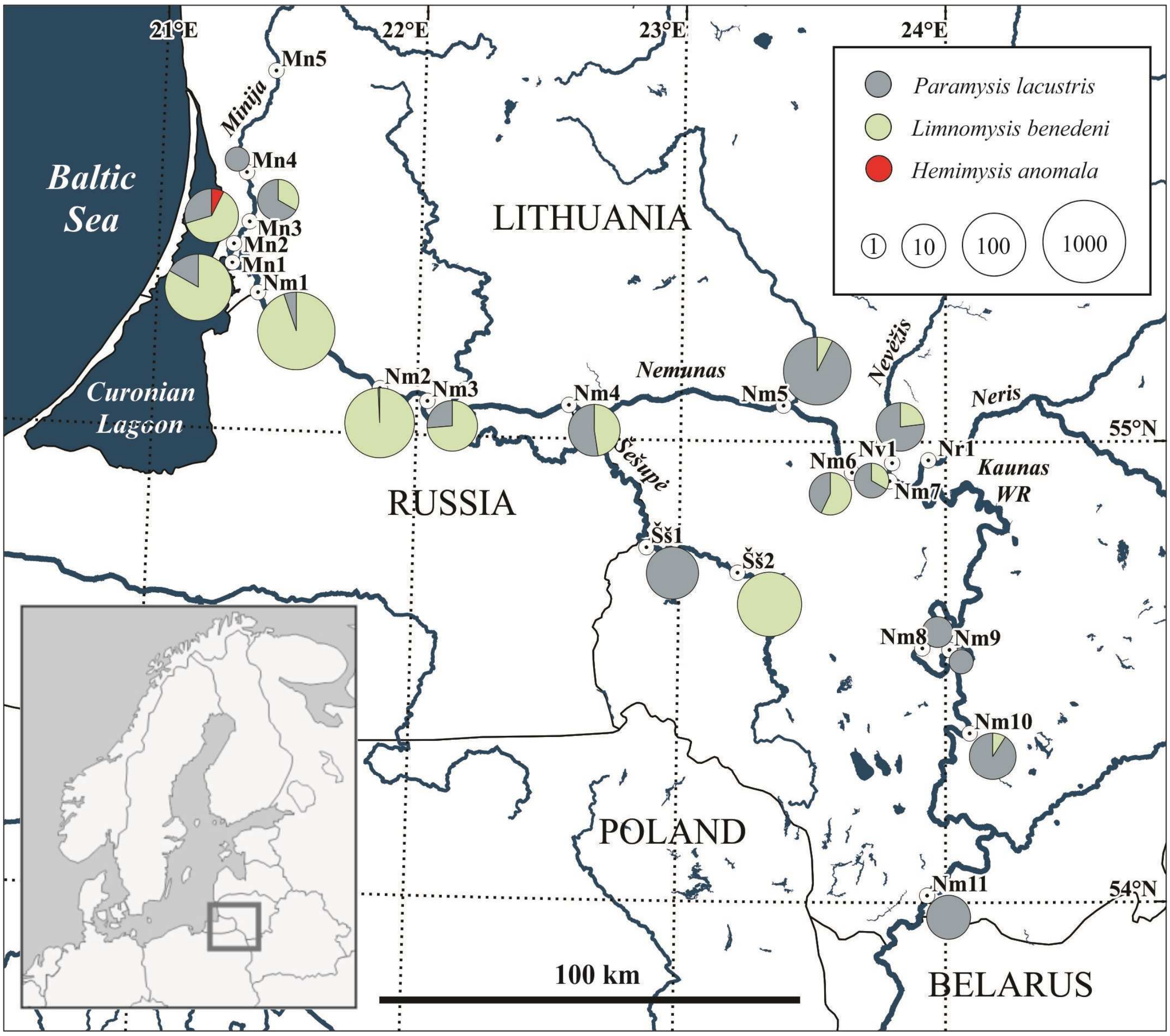

Figure 2. Occurrence and proportions of Ponto-Caspian mysids *Paramysis lacustris*, *Limnomysis benedeni*, and *Hemimysis anomala* in the Nemunas River basin according to the survey of 2015. The shown data were obtained from dredging. The size of the circles corresponds to mysid relative abundance in ind. per 10 m dredging (see Table S1).

The only night-time sampling during the 2015 survey was conducted at the Mn2 site (Minija River at Povilai), which is located near the original record site of *H. anomala* at the Kintai fish farm (55°23′49.44″ E, 21°15′59.95″ N). As expected, dredging at this site indeed resulted in detection of four juvenile specimens of the bloody-red mysid (Figure 3). *Hemimysis anomala* comprised 8% of all caught mysids, among which there were *L. benedeni* and *P. lacustris* (Table S1). On 21 June 2016, another nighttime dredge sampling was conducted from a boat in the Minija River. This resulted in the collection of 10 additional *H. anomala* specimens. The last record of the bloody-red mysid *H. anomala* comes from a dip net sample collected in the Šyša River, within the town of Šilutė (also adjacent to the Nemunas Delta) on 21 September 2018 (Figure 3). Three juvenile specimens of *H. anomala* were found alongside *L. benedeni* and *P. lacustris* (Table S1) in a sweep sample collected from underwater vegetation, including tree roots protruding from a steep riverbank.

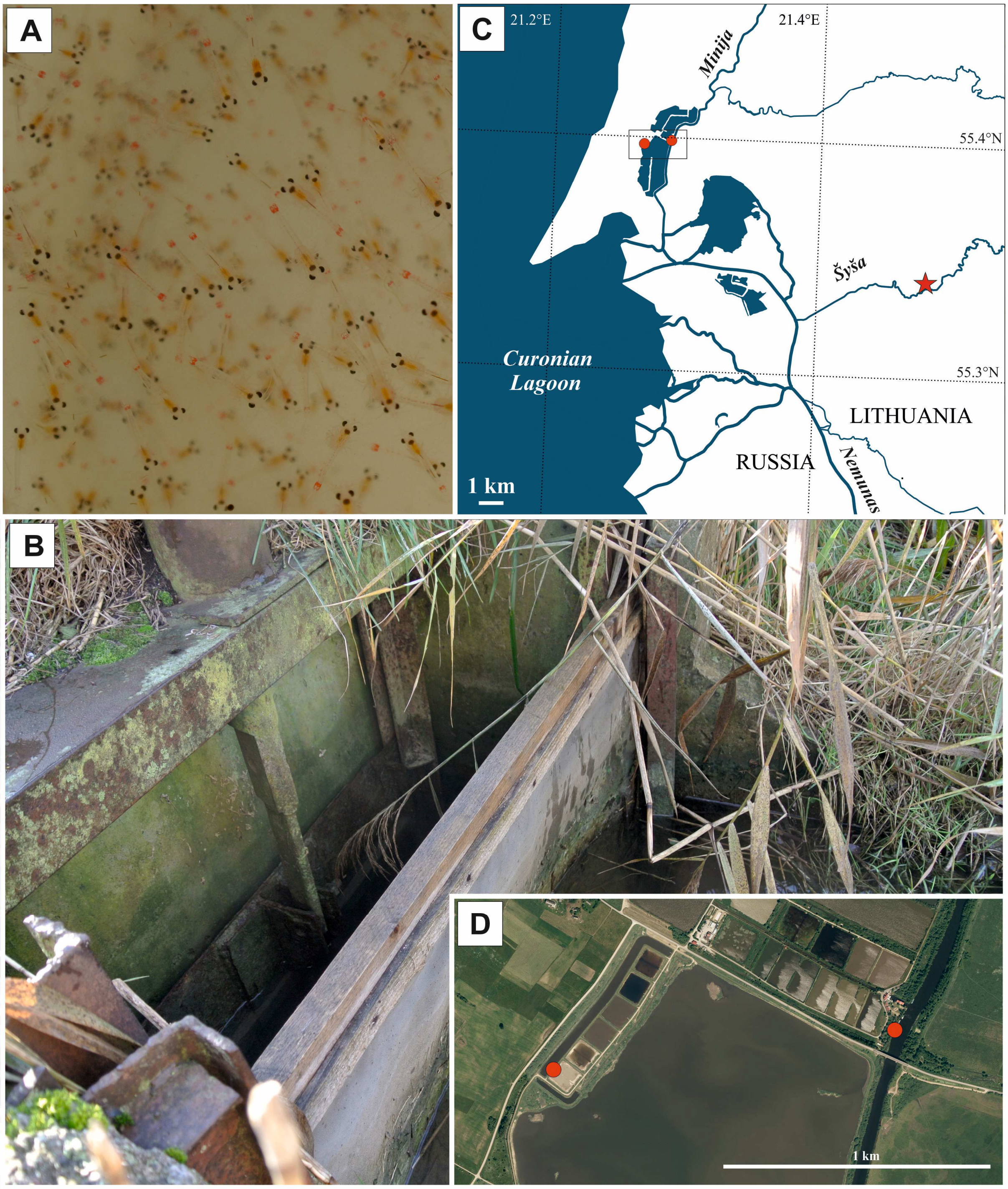

Figure 3. The bloody-red mysid *Hemimysis anomala* in Lithuanian waters: a swarm of mysids (**A**) from the monk water outlet (**B**) in the Kintai fish farm, and localities of the species in the Nemunas Delta area (**C**) and the fish farm area (**D**). Localities in the Minija (including the fish farm) and Šyša rivers are indicated with circles and a star, respectively. Photographs by J. Lesutienė.

To test the impact of the sampling method and relative abundance (as assessed by dredging) on the probability of detecting mysid species, classification tree analysis was first applied. The sampling method had a clear impact on the probability of detecting *P. lacustris* when its relative abundance was below 5 ind. per 10 m of dredging, whereas it had no effect on the detection of *L. benedeni* (see Figure 4). Based on the classification tree result, sampling data for *P. lacustris* were divided into two abundance categories (as a categorical predictor): fewer than 5 ind. and 5 or more ind. per 10 m of dredging (for comparison purposes, the same abundance categories were applied to *L. benedeni* as well). Presence–absence data were further modeled using logistic regression. Both sampling method and abundance had a highly significant effect on the detection of *P. lacustris*, whereas only abundance was significant for *L. benedeni* (Table 1). The modeling results also suggested that, in populations of *P. lacustris* with low abundance, the detection probabilities were 82% with an epibenthic dredge and 22% with a dip net.

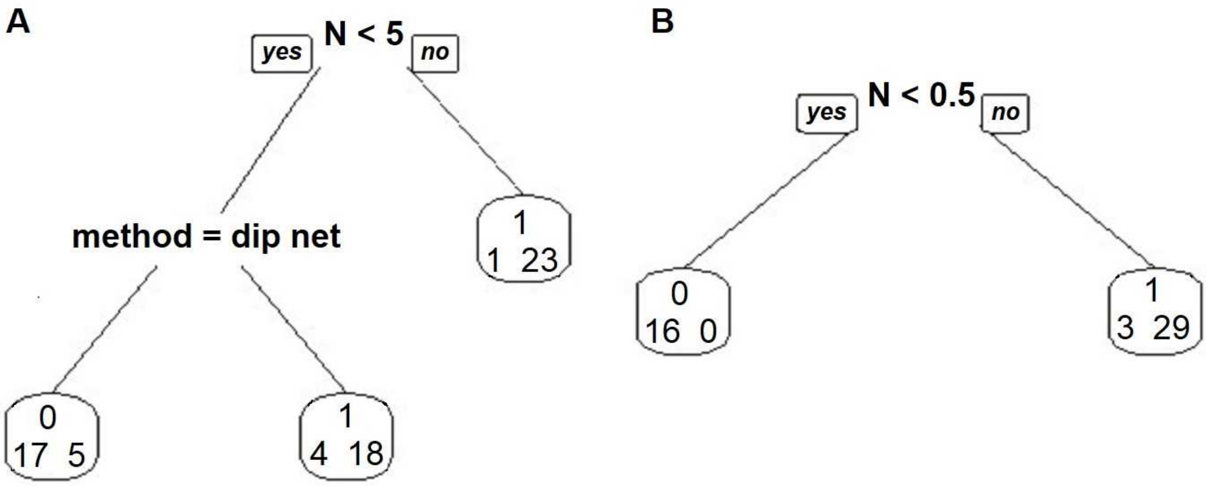

Figure 4. Classification trees showing impacts of species' relative abundance (estimated in ind. per 10 m dredging) and sampling method (dip net vs. epibenthic dredge) on the probability of detecting Ponto-Caspian mysids *Paramysis lacustris* (**A**) and *Limnomysis benedeni* (**B**). Numbers in boxes denote cases of species non-detection (left) and detection (right). Thresholds for classification abundances were set at 5 for *P. lacustris* and 0.5 for *L. benedeni*, based on the method.

Table 1. Results of logistic regression testing effects of relative abundance (Abund_category, two groups: <5 and ≥5 ind. per 10 m of dredging) and sampling method (Method, dip net vs. epibenthic dredge) on the probability of detecting Ponto-Caspian mysids *Paramysis lacustris* and *Limnomysis benedeni*.

Factor	$b \pm$ SE	Odds Ratio	p
Paramysis lacustris, *N* = 68			
(Intercept)	−1.24 ± 0.51	0.3	0.014
Abund_category	3.71 ± 1.14	40.8	0.001
Method	2.76 ± 0.74	15.9	<0.001
Limnomysis benedeni, *N* = 48			
(Intercept)	−0.60 ± 0.51	0.6	0.236
Abund_category	3.02 ± 1.10	20.4	0.006
Method	0.69 ± 0.69	2.0	0.315

The collected data also suggest that the efficiency of different sampling methods can vary between mysid species (Table S1). For *P. lacustris*, the relative abundance estimates obtained using a dredge tended to be higher than those from dip net sampling (mean = 15.4, median = 3.0 vs. 8.3 and 1.0, respectively; Wilcoxon matched pairs test, $T = 92.0$, $p < 0.004$). In contrast, for *L benedeni*, dredge estimates tended to be lower than those from dip net sampling (mean = 86.8, median = 4.0 vs. 868.5 and 31.0, respectively; Wilcoxon matched pairs test, $T = 12.5$, $p < 0.004$).

4. Discussion

The most recent survey of Ponto-Caspian mysids confirmed that mysids *P. lacustris* and *L. benedeni* are widely distributed throughout the Nemunas River basin. In addition to the previous overview of mysid distribution in Lithuanian inland waters [3], the newly obtained data indicate that *L. benedeni* is now also present in the Nemunas River upstream of the Kaunas Reservoir and is well established in the middle reaches of the Šešupė River. Both species were also recorded for the first time in the lower Šyša River. Notably, the bloody-red mysid *H. anomala* was recorded for the first time in Lithuanian inland waters outside the Kaunas Reservoir, having recently been detected in the Minija and Šyša rivers, tributaries of the Nemunas Delta.

A comparison of mysid catches using a dip net and an epibenthic dredge clearly demonstrated that larger, stronger-swimming mysid species, particularly when present at low abundance, are more likely to evade certain sampling gears. This was evident in the case of *P. lacustris*. Even with considerable effort, hand net sampling may be insufficient to reliably detect and quantify such species. These mysids require sampling devices specifically designed to efficiently capture well-swimming nectobenthic species, such as epibenthic dredges or sledges. Our data also suggest that the efficiency of different sampling techniques varies between mysid species. The epibenthic dredge proved more efficient for *P. lacustris*, while the dip net was more suitable for sampling *L. benedeni.* This pattern may be expected, as the former species tends to inhabit open bottom areas, whereas the latter typically dwells among macrophytes [23]. In general, late summer to early fall, when mysid populations are at their peak and represented by multiple cohorts [24], is the most favorable period for detecting and quantifying alien mysid species.

The detection of the bloody red mysid *H. anomala* in the tributaries suggests that the species has established itself in the Nemunas Delta region. It may, in fact, be more widely distributed, potentially inhabiting the entire lower Nemunas below the Kaunas Reservoir, as well as the lower reaches of its tributaries. *Hemimysis anomala* is virtually undetectable during conventional macroinvertebrates surveys due to its nocturnal behavior, which is driven by daylight avoidance [25]. Its presence in a daytime dip net sample from the Šyša River is likely an exception to the rule; a few individuals were probably captured incidentally while sweeping through substrates that provided suitable shelter. Therefore, dusk or nighttime sampling using devices designed to capture nectobenthic animals is recommended for monitoring *H. anomala*. Additionally, the use of specialized light-based traps may be a promising method for detecting its presence [26]. We also conducted a pilot trial to test the effectiveness of baited and light-based traps for detecting mysid species. Fresh fish we used as a bait, and chemical light sticks served as the light source. Two pairs of traps were deployed overnight at known *H. anomala* sites: the fishpond, where the species was first observed, and the site of its previous detection in the Minija River. The baited traps did not capture any mysids, whereas the light-based traps at both locations successfully captured mysids, including *H. anomala*. Notably, the third pair of traps was placed in a canal of the fish farm. There, the baited trap attracted only one specimen of *P. lacustris*, while the light-based trap captured 780 individuals of *P. lacustris* and 6 individuals of *L. benedeni* [27]. These pilot trial results suggest that light-based traps may be a highly effective tool for detecting not only *H. anomala* but also a range of other mysid species.

In this study, the bloody-red mysid *H. anomala* was generally found in habitats that differ from those typically reported in its native range or elsewhere in its invaded range. All known non-Lithuanian records of *H. anomala* come from lentic or very slow-flowing environments, such as lakes, reservoirs, ponds, canals, river backwaters, and coastal waters. Consequently, it has been suggested that discernable flow prevents the prolonged establishment of *H. anomala* [10,28]. In contrast, we detected this species in relatively fast-flowing waters, including medium and small rivers. At the Minija River sampling site, discharge ranged from 41 to 46 $m^3 s^{-1}$, with a flow velocity ~0.29 $m s^{-1}$, while in the smaller Šyša River (channel width ~5 m), discharge varied between 4 and 9 $m^3 s^{-1}$, and the flow velocity was ~0.46 m s^{-1}, during 2015–2017 [29]. These findings suggest that *H. anomala* is capable of tolerating, and potentially establishing populations in, at least, medium-fast flowing waters.

5. Conclusions

The Ponto-Caspian mysids *P. lacustris* and *L. benedeni*, which were deliberately introduced into Lithuanian waters, are now widely distributed across the Nemunas River basin. They occur throughout the Nemunas River itself, as well as the lower and middle reaches of its tributaries. The third introduced species, the bloody-red mysid *H. anomala*, has only recently been detected multiple times in the Nemunas Delta basin, suggesting that it is now well-established in the area and may also be present in the Nemunas River downstream of the Kaunas Reservoir and some of its tributaries.

Reliable detection and quantification of alien mysid species is crucial for the early stages of invasion and for documenting their local distributions. Due to differences in biological traits, behavioral patterns, and habitat preferences, different mysid species may require specific sampling methods. Detecting mysids with nocturnal behavior, such as *H. anomala*, requires sampling at dusk or nighttime, whereas larger, stronger-swimming species like *P. lacustris*, which may evade conventional sampling gear, require complementary methods using devices designed to capture active nektobenthic animals. In this regard, an epibenthic dredge or sledge is recommended. For mysid species which dwell among macrophytes, such as *L. benedeni*, sweep sampling with a dip net appears to be the most effective method. In addition to active sampling techniques, the use of light-based traps also shows promise for monitoring *H. anomala* and other mysid species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17050307/s1, Table S1: Occurrence and relative abundance of the Ponto-Caspian mysids *Paramysis lacustris*, *Limnomysis benedeni* and *Hemimysis anomala* within the Nemunas River basin, Lithuania, assessed using two sampling methods: an epibenthic dredge and a standard dip hand net.

Author Contributions: Conceptualization, K.A.; methodology, K.A. and E.Š.-C.; formal analysis, K.A. and E.Š.-C.; investigation, K.A., A.R., G.V. (Giedrė Višinskienė) and G.V. (Gintautas Vaitonis); writing—original draft preparation, all authors; writing—review and editing, K.A. and E.Š.-C.; visualization, E.Š.-C.; supervision, K.A. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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