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Title

The quick and the dead: copepods dominate as cladocerans decline following invasion by *Hemimysis anomala*

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Draft

Abstract

The North American invasion of a non-native mysid, *Hemimysis anomala*, has been expanding since 2006, with the first inland lake invasions detected for Oneida Lake in 2009 and Seneca Lake in 2010. Although we know that *Hemimysis* primarily consumes zooplankton, our ability to predict the community-level impact of an invasion is hampered by a lack of information on taxon-specific effects. To investigate the effects of *Hemimysis* on native zooplankton we conducted two mesocosm experiments that compared composition between communities with and without *Hemimysis*, and studied how the effects of this predator on zooplankton species composition varied across a natural gradient of low to high invader densities (0.01-0.1 individuals L⁻¹). Our first experiment found that *Hemimysis* primarily affected cladocerans, and particularly *Daphnia*, shifting communities towards dominance by copepods. Our second experiment showed that *Hemimysis* invasions may do little to suppress *Daphnia* abundances until between 0.067-0.11 individuals L⁻¹ or higher. Cladocerans are important links in freshwater trophic transfer and the nutrient cycle, and disruption of these linkages following *Hemimysis* invasion could result in both bottom-up and top-down impacts in nearshore food webs. However, *Hemimysis* can also fill a similar trophic role as the zooplankton they consume, and longer-term experiments are required to better assess their eventual impacts on native communities.

Keywords: non native, invasive, littoral, body size, prey preference, pelagic

Introduction

Hemimysis anomala (the ‘bloody-red shrimp’, hereafter referred to as *Hemimysis*) is a non-native Ponto-Caspian mysid that was first detected in Lake Ontario and Lake Michigan in 2006 (Pothoven et al. 2007). It has since spread to Lake Erie, Lake Huron, the St. Lawrence River, and two inland lakes in New York state (Brooking et al. 2010; Marty et al. 2010; Brown et al. 2012). *Hemimysis* has a broad range of environmental tolerances (Ricciardi et al. 2012; Sun et al. 2013), and can survive transport through a variety of active (e.g. recreational boats and angling, Minchin and Boelens 2010) and passive (e.g. dispersal through canal networks, Minchin and Boelens 2010; Brown et al. 2012) vectors. Additionally, *Hemimysis* is quite different from its native analogue, *Mysis diluviana* (formerly *Mysis relicta*). It has a much higher predatory functional response compared to the native mysid (Dick et al. 2013), potentially reaching feeding rates (per unit body mass of the predator) similar to those of other non-native zooplanktivores like *Bythotrephes longimanus* and *Cercopagis pengoi* (Halpin et al. 2013). *Hemimysis* also prefers primarily littoral instead of pelagic habitats, favouring rocky substrates and man-made structures (Brown et al. 2012; Sun et al. 2013), and therefore represents a novel invader with which native communities have little prior history. The littoral preferences of *Hemimysis* may also lead to greater impacts in inland lakes and ponds, compared to the Great Lakes, due to their higher perimeter:area ratios and tighter coupling between littoral and pelagic habitats (Schindler and Scheuerell 2002). The potential for *Hemimysis* to rapidly spread, its relative novelty to native communities, and its potential for large impacts in invaded systems, all suggest that *Hemimysis* could be an invader of particular concern.

However, little information is available on the effects of *Hemimysis* on invaded North American communities. Impact predictions commonly focus on their strong consumptive effects

on zooplankton (e.g. Ricciardi et al. 2012; Dick et al. 2013), with changes in zooplankton abundance and diversity potentially leading to shifts in phytoplankton biomass, along with alterations in nutrient cycling and upward trophic transfer (Ricciardi et al. 2012). Additionally, shifts in zooplankton community composition could translate to changes in the abundance and growth of planktivorous fish (Ricciardi et al. 2012), and there is evidence that *Hemimysis* is serving as a new food source for native fish in the Great Lakes (Lantry et al. 2010; Yuille et al. 2012). However, refinement of these predictions is limited by a lack of knowledge of the feeding ecology of *Hemimysis* (Walsh et al. 2012), specifically which zooplankton species they prefer to consume, making it difficult to predict their community-level impacts in North American systems.

Certain zooplankton groups or species play a critical role in the control of nutrient transfer and phytoplankton biomass in freshwater ecosystems (e.g. *Daphnia*, Sommer and Sommer 2006), and the impacts of *Hemimysis* could vary greatly depending upon which zooplankton they prefer. Both adult and juvenile *Hemimysis* can consume zooplankton (based on stomach and stable isotope analysis, Borchert et al. 2006; Marty et al. 2010; Pérez-Fuentetaja and Wuerstle 2014). Juvenile *Hemimysis* generally eat smaller zooplankton species (e.g. *Bosmina* and Rotifera, Ketelaars et al. 1999; Halpin et al. 2013), while the adults are better able to consume larger-sized zooplankton (e.g. *Daphnia*, Ketelaars et al. 1999; Halpin et al. 2013), and a preference for cladocerans has been reported in both laboratory and mesocosm experiments. For example, more cladocerans than copepods were consumed when Halpin et al. (2013) simultaneously offered *Bosmina longirostris*, *Daphnia pulex*, and mixed *Mesocyclops* spp. to juvenile and adult *Hemimysis*. Similarly, Iacarella et al. (2015) found that adult *Hemimysis* selected cladocerans over copepods in invaded mesocosm communities. *Hemimysis* also appears

to favour smaller-sized zooplankton prey. Pérez-Fuentetaja and Wuerstle (2014) offered different size classes of *Daphnia pulex* to juvenile and adult *Hemimysis*, and found positive selection for smaller *D. pulex* in both life stages.

However, these experimentally determined prey preferences may change depending upon the availability of alternate food resources and the light environment. Phytoplankton and detritus are often a larger component of the *Hemimysis* diet than zooplankton. Stomach content analysis of wild *Hemimysis* by Borcharding *et al.* (2006) and Pérez-Fuentetaja and Wuerstle (2014) found that their natural diets consisted of ~10-20% phytoplankton and 30-70% detritus. *Hemimysis* feeding experiments generally have not incorporated phytoplankton or detritus (but see Pérez-Fuentetaja and Wuerstle 2014 for a feeding experiment that included phytoplankton), and their inclusion could reduce the severity of zooplankton predation and alter selectivity for certain species (as was the case for *Limnomysis benedeni*, Fink *et al.* 2012). Additionally, *Hemimysis* is sensitive to daytime light levels, feeding near the substrate during the day and migrating up into the water column at night (Walsh *et al.* 2010; Boscarino *et al.* 2012). The previously discussed feeding experiments have included light and dark treatments, with the exception of Iacarella *et al.* (2015) which used opaque mesocosms, but they did not incorporate the potential for spatial movement. *Hemimysis* that have migrated downward during the day can have reduced impacts on cladoceran prey (Barrios-O'Neill *et al.* 2014), and incorporating this daytime spatial refuge is important for understanding their impacts on zooplankton communities.

The objective of our study was to investigate the effects of *Hemimysis* on native zooplankton while including a varied zooplankton community, phytoplankton and detrital food sources, a natural daily light cycle, and the potential for a daytime refuge. To accomplish this we conducted two field mesocosm experiments that incorporated these characteristics. The first

compared zooplankton composition and abundance between communities with and without a high density *Hemimysis* invasion. The second examined the effects of *Hemimysis* on zooplankton communities across a natural gradient of low to high invader densities. Lower density invasions may experience reduced intraspecific competition, which could change the diversity of prey incorporated into the diet (Svanbäck and Bolnick 2007). The spread of *Hemimysis* from the Great Lakes to inland lakes and canals in New York (Brown et al. 2012; Brown et al. 2014) suggests that other inland lakes in North America are at risk of invasion. Our experiments will help to improve predictions of the potential impacts of this spreading invasion on North American zooplankton by assessing their effects on native communities in a more natural setting.

Methods

Two field mesocosm experiments were conducted at the Queen's University Biological Station (QUBS, 44°34'00.7"N 76°19'29.1"W) in Ontario, Canada between June 11 and August 21, 2014. The field site was naturally shaded and received about 1-2 hours of direct sunlight per day. Average daytime air temperatures for the field site and sampled mesocosm water temperatures can be found in the online supplementary material (Fig. S1 and S2).

Experiment 1 – Hemimysis presence/absence

The first experiment (E_{PA}) compared zooplankton composition between communities with and without *Hemimysis*. Twelve 378L cattle tanks (134x78x63cm) were filled on June 11 with 350L of Lake Opinicon water (see online supplementary material Table S1 for details of all lakes used in the experiments) filtered through an 80µm mesh to remove large zooplankton but allowing most phytoplankton and some small zooplankton to pass through. Shards of two 14cm

diameter, unglazed ceramic pots were added to each mesocosm to provide a daytime refuge for the *Hemimysis*. Tanks were also covered with 1mm mesh to provide additional shade and to protect from insects and debris. Mesocosms were allowed to acclimate for one week prior to the addition of zooplankton. To create a diverse, native zooplankton community we filtered zooplankton from 1050L of water from the pelagic and littoral regions of four nearby, uninvaded lakes (totaling 4200L) on June 18 using an 80µm net. All zooplankton were condensed into cooled containers, transported to the mesocosm site, and added to the tanks on the same day they were collected. All tanks were inoculated with zooplankton from the equivalent of 350L of lake water from a condensed and constantly mixed pool, with this inoculum divided evenly over two randomly added aliquots. Tanks were then acclimated for one week prior to *Hemimysis* addition. *Hemimysis* was collected after sunset from the St. Lawrence River at Montreal, QC, CA (45°29'58.9"N, 73°33'01.7"W) on June 25 using vertical plankton net tows. All individuals were then transported that same night in cool, dark containers to a laboratory near the field site. Six tanks were haphazardly assigned to the 'Present' treatment, and 35 *Hemimysis* (0.1 individuals L⁻¹) were introduced to each of these tanks (n=6) on the same night they were captured. We chose a volumetric treatment density of 0.1 individuals L⁻¹ to approximate the higher *Hemimysis* densities observed in Lake Ontario between the spring and fall of 2009 by Taraborelli et al. (2012). Body size was standardized across replicates by using the same number of large (18 individuals between 9-11mm) and small (17 individuals between 6-8mm) *Hemimysis* in each replicate. Additionally, to standardize behaviour across replicates only adult male *Hemimysis* were used in the experiment. Different migratory behaviours have been reported between male and female *Hemimysis* (Ketelaars et al. 1999; Kipp and Ricciardi 2007), which could also be influenced by local abiotic and biotic conditions since different populations sampled around the

same period often have widely different sex ratios (e.g. Taraborelli et al. 2012). The diets of adult male and female *Hemimysis* are the same (Marty et al. 2010), so our use of only male *Hemimysis* would not have influenced the types of prey affected by our treatments.

The experiment ran for ten days. Initial phytoplankton and zooplankton samples (see Table 1 for initial zooplankton densities and biomass, sampling methodology is detailed below) were taken prior to *Hemimysis* addition, then again ten days following commencement of the treatments. When the experiment concluded, all ceramic cover was removed and we attempted to re-capture any remaining *Hemimysis* using aquarium nets (10cmx15cm) to estimate survival. Re-capture for the six 'Present' mesocosms ranged between 54-77%.

Experiment 2 – Hemimysis gradient

The second experiment (E_{Grad}) built upon the first by incorporating a gradient design to investigate how the community effects of *Hemimysis* invasion may differ across a range of low to high densities. Twenty 220L cylindrical tanks (84cm diameter x 53cm high) were filled on July 28 with 180L of Lake Opinicon water filtered through a 50 μ m mesh and ceramic shards were added to each tank as in E_{PA} . Tanks were covered with 1mm mesh and allowed to acclimate for one week prior to the addition of zooplankton. On August 4, zooplankton were filtered from 900L of water from each of the same four lakes as in E_{PA} (totalling 3600L) using a 50 μ m net. All tanks were inoculated with zooplankton from the equivalent of 180L of lake water following the same methodology as in E_{PA} and left to acclimate until August 15. *Hemimysis* were collected on August 15, then transported and introduced as in E_{PA} . Mesocosms were haphazardly assigned to *Hemimysis* treatment densities, with two replicates of each treatment of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 *Hemimysis* (0.011, 0.022, 0.033, 0.044, 0.055, 0.067, 0.078, 0.089, 0.1, 0.11

Hemimysis L⁻¹ respectively), corresponding to a range of low to high natural *Hemimysis* densities (0.01-0.1 individuals L⁻¹; Taraborelli et al. 2012). Body size was again standardized by adding 50% large and 50% small *Hemimysis* to each treatment, and only adult male *Hemimysis* were used.

The experiment ran for five days. Initial phytoplankton and zooplankton samples (see Table 1 for initial zooplankton densities and biomass) were taken prior to *Hemimysis* addition, and again five days post-treatment. To maintain our density gradient, every two days (after sunset and under red light) all cover was removed and *Hemimysis* in each mesocosm were captured with aquarium nets, counted, and returned to their tank. We replaced any dead individuals with live ones maintained in the lab (fed daily with Cyclop-eeze®). Total *Hemimysis* mortality was 23% by the second night, 28% by the fourth, and 63% by the sixth. The higher mortality by the sixth night necessitated the end of this experiment after five days.

Sampling Protocol

Zooplankton samples were taken using an 8cm diameter tube sampler that captured most of the depth of the water column in both experiments. Mesocosm water was gently stirred prior to sampling and samples were taken from three different points in each tank. For E_{PA}, three 3L samples were collected (9L total), and for E_{Grad} three 2L samples were collected (6L total). Samples were condensed on either 80µm mesh for E_{PA} or 50µm mesh for E_{Grad}, anaesthetized in carbonated water, and preserved in 70% ethanol. Enumeration was performed using a Leica MZ16 dissecting scope and a Leica DM E compound microscope. Cladocerans and copepods were identified to species, immature copepods as either nauplii or cyclopoid/calanoïd copepodids, and rotifers to genus. *Daphnia* that were too small for species identification

(generally $<0.7\text{mm}$) were recorded as 'Unidentified *Daphnia*', the majority of which were probably juvenile *Daphnia pulex/pulicaria* (*D. pulex/pulicaria*) based on the higher abundance of this species in the initial stocking of E_{PA} and E_{Grad} (Table 1). For analysis, rotifers were grouped by phylum for E_{PA} (due to the low number detected by its conclusion) and by genus for E_{Grad} . All zooplankton counts were converted to number per liter and biomass for E_{PA} was calculated based on published length-mass relationships (see Table S2 for regression details).

Body size of *Daphnia spp.* was measured from the top of the carapace to the base of the tail spine using up to the first 30 individuals encountered (this ranged from 1-30 individuals depending upon density) in each sample during counting. Only *D. pulex/pulicaria* occurred frequently enough for statistical comparisons of body size.

Phytoplankton was collected using a 500mL brown Nalgene bottle as a grab sample 0.1m below the water surface and split into two portions: 1) an unfiltered portion to estimate the total amount of chlorophyll a in all phytoplankton, and 2) a portion first filtered through a $30\mu\text{m}$ mesh to estimate the amount of chlorophyll a in only small phytoplankton. For simplicity we will hereafter refer to the filtered portion as small phytoplankton and the difference between the unfiltered and filtered portions as large phytoplankton. Phytoplankton samples were filtered, frozen, and later extracted in methanol for 24 hours and analysed following methods from Welschmeyer (1994) using a TD-700 fluorometer (Turner Designs, Sunnyvale, CA).

Statistical Analysis

All statistical analyses were performed using $\alpha=0.05$ in R 3.0.3 (R Core Team, 2014), along with the 'vegan' package (v.2.0-10, Oksanen et al. 2013). Normality and homoscedasticity for all linear models were checked using plots of residual vs. fitted values, normal quantile-

quantile, scale-location, and constant leverage. Log-transformation proved sufficient for any metrics that violated these assumptions. Significance of terms for all univariate models from E_{PA} was determined using analysis of variance (ANOVA), and for E_{Grad} using linear regression. Significance of zooplankton community composition differences between treatments was determined with the permutational multivariate analysis of variance (PERMANOVA) method described by Anderson (2001) using the function 'adonis' from the 'vegan' package (using Euclidean distance and 10,000 permutations). Rare species (those that occurred in only a single replicate) were removed prior to analysis and the abundance data was Hellinger transformed (Legendre and Gallagher 2001).

Response variables for E_{PA} were based on zooplankton and phytoplankton measurements from the final sampling date. Due to stocking variability in the initial zooplankton communities of E_{Grad} (Table 1), all zooplankton densities from the final sampling date were subtracted from the initial densities to estimate the change in species abundances between the start and end of the experiment. Selection of minimum adequate models was performed for all community metrics following the model simplification procedure described by Crawley (2005). Full models for E_{PA} contained only the categorical predictor term for *Hemimysis* presence (Absent/Present), while full models for E_{Grad} contained the interaction between the linear and quadratic terms (to test for non-linear effects) for *Hemimysis* density. Full models were simplified by comparing the deviance of successively reduced models using likelihood ratio tests. Terms were discarded if the deviance explained by a simpler model vs. a more complex model was not significantly different ($P > 0.05$), and terms were retained if the more complex model explained significantly more deviance ($P < 0.05$). Model simplification ended when a term was retained or the null model was

determined to be the minimum adequate model (see online supplementary material Table S3, S4, and S5 for all minimum adequate model details).

Results

Initial zooplankton community composition was not significantly different across treatments in both experiments (E_{PA} : PERMANOVA, $F_{1,10}=1.09$, $P=0.38$, $R^2=0.099$; E_{Grad} : PERMANOVA, $F_{1,18}=1.60$, $P=0.10$, $R^2=0.082$).

By the conclusion of E_{PA} , communities with *Hemimysis* had significantly lower total zooplankton abundance, biomass, and richness than communities without *Hemimysis* (Abundance: ANOVA, $F_{1,10}=15.59$, $P=0.003$, $R^2=0.61$, Fig. 1a; Biomass: ANOVA, $F_{1,10}=25.56$, $P<0.001$, $R^2=0.72$, Fig. 1e; Richness: ANOVA, $F_{1,10}=6.91$, $P=0.025$, $R^2=0.41$, Fig. 1i). This was primarily driven by declines in cladocerans (Abundance: ANOVA, $F_{1,10}=16.96$, $P=0.002$, $R^2=0.63$, Fig. 1b; Biomass: ANOVA, $F_{1,10}=32.12$, $P<0.001$, $R^2=0.76$, Fig. 1f; Richness: ANOVA, $F_{1,10}=39.87$, $P<0.001$, $R^2=0.80$, Fig. 1j). The *Hemimysis* treatment reduced cladoceran abundance by ~80% (from a mean of 27.3 down to 5.5 individuals L^{-1}), cladoceran biomass by ~88% (from a mean of 477 down to 56 μg dry weight L^{-1}), and richness by ~49% (from a mean of 4.3 down to 2.2 species). Although copepod abundances were also significantly reduced by ~52% (declining from a mean of ~62 to 30 individuals L^{-1}) in the presence of *Hemimysis* (ANOVA, $F_{1,10}=12.49$, $P=0.005$, $R^2=0.55$, Fig. 1c), these declines were almost entirely due to reductions in nauplii (declining from a mean of ~58 to 25 individuals L^{-1}) and there were no significant effects of the *Hemimysis* treatment on copepod biomass (Fig. 1g) or richness (Fig. 1k). *Hemimysis* presence also significantly reduced rotifer abundance (ANOVA, $F_{1,10}=6.98$, $P=0.025$, $R^2=0.41$, Fig. 1d) and biomass (ANOVA, $F_{1,10}=7.36$, $P=0.022$, $R^2=0.42$, Fig. 1h), and caused a marginally

non-significant decline in rotifer richness (ANOVA, $F_{1,10}=4.76$, $P=0.054$, $R^2=0.32$, Fig. 11), but this did not constitute a loss of a large portion of the total community abundance, biomass, or richness due to the low numbers of rotifers in this experiment (mean of 1.25 individuals L^{-1} and 1.3 species in mesocosms without *Hemimysis*). There were also no significant effects of the *Hemimysis* presence/absence treatment on large or small phytoplankton.

Changes in the above community metrics from E_{PA} corresponded to a significant shift in zooplankton community composition between treatments with and without *Hemimysis* (PERMANOVA, $F_{1,10}=5.23$, $P=0.002$, $R^2=0.34$, Fig. 2). Communities from treatments without *Hemimysis* were dominated mostly by *D. pulex/pulicaria*, as well as *Daphnia mendotae* (*D. mendotae*), *Ceriodaphnia lacustris* (*C. lacustris*), and rotifers, whereas communities with *Hemimysis* were dominated by adult (e.g. *Acanthocyclops robustus*) and juvenile copepods.

In E_{Grad} , increasing *Hemimysis* abundance resulted in significant declines in the abundance of *D. mendotae* (Linear regression, $t_{1,18}=2.75$, $P=0.013$, $R^2=0.29$, Fig. 3b) and the unidentified *Daphnia* (Linear regression, $t_{1,18}=2.93$, $P=0.009$, $R^2=0.32$, Fig. 3c), as well as a marginally non-significant decline in *D. pulex/pulicaria* (Linear regression, $t_{1,18}=2.04$, $P=0.056$, $R^2=0.19$, Fig. 3a) and a significant increase in cyclopoid copepodids (Linear regression, $t_{1,18}=2.28$, $P=0.035$, $R^2=0.22$, Fig. 3l). When *Hemimysis* abundance was lowest (0.011 individuals L^{-1}), the abundance of *D. pulex/pulicaria*, *D. mendotae*, and the unidentified *Daphnia* tended to increase between the initial and final sampling dates by ~ 3.7 (+82%), ~ 0.1 (+78%), and ~ 4.9 individuals L^{-1} (+780%) respectively, while cyclopoid copepodids decreased by ~ 0.7 individuals L^{-1} (-28%). However, at the highest *Hemimysis* densities (0.11 individuals L^{-1}), *D. pulex/pulicaria* and *D. mendotae* tended to decrease between the initial and final sampling dates by ~ 1.5 (-34%) and ~ 0.2 individuals L^{-1} (-100%) respectively, while the unidentified *Daphnia*

and cyclopoid copepodids increased by 0.47 (+74%) and 1.5 individuals L^{-1} (+51%) respectively. There was also a significant interaction between the linear and quadratic terms for *Hemimysis* density on *Euchlanis* abundance (Linear regression, $t_{3,16}=2.65$, $P=0.017$, $R^2=0.34$, Fig. S3), but this was not considered to be biologically important. There were no significant effects of the *Hemimysis* treatment gradient on any other cladoceran, copepod, or rotifer species, or on large or small phytoplankton.

Body size of *D. pulex/pulicaria* was also affected by *Hemimysis* in both E_{PA} and E_{Grad} . In the presence of *Hemimysis*, body size significantly increased by about 46% (from a median of 1.1 to 2.0mm)(ANOVA, $F_{1,275}=133.09$, $P<0.001$, $R^2=0.33$, Fig. 4a). In E_{Grad} , the *Hemimysis* treatment gradient increased body size by approximately 0.01mm per 0.005 increase in *Hemimysis* L^{-1} (E_{Grad} : Linear regression, $t_{1,371}=2.54$, $P=0.011$, $R^2=0.017$, Fig. 4b), corresponding to an increase of 18% from ~1.21mm at the lowest *Hemimysis* densities to ~1.37mm at the highest.

Discussion

In both experiments, *Hemimysis* primarily reduced the abundances of cladoceran zooplankton, particularly *Daphnia*, while copepods were generally unaffected by any of our *Hemimysis* treatments, with the exception of nauplii in the first experiment. Declines in cladoceran abundance, biomass, and richness resulted in a compositional shift away from cladoceran dominance in communities without *Hemimysis* towards copepod dominance when *Hemimysis* was present. Our results highlight the vulnerability of native cladoceran communities to non-native *Hemimysis*, the potential for large declines in overall zooplankton abundance and

richness in invaded systems, and indicate a potential shift towards copepod dominance for North American zooplankton communities invaded by *Hemimysis*.

Our first experiment illustrated the potential for *Hemimysis* to affect large compositional changes in invaded communities. The greatest effects of *Hemimysis* were observed on cladocerans, which declined in abundance, biomass, and richness. This was mostly due to reductions in *D. pulex/pulicaria* (the most abundant cladoceran in our experiment), but low density cladocerans like *D. mendotae* and *C. lacustris* were also affected by our *Hemimysis* treatment. The observed shift in composition away from cladoceran dominance and towards copepods is consistent with reports from Ketelaars et al. (1999), Halpin et al. (2013), and Iacarella et al. (2015) of the stronger effects of *Hemimysis* on cladocerans, which is more likely due to the greater escape abilities of copepods (Viitasalo and Rautio 1998; Ketelaars et al. 1999; Rudstam 2009) rather than the specific selection of cladoceran prey. Although reductions in cladocerans resulted in an overall decline in total zooplankton abundance and diversity, it is possible that over the course of a longer experiment a subsequent increase in copepods may have compensated for some of these losses (e.g. some species of copepods can increase following a mysid invasion, Spencer et al. 1999). However, *Hemimysis* can also effectively prey on copepods (Borcherding et al. 2006; Dick et al. 2013), and multi-year studies of the effects of *Hemimysis* (Ketelaars et al. 1999) and other non-native mysids (Ricciardi et al. 2012) have observed a persistent loss of total zooplankton abundance and diversity after invasion.

The results of our second experiment indicated a mostly linear correlation between the effects of *Hemimysis* and their density. Over five days, *D. pulex/pulicaria* and *D. mendotae* tended to increase between the start and end of the second experiment, likely because of maturing juvenile *Daphnia* from the initial stocking, until around 0.067-0.078 *Hemimysis* L⁻¹.

After this point the density of both adult *Daphnia* species tended to decline between the initial and final sampling dates. It is also around this point that the cyclopoid copepodids switched to a tendency to consistently increase in density over the course of the experiment (probably due to increased copepod reproduction as competition with *Daphnia* lessened). Additionally, while the unidentified *Daphnia* always tended to increase during the experiment, the magnitude of these increases became much smaller at higher *Hemimysis* densities (either due to higher predation or lower *Daphnia* reproduction). These results suggest that the higher treatment densities used in our experiment (between 0.067-0.11 *Hemimysis* L⁻¹) could be around the density at which the effects of *Hemimysis* begin to exceed the reproductive capabilities of *Daphnia*. If all our initially stocked unidentified *Daphnia* matured enough over five days to become identifiable (~113 individuals in each 180L tank), about 23 *Daphnia* per day would have had to be consumed to reduce adult abundance between the initial and final sampling dates. This is possible for even a single *Hemimysis* based on reported laboratory feeding rates (individuals have consumed ~40 *Daphnia* in 12 hours, Dick et al. 2013; ~6 *Daphnia* in 2 hours, Halpin et al. 2013; ~1.6 *Daphnia* per hour, Pérez-Fuentetaja and Wuerstle 2014), but we did not observe declines in *Daphnia* until around 12-14 *Hemimysis* per mesocosm. This difference in predicted vs. observed effects of *Hemimysis* on *Daphnia* could have occurred because other food sources were available in our experimental mesocosms, such as phytoplankton and detritus. Natural light cycles may also have restricted feeding periods to only the hours between sunset and sunrise (the times during which *Hemimysis* migrates up from the sediment, Walsh et al. 2010). Although our mesocosms were too small to allow for a natural vertical migration, the refuge provided by our ceramic cover still served as effective spatial isolation since the *Hemimysis* would not emerge from beneath it during the day. However, while we did observe short-term declines in *Daphnia* abundance above

about 0.067 individuals L^{-1} , longer term studies are needed to determine how these density-related effects might translate to shifts in the zooplankton community. The patchy nature of *Hemimysis* results in widely variable densities reported across North America (0.01-6.2 individuals L^{-1} , Ricciardi et al. 2012; Taraborelli et al. 2012), and impact predictions would benefit from a better understanding of the density at which a *Hemimysis* invasion begins to exert changes on zooplankton community composition.

Both experiments showed similar declines in *Daphnia* abundance and an increase in *D. pulex/pulicaria* body size due to the *Hemimysis* treatments, though the magnitude of these changes were much smaller in the second experiment (probably due to its shorter duration). These effects on *Daphnia* were likely not due to differences in relative abundances of cladocerans and copepods, since both zooplankton groups were initially stocked in similar amounts (though *Daphnia* biomass was higher, Table 1), but instead by the greater ease of capturing *Daphnia*. The shifts towards larger body size in *D. pulex/pulicaria* could have been caused by size-selective predation. Feeding experiments by Pérez-Fuentetaja and Wuerstle (2014) found that *Hemimysis* preferentially ate smaller bodied prey when offered a variety of *Daphnia* sizes. They attributed this to handling difficulties as consumption of larger *Daphnia* may have required longer handling and manipulation times, allowing for potential escape and non-lethal damage, while smaller *Daphnia* could be quickly consumed whole. Our detected shift towards larger bodied *D. pulex/pulicaria* suggests a similar mechanism, though this could have also be due to reduced reproduction at lower *Daphnia* abundances, causing a general shift in overall size composition.

While the negative effects of *Hemimysis* on *Daphnia* were consistent between experiments, there were some inconsistent effects on other zooplankton. The first experiment

indicated effects of *Hemimysis* not only on *Daphnia*, but also on *C. lacustris*, rotifers, and copepod nauplii, while the second experiment only showed effects on *Daphnia*. This could have occurred if the longer period of the first experiment resulted in larger effects of *Hemimysis*, and therefore better allowed us to statistically detect differences in other species. *Hemimysis* can also shift their diet depending upon available food (Halpin et al. 2013; Ives et al. 2013), and can utilize a wide variety of zooplankton prey, including rotifers (Borcherding et al. 2006; Pérez-Fuentetaja and Wuerstle 2014) and *Ceriodaphnia* (Dick et al. 2013). While there is little evidence of *Hemimysis* consuming nauplii (e.g. only a few were found in *Hemimysis* gut contents by Borcherding et al. 2006), the adult copepod declines that can occur following invasion (e.g. Spencer et al. 1999) could be partly driven by nauplii consumption. The longer duration and larger *Daphnia* declines in the first experiment may have more strongly necessitated the diversification of the *Hemimysis*' diet, thus better capturing their predatory effects on other zooplankton species.

Interestingly, although both experiments contained littoral cladocerans (*Scapholeberis mucronata* in the first and second experiments, with *Chydorus sphaericus* in the second only) and *Hemimysis* can consume littoral species (Dick et al. 2013), no effects of our *Hemimysis* treatments on these zooplankton were detected. This suggests that *Hemimysis*, a primarily littoral predator, could have larger effects on pelagic zooplankton species (i.e. *Daphnia*). The difference in effect on pelagic vs. littoral prey could be due to differences in encounter rate, defense, or preference. *Chydorus sphaericus* clings to the surface of algae (Fryer 1968), while *Scapholeberis mucronata* feeds on surface film (Olsen et al. 2001). These preferred habitats might reduce contact with *Hemimysis* in comparison to the open water preferences of the *Daphnia*. *Chydorus sphaericus* also exhibits a 'dead-man' response to predators (Fryer 1968). This is adept at

avoiding predators that use mechanoreception (which mysids do, Viherluoto and Viitasaloa 2001) since it produces little turbulence, making them difficult to detect. *Scapholeberis mucronata* also appears to have some defense against littoral predators (as suggested by Chang and Hanazato (2005) based on the lower vulnerability of *Scapholeberis mucronata* to littoral copepod predation), but the mechanism used is not known. In contrast, the rapid swimming escape response of *Daphnia* (Barnett and Beisner 2007) would not be particularly effective against mysids as they are very fast swimmers (Borcherding et al. 2006). Finally, *Hemimysis* may just prefer to eat pelagic instead of littoral cladocerans. However, we cannot determine which of these possible mechanisms is responsible for why littoral cladocerans are less affected by *Hemimysis* compared to pelagic species.

Implications

Our experiments have shown the strong, negative effects *Hemimysis* can have on native cladoceran zooplankton, and particularly *Daphnia*. Reductions in *Daphnia* abundance, increases in body size, and an overall shift to copepod dominance in invaded systems could have numerous direct and indirect consequences in invaded communities. *Daphnia* are key grazers in freshwater communities (Sommer and Sommer 2006), and their reduction could lead to persisting declines in ecosystem functioning and cascading trophic effects. Depauperated systems cannot fully utilize all available ecosystem resources (Hooper et al. 2005), and declines in the abundance and diversity of aquatic grazers could reduce the transfer of primary productivity to higher trophic levels (e.g. Mallin and Paerl 1994; Duffy et al. 2003). It could also release the primary producers from consumptive control, potentially leading to large increases in phytoplankton biomass and a shift away from larger phytoplankton species as *Daphnia* become less dominant (Sommer et al.

2001). However, the resulting bottom-up or top-down effects of a *Hemimysis* invasion are difficult to predict. *Hemimysis* juveniles, which were not included in our study, fill a similar trophic position as cladoceran zooplankton (Ricciardi et al. 2012), and feed mostly on phytoplankton rather than zooplankton (Ketelaars et al. 1999; Borchertding et al. 2006). *Hemimysis* could also compensate for some of the losses of zooplankton prey by serving as a new, high-quality food source for fish (Lantry et al. 2010; Ricciardi et al. 2012; Yuille et al. 2012).

The negative effects of *Hemimysis* on *Daphnia* could also interact additively or non-additively with other native zooplanktivores. *Hemimysis* is a novel, littoral predator with which North American *Daphnia* have little experience. Although *Daphnia* are often considered a primarily pelagic group, there is no clear boundary between the pelagic and littoral regions of lakes. 'Pelagic' species can occur in equal abundance in the littoral region (Walseng et al. 2006), which also serves as an important refuge for *Daphnia* from pelagic predators (Burks et al. 2001; Van de Meutter et al. 2005). While *Hemimysis* would likely compete with native littoral zooplanktivores, there would be limited competition with native mysids (i.e. *Mysis diluviana*) and pelagic zooplanktivorous fish since there is little overlap in preferred habitat. *Hemimysis* invasion could therefore lead to an increase in total zooplankton predation by combining a novel littoral predator with native pelagic predators. Alternatively, similar interactions could result from invasion into communities with non-mysid zooplanktivores more capable of capturing copepods (e.g. *Chaoborus*, Rudstam 2009). The presence of a native copepod predator in invaded systems could prevent any potential for a compensatory increase in the copepods, resulting in larger overall declines in zooplankton abundance and diversity.

The potential complexity of the bottom-up-top-down trophic effects of *Hemimysis*, and its possible interactions with other native predators, illustrates the need for further investigation of this non-native species. While we have shown the strong, negative effects *Hemimysis* can have on zooplankton abundance, diversity, and composition, our experiments only represent short-term impacts. Longer-term community experiments that incorporate adult, juvenile, and male/female *Hemimysis* (and other zooplanktivorous predators) are required to investigate how *Hemimysis* might alter native zooplankton community composition, and how these changes may subsequently affect phytoplankton composition, primary production, and overall community function. The ecological impacts of *Hemimysis*, and the factors that influence its ability to alter native community structure and trophic transfer, are not well understood (Ricciardi et al. 2012; Walsh et al. 2012). It is important to investigate how this non-native, littoral mysid may further disrupt nearshore communities. Littoral regions in freshwater systems are integral to aquatic productivity, and are already inundated with multiple anthropogenic stressors (Strayer and Findlay 2010). Our experiments, and previous work on the prey preferences and effects of *Hemimysis*, have shown the potential for dramatic community shifts in invaded aquatic systems. Therefore, reducing the spread of this species into inland lakes should be a high priority for preventing further expansion and protecting native communities.

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Figure Captions

Fig. 1 Abundance +1 (individuals L⁻¹, a-d), biomass +0.1 (µg dry weight L⁻¹, e-h), and richness +1 of (aei) the total adult and juvenile zooplankton community, (bfj) cladocerans, (cgk) copepods, and (dhl) rotifers from absent (unshaded, 0.0 *Hemimysis* L⁻¹) and present (shaded, 0.1 *Hemimysis* L⁻¹) treatments (n=6) for E_{PA}. Bottom and top edges of boxes represent the first and third quartiles, respectively, with a dark, horizontal line as the median. Whiskers encompass data

points that lie within 1.5 times the inner quartile range; data outside this range are shown as separate points. R^2 and P -values are shown in the top-right panel corners for metrics where the *Hemimysis* treatment had a significant effect ($P < 0.05$). Note the log scale on all y-axes.

Fig. 2 Biplot of principal component scores for PCA axis 1 and 2 for community data from the final sampling date for E_{PA} . Each point represents a zooplankton community from mesocosms in which *Hemimysis* was absent (white circles, 0.0 individuals L^{-1}) or present (black circles, 0.1 individuals L^{-1}). Arrows and species name locations show which species are associated with which areas of the figure, with the direction and length of the arrow indicating the importance of that species to differentiating between communities. Note that the ends of the species arrows for *Daphnia pulex/pulicaria* and *Scapholeberis mucronata* lie outside the figure. R^2 and P -values were determined from the PERMANOVA analysis.

Fig. 3 Changes in abundance (initial – final individuals L^{-1}) of (a-g) cladocerans and (h-l) copepods from E_{Grad} across the *Hemimysis* treatment gradient (0.011, 0.022, 0.033, 0.044, 0.055, 0.067, 0.078, 0.089, 0.01, 0.011 individuals L^{-1}). Abundance values below zero indicate species that increased in density between the initial (day 0) and final (day 5) sampling dates, while values above zero indicate a decrease in density. Best fit lines are only shown for species whose density was related to *Hemimysis* abundance ($P < 0.1$, plotted based on the minimum adequate models for these species). Note the changing y-axes scales for each panel.

Fig. 4 *Daphnia pulex/pulicaria* body size (mm) from the final sampling date for (a) absent/present mesocosms from E_{PA} (Absent: $n=180$, Present: $n=97$), and (b) across the

Hemimysis abundance gradient from E_{Grad} . Sample size for the present treatment from E_{PA} was lower since there was generally less *D. pulex/pulicaria* in the presence of *Hemimysis*. Bottom and top edges of boxes represent the first and third quartiles respectively, with a dark, horizontal line as the median. Whiskers encompass data points that lie within 1.5 times the inner quartile range, data outside this range are shown as separate points. In (b), the grey line is the best fit line representing the significant, positive relationship between body size and *Hemimysis* density ($P < 0.05$, plotted based on the minimum adequate model for this species). Note log-scale used on the y-axis.

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Table 1. Initial abundances (mean $\pm$ SD, ordered from highest to lowest) and biomasses (mean $\pm$ SD) of cladocerans, copepods, and rotifers from the Day 0 samples for E_{PA} and E_{Grad}.

Experiment	Taxonomic Group	Species	Abundance (individuals L ⁻¹)	Biomass (µg dry weight L ⁻¹)
E _{PA}	Cladoceran	<i>Daphnia pulex/pulicaria</i>	1.93 $\pm$ 1.39	47.8 $\pm$ 34.34
		<i>Bosmina freyi/liederi</i>	1.49 $\pm$ 1.72	1.87 $\pm$ 2.15
		<i>Ceriodaphnia lacustris</i>	1.34 $\pm$ 0.92	3.75 $\pm$ 2.59
		<i>Daphnia mendotae</i>	0.64 $\pm$ 0.73	12.55 $\pm$ 14.33
	Copepod	Nauplii	10.56 $\pm$ 6.41	0.56 $\pm$ 0.34
		<i>Mesocyclops edax</i>	3.85 $\pm$ 3.89	16.75 $\pm$ 16.92
		Cyclopoid copepodid	1.96 $\pm$ 1.62	1.93 $\pm$ 1.59
		<i>Skistodiaptomus oregonensis</i>	1.26 $\pm$ 1.01	13.15 $\pm$ 10.51
		Calanoid copepodid	1.60 $\pm$ 2.51	1.6 $\pm$ 2.51
		<i>Acanthocyclops robustus</i>	0.64 $\pm$ 0.63	9.99 $\pm$ 9.79
	Rotifer		21.35 $\pm$ 12.11	4.27 $\pm$ 2.42
E _{Grad}	Cladoceran	<i>Daphnia pulex/pulicaria</i>	4.50 $\pm$ 3.27	111.38 $\pm$ 80.97
		<i>Bosmina freyi/liederi</i>	2.05 $\pm$ 1.46	2.57 $\pm$ 1.84
		<i>Scapheloberis mucronata</i>	1.35 $\pm$ 4.40	4.45 $\pm$ 14.51
		<i>Ceriodaphnia lacustris</i>	0.77 $\pm$ 0.46	2.15 $\pm$ 1.29
		<i>Chydorus sphaericus</i>	0.11 $\pm$ 0.24	0.17 $\pm$ 0.37
		<i>Daphnia mendotae</i>	0.092 $\pm$ 0.13	1.79 $\pm$ 2.47
	Copepod	Nauplii	15.12 $\pm$ 3.72	0.80 $\pm$ 0.19

<i>Acanthocyclops robustus</i>	4.64 ± 1.51	72.45 ± 23.62
Cyclopoid copepodid	2.83 ± 1.30	2.78 ± 1.28
Calanoid copepodid	1.46 ± 0.58	2.90 ± 1.16
<i>Skistodiaptomus oregonensis</i>	1.28 ± 0.59	13.28 ± 6.20
<i>Mesocyclops edax</i>	0.87 ± 0.53	3.77 ± 2.30
<i>Diacyclops thomasi</i>	0.083 ± 0.10	0.38 ± 0.46
Rotifer	16.58 ± 7.26	3.31 ± 1.45

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Hemimysis (individuals L⁻¹)

Hemimysis (individuals L⁻¹)

Hemimysis (individuals L⁻¹)