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Ecology and Behavior of the Invasive Mysid Hemimysis Anomala in Milwaukee Harbor

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ECOLOGY AND BEHAVIOR OF THE INVASIVE MYSID *HEMIMYSIS ANOMALA* IN MILWAUKEE HARBOR

by

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ABSTRACT

ECOLOGY AND BEHAVIOR OF THE INVASIVE MYSID *HEMIMYSIS ANOMALA* IN MILWAUKEE HARBOR

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Aquatic invasive species, typically introduced in ballast water, are a concern in many ecosystems. In the Laurentian Great Lakes, the Ponto-Caspian mysid, *Hemimysis anomala,* has established and is especially abundant in harbor breakwall environments in Lake Michigan, forming large swarms. Predicting the effects of the invader depend on whether *Hemimysis* is competing for zooplanktonic prey or exploiting other benthic resources. To understand population dynamics (seasonality, size distribution, sex ratios, abundance, etc.) and food web position, weekly to monthly sampling of breakwall environments was conducted using lighted funnel traps in Milwaukee Harbor, WI. In addition to time series sampling, we also sampled from within a swarm to compare individuals in traps to individuals in the swarms. Preserved samples were quantified, body size measured, and sexes determined. In addition, subsamples of at least 6 females, males, and juveniles from each collection were stored at -70°C for gut content analysis using purified antibodies raised against potential prey species (*Bosmina longirostris, Bythotrephes longimanus*, *Cercopagis pengoi*, *Daphnia mendotae*, *Daphnia pulex, Keratella cochlearis*, *Leptodiaptomus ashlandii, Limnocalanus macrurus*, and veliger larvae of *Dreissena* mussels). Samples from mid to late summer indicate a population dominated by juveniles (< 6mm with no obvious secondary sexual characteristics), the majority (80-90%) of adults being males, and very few sexually reproductive females (5-10%). Adult sizes during this period were

smaller (average 7 mm, maximum 8.5 mm) than those in winter months or reported in other Great Lakes studies. Late fall and winter samples indicate lower overall abundances composed predominantly of females (55-75%) with fewer males (15-25%) and rarer juveniles (~5%). Individuals were also typically larger in winter samples (average 10 mm, maximum 12 mm). These data suggest that *Hemimysis* populations in Milwaukee Harbor breakwall environmental show quite distinct characteristics from those described in other Great Lake ecosystems. Gut content revealed that *Hemimysis* appear to be generalist feeders and all prey items were found in their guts. many individuals (39.7%) had empty guts, most likely due to the bias of the traps with time for *Hemimysis* to clear their gut before the traps are pulled. Additionally, in the guts that were not empty, the type and abundance of a particular prey item show that there appear to be no differences in dietary differences between sexes, or between adults and juveniles (measured as a proxy of length). There was also no difference in size of individuals present in the swarm, however individuals in the swarm were less likely to consume *D. pulex* and *B. longimanus*, but more likely to consume *C. pengoi*. These results support only the hypotheses related to population dynamics, and do not support the hypotheses related to dietary differences.

To my grandpa,

who always said he wouldn’t live long enough to see me graduate,

to jest dla ciebie

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Introduction

Background

The mysid *Hemimysis anomala* (hereafter referred to as *Hemimysis*), also known as the "bloody red shrimp", is one of many invasive species that has colonized the Laurentian Great Lakes. Native to the Azov, Black, and Caspian Seas, *Hemimysis* is a halo-tolerant species that has propagated itself in several non-native regions by traveling in the ballast water of cargo ships via popular shipping routes (Salemaa and Hietalahti 1993; Pothoven et al. 2007; Audzijonyte et al. 2008). The first *Hemimysis* specimens found in the Great Lakes were collected from a nuclear powerplant on the far east side of Lake Ontario in May of 2006. Later in November of that same year, specimens were collected from a channel that connects Muskegon Lake in Grand Haven, Michigan, to the greater Lake Michigan water body (Pothoven et al. 2007). Although these are the first recorded incidences of *Hemimysis* in the Great Lakes, it is hypothesized that the *Hemimysis* were present in the Great Lakes prior to these collections and were not being caught in regular sampling techniques because of sampling biases (Borcherding et al. 2006; Reid et al. 2007).

Hemimysis differs from the Lake Michigan native mysid, *Mysis diluviana*, in several ways. First, *Hemimysis* have red chromatophores, which gives it the nickname "bloody red shrimp", concentrated on the carapace, the junction of abdominal segments, and at the base of the telson (Salemaa and Hietalahti 1993; Ioffe 1973; Pothoven et al. 2007; Marty 2007). In contrast, *M. diluviana* does not have any chromatophores. Other physical differences between the species include the shape of the telson: *Hemimysis* telsons are flat whereas *M. diluviana* telsons are forked (Pothoven et al. 2007; Marty 2007). In addition to physical differences, the two species occupy different regions of the lake which gives them two entirely separate species

Figure 1: Image of male *Hemimysis* caught using light-based funnel traps. In this image, the digestive tract containing a full stomach and forming fecal pellets can be seen (a) as well as the elongated 4th and 5th pleopods (b) that allow for easy sexing of males.

ranges (Salemaa and Hietalahti 1993; Boscarino et al. 2007). This means they are not likely to be collected within the same sample and allows for higher confidence of correct species identification.

Hemimysis, in comparison to the native *M. diluviana*, occupy shallower and warmer parts of the Great Lakes. *Hemimysis* have been found in depths as shallow as 0.5 m and as deep as 50 m, but typically occupy waters less than 10 m depth (Salemaa and Hietalahti 1993). In contrast, *M. diluviana* are typically not found in waters less than 25 m and can be found as deep as 170 m (Boscarino et al. 2007). When it comes to temperature, *Hemimysis* prefer temperature between 9 and 20 °C (Ioffe 1973) but can survive in temperatures as low as 0 °C (Borcherding et al. 2006) and as high as 28 °C (Marty 2007). In contrast, *M. diluviana* prefers temperatures that range from 6-8 °C, and completely avoided temperatures over 14 °C, unless there were extremely high prey densities (Boscarino et al. 2007). This data further supports the lack of geographical overlap between the two Mysidae species.

General Life History

Hemimysis found in fresh waters typically range in size from 6 mm to 13 mm in length, with mature females (~11 mm) being slightly larger than males (6-10 mm) (Borcherding et al. 2006; Janas and Wysocki 2005; Salemaa and Hietalahti 1993). However, *Hemimysis* in marine and brackish waters reach larger sizes than their freshwater counterparts. Male *Hemimysis* (Fig. 1) are around 11 mm in length while the larger females reach up to 16 mm in length (Komarova 1991).

Hemimysis have a relatively short lifespan, 9 months, compared to the native *M. diluviana'*s lifespan of up to two years (Sell, 1982). Because of *Hemimysis'*s short lifespan,

Figure 2: Images of two female *Hemimysis* caught during the reproductive season using light-based funnel traps. One female (top) has a fully developed young in her marsupium that is ready to emerge, as well as full ovaries that are ready to secrete more eggs once the marsupium is empty. The other female (bottom) has several developing embryos (with visible eyespots) inside her marsupium and has visibly enlarged ovaries.

individuals reach sexual maturity in less than 45 days (Ioffe 1973). Females begin carrying eggs when water temperatures reach 8-9 °C (Pothoven et al. 2007). Females in the Great Lakes have been seen with young in their pouches as early as February and as late as November (Pothoven et al. 2007). However, their typical reproductive season ranges from April through September (Borcherding et al. 2006). Females can produce up to 4 broods in a single reproductive season, but usually produce at least 2 broods (Pothoven et al. 2007). Brood size can range anywhere from 2-70 embryos that emerge as fully developed individuals (Fig. 2), as they undergo nauplii stages within their mother's marsupium (Ketelaars et al. 1999; Borcherding et al. 2006; Pothoven et al. 2007; Marty 2007). It has also been reported that males die shortly after mating, leading to a female dominated population after the breeding season (Ketelaars et al 1999; Pothoven et al. 2007; Marty 2007).

Behavior: Habitat Usage

Many Mysidae species, including *Hemimysis*, are known to be diel migrators, meaning their position in the water column is dependent on the time of day (Salemaa and Hietalahti 1993; Dumont 2006; Boscarino et al. 2009). During the day, *Hemimysis* tends towards the benthos and seeks refuge on top of the substrate. At night, *Hemimysis* migrates towards the surface to feed on other zooplankton and phytoplankton (Janas and Wysocki 2005; Borcherding et al. 2006). This pattern of movement seen in many mysid species, including the native *M. diluviana*, minimizes predation risk while maximizing feeding rate. However, there are differences seen in these migration patterns depending on the organisms age, size, and sex. Juveniles, who lack the developed red chromatophores and are more transparent than the adults, do not strictly follow this migration pattern because their transparency makes them less visible to predators (Ketelaars

et al. 1999; Borcherding et al. 2006). In addition, it is typically only the male adult individuals who participate in these migrations, thus affecting the size and sex distribution of these nightly swarms.

Several studies have been done on *Hemimysis* habitat preference, particularly when it comes to substrate and water column occupation. In terms of substrate, *Hemimysis* most prefer large cobble that create large enough spaces between the rocks to allow *Hemimysis* to accumulate, but small enough to protect *Hemimysis* from predatory fish (Boscarino et al. 2020). Previous studies showed a lower proportion of *Hemimysis* on sandy or muddy substrate, however, later studies showed that the substrate preference is heavily dependent on light availability and predator preference (Claramunt et al 2012; Boscarino et al. 2020). *Hemimysis* have also been seen swarming in very large densities (>1,500 indiv./L) in small "caves" created by large boulders on the shoreline (Pothoven et al. 2007; Geisthardt et al. 2021). This behavior is similar to that of *Hemimysis*'s closest relative, a cave dwelling species in the Mediterranean, *H. margalefi* (Lejeusne and Chevaldonné 2005).

Behavior: Feeding

Hemimysis feeding behavior is dependent on the organism's size and age and therefore exhibit ontogenetic shifts in their dietary preference. Smaller individuals and juveniles (<4 mm) feed primarily on phytoplankton and detritus while the larger adults (>4 mm) rely on zooplankton to makeup a greater portion of their diet (Ketelaars et al. 1999; Borcherding et al. 2006; Dumont 2006). The primary zooplankton consumed by adult *Hemimysis* are Cladocera. In fact, *Hemimysis* are so efficient at hunting and consuming cladocerans that mesocosm experiments as well as observations in the field show *Hemimysis* can reduce the Cladocera

abundance by 80% and biomass by 88% (Sinclair et al. 2015). *Hemimysis* tend to feed on adult copepods at a lower rate because of copepods' fast escape response, however, they are still capable of reducing copepod nauplii abundance by 52% (Sinclair et al. 2015). Despite the ontogenetic shift from a "herbivorous diet" to a "carnivorous diet" with age, there was still a large portion of the gut content (59-72%) that was detrital material, regardless of animal size, particularly during day-time sampling (Perez-Fuentetaja and Wuerstle 2014). A study by Evans et. al (2018) in Lake Ontario showed that both adults and juveniles feed on primary producers such as diatoms, dinoflagellates, and cyanobacteria when they are abundant during the spring bloom, and then make the switch to zooplankton as their numbers increase. Direct examination of gut contents showed a preference for *Bosmina* and *Daphnia*, as well as the less expected invasive species *Bythotrephes* and *Cercopagis* (Evans et al. 2018). This dietary composition further supports the observations of *Hemimysis* remaining mostly in the benthos during the day, feeding on detritus, and emerging at night to feed on plankton. *Hemimysis*'s voracious appetite can have significant impacts on the nearshore community structure and ultimately food web dynamics and transfer of energy in the system.

Current and Proposed Methodologies for Sampling Hemimysis and Determining Diet

Different sampling methodologies for *Hemimysis* have each been found to have particular biases. For example, vertical plankton nets rely on *Hemimysis* being within the water column, rather than close to or on the benthos like many studies have found (Brown et. al, 2017; Brown et al. 2012; Boscarino et al. 2012; Borcherding et al, 2006). Because of this, plankton tows, particularly ones done during the day, risk underestimating local abundances and densities of *Hemimysis* unless they are performed at night during which the species is known to vertically

migrate (Brown et. al, 2017). Another method more recently developed for catching *Hemimysis* is the use of lighted funnel traps. Brown et al. (2017) constructed two separate trap types: a bucket trap, and a funnel trap, both which contained a light source. The traps were tested in both the field and laboratory environment to see if there was a difference in the number of individual *Hemimysis* caught by each trap. The results showed that the funnel traps caught significantly more individuals than the bucket trap, and that traps with light caught more individuals that traps without light (Brown et al. 2017). However, because the lighted traps are attracting *Hemimysis* from the area around the trap and not a particular volume of water being sampled, densities of organism in the environment cannot be directly calculated using traps, only inferred.

Because the goal of this thesis was to get the best understanding of the relative number of *Hemimysis* in select locations within Milwaukee Harbor (rather than absolute densities), we decided to construct our own light-based funnel traps to collect *Hemimysis* rather than using plankton nets.

When it comes to determining gut content of the *Hemimysis*, several methods have been previously published in the literature including stable isotope analysis (Evans et al. 2018; Marty et al. 2012: Yuille et al. 2012), dissection and visual identification (Evans et al. 2018; Borcherding et al. 2006), metabarcoding of the stomach contents (Mychek-Londer et al. 2020), and laboratory feeding experiments (Perez-Fuentetaja and Wuerstle 2014). Stable isotope analysis will show where the organism lies in the food web and will not give specifics of what the organism is consuming down to the genus level. Dissection and visual identification are not only time consuming and tedious but are also dependent on proper identification of small fragments on a prey item that has already been partially digested and therefore decomposed. Metabarcoding and other DNA type of analyses relies on a DNA segment being identified that is

specific enough to the species of interest, but not so specific that it only gets a hit on some individuals and not others. This requires a large database of DNA samples of both the species of interest and the prey items, which do not currently exist for planktonic zooplankton in Lake Michigan (see discussion in Berges et al. 2020). Lastly, laboratory experiments only mimic feeding behavior of organisms in a controlled environment when fed a specific species, which are often prey species that are easy to culture rather than ones that are representative of diversity and abundances in the organism's natural environment.

Because of all the reasons previously listed, it we decided that the method to identify *Hemimysis* gut content in this thesis would be the use of polyclonal antibodies. The use of this method to determine *Hemimysis* gut content is relatively inexpensive, easily quantified, sensitive, specific, and has been applied in many aquatic studies (Haberman et al. 2002; Ohman et al. 1991; Hoving et al. 2005). In addition to these reasons, to our knowledge, this use of this method in the *Hemimysis* system is novel.

Objectives and Hypotheses

The objective of this thesis is to establish a time series of population parameters (sex, length, abundance) for *Hemimysis anomala* at selected locations within Milwaukee Harbor. Additionally, collected individuals will be probed to access gut contents using immunochemical methods indicating the presence of nine potential prey species for the novel invader. These data will allow us to distinguish dietary preferences between life stages, sex, and size across time, as well as identify any differences in the population or dietary preferences when comparing trap time series samples to swarm samples.

From the literature, it appears that the males die off shortly after mating, and that population overwinters mostly as females which then begin reproducing in the spring. Thus, we hypothesize:

H1: Juveniles will be the most abundant group in the population during the reproductive season.

H2: Females will be the most abundant group in the population during the non-reproductive season and winter months.

H3: After the reproductive season, the number of males will decline.

Based on previous literature, juveniles are more likely to consistently occupy the upper water column, while the adults are the ones who vertically migrate, and of the adults, females are more likely to remain closer to the bottom to avoid predation. Thus, we hypothesize:

H4: Juvenile diet will be different from adult diet.

H5: Female diet will be different than male diet.

Previous studies show that reproductive individuals within a population are often larger than non-reproductive individuals within the same population. This is because larger individuals do not need to allocate resources to growth to outcompete others, and therefore can allocate more resources to reproduction. Conversely, when an individual cannot reproduce, it allocates those resources to growth. Thus, we hypothesize:

H6: Reproductive females will be longer than non-reproductive females.

H7: Average length of adults will increase in winter months (non-reproductive season) compared to the summer months (reproductive season).

Because swarms are removed from the benthos and are within the water column, and are also seen during the day at the surface, we hypothesize that:

H8: Diet of individuals captured within swarms will be different than that of individuals caught in traps.

Methodology

Trap Construction and Use

Light traps (Fig. 3) were constructed using 4" black PVC pipe, a ChaseLight diving light (with T6 LED bulbs rated at 600 lumens). These flashlights had a 470-ohm resistor placed in the circuit, reducing output, and extending battery life. Two pieces of black mesh and one piece of 1μm white filter were placed over the light source so that all traps had a light reading of 0.022 – 0.035 μmol m^2 s^{-1} on the low setting and 0.38 – 0.54 μmol m^2 s^{-1} on the high setting. A 4.5" clear plastic funnel was placed at the mouth to guide entry into the trap and to allow the maximum amount of light to shine into the water column with a 0.5 cm plastic mesh glued over the aperture to restrict the size of the organisms that can enter the trap. Additionally, silicone stoppers were used for easy emptying of the traps, and bricks were used to weigh the trap down. A retrieval hook was screwed into the top of the trap to add different removal apparatuses throughout the sampling period. During the warmer months when traps was deployed via diving, a braided rope was tied to the retrieval hook along with a 3" fluorescent, round, foam fishing bobber to easily locate the traps from the surface. During colder months where water is not accessible, wire cables were attached to the removal hook and traps were hung off docks or breakwalls and tethered above water for easy locating and removal. Rechargeable batteries were used for each deployment.

Permission to conduct sampling within Lakeshore State Park was given by the State of Wisconsin Department of Natural Resources, and the project was carefully reviewed by the Wisconsin State Park Ecologist, Craig Anderson. There was also communication with the Lakeshore State Park Manager, Angela Vickio. Traps were deployed weekly starting on July 28, 2021. Some deployments were adjusted based on local weather or water conditions. Field sites

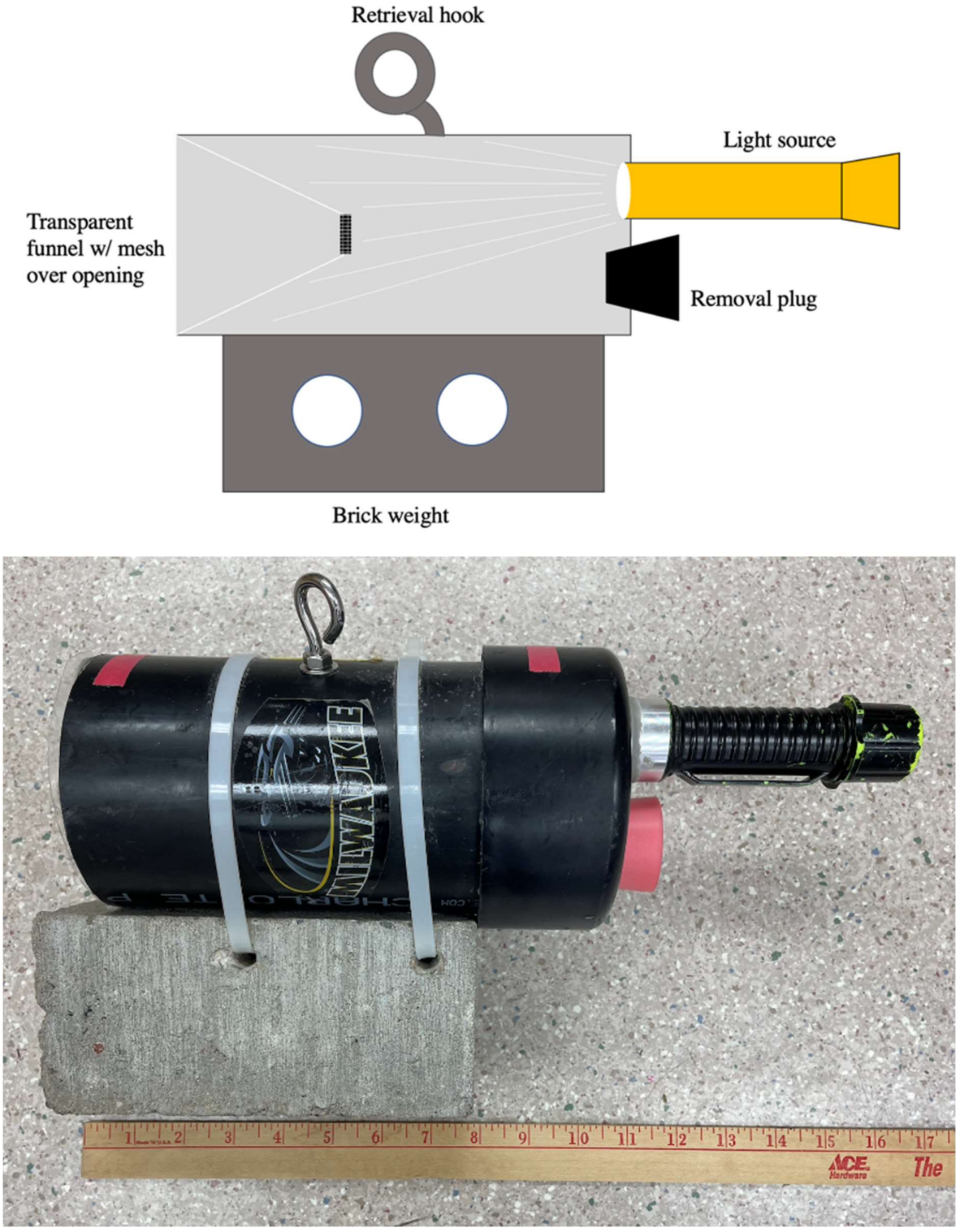

Figure 3: Diagram and photo of light trap designed for capture of *Hemimysis* based on input from Brown et al. 2017.

Figure 4: Map of Lakeshore State Park in Milwaukee, WI showing approximate location of trap deployments on the harbor side of the park. Picture provided by Angela Vickio, Lakeshore State Park Manager - DNR

were located in Lakeshore State Park in Milwaukee, WI (43° 1' 51.6216" N, 87° 53' 44.1276" W, Fig. 4) during open water, traps were deployed directly off the breakwall, with some adjustments due to water temperature, surface conditions and overall accessibility. When ice cover forms, traps were deployed either on the transient docks at the North end of the park, or at the South end of the park where there is less ice cover if any at all.

Traps were deployed between 30 min and 1 h before sunset, to have them placed before the start of vertical migration. Upon arrival to the park, the water temperature was recorded, as well as deployment time, sunset time, sunrise time, and moon phase. The traps were placed so that they are within the underwater rocky grottos (an artificial or man-made recess or structure that resembles a naturally occurring cave) where they can be easily deployed and retrieved. The trap was then flooded with water, checked to ensure light was functioning properly, and placed (via diving or dropping depending on the season). After the trap was settled into position, the depth is recorded by taking a weighted line to the trap mouth opening and measuring how far from the trap mouth to the surface. Traps were placed between 0.5 m and 3 m depth in open water season and between 0.5 m and 5.6 m depth during winter months.

Traps were retrieved between 30 min and 2 h after sunrise to reduce cannibalism within the trap and to ensure the individuals do not clear their gut before they can be processed. Preliminary experiments showed that gut clearance times were between 2 h and 5 h, with an average gut clearance time of 3.5 h (Jenrette, Wachala, and Berges, unpublished). During retrieval, traps were kept funnel-side up once out of the water to ensure minimal loss of samples. The traps were swirled for 5-10 s so that *Hemimysis* are suspended in the water and not stuck to the sides of the trap. Once all of the sample was in the receptacle, the trap was washed at least once with more lake water to make sure all the *Hemimysis* are removed from the trap. Once one

trap had been emptied and washed, its contents are placed on ice to slow down the *Hemimysis* metabolism to keep their gut content inside them. Putting them on ice also makes them easier to handle and process, which results in less damage to the organism.

Swarm Sampling

On 10/19/21, a dive was conducted near the breakwall within Milwaukee Harbor on the inner side of the outer harbor breakwall. The purpose of this dive was to collect *Hemimysis* from within a swarm to analyze differences between and among swarms. A small handheld net estimated at 15 cm by 8 cm was used by SCUBA divers to collect *Hemimysis* from the top and the bottom of the swarm using a slow fanning motion. The individuals captured were then brought back to the surface with the collection depth recorded (indicating either the top or the bottom of a swarm), as well as the swarm number. A total of 10 swarms were sampled, of which three were randomly selected for processing. Samples were held on ice after capture to slow down *Hemimysis* metabolism and reduce gut clearance time. Samples were on ice for approximately 1 h to 2 h, depending on the order the samples were collected, before processing began back at the lab.

Retrieval Day Processing

At the lab, traps were processed one at a time so as not to cross-contaminate between replicates. The samples were filtered through a 64 μm sieve, and the process was repeated 3 times so that no *Hemimysis* were left behind. From there, the *Hemimysis* were poured into a quadrat petri dish, where all the small juveniles (too small to handle individually for gut analysis ~< 3 mm) were sucked out using a pipette and moved to a beaker. Once all the smallest juveniles

had been removed, the beaker containing the juveniles was sieved twice through a 64 μm Nitex mesh, and the sample was stored in a glass jar preserved with 90% ethanol and marked with the trap date and color so that they could be counted later. The remaining *Hemimysis* that were large enough to handle and put into individual cryotubes were aged and sexed. The distinctions, abbreviations, and descriptions can be found in Table 1 (Borcherding et al. 2006; U.S. EPA, 2015; K. Bowen, Dept. Fisheries and Ocean Canada, 2/8/2022, personal communication). The process was repeated for all trap replicates, and the individual cryotubes were stored at -70°C to halt all metabolic processes. After enough time to allow for adequate freezing, the individuals were removed from their tubes and total length (from the tip of the rostrum, which is almost entirely absent in *Hemimysis*, to the base of the telson) was measured to the nearest 0.5 mm using a dissecting microscope. Individuals that were fragile and broke (typically juveniles) did not receive a length measurement. The cryotubes were kept on a blanket of ice inside a cooler and were never out of the -70°C freezer for more than 2 h to minimize damage and degradation to the proteins.

Table 1: Classification of individual *Hemimysis* caught by type with abbreviations and descriptions that were used across sample dates for classification continuity

Type	Abbreviation	Description
Juvenile	J	*less than 6mm and has no developing limb buds or pouch*
Immature male	IM	*development of IV pleopod starting, but does not have all segments and legs do not extend past caudal peduncle*
Male	M	*developed IV pleopod, generally larger than 6mm, but may be slightly smaller (Fig. 1)*
Female	F	*larger than 6mm, no enlarged IV pleopod and visibly empty marsupium*
Female w/ ovaries	FO	*fits female criteria, and has full/colored ovaries (Fig. 2)*
Female w/ eggs	FE	*fits female criteria, but marsupium contains eggs*
Female w/ embryos	FEM	*fits female criteria, but marsupium contains embryos (Fig. 2)*
Female w/ young	FY	*fits female criteria, but marsupium contains fully developed young (Fig. 2)*

Gut Content Analysis

The methods in this section were adapted from Berges et al. (2020). Antisera previously raised against nine potential prey items in New Zealand White rabbits (*Bosmina longirostris, Bythotrephes longimanus*, *Cercopagis pengoi*, *Daphnia mendotae*, *Daphnia pulex, Keratella cochlearis*, *Leptodiaptomus ashlandii, Limnocalanus macrurus*, and veliger larvae of *Dreissena* mussels) were purified against *Hemimysis* to reduce cross reactions between *Hemimysis* and potential prey items. A sample of fifteen large *Hemimysis* which had been starved for at least one day to allow for gut clearance were used to purify the antisera. Antibodies were purified using the reverse affinity method and a Thermo Scientific AminoLink Plus Immobilization Kit (44894) (as described in Berges et al. 2020).

To probe the gut contents, individual previously identified *Hemimysis* were homogenized using a sonicator in 4% (w/v) SDS 0.2 M carbonate buffer. After sonication, 2 µL and 5 µL sub-samples were taken to determine the total protein concentration using a Pierce BCA Protein Assay Kit (23225) with a bovine serum albumin standard. A VersaMax microplate reader (Molecular Devices, San Jose) was used to read absorbance at 562nm, from which protein concentration of the samples were calculated. The average of the two samples was taken to calculate the necessary volume that needed to be added so that the sample could be diluted to 1 µg μL^{-1} using SDS carbonate buffer (Berges et al. 2020). Once the samples were diluted, 2 µg of sample (equivalent to µL unless the sample had less than 1 µg μL^{-1} protein concentration) was blotted into a black 96 well plate (ThermoFisher Scientific), which was dried overnight. On each of the 96 well plates, the first four wells were used as controls. Well #1 had no sample but received every other step in the protocol. The second well did not receive primary antibody, the third well received no detection agent, QuantaBlu NS/K Fluorogenic Substrate (15162), and the

fourth well was run as normal. A single control sample (chosen based on the volume it contained so that there would be enough sample to run all of the 36 plates) was used for all plates across all dates in the second through fourth wells. The plates were incubated in SuperBlock Blocking Buffer in PBS (37515) for one hour. The primary antibody was then added and incubated for an hour, then was washed three times using a wash buffer (6.35 g Tris HCl, 1.18 g Tri Base, 8.76 g NaCl, and 5 mL Tween-20 topped off to 1 L using DI water). Then the secondary antibody of Pierce Goat Anti-Rabbit IgG (H+L), Horseradish Peroxidase conjugated (31460) was added, and plates were incubated for an hour before receiving three washes using the same wash buffer from the previous step. QuantaBlu was then added before loading the plate into a SpectraMAX Gemini XS microplate reader (Molecular Devices, San Jose) to measure relative fluorescence at excitation of 325 nm and emission of 420 nm (Berges et al. 2020).

In order to determine the correct 'background' to subtract from assay responses, we used the approach of Berges et al. (2020), assuming that the true signal came from a normal distribution, while the background did not and applying a variation of a Gaussian mixture model to establish the zero. It should be noted that because different antibodies have different sensitivities, among-prey-item comparisons must be made cautiously; only comparisons within a single prey item are truly quantitative.

Statistical Analysis

For time series data, linear regression analyses for each sex classification (J, IM, M, NRF, and RF) of length versus day-of-year were run. Two-way ANOVA tests were then run to see if reproductive female (RF) size differed from non-reproductive-female (NRF) size by day-

of-year, as well as another two-way ANOVA to see if immature male (IM) size differed from male (M) size by day-of-of year.

For gut content data, a logistic regression model was created using day-of-year, day-of-year^2 (to test for a quadratic effect), sex, length, and sample type (time series vs. swarm samples) to see which, if any, were a determining factor in presence or absence of any particular prey item in the gut. A quantitative linear model was also run with the same parameters for each prey item, and only included individual *Hemimysis* where prey items were detected in the gut. Because there were nine potential prey items and multiple comparisons were made, a Bonferroni correction ($corrected\ p-value = \alpha/\eta$, where α is the original p-value and η is the number of tests performed, i.e., 9) was used to ensure that the Type I error remained at 5%. This resulted in a corrected p-value = 0.0056.

One way ANOVA tests were run on all sex classifications to see if there was a significant difference in the size of individuals in the swarms versus the size of individuals in the time series data. Additionally, another one-way ANOVA was run to test for a significant difference in length individuals in the top versus the bottom of the swarm.

Results

Population Dynamics

For analysis, females were grouped into reproductive and non-reproductive females. Reproductive females were counted as females with ovaries, eggs, embryos, young, or any combination of the previous. Non-reproductive females were all other females that did fit those characteristics.

Average male size significantly increased over the sampling period from just above 6mm in the summer to over 10mm by the end of Winter ($F = 1174.61$; $R^2 = 0.7417$; $n = 411$; $p < 0.00001$) (Fig. 5). However, the average size of immature males did not increase over time, but rather stayed constant between 7 and 8mm ($F = 0.4115$; $R^2 = 0.0036$; $n = 116$; $p = 0.5225$) (Fig. 5). The one-way ANOVA looking at differences in male and immature length showed that males were not significantly larger than immature males ($F = 0.3836$; $df = 1, 114$; $p = 0.5369$).

The one-way ANOVA for reproductive and non-reproductive females revealed that reproductive females were significantly larger than non-reproductive females on all dates where both groups were present ($F = 6.7813$; $df = 1, 132$; $p = 0.01$) (Fig. 5), thus supporting H6 that reproductive females will be larger than non-reproductive females. Additionally, both reproductive and non-reproductive females increased significantly over time: reproductive females ($F = 54.22$; $R^2 = 0.4665$; $n = 64$; $p < 0.00001$), non-reproductive females ($F = 320.535$; $R^2 = 0.4187$; $n = 447$; $p < 0.00001$). Juvenile length was also dependent on date and showed a significant relationship, with juveniles in summer being smaller than juveniles at the end of the reproductive reason ($F = 5.056$; $R^2 = 0.0218$; $n = 229$; $p = 0.0255$). Juveniles were also significantly smaller than any other group ($F = 154.4913$; $df = 4, 367$; $p < 0.0001$) (Fig. 5).

When looking at adult length over time, males and females collected in November-April were significantly longer than those collected in July-October, thus supporting H7 that "average length of adults increases in the non-reproductive season compared to the reproductive season."

Linear regression analyses showed significant positive relationships between length and day-of-year for, males, reproductive females, non-reproductive females, and juveniles, but not immature males. All significant linear regressions yielded equations, which included a growth rate based on day-of-year (slope). For males the equation was $y = 0.0149 (\pm 0.0004) X + 3.6477 (\pm 0.138)$, giving a growth rate of 0.0149 mm d^{-1}. For reproductive females the equation was $y = 0.0111 (\pm 0.0015) X + 5.3862 (\pm 0.398)$, giving a growth rate of 0.0111 mm d^{-1}. For non-reproductive females the equation was $y = 0.0129 (\pm 0.0007) X + 3.387 (\pm 0.261)$, giving a growth rate of 0.0129 mm d^{-1}. While the growth rates for reproductive females was not significantly different from the growth rate for non-reproductive females, the growth rate of males was significantly different than that of females. Lastly, for juveniles the equation was $y = 0.005 (\pm 0.0022) X + 3.1774 (\pm 0.571)$, giving a growth rate of 0.005 mm d^{-1}.

During the reproductive season, the juvenile abundance was orders of magnitude higher than any other group. The highest juvenile count was from 8/13/21 yielding a count of 6,572 juveniles, which was 99.47% of the total abundance (Fig. 7). Starting in late October, there was a decline in juvenile abundance that gradually decreased until December were no juveniles were caught thus supporting H1 that, "juveniles will dominate the population composition during the reproductive season." When looking at the adults, within the reproductive season, males were the most abundant, composing anywhere from 92-44% of the adult population. Reproductive females were the next most abundant adult group during the reproductive season, followed by non-reproductive females (Fig. 6). During the non-reproductive season, starting in October (no

reproductive females were identified past 9/28/2021 and were not seen again until 3/25/2022), non-reproductive females began dominating composing anywhere from 40-100% of the adult population on any sample date. Males and immature males were present in smaller numbers comparatively, with mature males usually outnumbering immature males (Fig. 6). This supports H2 that, "females will dominate the population during the non-reproductive season," and simultaneously supports H3 that, "after the reproductive season, the number of males with decline." Overall abundance also decreased in the non-reproductive season from several thousand individuals in the reproductive season, to not more than 200 starting in October (with the exception of 3/4/2022, which had a total of 483 individuals) (Fig. 7).

Swarm Data

ANOVA tests of length comparing individuals in the swarm to individuals in the time series data showed that there was no significant difference in length of individuals between the two sample types ($F = 2.8124$; $df = 1, 367$; $p = 0.0944$). Additionally, among swarms there was no significant difference in length of individuals who were at the top of the swarm compared to those who were at the bottom of the swarm ($F = 0.0131$; $df = 1, 2.147$; $p = 0.9188$) (Fig. 13b)

Gut Content

Of the 368 individuals probed, 143 (38.9%) did not have anything detectable in their gut, 99 (26.9%) had only one prey species detectable in their gut, 54 (14.7%) had two prey species detectable in their gut, and 72 (19.6%) had three or more prey species detectable in their gut. (Fig. 11). The highest values for *Keratella cochlearis* and *Limnocalanus macrurus* occurred on 8/4/21 (Fig. 8b). The highest value for *Daphnia galatea mendotae* occurred on 8/13/21 (Fig. 8c).

The highest veliger larvae value occurred on 9/1/21 (Fig. 8d). The highest values for *Daphnia pulex* occurred on 9/28/21 (Fig. 8g). The highest value for *Leptodiaptomus ashlandii* occurred on 10/19/21 (Fig. 9b). The highest values for *Bosmina longirostris* and *Cercopagis pengoi* occurred on 10/20/21 (Fig. 9a) in Swarm 1 within the gut on the same individual, #7. Lastly, the highest value for and *Bythotrephes longimanus* occurred on 2/18/22 (Fig. 9g).

The results from the logistic regression model based on presence or absence of a particular prey item in the gut are displayed in Table 2. There were no significant relationships for DOY ($p > 0.025$ for all prey items), sex ($p>0.12$, for all prey items) or length ($p > 0.01$ for all prey items) (Table 2). *Daphnia pulex* showed a significant relationship for DOY^2, indicating a quadratic relationship over time ($df = 1$; $X^2 = 11.324$; $p = 0.008$). *Daphnia pulex* also showed a significant relationship for sample type ($df = 1$; $X^2 = 30.084$; $p < 0.0001$) with more being present in the time series samples and almost no *D. pulex* in the swarm samples. However, *D. pulex* did not have any significant relationships for DOY, sex, or length ($p > 0.05$) (Table 2, row 4). *Bythotrephes longimanus* also showed a significant relationship for DOY^2 ($df = 1$; $X^2 = 16.746$; $p < 0.0001$) with more appearing in the guts in the summer and late winter and less appearing in the guts in late fall. *Bythotrephes* also had a significant relationship for sample type ($df = 1$; $X^2 = 8.3602$; $p = 0.0038$) with higher values occurring in the time series samples than in the swarm samples. However, *Bythotrephes* did not have significant relationships for DOY, sex, length, or sample type ($p > 0.025$) (Table 2, row 5). Additionally, there were no significant relationships with DOY^2 or sample type for *Keratella cochlearis*, *Leptodiaptomus ashlandii*, *Limnocalanus macrurus*, *Dreissena* veliger larva, *Daphnia galatea mendotae*, *Bosmina longirostris*, or *Cercopagis pengoi* ($p > 0.01$ for all prey items, Table 2). These results show that there are no significant relationships between sex and prey item consumed, and therefore does not support the

hypothesis, H4 and H5 that, “juvenile diet is different from adult diet” and that, “male diet is different from female diet.” These results also partially support H8 that, “diet of individuals in the swarm is different that the diet of individuals caught in traps,” depending on the prey item in question.

The results from the qualitative linear model based on the amount of a particular prey item present in the gut are displayed in Table 3. All tests for DOY, DOY^2, sex, and length yielded non-significant results ($p > 0.009$ for all prey items, Table 3). *Cercopagis pengoi* was the only prey item to have a significant relationship for sample type ($df = 1, 43$; $X^2 = 14.859$; $p = 0.0004$), with higher *Cercopagis* signals being found in the swarm samples than in time series samples, while all other prey items had no significant relationship for sample type ($p > 0.02$, Table 3). These results further suggest that H4 (“juvenile diet is different from adult diet”) and H5 (“male diet is different from female diet”) are not supported by the data. Furthermore, these results also partially support H8 (“diet of individuals in the swarm is different that the diet of individuals caught in traps”) depending on the prey item in question.

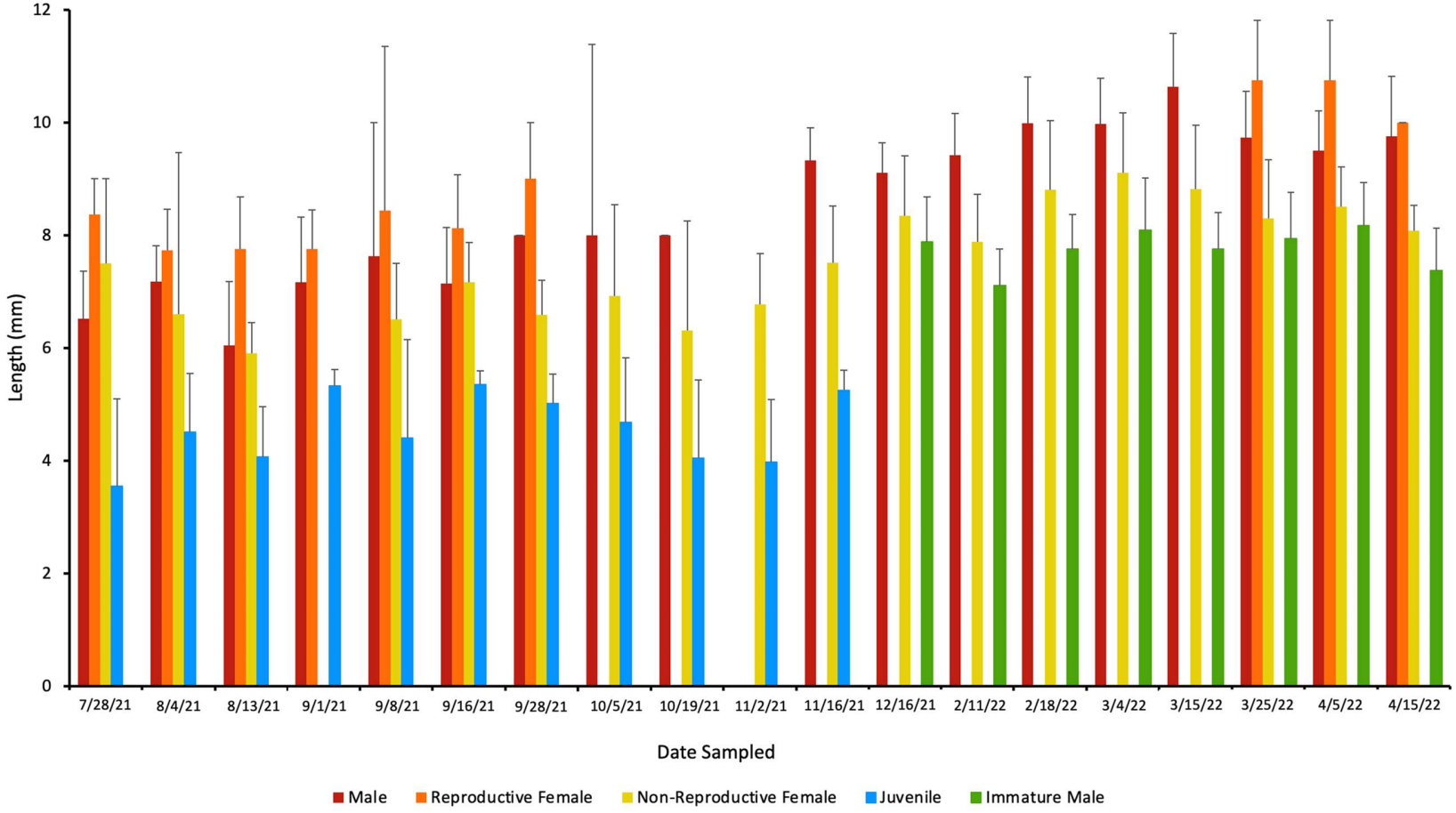

Figure 5: Average *Hemimysis* length in mm for males, reproductive females, non-reproductive females, juveniles, and indeterminates for each sample date collected from underwater lighted funnel traps. Error bars indicate standard error. For dates where there is no bar for that category means no individuals in that category were found on that date. Error bars with values of zero indicate only one individual in that category was sampled.

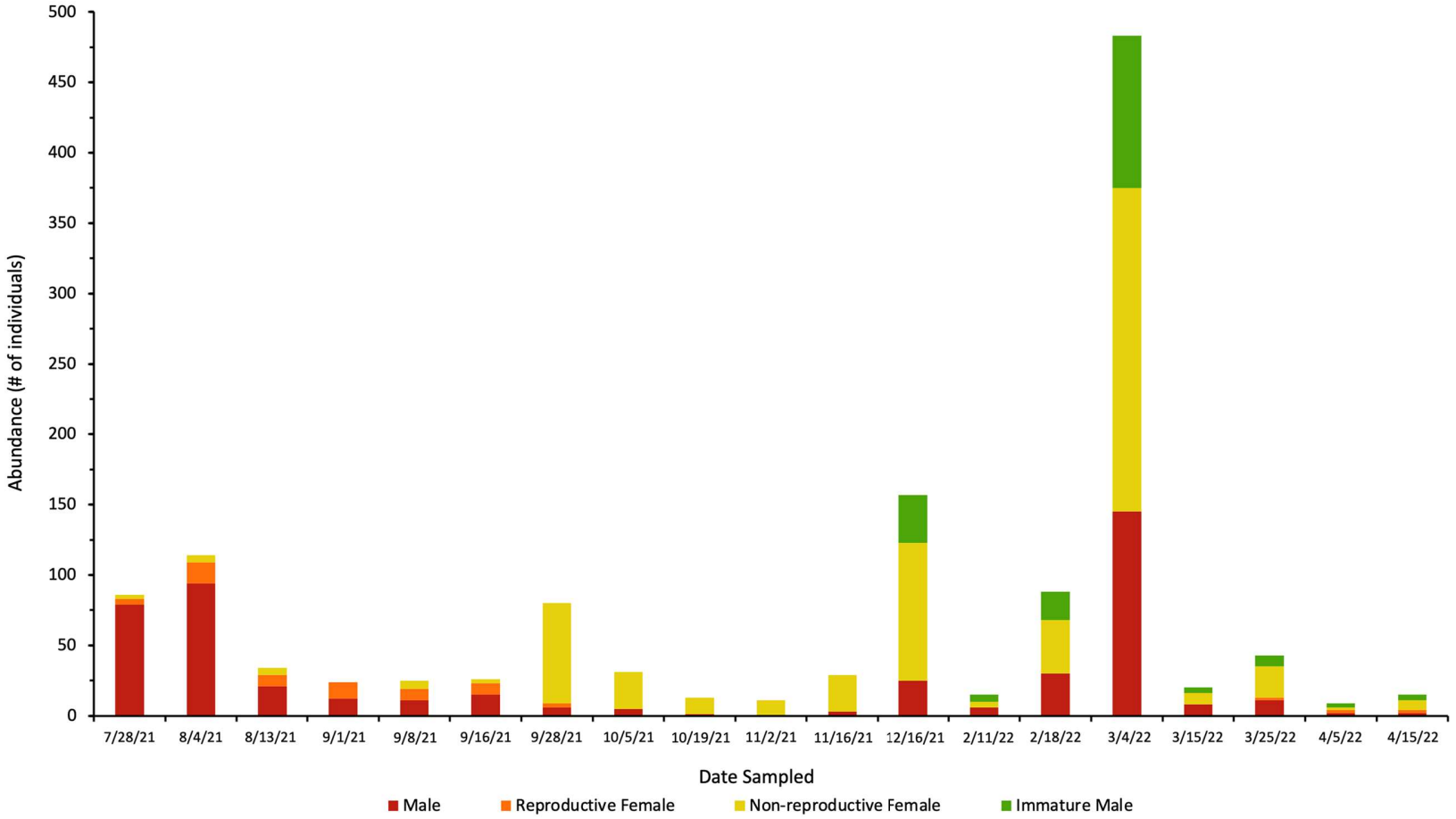

Figure 6: Relative abundance of males, reproductive females, non-reproductive females, and indeterminate *Hemimysis* as number of individuals by sample date collected from underwater lighted funnel traps. Dates where a category is not present means no individuals within that category were found for that sample date. Juvenile abundance was not included in this graph.

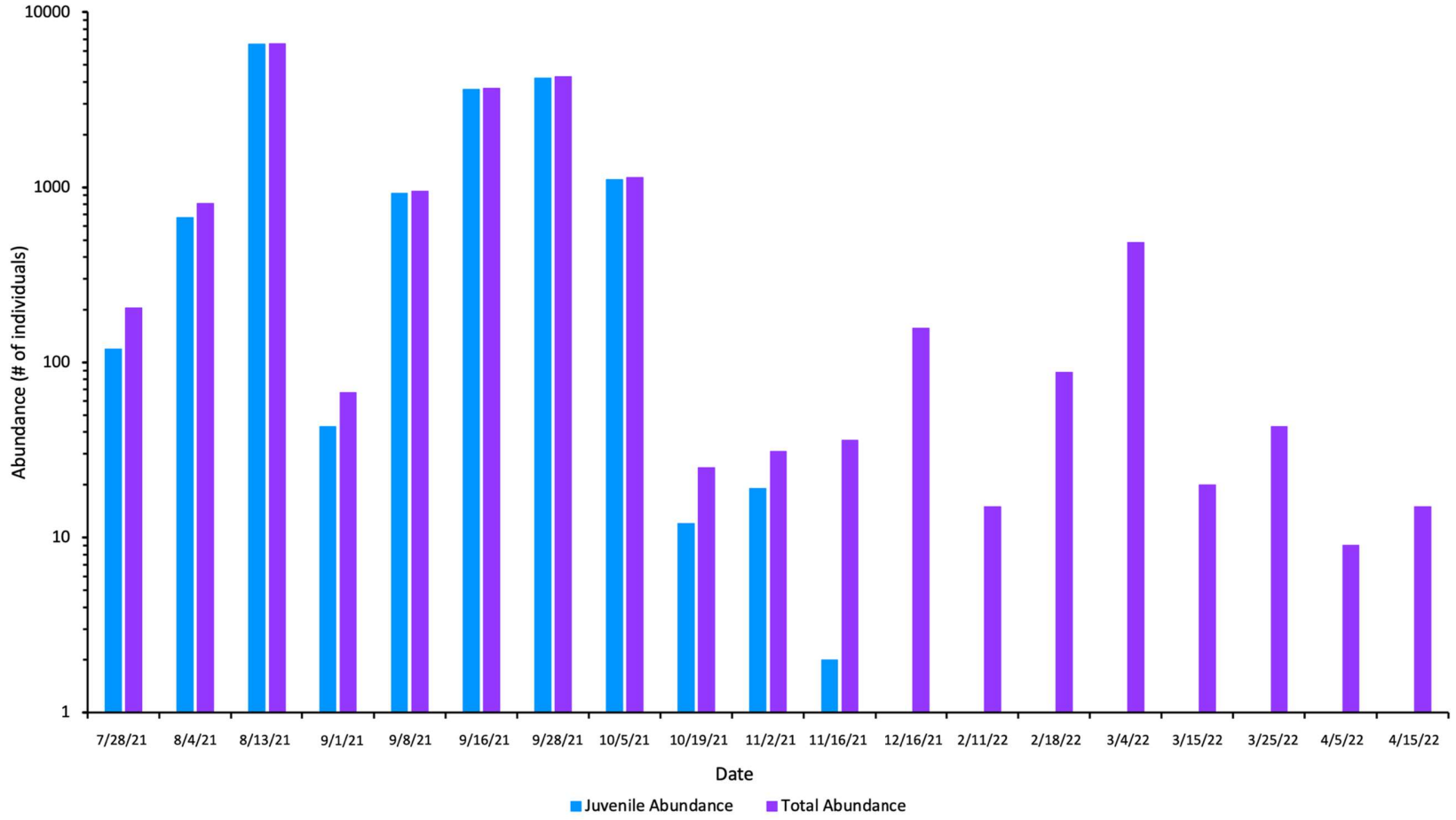

Figure 7: Juvenile and total abundance of *Hemimysis* as number of individuals by date collected from underwater lighted funnel traps displayed on a log scale.

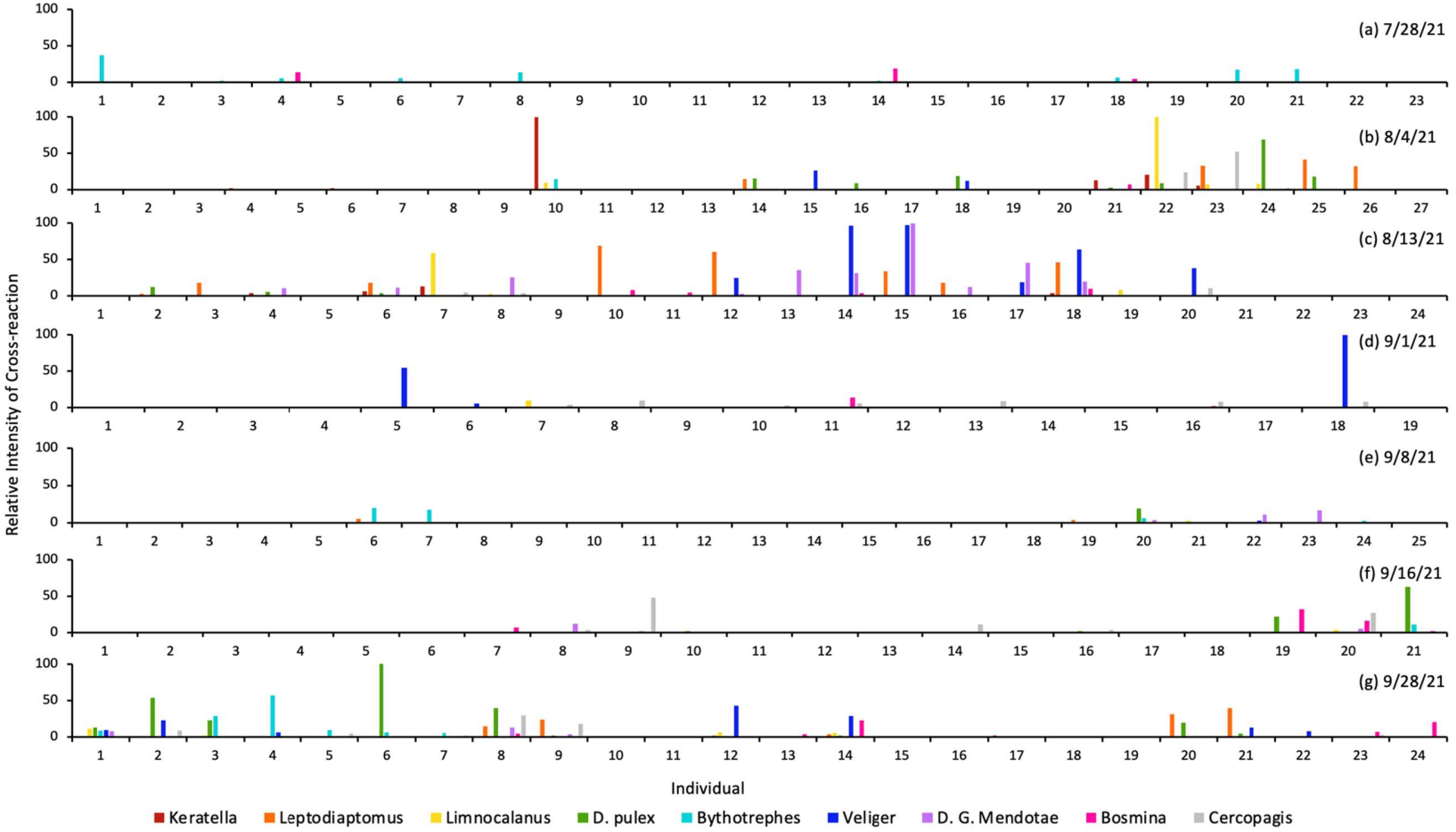

Figure 8: Results of probed gut content for sonicated samples from 7/28/21 to 9/28/21. One hundred and sixty-three individual *Hemimysis* were probed with antibodies against *Keratella*, *Leptodiaptomus*, *Limnocalanus*, *D. pulex*, *Bythotrephes*, Veliger, *D. G. Mendotae*, *Bosmina*, and *Cercopagis* using the immunochemical methods described in Berges et al. 2020. Results were expressed as a percentage of the highest value recorded for each species.

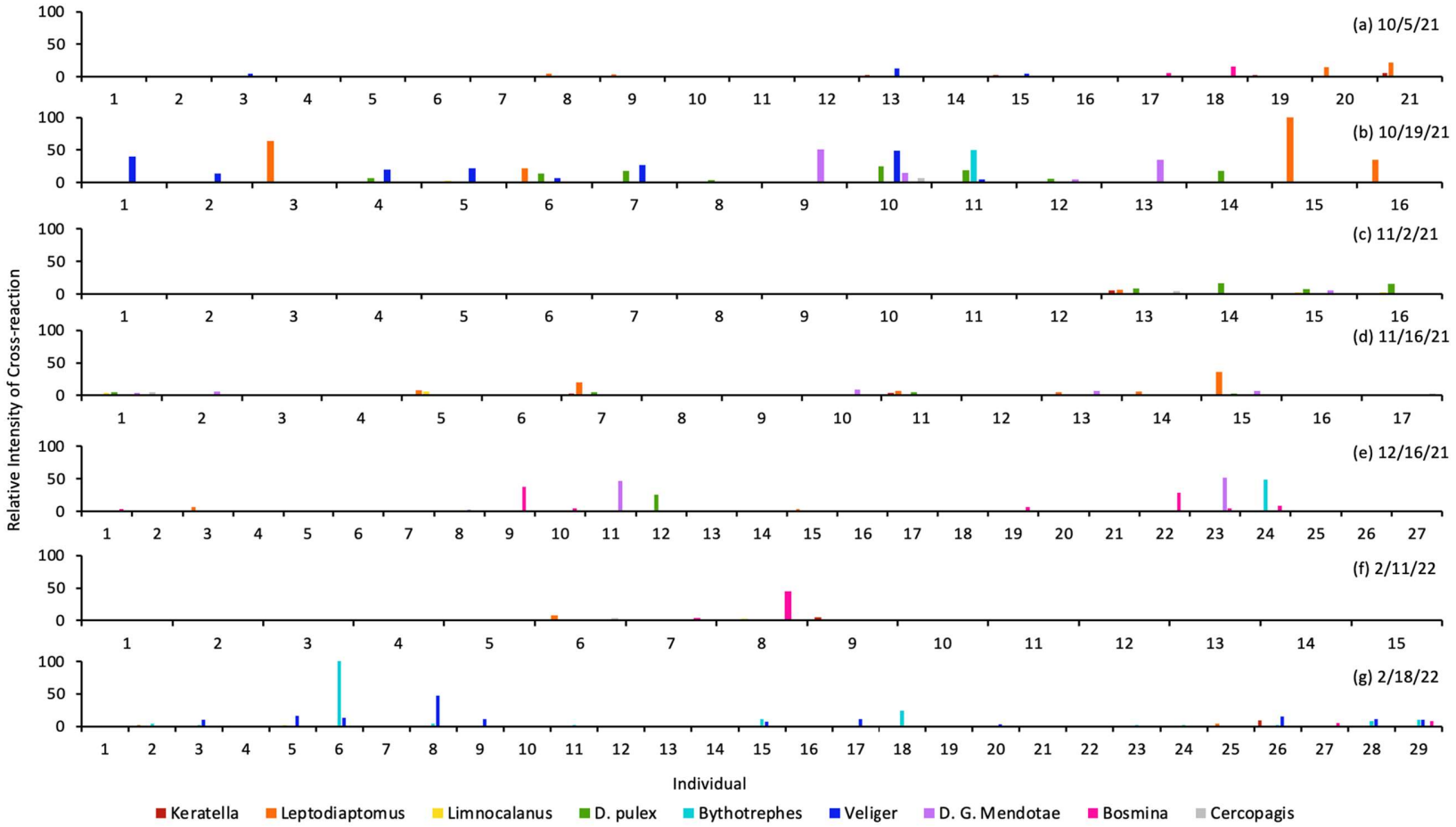

Figure 9: Results of probed gut content for sonicated samples from 10/5/21 to 2/18/22. One hundred and forty-one individual *Hemimysis* were probed with antibodies against *Keratella*, *Leptodiaptomus*, *Limnocalanus*, *D. pulex*, *Bythotrephes*, Veliger, *D. G. Mendotae*, *Bosmina*, and *Cercopagis* using the immunochemical methods described in Berges et al. 2020. Results were expressed as a percentage of the highest value recorded for each species.

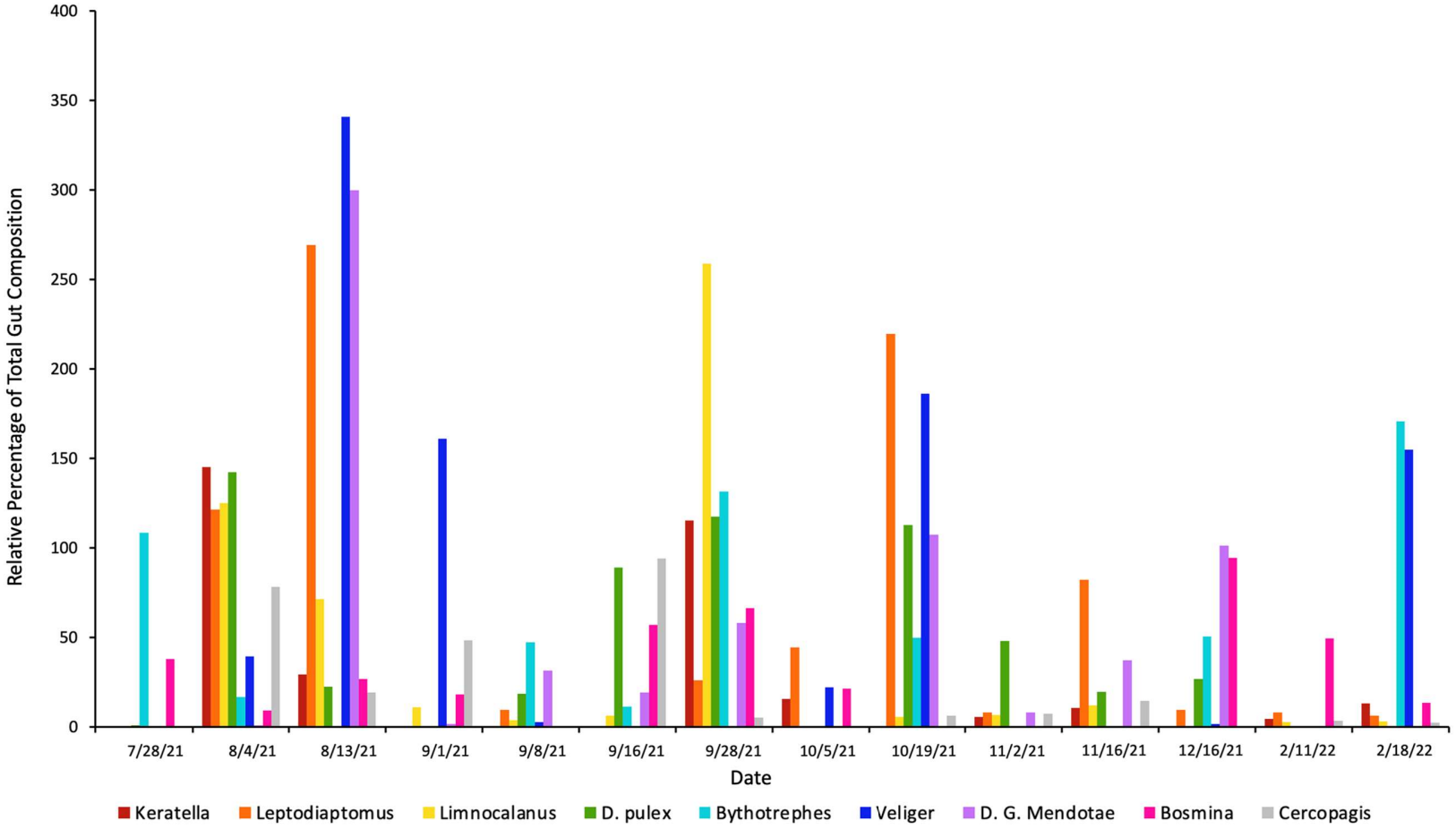

Figure 10: The percentages of each prey item for a particular sample date totaled to give a proportion of that prey item compared to others on the same day across all sample dates.

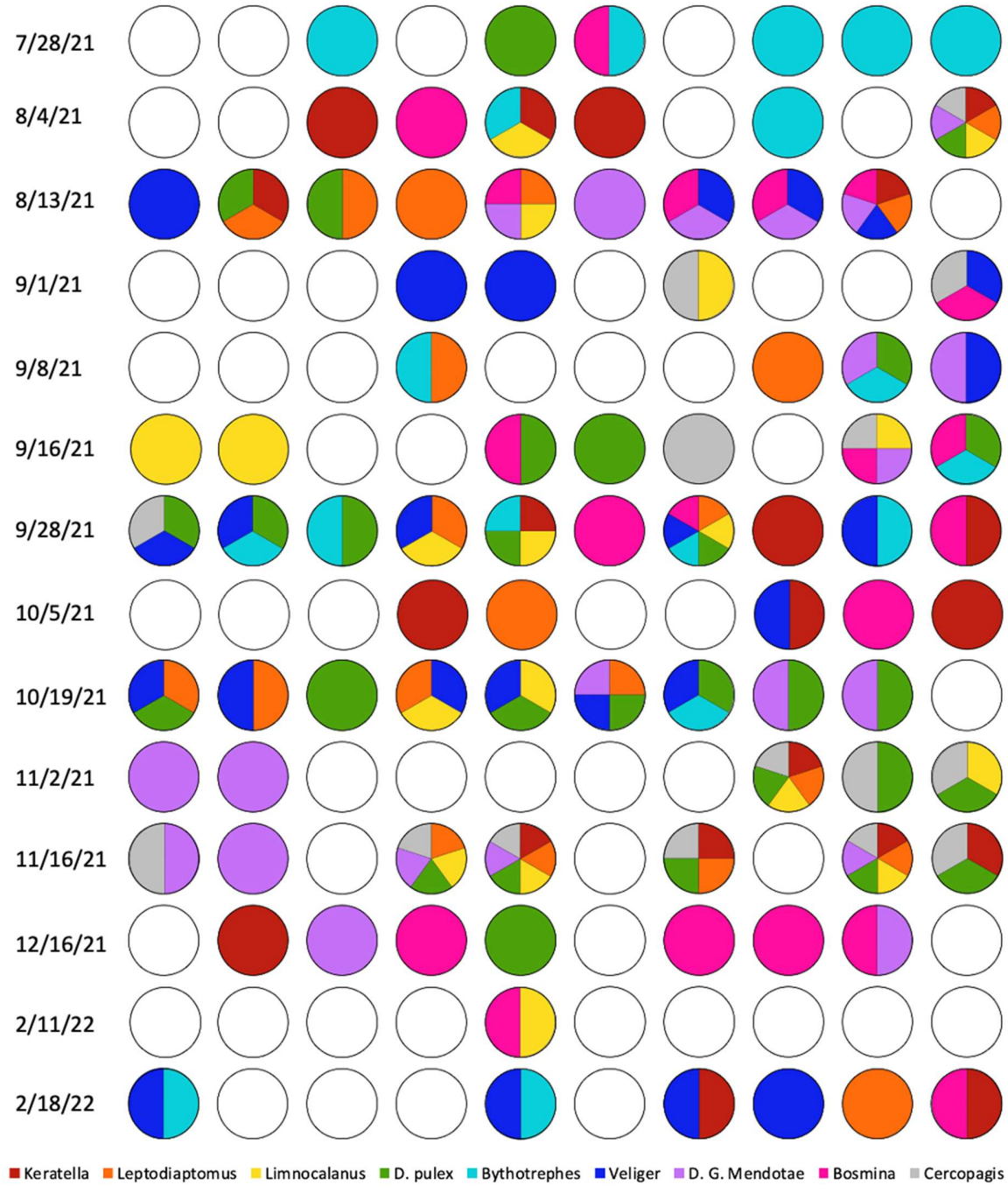

Figure 11: Ten individuals from each sample day were chosen at random using a random number generator. The individual pie charts represent the prey items that were in the gut on a presence/absence basis, not the quantity present. White circles indicate individuals who had no detectable prey items in their gut.

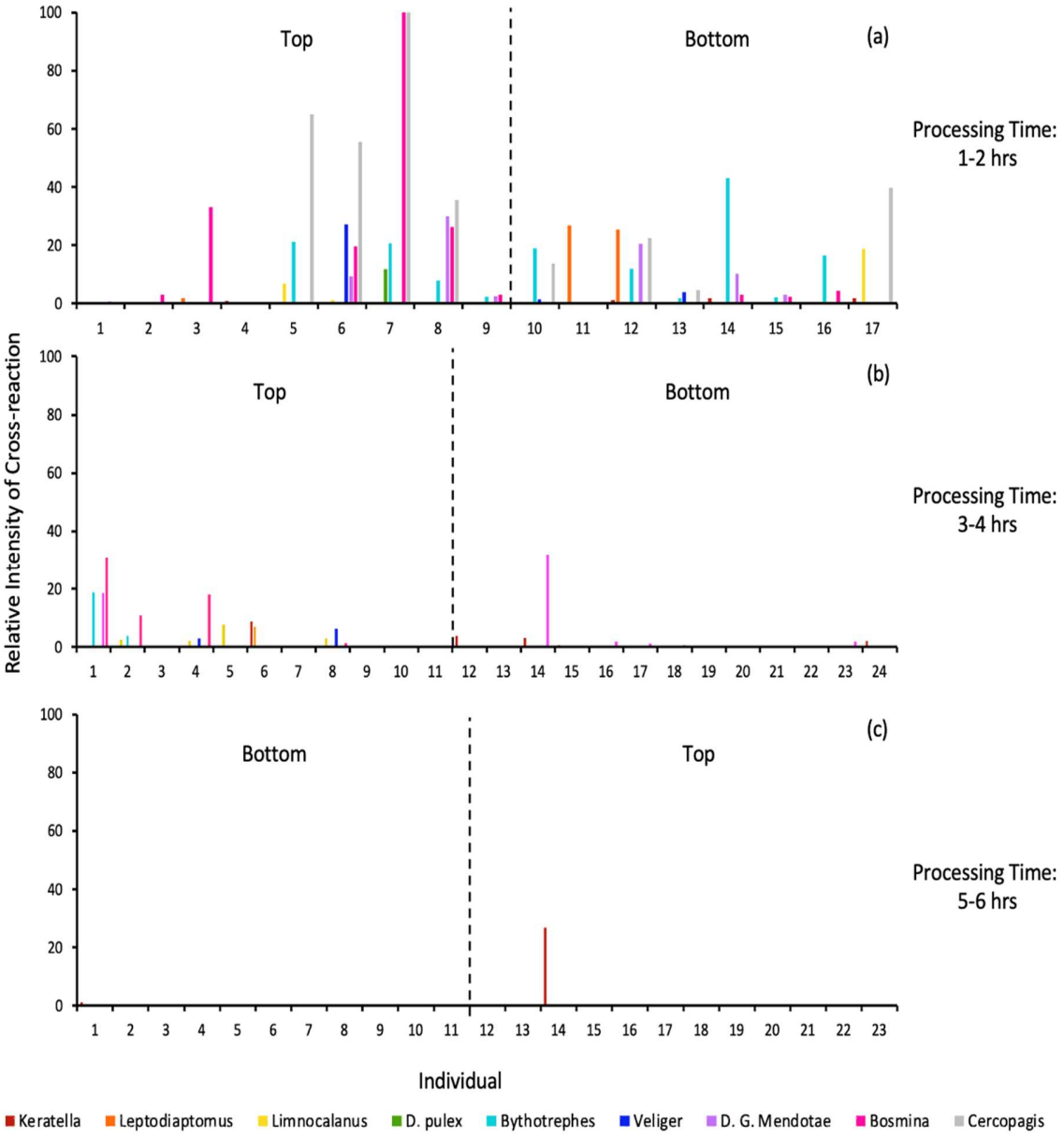

Figure 12: Results of probed gut content for sonicated samples from three swarm replicate samples. Dotted lines on the graph indicate the separation between individuals that came from the top and bottom of the swarms. Processing time in on the right indicates the amount of time passed from the moment of sampling to the moment Hemimysis were put into the -70 °C freezer. A total of sixty-four individual *Hemimysis* were probed with antibodies against *Keratella*, *Leptodiaptomus*, *Limnocalanus*, *D. pulex*, *Bythotrephes*, Veliger, *D. G. Mendotae*, *Bosmina*, and *Cercopagis* using the immunochemical methods described in Berges et al. 2020. Results were expressed as a percentage of the highest value recorded for each species.

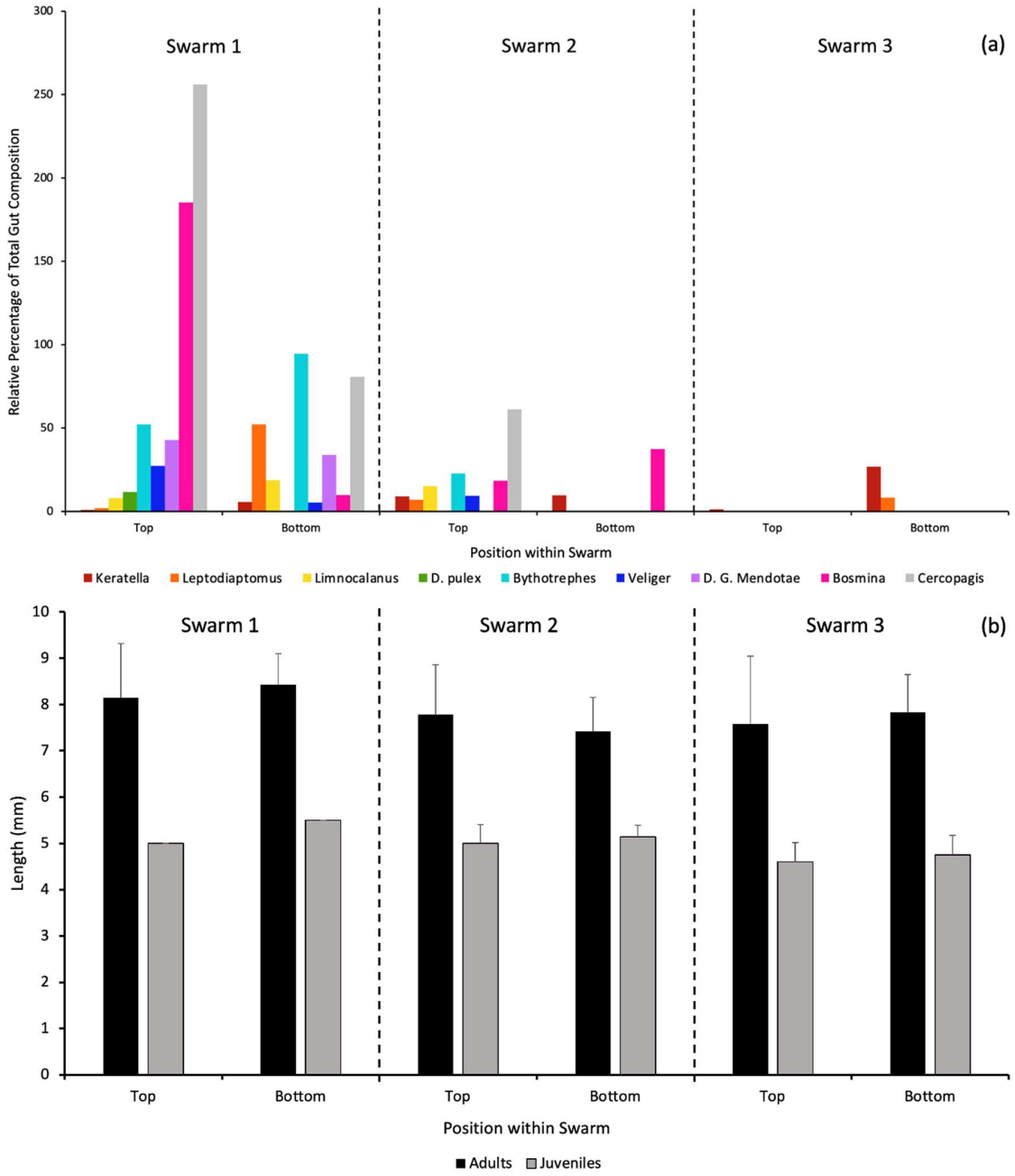

Figure 13: (a) Results of probed gut content for sonicated samples from three swarm replicate samples. Dotted lines on the graph indicate the separation between individuals swarm replicates. A total of sixty-four individual *Hemimysis* were probed with antibodies against *Keratella*, *Leptodiaptomus*, *Limnocalanus*, *D. pulex*, *Bythotrephes*, Veliger, *D. G. Mendotae*, *Bosmina*, and *Cercopagis* using the immunochemical methods described in Berges et al. 2020. Results were expressed as the percentages of each prey item for a particular swarm totaled to give a proportion of that prey item compared to others across swarms. (b) Length in mm of adults and juveniles at the top and bottom of the swarm for all three replicates with standard deviation bars.

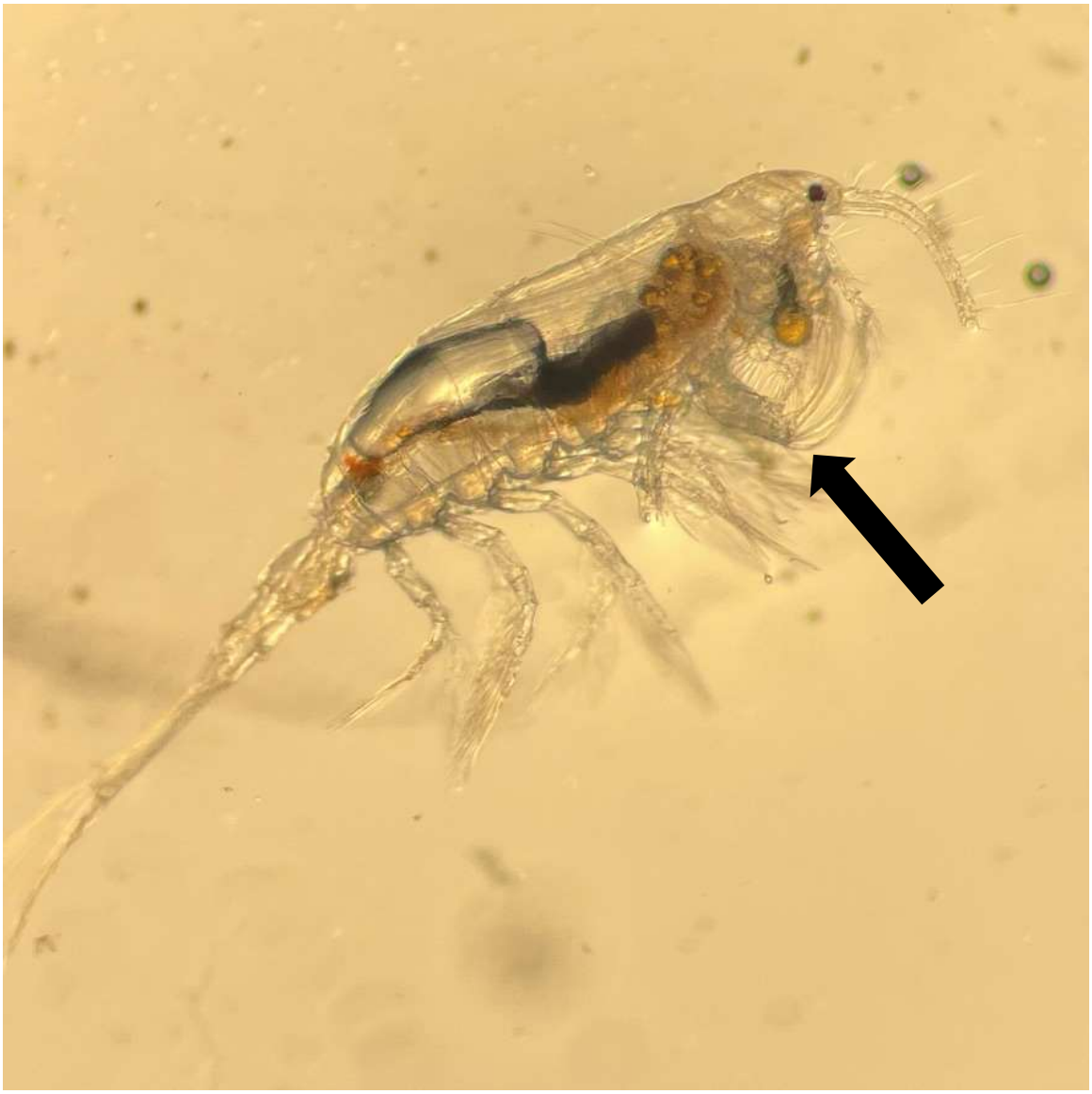

Figure 14: Photo of *Limnocalanus macrurus* caught in light-based funnel trap on 2/11/22. Individual was easily identified because of large mouth parts, as indicated with arrow.

Table 2: Results of logistical regression model of presence versus absence of individual prey items in the gut run on JMP reporting degrees of freedom, F-ratio, and the p-value for day-of-year (DOY), DOY^2, sex, length, and sample type for 368 individual *Hemimysis* probed against nine antibodies. Red values indicate significant relationships. Adjusted using a Bonferroni correction, a 5% probability of a type I error corresponds to p = 0.0056.

	Day of Year			Day of Year ^{2}			Sex			Length			Sample Type		
Antibody probed	df	x^2	p-value	df	x^2	p-value	df	x^2	p-value	df	x^2	p-value	df	x^2	p-value
Keratella cochlearis	1	0.9377	0.3329	1	0.8695	0.3511	4	2.7172	0.6062	1	0.4891	0.4843	1	1.2371	0.2660
Leptodiaptomus ashlandii	1	0.0026	0.9589	1	1.8234	0.1769	4	5.7033	0.2224	1	1.6389	0.2005	1	2.5761	0.1085
Limnocalanus macrurus	1	0.0065	0.9357	1	0.7773	0.3780	4	2.3898	0.6645	1	1.1158	0.2908	1	0.4332	0.5104
Daphnia pulex	1	0.5211	0.4707	1	11.324	0.0008	4	7.6168	0.1067	1	3.7531	0.0527	1	30.084	<0.0001
Bythotrephes longimanus	1	4.9648	0.0259	1	16.746	<0.0001	4	1.5904	0.8105	1	0.0942	0.7589	1	8.3602	0.0038
Dreissena veliger larvae	1	0.5586	0.4548	1	1.0052	0.3161	4	7.2914	0.1213	1	6.0502	0.0139	1	3.1259	0.0771
Daphnia galatea mendotae	1	0.1657	0.6839	1	6.4313	0.0112	4	6.6185	0.1575	1	1.2529	0.2630	1	4.6213	0.0316
Bosmina longirostris	1	0.1146	0.7350	1	0.0024	0.9613	4	2.3174	0.6776	1	0.1566	0.6923	1	5.7385	0.0166
Cercopagis pengoi	1	0.1596	0.6895	1	2.4457	0.1179	4	1.4251	0.8398	1	0.8090	0.3684	1	0.0119	0.9133

Table 3: Results of Quantitative linear regression model of amount of an individual prey item in the gut run on JMP reporting degrees of freedom, chi-squared, and the p-value for day-of-year (DOY), DOY^2, sex, length, and sample type for 368 individual *Hemimysis* probed against nine antibodies. Red values indicate significant relationships. Adjusted using a Bonferroni correction, a 5% probability of a type I error corresponds to p = 0.0056.

	Day of Year			Day of Year ^{2}			Sex			Length			Sample Type		
Antibody probed	df	F	p-value	df	F	p-value	df	F	p-value	df	F	p-value	df	F	p-value
Keratella cochlearis	1, 48	2.8297	0.0090	1, 48	1.1753	0.2837	4, 48	0.3352	0.8529	1, 48	0.0068	0.9346	1, 48	0.1664	0.6852
Leptodiaptomus ashlandii	1, 43	4.9186	0.0319	1, 43	1.2699	0.2660	4, 43	0.1524	0.9606	1, 43	0.1081	0.7439	1, 43	5.6544	0.0219
Limnocalanus macrurus	1, 40	1.1600	0.2879	1, 40	06841	0.4131	4, 40	0.5331	0.7121	1, 40	0.2220	0.6400	1, 40	0.4247	0.5183
Daphnia pulex	1, 45	1.2525	0.2690	1, 45	1.5607	0.2180	4, 45	0.4092	0.8011	1, 45	0.0571	0.8123	1, 45	0.0340	0.8545
Bythotrephes longimanus	1, 47	0.1699	0.6821	1, 47	2.0564	0.1582	4, 47	1.1198	0.3585	1, 47	2.0407	0.1598	1, 47	0.3788	0.5412
Dreissena veliger larvae	1, 44	1.3689	0.2483	1, 44	1.5812	0.2152	4, 44	0.9607	0.4386	1, 44	0.4189	0.5209	1, 44	0.4465	0.5075
Daphnia galatea mendotae	1, 45	0.5252	0.4724	1, 45	0.1480	0.7023	4, 45	0.6715	0.6152	1, 45	0.4946	0.4855	1, 45	0.0546	0.8164
Bosmina longirostris	1, 43	4.2095	0.0463	1, 43	0.1750	0.6778	4, 43	1.1407	0.3503	1, 43	1.2240	0.2747	1, 43	1.6016	0.2125
Cercopagis pengoi	1, 43	3.5772	0.0653	1, 43	1.0270	0.3165	4, 43	0.4522	0.7702	1, 43	0.0777	0.7818	1, 43	14.859	0.0004

Discussion

Comparing to Literature and Environmental Availability

One of the most striking results from our study is the juvenile dominance of abundance in the reproductive season. While other studies have also found that juveniles were the most abundant group during this season, it was not nearly the dominance that we found in ours. For example, Borcherding et al. (2006), whose study was conducted in parts of the Rhine River found that juveniles made up 60% of samples in September. Additionally, a separate study done in Lake Ontario (Taraborelli et al. 2012) found that juveniles comprised 53.3% of the sample in Summer (July 20th to August 28th). However, during these sample times, we found that juveniles made up more than 95% of the samples until mid-October (Fig. 5). While there are possible biases in the different sampling methods (both Borcherding et al. and Taraborelli et al. used plankton nets), our sampling methods would be biased against juveniles rather than in favor of them (see Limitations section). Additionally, this may be a factor of habitat preference and selection. Borcherding et al. (2006) sampled in a gravel pit lake, and while gravel is more preferred that sandy and muddy substrates, it is still not the most preferred substrate among *Hemimysis* (Boscarino et al. 2020). Taraborelli et al (2012) sampled around a pier, which while it is a man-made structure, it is still more exposed in three-dimensional space compared to a large boulder environment, which is the most preferred substrate by *Hemimysis* (Boscarino et al. 2020), and to a breakwall environment where we sampled. A recent study shows that the breakwall environments may truly be a novel ecosystem for *Hemimysis* (Geisthardt et al. 2021), which may be why we see such a clear dominance of juveniles in the area that is not seen in habitat types that have been previously sampled. In terms of adult abundance, our results match closely with both Borcherding et al. and Taraborelli et al. where males are the most abundant

adult during the reproductive season, and that females become the most abundant adult in the non-reproductive season (Fig. 5 & 6). This may be because it is well documented that some male mysids die shortly after mating (Mauchline, 1960; Amaratunga and Corey, 1975; Hakala, 1978; Astthorsson, 1990).

While there are many comparisons and regressions for length in the literature to brood size, mass (both dry and wet weights) and prey item consumed, there is less (if any) data and equations available for growth rates of *Hemimysis* over time of year. Prior studies only focused on sampling during the most active season (May through November), and we found no differences in length during those months but did find that those months were significantly different than overwintering months (November through March) where we saw increase in growth over time. This may explain why there are no published growth rates for *Hemimysis*. During the reproductive season, individuals are allocating energy and resources to reproduction, not to growth, so during the reproductive season there is not a significant change in length, which may explain why there are not any linear regressions and growth rates reported (because they were non-significant). However, because we sampled through the winter months and saw an increase in size of both sexes, our results were significant, hence why we report growth rates over time.

Zooplankton are an important link between the base of the food chain, such as phytoplankton and benthic algae (i.e., *Cladophora*), and the higher trophic levels within the system (i.e., fish) (Levington 2018). To properly understand food web dynamics, energy flow within a system, and how these systems will be impacted by the decline of some species and the addition of new species, like *Hemimysis*, it is essential to understand the behavior and distribution of zooplankton. In particular, for *Hemimysis*, while their position is controlled by

vertical migration, as it is in many planktonic species, the formation of swarms also plays a huge role in *Hemimysis* distribution. Ritz (2000) found that the individual food intake and individual growth rate of the swarming mysid, *Paramesopodopsis rufa*, was well correlated to the size of the swarms. At low densities, there is less overall food intake, and thus less overall growth, likely due to increased predation. At intermediate densities, the individuals gain protection in numbers and are able to subsist with greater food intake and therefore higher growth rates. However, at high densities, competition with other individuals becomes a factor and overall food intake decreases and so does growth, but it still is higher than that of individuals who congregate in small groups, or do not congregate at all (Brockett and Hassall 2005; Ritz 2000). Because these *Hemimysis* swarms tend to be closer to the surface (Geisthardt et al. 2021; Pothoven et al. 2007; Ketelaars et al. 1999), even during the day, it was hypothesized that gut content of individuals within the swarm would be different than gut content of individuals caught in traps, which were placed on the benthos. This hypothesis was partially supported, particularly when looking at the presence or absence of *Daphnia pulex* and *Bythotrephes longimanus* (Table 2), as well as the amount of *Cercopagis pengoi* present in the gut (Table 3).

Results of polyclonal antibody gut content probing show that *Hemimysis* is a generalist feeder consuming all nine prey items tested, and that there is no difference in type of prey consumed based on sex, age, or body length. There are a few studies in the literature which have looked at *Hemimysis* gut content, but none have used the polyclonal antibody method. Evans et al. (2017) used plankton nets (0.5 m diameter, 500 μm mesh) at night to capture *Hemimysis* rather than traps. Whereas almost 40% of our *Hemimysis* had empty guts, less than 5% of their *Hemimysis* had empty guts. This leads us to believe that our traps had a bias which allowed for more gut clearance time. Evans et al. (2017) collected 156 individuals from May through

November and performed dissections of the guts to identify prey items. They found *Leptodora* (which we did not have an antibody for) in adult (10-36%) and juvenile guts (8-20%) in July and August. They also analyzed the presence of phytoplankton in the gut (which we did not do) and found that phytoplankton was a sizable amount of the gut content in September through November for juveniles, and for adults, phytoplankton in the guts was high in June, as well as September through November. This diet shift corresponded well with abundance in the lake during the time of year, with both juveniles and adults eating more zooplankton when phytoplankton are less abundant in the gut (i.e., July and August). However, there were several species that we both found. While we found *Cercopagis* in both swarm and time series samples from August through November, they only found *Cercopagis* in adult (45-90%) and juvenile (10-30%) guts in July. This is similar to *Cercopagis* abundances reported in Lake Michigan by Witt et al. (2005), where the population appeared in the water column starting in July and persisted through October, which explains why we say high consumption of *Cercopagis* even in October (Fig. 13a). While we found *Bythotrephes* in July through December as well as February in both adult and juvenile guts, they only found *Bythotrephes* in 36% of guts in July and 17% of guts in September for adults only, and there were no *Bythotrephes* found in the gut after that. In the nearshore environment in Lake Michigan, *Bythotrephes* are most abundant in October, but can be found July through December, and even in March (Pothoven and Vanderploeg, 2019). One possibility for the discrepancy between our study and Evans et al. (2017) is that *Hemimysis* are eating *Bythotrephes* eggs/resting cysts which settle on the benthos (Yurista, 1997), and which would likely be detected by the polyclonal antibody method but could be otherwise missed by dissection and visual inspection. We found *Kertella cochlearis* in the gut from August through November, as well as in February. Evans et al. (2017) found rotifers (including *Keratella*)

present in May through November, with lower values in May (13-33%) and November (30-40%) but made up anywhere from 75-100% of the gut content in August. Because we did not see this high of abundance in the guts of our *Hemimysis*, it is possible that Evans et al. was counting more of the other *Keratella* species they looked at besides *cochlearis*, while our antibody probe only had a lower degree of cross reaction and only reacted with *cochlearis*. This is consistent with the fact that *Keratella cochlearis*'s highest abundances in Lake Michigan are in June, and not in August (Duffy and Liston, 1978) when they found *Keratella* composing as much as 100% of the gut content of individuals (which may suggest selective feeding by the *Hemimysis*). Evans et al. found that *Bosmina* and *Daphnia* spp. dominated the gut (>90%) of both adults and juveniles in July, whereas we saw both *Daphnia* species (*galatea mendotae* and *pulex*) present in the guts from August to December, but it never made up 90% of the gut content. Additionally, we found *Bosmina* in the guts form July through December, but it also was never 90% of the gut content. These findings are consistent with *Daphnia galatea mendotae* being absent or in low abundances in the winter and spring months, but highest in abundance in late summer and early fall (see 8/13/21 in Fig. 10) (Gannon, 1972). These findings are also consistent with abundances of *Daphnia pulex*, which are most abundant in the summer, and whose abundance rapidly declines in November (Gannon, 1974). While we did find higher abundance of *Bosmina* in the guts in later months (October and December) as well as in February, this mismatches with the abundances of Bosmina in the water column, which are highest in late summer and early fall (Gannon, 1972). However, it is possible that the *Hemimysis* are consuming the overwintering resting eggs, as is possible with *Bythotrephes*. Evans et al. (2017) also looked at copepod abundance in the gut (including *Limnocalanus*) and found adults had between 20-78% of gut content and juveniles had 14-55% from June to November. Nauplii abundant in water column in

mid-June in Lake Ontario (~25indiv/L) (Evans et al. 2017). However, we found *Limnocalanus* in the guts throughout the year, including in February, with the highest abundances in September. Given the fact that *Hemimysis* prey on copepod nauplii because the adults have too rapid of an escape response (Green, 1986), it makes sense to find *Limnocalanus* in the gut during the entire sampling period because, in Lake Michigan, nauplii are of greatest abundance in November through March, with adults having spermatophores as early as September (Gannon, 1972). Additionally, we were catching Limnocalanus in the traps all winter, which would also explain why we were finding them in the gut (see Fig. 14). Lastly, we looked at presence of *Dreissena* veliger (larvae of the invasive Quagga and Zebra mussels) in the gut, and saw the highest peaks in August, October, and February (Fig. 10). While August and October fit the distribution of abundance of veliger in the water column, highest in August and September (Bowen et al. 2018), it does not explain the peak of veliger in the gut in February. A potential hypothesis here is that because *Hemimysis* consume such a large portion of detritus (as much as 50% found by Borcherding et al. 2006), they are consuming dead and decaying adult mussel material that have settled out in the benthos, where the *Hemimysis* remain in the winter. Overall, these results conclude that *Hemimysis* are omnivorous generalist feeders, who are capable of making dietary shifts based on seasonal prey item abundance (Evans et al. 2017; Sinclair et al. 2016; Perez-Fuentetaja and Wuerstle 2014; Borcherding et al. 2006).

Implications

As our results suggest *Hemimysis* are overall generalist feeders and other studies have confirmed this, other studies have taken it a step further and looked at how the introduction of *Hemimysis* has impacted the abundance of other zooplankton in the water column. Several

studies found that many other zooplankton species had greatly reduced abundances within one to two decades after a Mysidae invasion (includes *Mysis diluviana* – like their invasion in Flathead Lake, Montana - and *Hemimysis* invasions). Ketellars et al. (1999) saw a significant decrease ($p < 0.0001$) in Rotifer, *Daphnia* spp., and *Bosmina* spp. abundance in just three years after the *Hemimysis* invasion in the Netherlands. Additionally, Ricciardi et a. (2012) conducted a review of Mysidae invasions to better understand the impacts of *Hemimysis* invasions, and they found that in several locations around the world including Sweden, Norway, Netherlands, and in several location within the United States, Mysidae invasions are capable of reducing the Cladoceran abundance by 55-99%. When other zooplankton such as Cladocerans, a common food source for many fish, particularly young-of-the-year and juveniles, are greatly reduced, local fish populations can switch their diet to consume more *Hemimysis* (Borcherding et al. 2006), which may not be a bad alternative considering that *Hemimysis* have a wet caloric density of 611 cal g^{-1} and can be just as high in lipid content as the native *Mysis diluviana* if not higher (Walsh et al. 2010). However, with the introduction of *Hemimysis* into the system and them now consuming common prey items of the local fish populations, they are causing an elongation of the food web (Ricciardi et al. 2012). This not only reduces trophic efficiency and energy transfer up to higher trophic levels, but also increases the risk of biomagnification within the system. A newly published study by Brown et al. (2022) found that adult and juvenile *Hemimysis* had higher methylmercury concentrations (187.0 ng g^{-1} dry weight and 57.2 ng g^{-1} dry weight respectively) than other littoral invertebrates such as amphipods (26.6 ng g^{-1} dry weight), *Dreissena* mussels (46.0 ng g^{-1} dry weight), and crustaceous zooplankton (5.1 ng g^{-1} dry weight). This suggests that methylmercury has a greater ability to biomagnify in fish that make the shift to feeding on *Hemimysis* (Brown et al. 2022).

In the present study, *Hemimysis* size was estimated using length, but measures like dry mass are more useful for food web modeling. However, there are a number of published length/weight regressions for *Hemimysis*, for example Marty et al. (2012):

$$Dry\ weight\ (mg) = 0.0024 \bullet [Length(mm)]^{3.178}$$

This allows a total dry weight biomass to be calculated from our data. For example, using this equation for the 8/13/21 sample date with 6,572 juveniles (average length = 4.07 mm), 21 males (average length = 6.04 mm), 8 reproductive females (average length = 7.75 mm), and 5 non-reproductive females (average length = 5.9 mm), we get a total dry weight of 1.4 x 10^3 mg where juveniles make up 97.7% of the weight. The size range of individuals in our study is comparable to Marty et al. (2012) as their body lengths ranged from 3 mm to 10 mm with an average length of 6.75 mm. Other studies have shown that *Hemimysis*, particularly juveniles who are smaller and have lower escape responses and are therefore easier to catch, are a popular prey item among many species in the Great Lakes including adult and juvenile alewife (*Alosa pseudoharengus*), rainbow smelt (*Osmerus mordax*), yellow perch (*Perca flavescens*), rock bass (*Ambloplites rupestris*), and largemouth bass (*Micropterus salmoides*) (Geisthardt et al. 2021). In fact, Geisthardt et al. (2021) found that *Hemimysis* were the primary food item consumed by young-of-the-year yellow perch, young-of-the-year largemouth bass, and juvenile rock bass. Using this information in congruence with the bioaccumulation of methylmercury in *Hemimysis* as mentioned above, it can be deduced that populations of fish species that eat *Hemimysis* (some of which are commercially important species) may begin experiencing methylmercury toxicity which could have huge economic impact of the fishery needs to be shut down.

Limitations

As with any study, there are issues that need to be mentioned in order to better understand the limitations of the results. First, it should be noted that trap design was changed and modified several times over the course of sampling. The traps were initially run on single-use batteries, which consistently drained during the time of deployment and the lights were off at the time of retrieval. So, for the first two sample dates (7/28/21 and 8/4/21), we cannot be sure that the lights within the traps were on for the entire time, and therefore actively attracting *Hemimysis* during the entire deployment. We switched to rechargeable batteries to combat this; however, the rechargeable batteries did not last as long, and a resistor needed to be installed to elongate battery life, which also reduced light output. Thankfully, the reduction in light was not a huge setback, because we had initially been darkening the lights with pieces of black felt, which were just removed so that the light intensity could be close to the original light used on the first two deployment dates. It should also be noted that two additional traps were made during sampling to increase the number of replicates. However, these traps were about 50% longer than the original traps. Consistently over the sampling dates, these two newer, longer traps caught more *Hemimysis* than the older, shorter traps. The theory here is that the longer traps lured the *Hemimysis* further away from the mouth of the trap, thus making them less likely to escape out through the mesh on the opening of the funnel, and more were retained inside the trap. Additionally, starting in early February, the mesh cover over the mouth of the funnel was removed. This was done for two reasons. One, because when pouring water into the traps at the surface to flood them and help them sink, the air and water temperatures were so low that ice began to form over the mesh, blocking it off from water flow and *Hemimysis* from entering. Two, because we noticed that the overall size of the *Hemimysis* was increasing, and we were

worried that the *Hemimysis* would no longer be small enough to fit through the gaps in the mesh. This of course would lead to a higher likelihood of individuals exiting the trap after being captured since the size of the opening to get out was larger.

In addition to change in trap design over the course of deployments, the location and method of deployment also had to be adjusted. For example, in the summer and early fall dates when the water temperature was over 14 °C, traps were deployed via freediving on the large boulder region of the park (indicated with red dots on Fig. 4). However, as weather condition became less conducive to diving (i.e. colder temperatures, windier conditions with higher waves and lower visibility, and eventually ice cover), the traps were shifted approximately 150-200 m South towards the lighthouse at the mouth of the Milwaukee River. This meant the traps were no longer being deployed in or over a rocky substrate and were instead being deployed off the side of the breakwall over and muddy/sandy bottom, which have been previously shown to be the least preferred bottom type habitat for *Hemimysis* (Boscarino et al. 2020).

Another issue we ran into was high rates of cannibalism among the *Hemimysis*. On several occasions, a culture was started in order to maintain several in-house *Hemimysis* that could be used as needed for experiments. However, every time a culture was started, it ultimately crashed as a result of the *Hemimysis* attacking and eating each other; on multiple occasions the *Hemimysis* were witnessed carrying another dead *Hemimysis* around with its mandibles, and cannibalism among crustaceans has been well documented on the literature (Romano and Zeng, 2016; Bleakly et al. 2018). The only time we were able to keep *Hemimysis* alive for extended periods of time (> 1 week), was when they were held individually in separate beakers. Because of this predation and cannibalism in a culture tank with ample habitat, great water quality, and an excessive food supply, we suspect that cannibalism was also taking place within the traps during

deployment, especially during the reproductive season, when there are a tremendous number of juveniles for the adults to prey on. It could also be taking place during the colder months when typical prey species from the summer are less abundant, and the *Hemimysis* are now in close proximity to other potential prey items: other *Hemimysis*. This consumption of *Hemimysis* within the traps would not only lead to a lower abundance overall, but also potentially lower signals in the gut content as consumption of new food speeds up digestive processes and clears the gut of prey consumed before entering the trap. And while the *Hemimysis* may be preying on each other, this is not something that could be detected using the polyclonal antibody method, as it cannot separate *Hemimysis* proteins in the homogenized animal itself from *Hemimysis* protein found in the gut.

Additionally, preliminary feeding experiments showed that gut clearance time for *Hemimysis* at room temperature in a laboratory (potentially high stress) environment is around 3.5 h (Jenrette, Berges, and Wachala, unpublished). Assuming this is true for all individuals, once they were removed from the trap, we had approximately 3.5 h until they cleared their gut, and we were unable to detect prey signals. Most of the *Hemimysis* were processed (sex identified and placed in an individual cryotube) in less than 3 h. However, on days where there were a large number of adult *Hemimysis* present (ex: 3/4/22 and swarm collection), individuals that were processed towards the end had the most time to sit and empty their gut. Although they were on ice, the high stress from being handled, passed onto a sieve, and held at high densities in a quadrat petri dish, may have negated the metabolism-slowing effects of being kept in a cooler. This may also help to explain why there are a relatively large number of *Hemimysis* (38.9%) with nothing detectable in their guts. If entered the trap more than 3.5 h prior to trap recovery, they may have already cleared their gut by the time the traps were pulled.

Ultimately, all these limitations result in the possibility that total abundances and gut content were underestimated rather than overestimated. This means there is potential for not only *Hemimysis* to be present in larger numbers, but also that they could be consuming more prey than previously estimated, leading to a higher ecological impact than ones previous discovered or identified.

Lastly, while we can say which prey items were present or absent in the gut on a particular day and give relative comparisons within the same antibody readings, what we cannot do is identify the amount of organism that was consumed for that individual. Because of this, we can also not quantifiably compare between prey items, saying that the *Hemimysis* ate more or less of one item than they did of another. This leads to gaps in knowledge of the amount of a particular prey being consumed, and how those impact ecosystem models to predict *Hemimysis*'s effects on local zooplankton populations and ultimately local food webs. However, it is possible to develop a quantitative method for the antibodies.

Next Steps

In order to better understand and interpret polyclonal antibody gut analysis results, the next step is to conduct feeding experiments with each prey item in question. In these experiments, only one prey item would be available for consumption by the *Hemimysis*, and the individual would be monitored over time measuring how much of that particular prey item was consumed. Then, that *Hemimysis* would be sonicated and processed exactly like a wild sample. However, with the relative fluorescence units' slope, one can say, "this value is from an individual who ate x number of x prey item in x amount of time". This would allow for calibration of the gut content results and allow us to say just how many of a particular prey item

was in the gut. This, in turn, allows us to give quantitative comparisons between antibodies, and also allows us to input quantitative values into ecosystem models to help understand the impact *Hemimysis* has on local food webs, energy transfer within the system, and local biodiversity.

During sampling throughout the year, we observed various stages and expression of the red chromatophores that give *Hemimysis* the name "bloody red shrimp." For example, the first trap caught on 10/20/20 (which was later used to purify antisera), was placed at 4 °C post-retrieval before being returned to that lab. Upon viewing the container several hours later, we noticed that the *Hemimysis*, which had been almost transparent upon retrieval, were not bright "bloody" red. This led us to ask the question of how chromatophore expression is affected by environmental ques (i.e., light and temperature) and how the chromatophores are used for communication between individuals and predator avoidance. Preliminary experiments extracting pigments from individuals in ethanol or DMSO and running absorption suggest the presence of a carotenoid-like pigment (Bauer, Berges, and Wachala, unpublished). We also conducted preliminary observations during processing and culturing that adult male and female Hemimysis can go from bright red being held in the dark to completely transparent in less than 2 min 30 s. Additionally, we noticed that a female when kept alone had very little chromatophore expression, but when paired with a male, she became much redder for a period of several hours, until the ultimately killed the male. So, we suggest that experiments be conducted on chromatophore expression in the different sexes under varying light, temperature, density, prey, predator, and mating conditions. This would allow for a greater understanding of the ecological purpose and benefit of a transparent organisms producing a red chromatophore.

Conclusion

The population of *Hemimysis anomala* within the Milwaukee Harbor is smaller in average length compared to other populations within the Laurentian Great Lakes and other parts of its invaded areas in Europe. Additionally, the summer population is dominated by juveniles, while the overwintering population is predominantly female. This population also exhibits a more generalist feeding style with no obvious differences in diet between sexes and life stage.

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Appendix A: Vertical Migration Experiments

Introduction

Hemimysis, like many other crustaceous zooplankton are known to vertically migrate, aggregating towards the benthos during the day, and entering the upper portion of the water column at night (see thesis introduction). However, this behavior can be "disrupted" by the formation of swarms, that have been seen at the surface during the day. These experiments were originally intended on being a part of the body of the thesis, however, complications prevented us from obtaining any definitive results.

Hypothesis

In vertical migration experiments, juveniles will be found in the upper part of the columns, females will be more likely to be found in the lower part of the columns and distributions of males will be random. This is because juveniles are attempting to avoid the adults, who may cannibalize them, females are staying closer to the benthos in an act of self-preservation and increasing reproductive success, while males are able to follow the typical vertical migration pattern and are not confined to any particular region of the water column.

Methods

The following methods were adapted from Boscarino et al. (2007). Two vertical migration columns were constructed using clear acrylic plexiglass tube (89 mm diameter) designed to fit 3" PVC fittings, Valtera 6301 3" PVC gate valves placed in the middle of the column to control acclimation of the *Hemimysis*, and 3" PVC end caps (Fig. 5). These columns were used in a dark room, with a small light hanging overhead to simulate moonlight. Several different variations of the experiment were conducted, with the main goal being to determine if there is a difference between males, females, and juveniles when it comes to migration behavior.

First, before starting the experiment, the bottom half of the column below the gate valve was filled with filtered lake water. Then, three *Hemimysis* of a particular category (i.e., male, female, or juvenile) were added to this half of the column, and the gate valve was closed. This allowed the *Hemimysis* to acclimate being in the column before the experiment, and therefore potentially skewing the results. More filtered lake water was added to the top half of the column, all lights were turned off, except for the simulated moonlight, and the column was left for 30 min to acclimate to room temperature. After the 30 min acclimation, the gate valve was opened, and the movement behavior of the *Hemimysis* was recorded using an infrared video camera. Infrared is a great way to track *Hemimysis* movement because they are not sensitive to this region of the light spectrum, and the rays bounce off the back of their eyes, causing a bright "glow" that is easily distinguishable from the background. Over the course of 1.5 h, the position and movement within the water column of the three individuals was recorded every minute. The experiment was repeated at least three times for each of the various groups. The columns were also split into quadrats every 0.5 m depth. The quadrat the individual was in was recorded at each time point to determine if there was a preferred depth within the vertical migration. This number was then compared across the different groups to determine if there were differences in preferred depth and distribution between juveniles, males, and females.

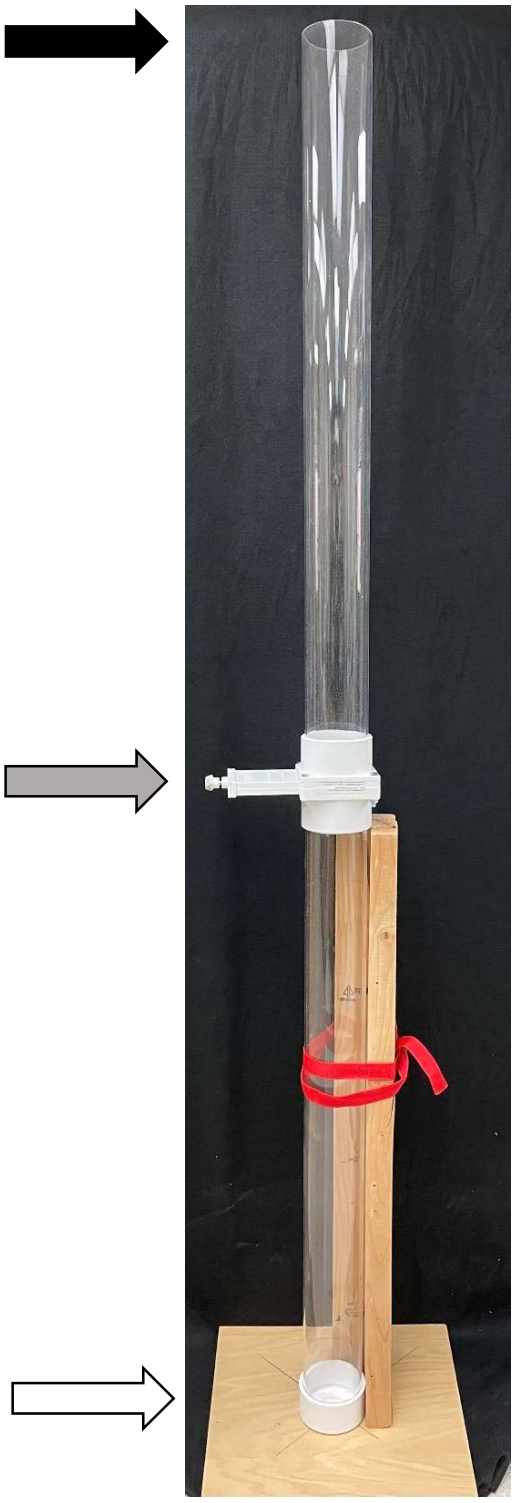

Figure 5: Approximately two-meter-tall vertical migration column constructed using methods adapted from Boscarino et al. 2007. Two identical columns were created for the vertical migration experiments. The black arrow indicates the top of the column with the opening where water and *Hemimysis* enter the tube. The grey arrow indicates the presence and position of the gate valve separating the two halves of the column, and the white arrow indicated the presence and position of the end cap.

Results and Discussion

Vertical migration experiments were conducted in early February. The first replicate was run with three males in one column and three females in the other column (given that it was February there were no juveniles in the population to use in the experiments). They were given 30 minutes to acclimate in the lower half of the column below the gate valve, which was closed, (grey arrow in Fig. 5) and the flashlight was turned on, giving a reading of between 0.2 and 0.5 μmol m^2 s^{-1}, which was comparable to the traps on the high setting. The water temperature was 18°C, which was consistent with the temperature of the walk-in where the *Hemimysis* were being held. After the 30-minute incubation, and the gate valve was opened, we noticed that all six *Hemimysis* remained on the bottom of the column near the end cap (white arrow in Fig. 5). The experiment was run for more than 1.5 h, and in that time all 6 *Hemimysis* never left the bottom quadrat of the column. We reran the experiment the following day with three new males and females in their separate columns. They were given 1 h to acclimate, because we thought perhaps the *Hemimysis* in the previous replicate were not given enough time to acclimate. The second replicate was also run for 1.5 h, and yielded the same result, with all *Hemimysis* not leaving the bottom quadrat (bottom 0.5 m) of the column. Previous vertical migration experiments were run with *Mysis diluviana* (from which we adapted our methods, Boscarino et al. 2007) did not have this issue, we concluded that there was something wrong with the experimental setup and abandoned the experiment.

References

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Appendix B: Feeding Experiments

Introduction

As mentioned in the "Next steps" portion of the thesis, being able to translate relative fluorescence units over time into number of prey items consumed for a particular species would allow us to better quantify *Hemimysis* predation and better estimate the effect *Hemimysis* has on the abundances of other zooplankton species in the local food web. These experiments were originally intended on being a part of the body of the thesis, however, complications prevented us from obtaining any definitive results.

Hypothesis

These experiments will allow us to quantify the signal in the gut from *Daphnia pulex* and *Daphnia galatea mendotae* to allow determination of number of prey items consumed rather than an arbitrary relative fluorescence over time.

Methods

Previous studies have suggested that there are predatory behavioral differences between males, females, and juveniles. Because it is believed that the majority of *Hemimysis* hunting takes place at night, these experiments were also conducted in the dark. "Dark boxes" were constructed using a cardboard box lined with black felt. A small hole was poked in the top of the box for a light to be added that will simulate moonlight. Additionally, a small hood was placed over the mouth of the box so that the recorder can insert the Underwater Infrared Camera Model 91309 (Harbor Freight Tools, Camarillo) in and out of the box without introducing a significant amount of light to the enclosure (Fig 6). A USB RCA composite adaptor was used to connect the camera to a 2019 Macbook Pro (Apple, Cupertino) where the video feed was recorded and analyzed at a later time.

A 1 L clear beaker housed the individual *Hemimysis* for the experiment. Each individual was given 30 min to acclimate to its environment before starting the experiment. There were several treatments: one with varying amounts of *D. magna*, one with varying amounts of *D. pulex*, and one with varying amounts of both *D. magna* and *D. pulex*. The feeding experiment continued for 3 h each, or until all the prey items have been consumed, whichever happened first. The quantitative parameters being recorded were number (and percent) of prey consumed and how long it took for the prey to be consumed. The qualitative parameters recorded were predation behaviors such as lunging, shredding of prey, and swimming behavior during the experiment. This was then be repeated multiple times with multiple individuals for males, juveniles, and females, allowing for comparison of feeding behaviors and consumption rates among and between the different groups.

Figure 6: Dark box set up composed of cardboard box lined with black felt. A small flashlight was placed on the top to simulate moonlight. An elongated 1L beaker was placed in the middle. This is where the *Hemimysis* were held during the experiment.

Results and Discussion

Feeding experiments were also conducted in February. Three replicates of each sex (male, immature male, and female) were run in separate dark boxes in separate 1L glass beakers, with a flashlight overhead to simulate moonlight (Fig. 6). Given that all *Hemimysis* were all over 8 mm in length, each individual was given five *Daphnia magna* as potential prey items. All *Hemimysis* were given 30 minutes to acclimate to the dark box and beaker before the *Daphnia* were added. The *Hemimysis* were given 2 h to consume prey, and counts were conducted. After 2 h, all five *Daphnia* were still present in all nine beakers. Because of this, it was decided to leave the *Hemimysis* in their beakers with the Daphnia for another 2 h to see if there was any predation over a longer period. At the end of the additional 2 h, all five *D. magna* were still present. We concluded that the metabolism of the individuals was too low because of the cold waters in February (between 0-1 °C taken during sampling). This lower metabolism meant a lower overall drive and necessity for prey, which is why we saw no predation of *D. magna*. Because of this, feeding experiments were discontinued.

Appendix C: Enlarged Gut Content Figures

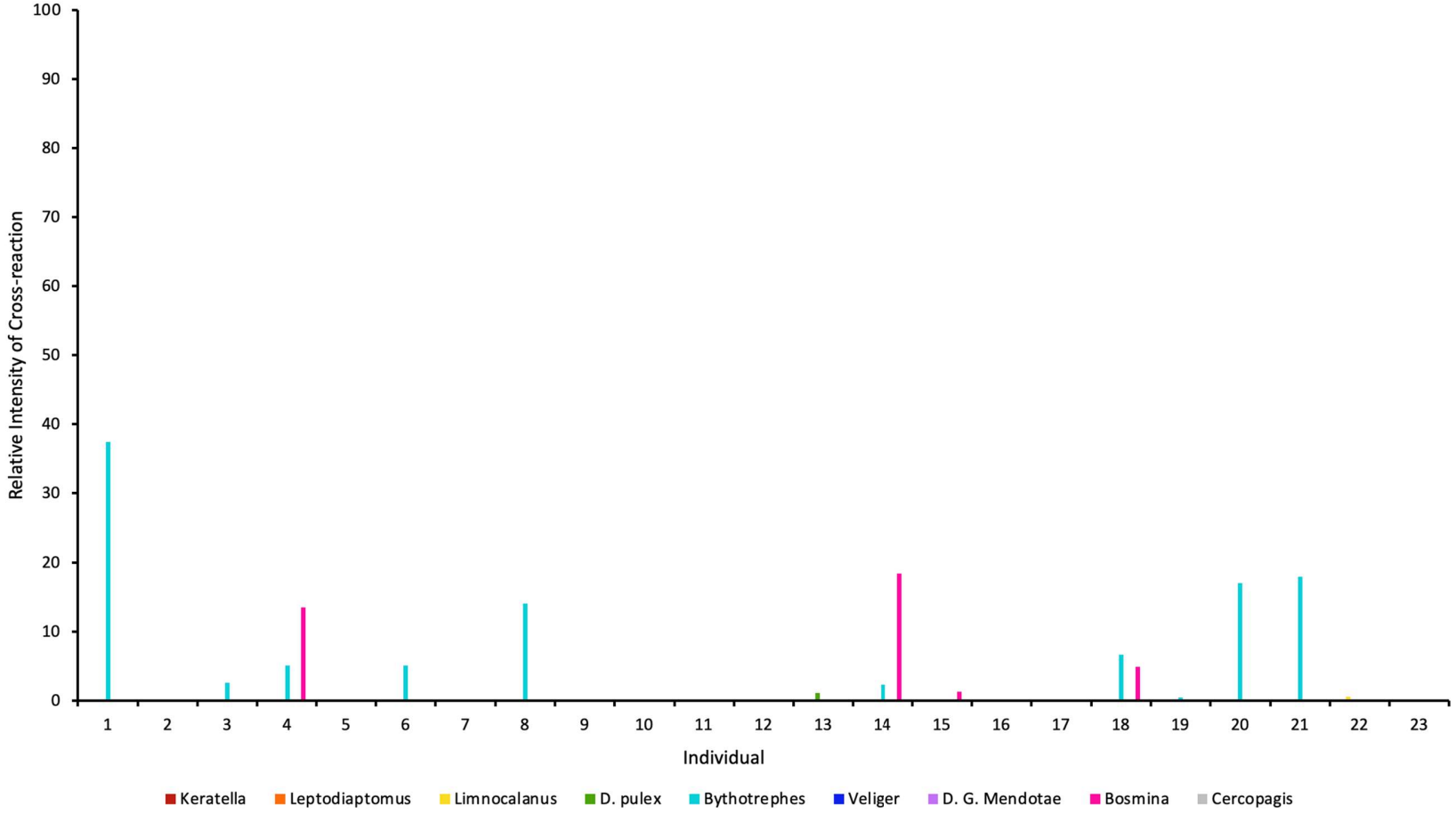

Figure 1: Results of probed gut content for sonicated samples from 7/28/21. Twenty-three individual *Hemimysis* were probed with antibodies against *Keratella*, *Leptodiaptomus*, *Limnocalanus*, *D. pulex*, *Bythotrephes*, Veliger, *D. G. Mendotae*, *Bosmina*, and *Cercopagis* using the immunochemical methods described in Berges et al. 2020. Results were expressed as a percentage of the highest value recorded for each species.

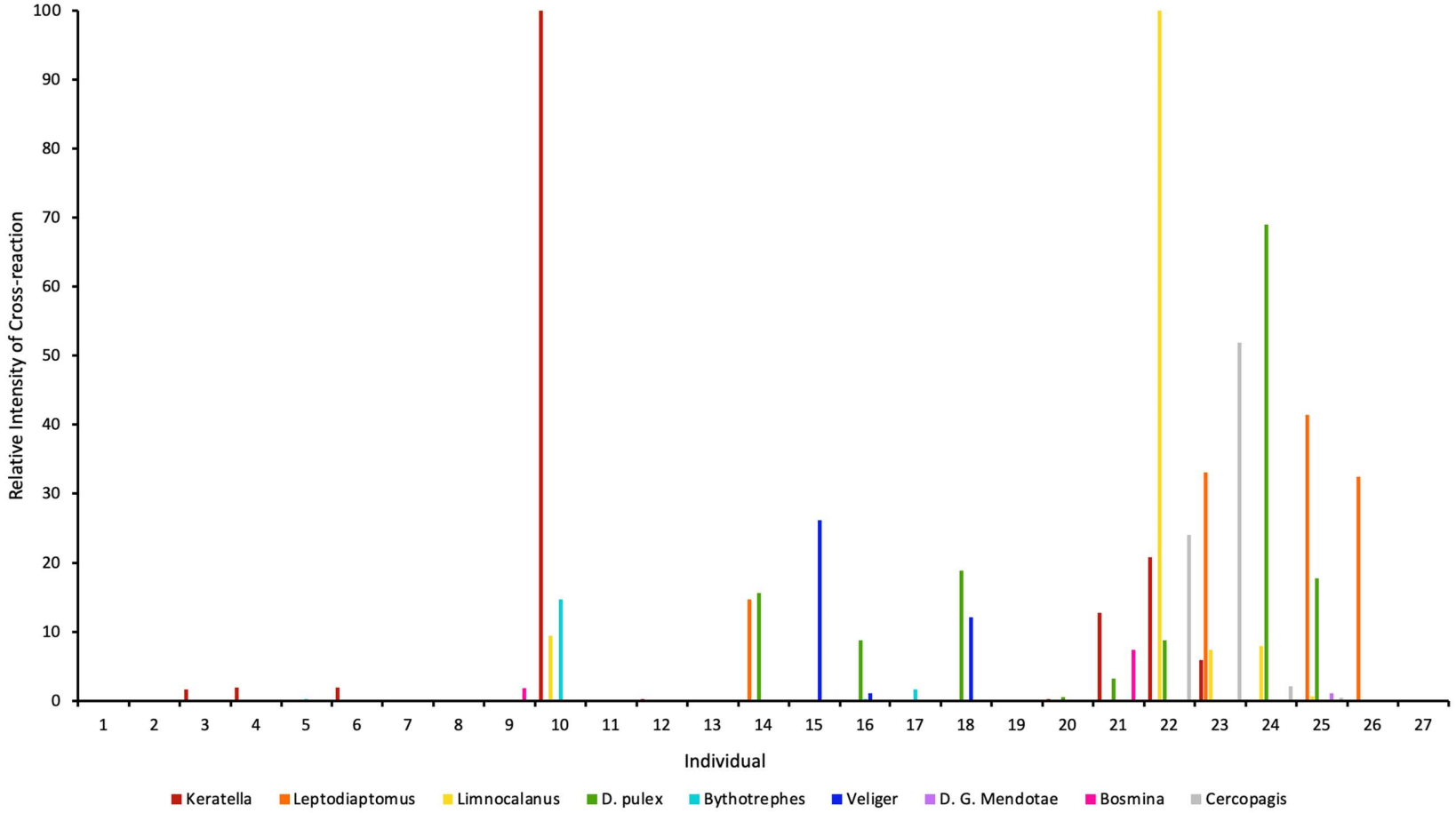

Figure 2: Results of probed gut content for sonicated samples from 8/4/21. Twenty-seven individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

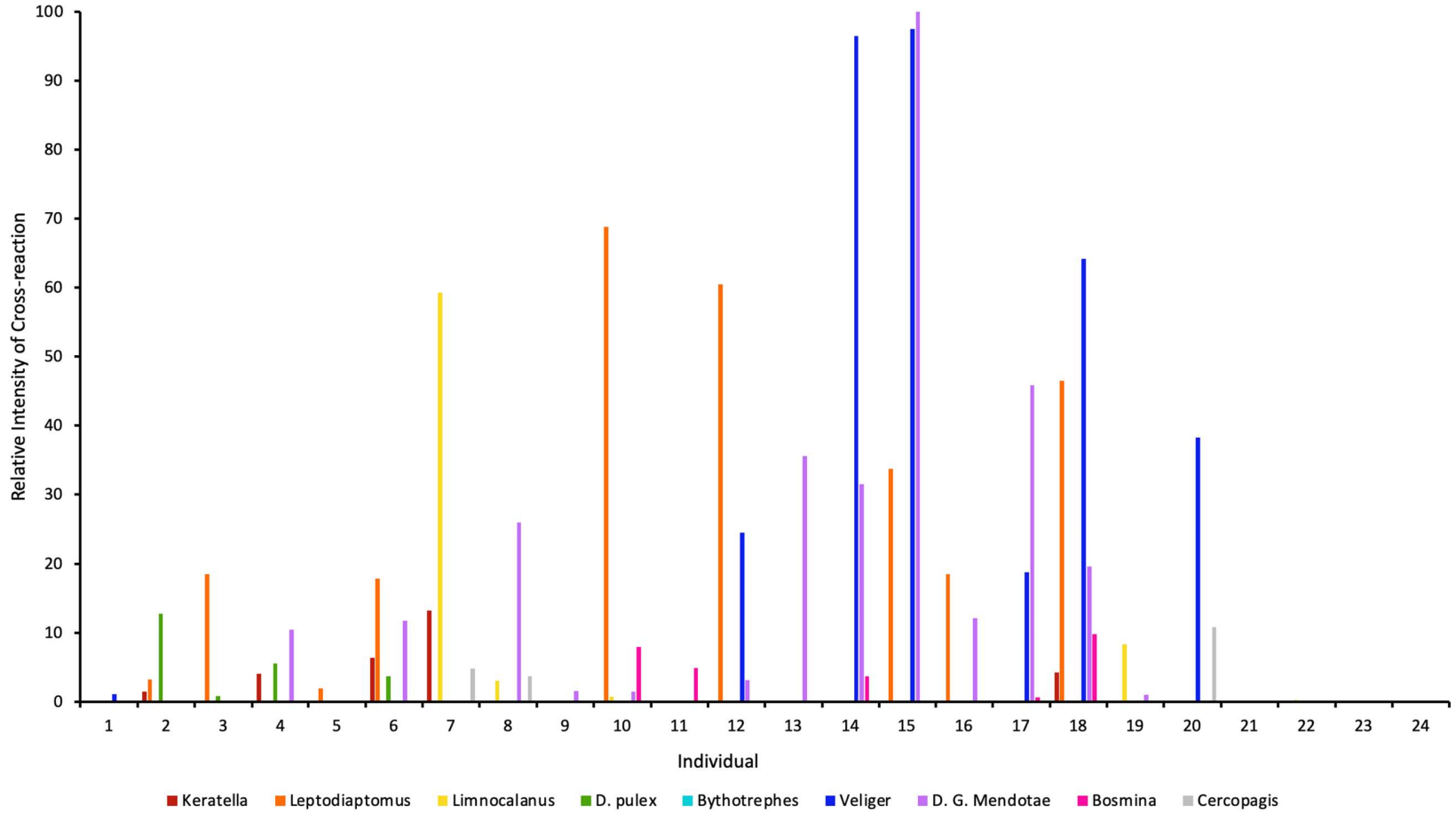

Figure 3: Results of probed gut content for sonicated samples from 8/13/21. Twenty-four individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

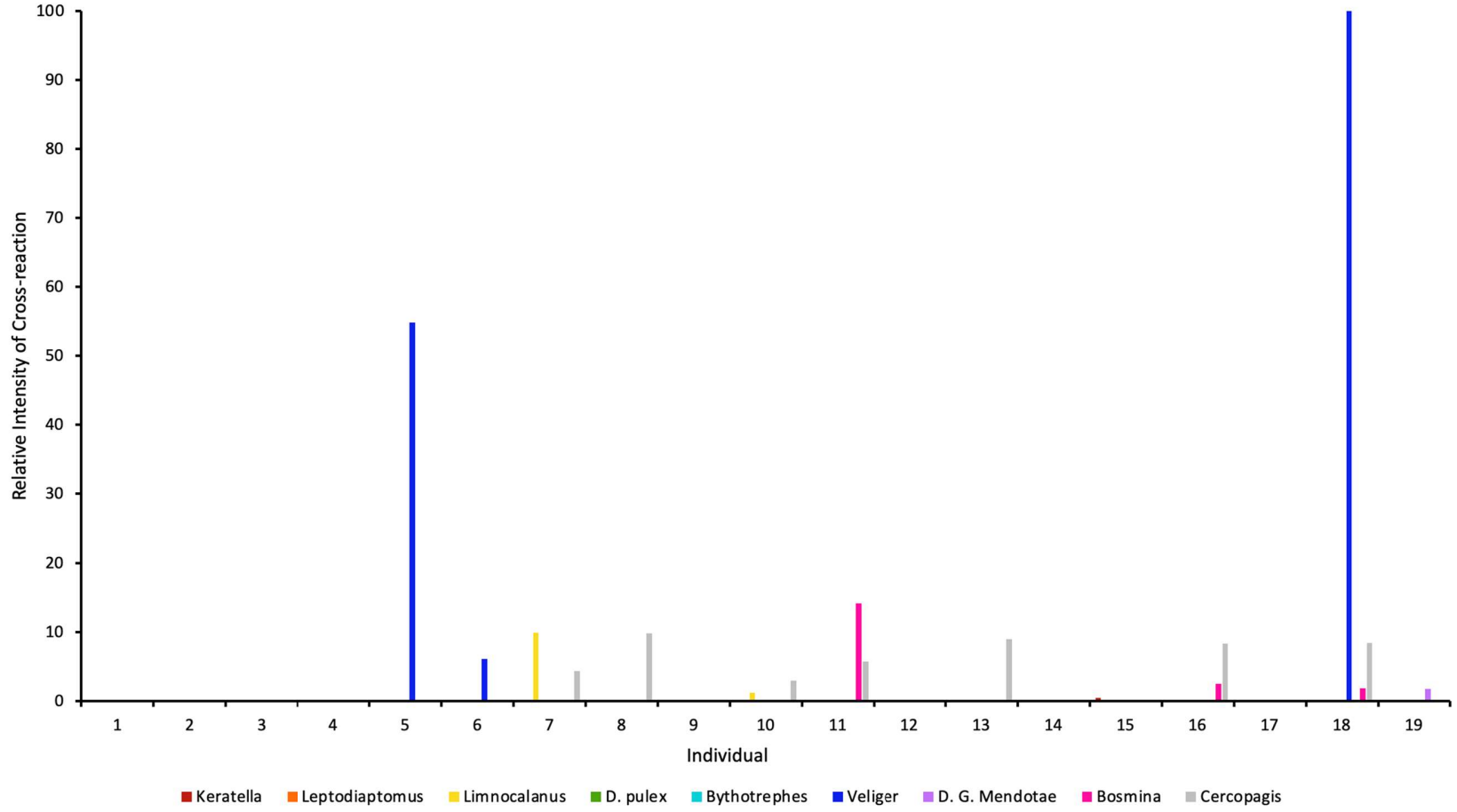

Figure 4: Results of probed gut content for sonicated samples from 9/1/21. Nineteen individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

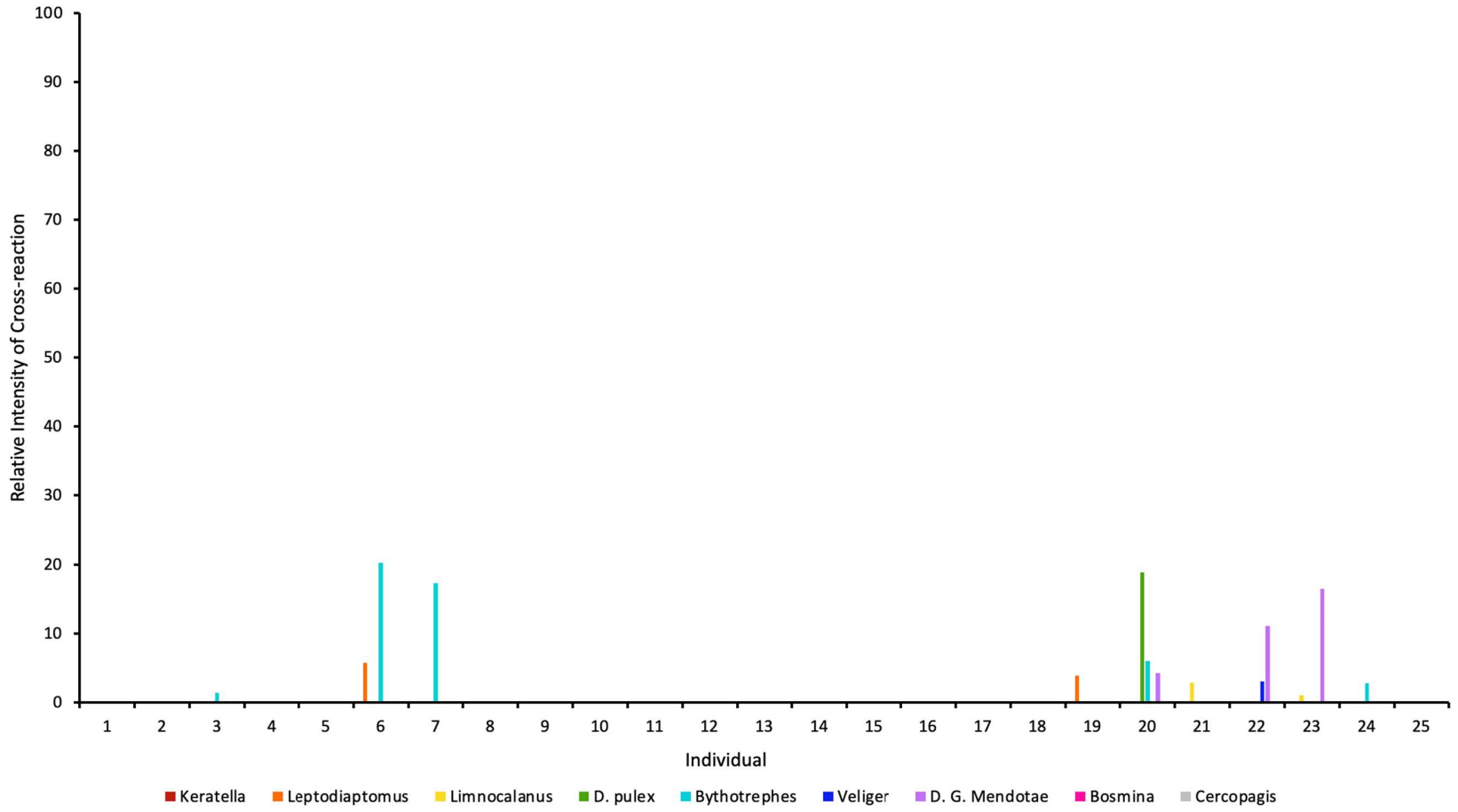

Figure 5: Results of probed gut content for sonicated samples from 9/8/21. Twenty-five individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

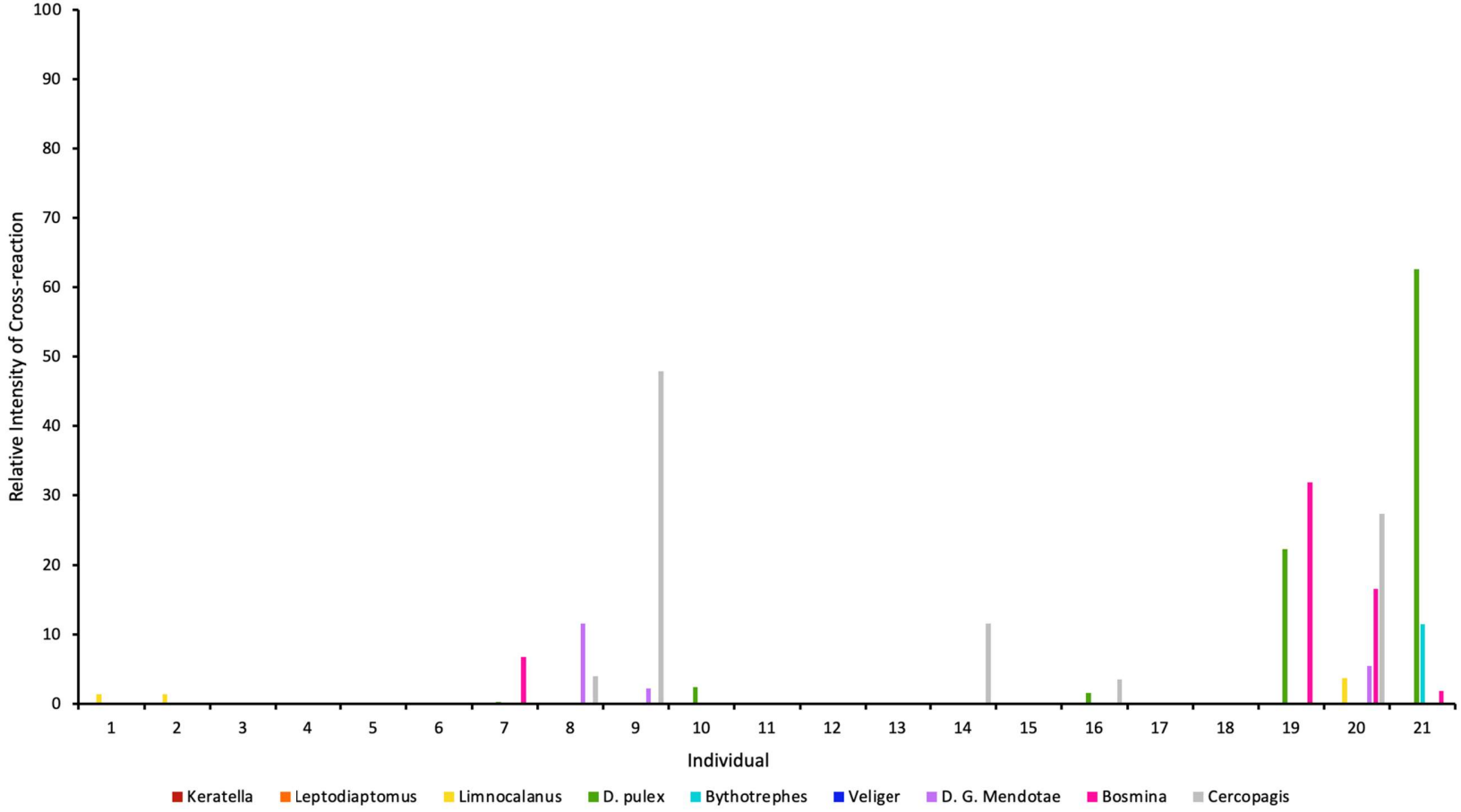

Figure 6: Results of probed gut content for sonicated samples from 9/16/21. Twenty-one individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

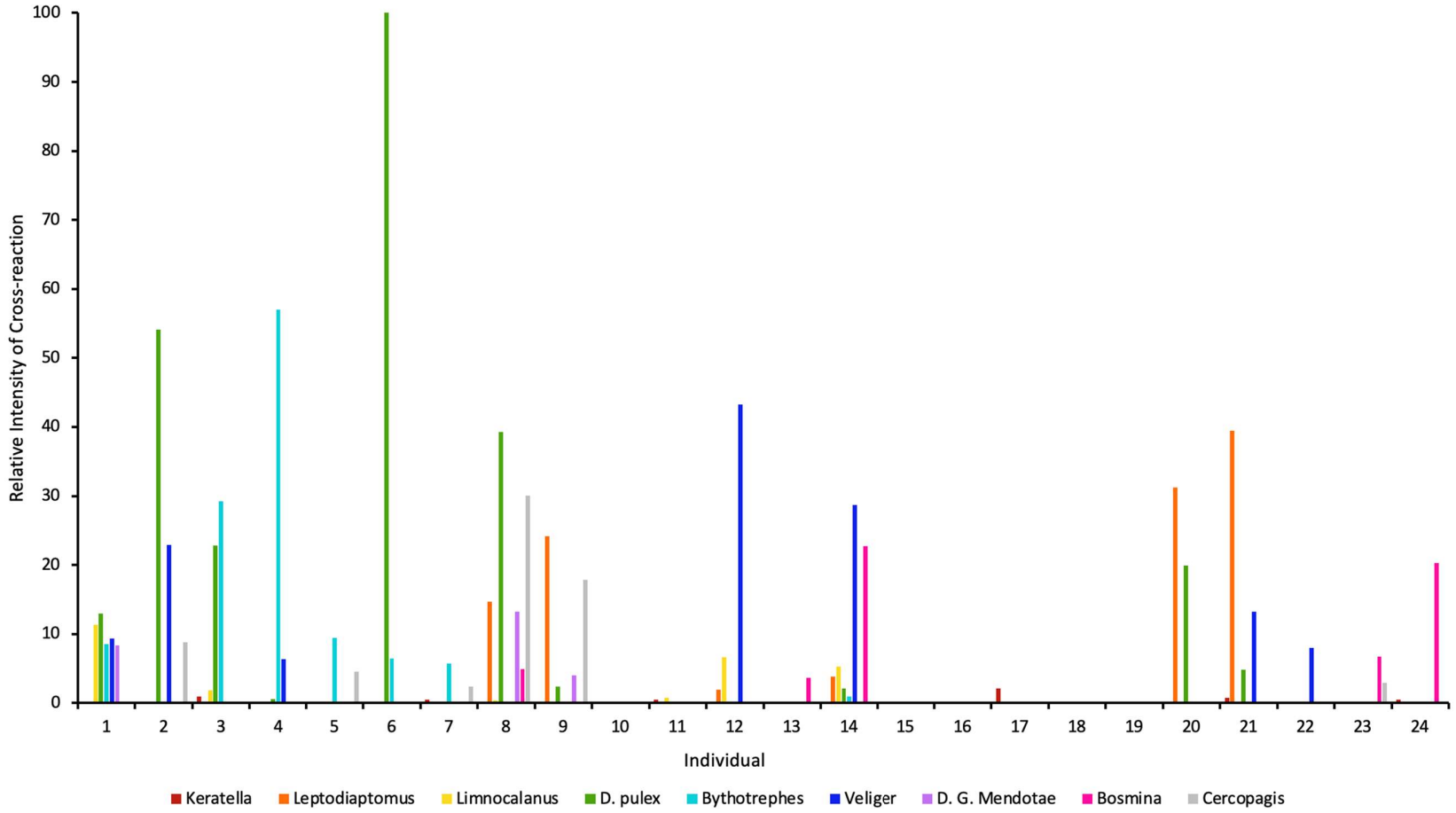

Figure 7: Results of probed gut content for sonicated samples from 9/28/21. Twenty-four individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

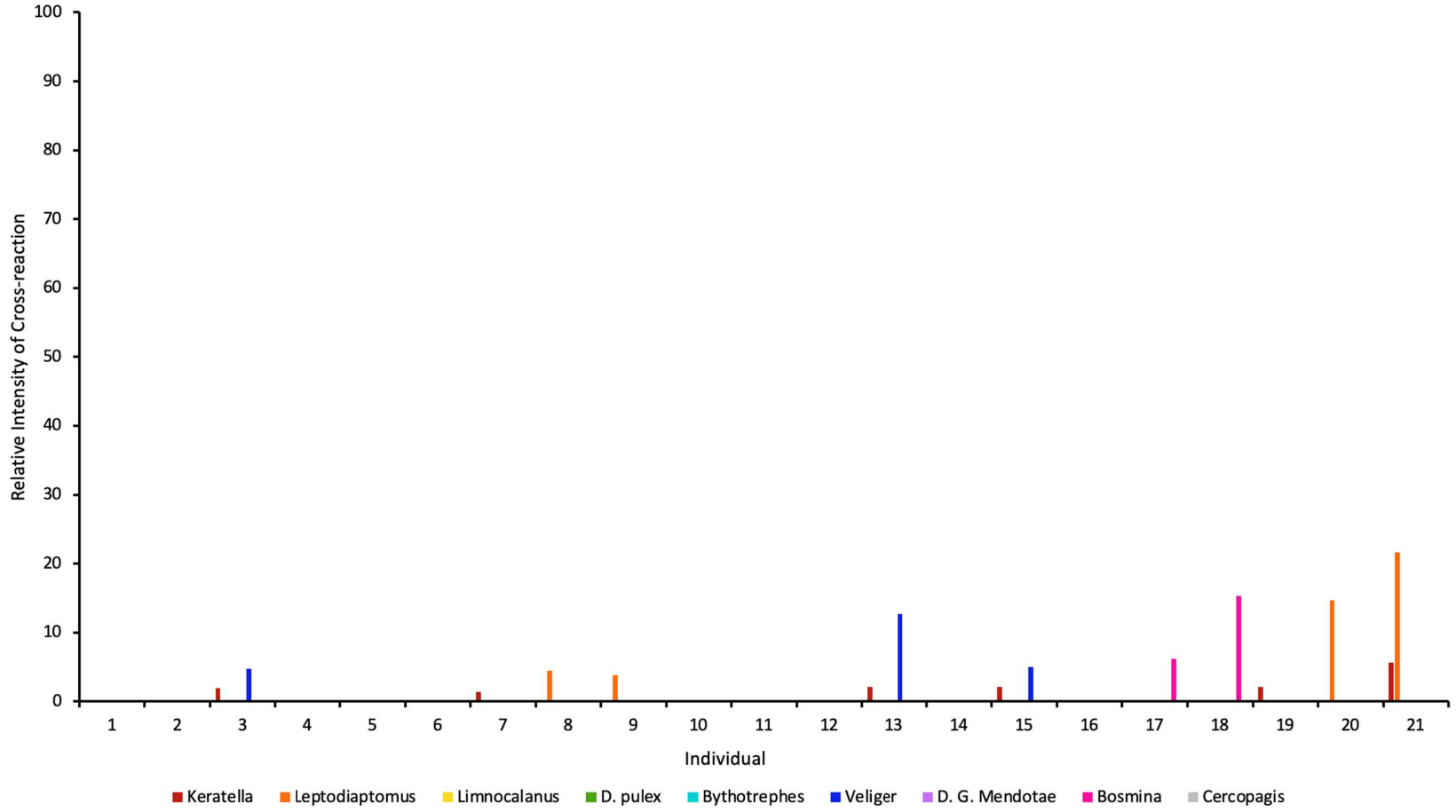

Figure 8: Results of probed gut content for sonicated samples from 10/5/21. Twenty-one individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

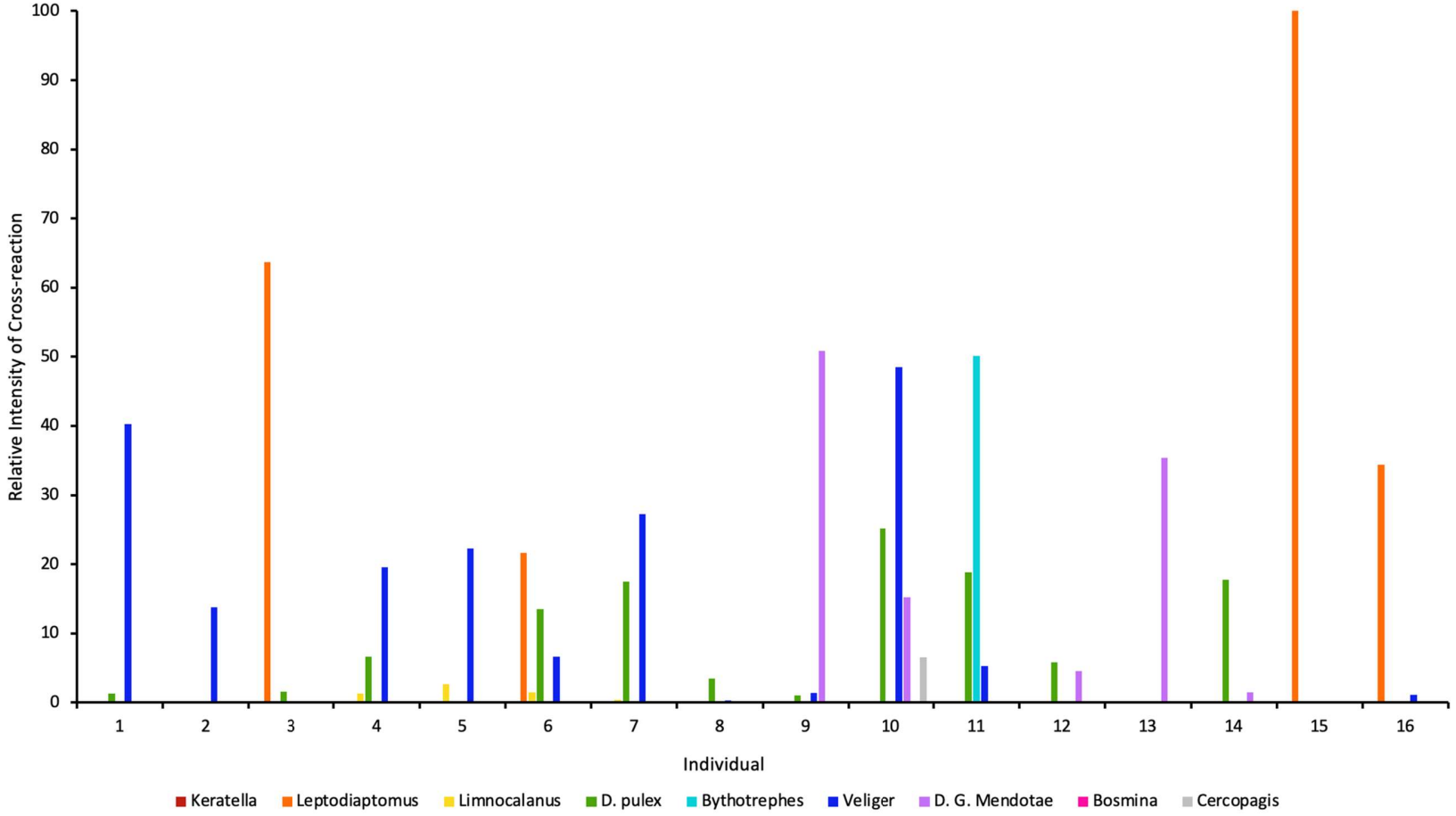

Figure 9: Results of probed gut content for sonicated samples from 10/19/21. Sixteen individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

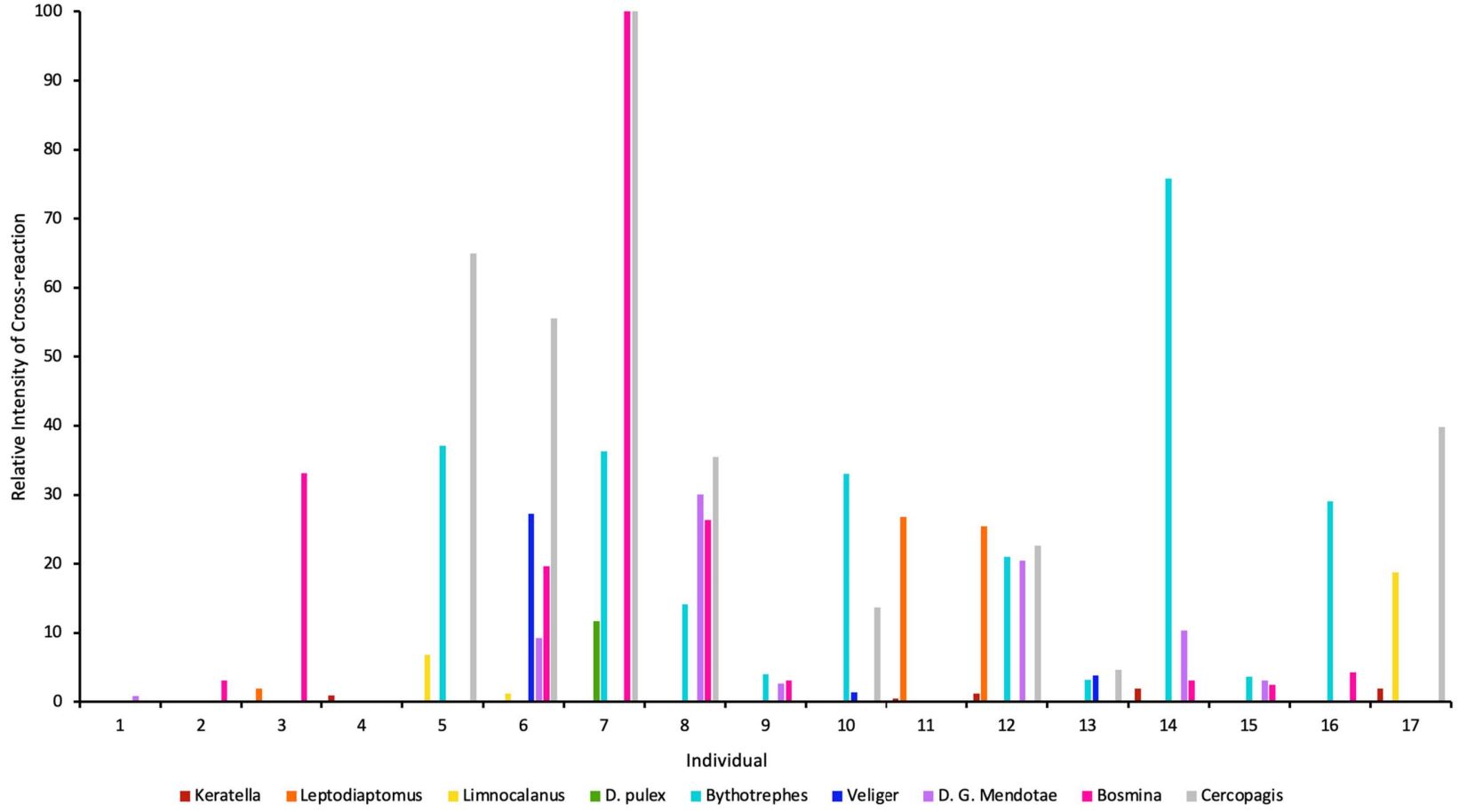

Figure 10: Results of probed gut content for sonicated samples from Swarm 1 on 10/20/21. Seventeen individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

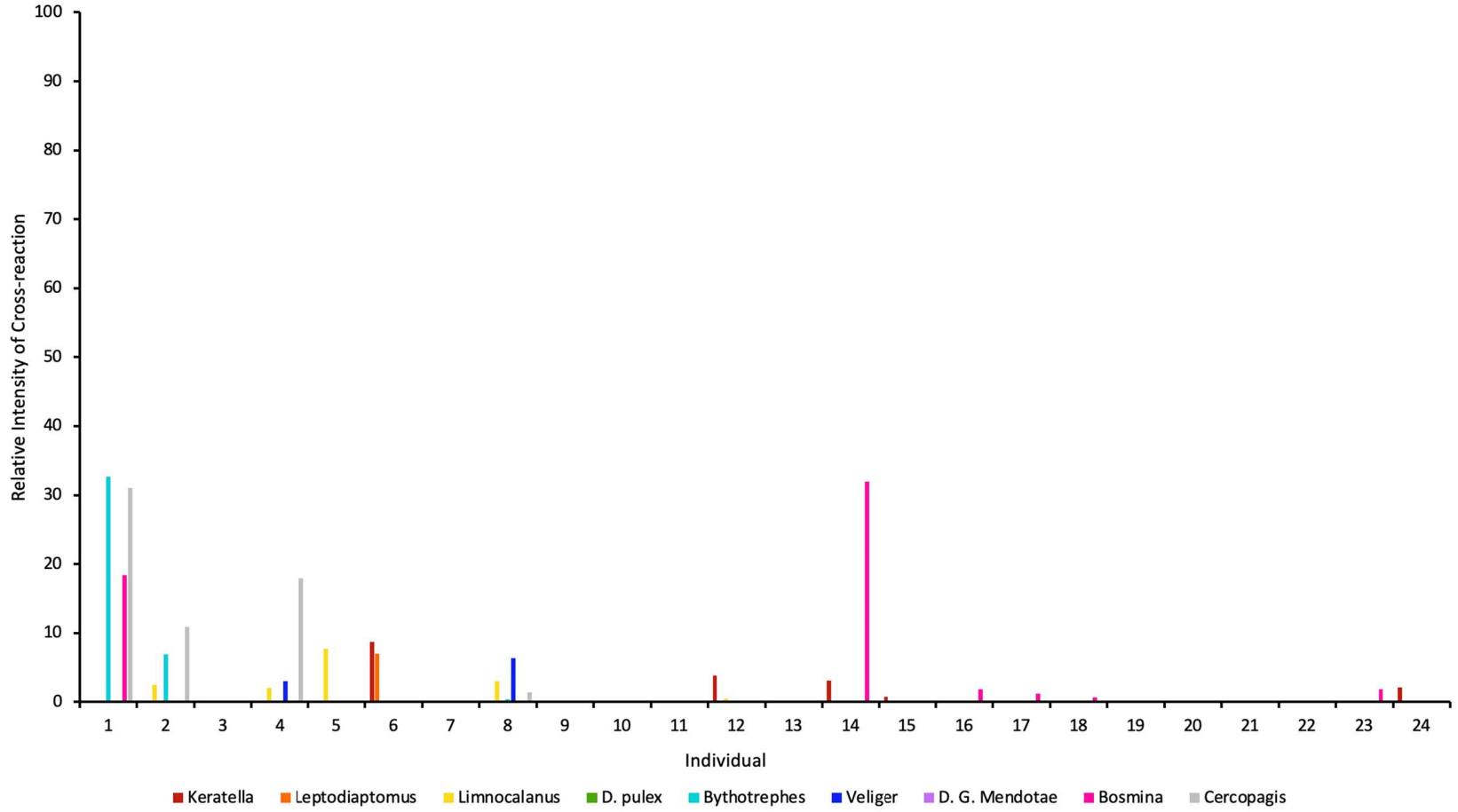

Figure 11: Results of probed gut content for sonicated samples from Swarm 2 on 10/20/21. Twenty-four individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

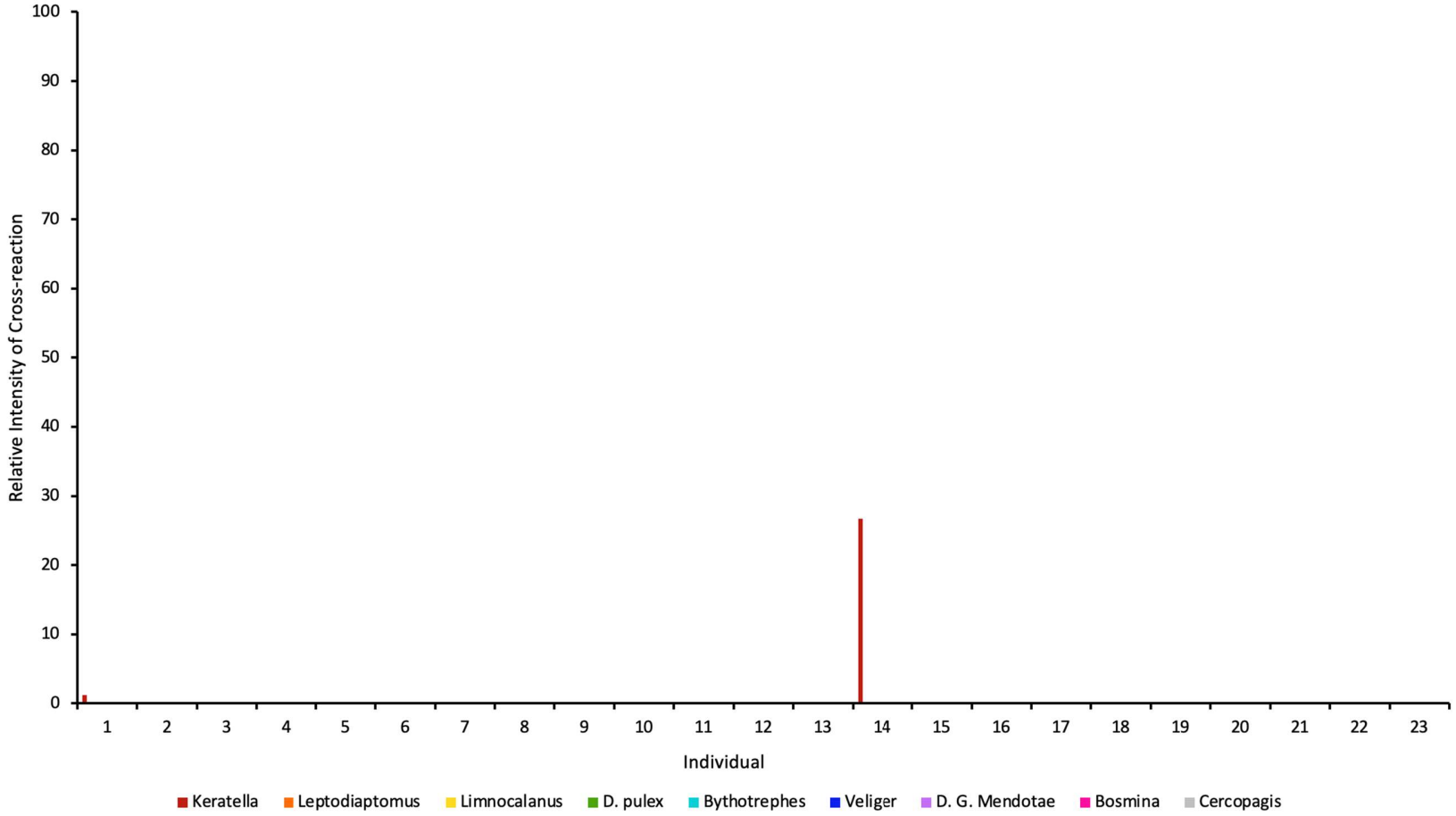

Figure 12: Results of probed gut content for sonicated samples from Swarm 3 on 10/20/21. Twenty-three individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

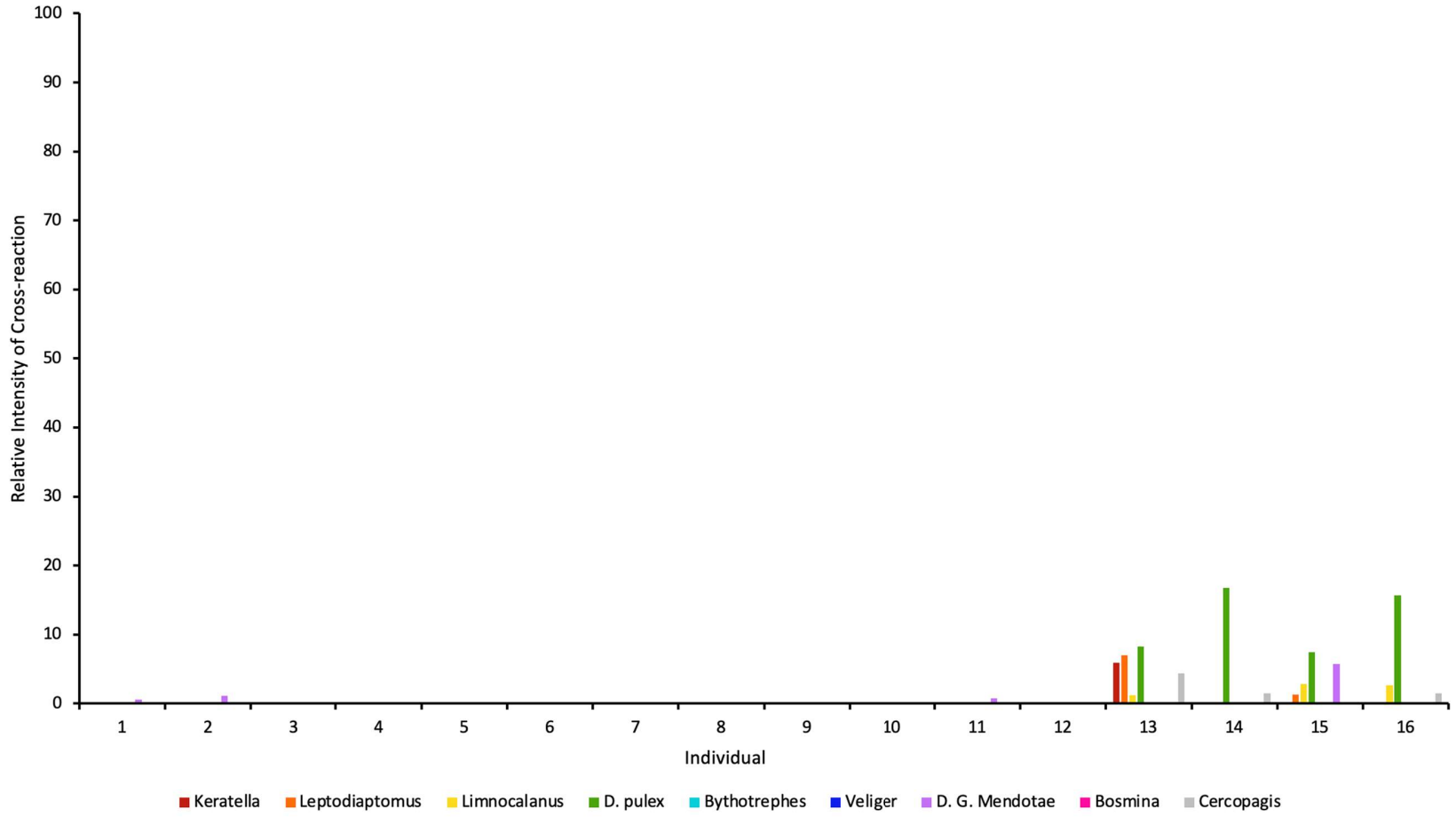

Figure 13: Results of probed gut content for sonicated samples from 11/2/21. Sixteen individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

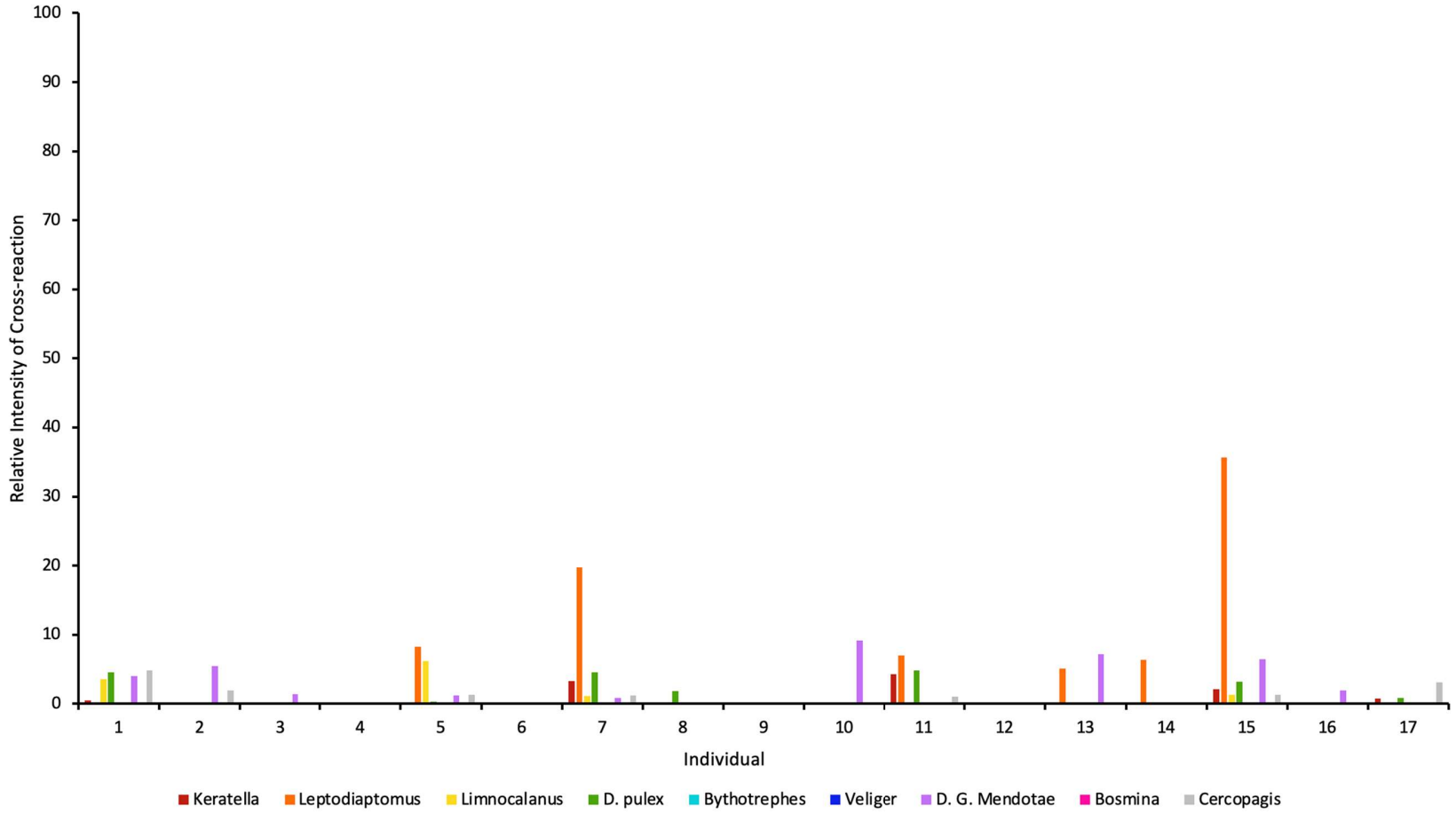

Figure 14: Results of probed gut content for sonicated samples from 11/16/21. Seventeen individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

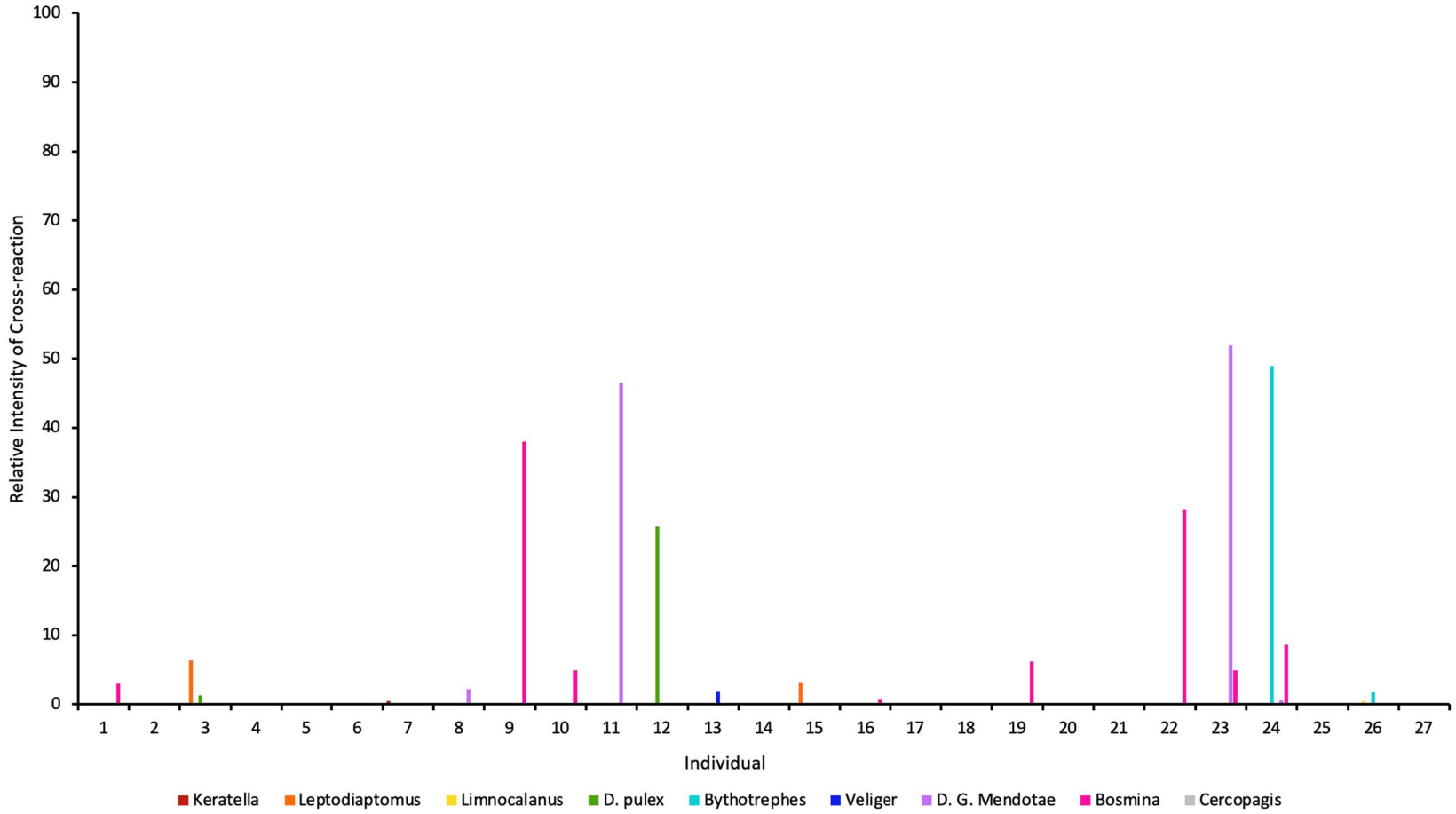

Figure 15: Results of probed gut content for sonicated samples from 12/16/21. Twenty-seven individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

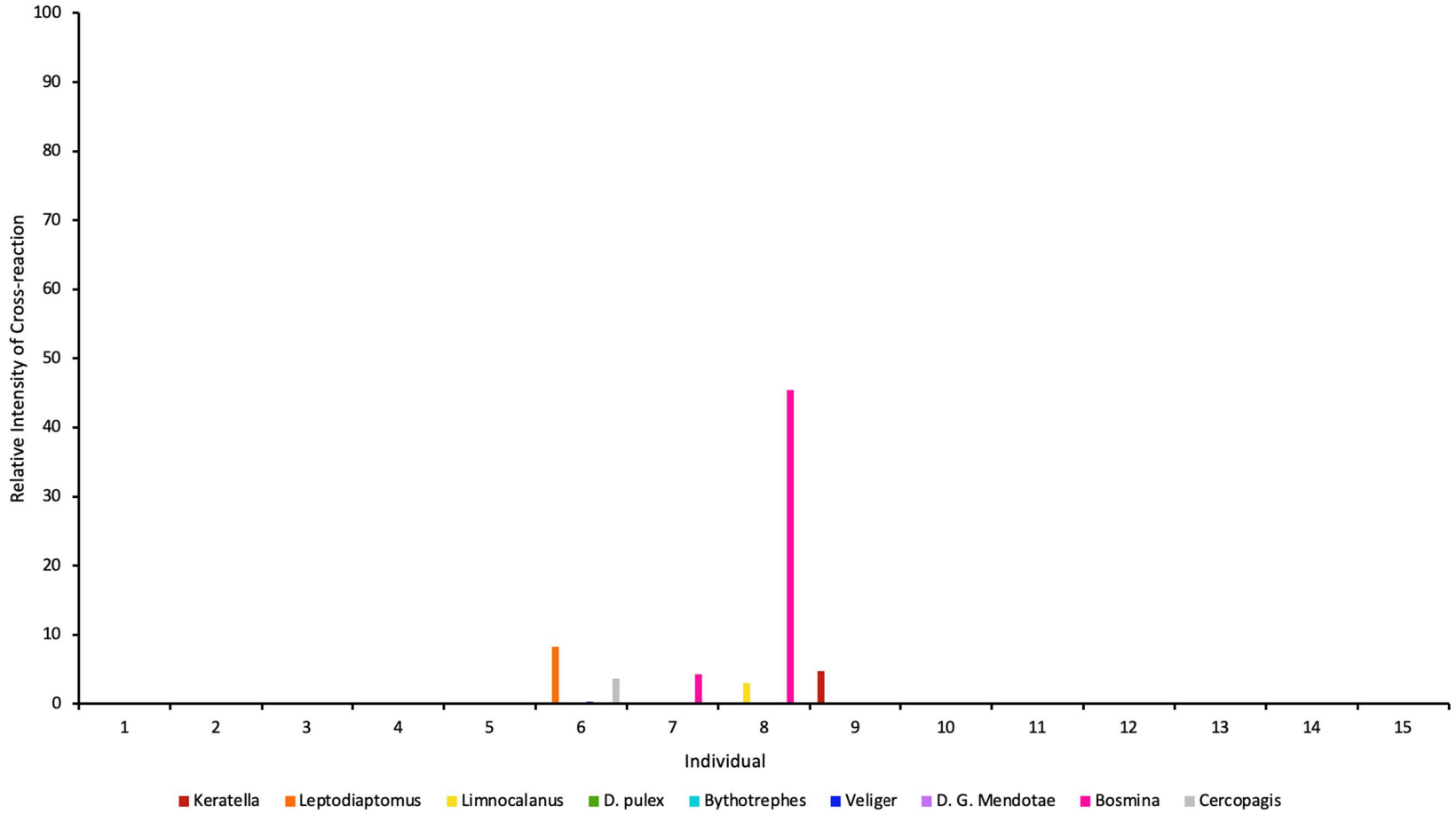

Figure 16: Results of probed gut content for sonicated samples from 2/11/22. Fifteen individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.

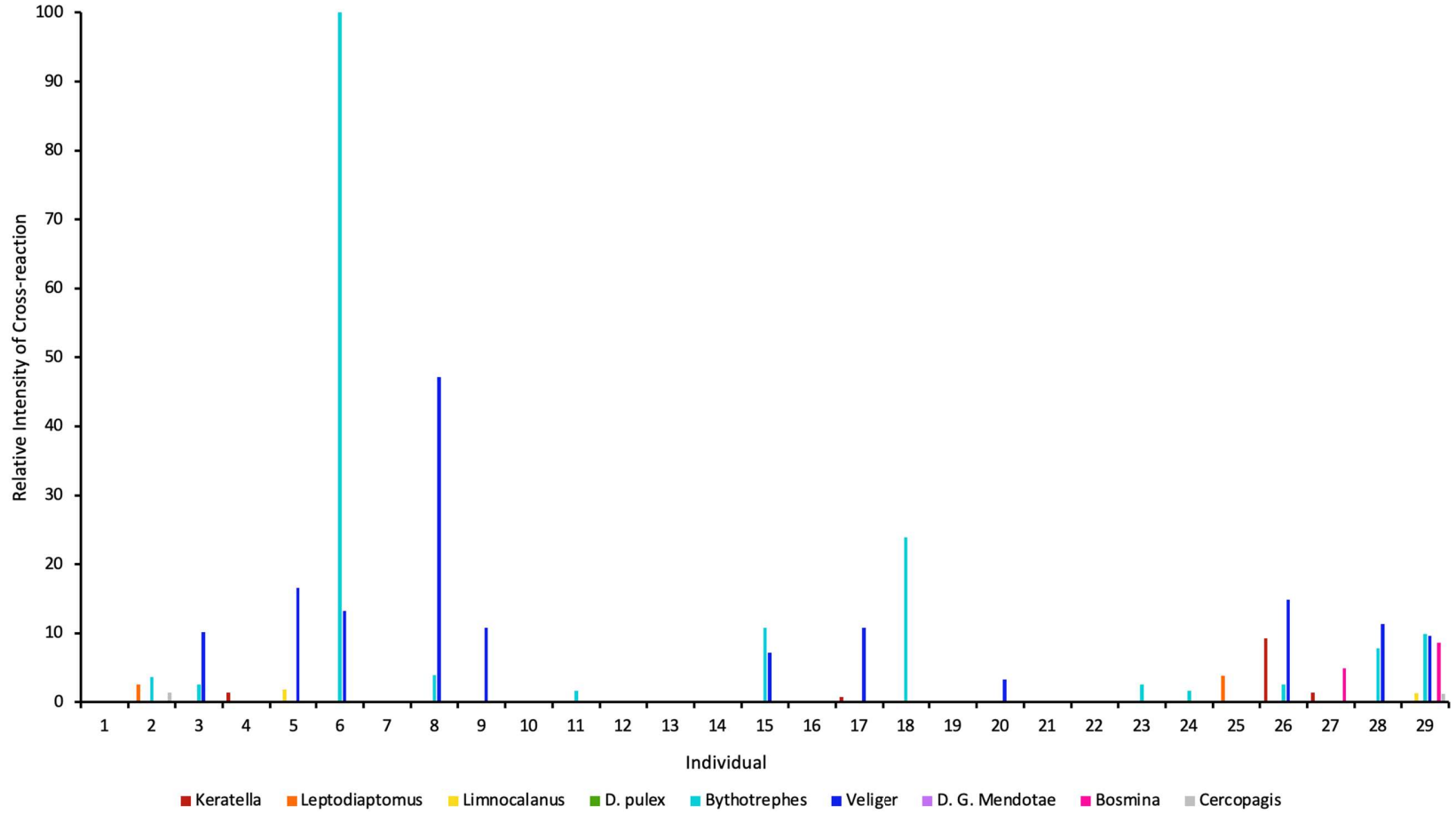

Figure 17: Results of probed gut content for sonicated samples from 2/18/22. Twenty-nine individual *Hemimysis* were probed with nine antibodies. Details in Figure 1.