Trophic Assessment of the Invasive Hydrozoan Gonionemus vertens Using Stable Isotope Analysis: You are What You Eat

Diana Sisk-Gritz
Montclair State University

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Abstract

*Gonionemus vertens*, commonly known as the clinging jellyfish, is a small hydrozoan known for their conflicts with humans due to their neurotoxins that can lead to paralysis. Native to the Pacific Ocean, it has now invaded numerous ecosystems in the coastal waters of the Mediterranean Sea, Europe, and the United States. In the United States, it has flourishing populations from New Jersey to Maine. However, many questions remain as to how it arrived and spread, but equally important is what impact this species may have in the invaded communities they now reside. Research suggests that *G. vertens* actively feeds on zooplankton during the evening, but direct evidence is lacking. Field observations of *G. vertens* have demonstrated a variety of macrofauna in their gastrovascular pouch including fish and peracarid crustaceans. As these organisms are extremely abundant in the aquatic vegetation where they live, it is highly probable that they contribute to their diet. We collected specimens and potential food resources to conduct stable carbon (C) and nitrogen (N) isotope analyses from three locations (Rhode Island or RI, Connecticut or CT, and New Jersey or NJ). The NJ and CT populations had similar δ¹³C values of -19.16 ± 1.77‰ and -17.3 ± 0.3‰ respectively, while the average RI *G. vertens* δ¹³C value was significantly higher at -13.27 ± 0.28‰. However, with regards to δ¹⁵N values, NJ populations were significantly higher with an average δ¹⁵N value of 12.45 ± 1.3‰, compared to CT (10.25 ± 0.1‰) and RI (9.22 ± 0.48‰). Consequently, three trophic structures may be present among these three regions with NJ δ¹⁵N values being the highest suggesting that the *G. vertens* in NJ is feeding potentially one trophic level higher than the populations from RI and CT. With respect to δ¹³C data, RI populations (-13.28‰ δ¹³C) may be gaining greater energy resources from the vascular plant *Zostera marina* (-8.65‰ δ¹³C), while CT (-17.30‰ δ¹³C) and NJ (-19.16‰ δ¹³C) may have greater inputs from macroalgal (-16.35‰...
Continued evaluation will help elucidate the trophic status of this invasive species and shed light on its actual diet. This includes, but is not limited to, collecting more samples to have a robust data set for fuller evaluation of their diet and trophic status. Collecting additional samples from the RI and CT *G. vertens*, prey items in all locations (e.g. phytoplankton), and primary producers would only strengthen the preliminary conclusions generated from this research.
Trophic Assessment of the Invasive Hydrozoan Gonionemus vertens using Stable Isotope Analysis: You are What You Eat

By

Diana Sisk-Gritz

A Master’s Thesis Submitted to the Faculty of Montclair State University
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College of Science and Mathematics
Biology Department

Thesis Committee:

Dr. Paul Bologna
Thesis Sponsor

Dr. Ying Cui
Committee Member

Dr. Xiaona Li
Committee Member
Trophic Assessment of the Invasive Hydrozoan *Gonionemus vertens* using Stable Isotope Analysis: You are What You Eat

A THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Sciences

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Diana Sisk-Gritz

Montclair State University

Montclair, NJ

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Introduction

According to the United States Department of Agriculture Invasive Species Center, invasive species are non-native and introduced species to an ecosystem that have the potential to cause some form of harm to the other organisms or environment (Beck et al., 2008). With a consistently changing world, some of these invasions are unpreventable and can be too costly to adequately eradicate. Yet, it is equally important to observe the changes that occur in an ecosystem that has recently experienced an introduction of invasive species. Research can show alterations to the ecosystem in a number of ways, including, but not limited to, food webs, nutrient cycles, species presence, and community dynamics influenced by invasive species (Gaynor et al. 2016, McCormack et al. 2019, Rigby 2020). Marine environments are particularly susceptible to invasions through transport by humans and cargo from various global ports (Gallardo et al., 2016). High invasion risk is often associated with human migration patterns, making it easier for these invaders to expand to new locations (Hulme, 2009). Unfortunately, the marine environments in the United States are no exception to these potential threats and dangers associated with invasive species.

*Gonionemus vertens* (A. Agassiz, 1862), a small hydrozoan, is only about 25 mm in adult size, but can deliver a powerful sting to the victims it encounters. Like all jellyfish, they can reproduce sexually through egg development or asexually, through various clonal pathways (Uchida, 1976). Many jellyfish also have a biphasic life history with a mobile medusa phase and a semi-sessile polyp phase. In sexual reproduction, fertilized eggs develop into planula larvae that settle onto hard substrates where they metamorphose into polyps. These polyps grow tentacles and eventually repeat the cycle, multiplying by cloning themselves. Some clonal mechanisms for *G. vertens* include the production of motile frustules (Uchida, 1976), but more
recently evidence of budding and binary fission have been observed (Booker, 2022). Polyps can also generate medusa, which progressively develop into mature adults, ultimately repeating the cycle (Uchida, 1976).

*Gonionemus vertens* is native to the Pacific Ocean, but has been introduced to numerous global locations (Rodriguez et al., 2014; Gaynor et al., 2016; Marchessaux et al., 2017). In 2016, the first *G. vertens* was documented in the Manasquan River estuary in New Jersey (Gaynor et al., 2016). That discovery has led to continuous research identifying invaded regions of New Jersey, which in turn develops into analyzing biological and ecological changes of these impacted sites. Habitats that include macroalgae and seagrass beds are primary areas where *G. vertens* have been found. Through personal observations, the species has been seen closer to the shore in shallow marine environments and surrounding areas (Clinging Jellyfish-*G. vertens* GIS mapping data, *Gisdata-njdep.opendata.arcgis.com*). This poses an increasing risk of potential harm to humans and human activity occurring near these sites. The primary time when free swimming medusae can be found is from mid-May until late June in New Jersey, but by early July the medusae are minimally present. The preferred temperature range for the medusae can range between 20 to 25°C, but when temperatures start to exceed 28°C, high mortality rates are observed (Rigby, 2020), but lower temperature thresholds for mortality (25°C) were observed in France (Marchessaux et al., 2017).

Prior literature regarding *G. vertens* describe how the species swim close to the surface of the water column and then drift down. As they free float down, if they encounter prey in the water column they sting and consume them (Mills, 1983; Houghton, 2010). The food source of *G. vertens* has been recorded to be copepods, larval fish, and other microscopic organisms (Todd, 1966; Bakker, 1980). However, one challenge facing this viewpoint is that the presence
of medusae within vegetation opens the possibility of other trophic pathways. Specifically, both eelgrass and macroalgae support high concentrations of fish and macro-invertebrates like amphipods (Nelson, 1980). Given that the swimming and feeding behavior of *G. vertens* is stimulated at night (Mills, 1983), they spend a greater amount of time among the vegetation they cling to. As a result, there could be a clear trophic pathway where *G. vertens* is actively encountering these vegetation related organisms and feeding on them during the day; disrupting the notion of the medusae as solely planktonic feeders. If this is true, there should be evidence of these trophic resources in their diet indicating a broader trophic niche than once believed, as observed from field collected individuals (Bologna pers. comm., Fig. 1).

**Figure 1:** Photos taken by Dr. Paul Bologna, *G. vertens* collected from field sampling that have come up with non-zooplankton prey in their grasp and gastrovascular cavity. A: Adult medusa feeding on *Apeltes quadracus* B: Adult medusa feeding on *Apeltes quadracus* C: Adult medusa feeding on amphipod D: Adult medusa feeding on amphipod E: Adult medusa feeding on *Fundulus*
Food web dynamics require that we answer the question: what does *G. vertens* consume? The use of these dynamics is to understand and map out trophic interactions occurring within an ecosystem. Some of these include: how energy is transferred up a trophic level, feeding relationships, population control and much more (Fig. 2). Figure 2 is simplified to try and best explain what species may be involved but marine food webs can, and are usually more complex. An organism that may be a lower level consumer at juvenile life stage can possibly be a higher level consumer at an adult stage. Factors such as age, size, and spatial placement (i.e., benthic versus pelagic food webs) can all contribute to mapping out a food web. There are a number of factors that can alter a current food web system in any given environment. Many human stressors, such as pollution, overfishing, and eutrophication, whether it be direct or indirect, can increase the chance of overpopulation of one species or invasion and survival of non-native species (Richardson et al., 2009). Hulme (2009) reviewed the spreading of ‘alien’ species into Europe over a span of 500 years and found that there was a drastic increase in non-native plants, invertebrates and mammals as time moved forward. As human demands for economic and material goods continue to rise, so will the rate of invasions.
In New Jersey coastal bays, the top pelagic predators in the system are the ctenophore *Mnemiopsis leidyi* and the scyphozoan *Chrysaora chesapeakei*. Between these two, *C. chesapeakei* exerts significant top-down pressure on *M. leidyi* and other zooplankton (Bologna et al., 2017). When Hurricane Sandy struck the mid-Atlantic region and destroyed polyp habitat for *C. chesapeakei*, significant reductions occurred for this top predator. With the release from this top-down pressure, numerous gelatinous zooplankton species increased in the following years (Bologna et al., 2018). This event could be the reason that *G. vertens* began to increase in New
Jersey, ultimately leading to their discovery four years later (Gaynor et al., 2016), as C. chesapeakei is a known predator of G. vertens (Rigby, 2020).

Food web modeling can be used as a way to graph what is occurring in marine ecosystems in current times or as a predictor for future dynamic assessments. The isotopic ratio within any given consumer species is traced from the base food choice within a particular region, specifically the primary producer (McCormack et al., 2019). Stable isotope analysis is currently a powerful tool that can reveal consumption patterns. It allows us to assess how a food web is being shaped and how energy is being transferred throughout the food chain. The most commonly used isotopes are stable carbon and nitrogen isotopes (δ^{13}C and δ^{15}N,

\[ \delta^{13}C = \left(\frac{^{13}C/^{12}C \text{ Sample}}{^{13}C/^{12}C \text{ Standard}} - 1\right) \times 1000, \delta^{15}N = \left(\frac{^{15}N/^{15}N \text{ Sample}}{^{15}N/^{15}N \text{ Standard}} - 1\right) \times 1000 \]). Both δ^{13}C and δ^{15}N values increase as the trophic level goes up in the food web and can be used to discriminate trophic pathways from several primary producers who may contribute solely or in combination to primary grazers (Post, 2002). A 2-4‰ increase in δ^{15}N values is commonly attributed an increase in trophic level (de Lecea et al., 2011). When δ^{13}C and δ^{15}N values are plotted against each other, the δ^{15}N can help determine trophic placement and the δ^{13}C helps understand the origins of dietary sources with distinctly carbon isotope signature, their consumption patterns of lower trophic levels and/or photosynthetic pathway of the primary producers.

There are a number of different and abundant options for potential prey for G. vertens (e.g., Amphipoda, Isopoda, Decapod, Copepoda, and Osteichthyes). Analyzing the δ^{13}C and δ^{15}N of G. vertens and possible food sources will help clarify and accurately document the generalized food choices of G. vertens. Through the use of δ^{13}C and δ^{15}N, we can quantify food web dynamics and probable trophic pathways to G. vertens. This research took an interdisciplinary
approach to assess the trophic standing of the invasive clinging jellyfish, *G. vertens*, to determine if it poses a threat to food web dynamics in this invaded community.

**Methodology**

53 samples were collected in northern Barnegat Bay, NJ (40.055023°N, 74.119270°W) from May to July in 2022, with additional *G. vertens* samples collected in Mumford Cove, Connecticut (41.321963, -72.015985) and Potter Pond, Rhode Island (41.396425°N, 71.536334°W). Field collection of *G. vertens* occurred using dip nets swept into their primary habitats, including seagrass and macroalgal beds. Samples were brought into the boat, with *G. vertens* isolated as a group and placed into glass jars to be returned to the lab. A sample of macroalgae was also returned to the lab in seawater to collect various fauna within and to assess the δ¹³C and δ¹⁵N values of the primary producers. To assess potential planktonic food resources, 363 micron plankton nets were towed in proximity to the beds where the *G. vertens* was located. These samples were collected and returned to the lab live in seawater (Fig. 3). All samples were then brought back to Montclair States to be identified and prepared for analyses. Larger organisms were isolated individually, rinsed in deionized (DI) water, and then frozen at -20°C until they were ready to be analyzed (Jardine et al., 2003; MacKenzie et al., 2017). Prior to isotopic analyses, samples were removed from the freezer, thawed, and then oven dried at approximately 50°C for 24 hours, following the protocol of de Lecea et al. (2011), who found no significant isotopic difference between oven-drying or freeze-drying.
**Figure 3.** Some of the proceed and identified species used in analysis. A: Crab Larvae (approximately 0.5 mm as seen in black above), B: Amphipod (approximately 1.0 mm as seen in red above), C: *G. vertens* taken by Mike Peters / Montclair State University (approximately 1.5 cm as seen in blue above), D: Fish Tail (approximately 1.0 cm as seen in blue above), E: Corophiidae Amphipod (approximately 1.0 mm as seen in red above), F: Melitidae Amphipod (approximately 1.0 mm as seen in red above), G: Copepod (approximately 0.5 mm as seen in black above)

**Stable Carbon and Nitrogen Isotope Analyses**

Prior to stable carbon and nitrogen isotope analyses, each sample was washed with DI water then placed into a tin capsule to best remove any lingering waste excreted from the sample (MacKenzie et al., 2017). Minute individuals (e.g., amphipods, copepods etc.) were placed into tins, while larger samples (e.g. fish) were ground using a mortar and pestle prior to being placed into tin. To ensure no cross contamination occurred, the mortar and pestle were cleaned with DI water in between uses. The tin capsules were left in a drying oven at 50°C for twenty-four hours. Samples were then weighed and recorded to assess the actual dry weight (Table 1). Samples assessed for isotopic analysis included *G. vertens* (*n* = 28), Osteichthyes (*n* = 3), Amphipoda (*n* = 16), Decapoda (*n* = 3), Calanoida (*n* = 2), and Harpacticoida (*n* = 1); Total *n*=53; Table 1). In
addition to these samples, previously analyzed samples by Dr. Bologna (unpublished data) were added to be analyzed and compared, making the total sample n=140.

Table 1. Taxonomic groups collected, identified and analyzed for stable isotopes. Data provide lowest reasonable taxonomic identification and the number of samples used in the analyses.

<table>
<thead>
<tr>
<th>Major Group</th>
<th>Taxa (Abbreviation in Graphs)</th>
<th>Number of samples collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td>Ulva lactuca (Ulva)</td>
<td>6</td>
</tr>
<tr>
<td>Plantae</td>
<td>Zostera marina (Z MAR)</td>
<td>6</td>
</tr>
<tr>
<td>Cnidaria</td>
<td>G. vertens NJ (NJ GV)</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>G. vertens RI (RI GV)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>G. vertens CT (CT GV)</td>
<td>2</td>
</tr>
<tr>
<td>Amphipoda (APOD)</td>
<td>Aoridae (AOR)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Caprellidae</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Corophiidae</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Melitidae (MELIT)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Unidentified Amphipod Spp.</td>
<td>28</td>
</tr>
<tr>
<td>Copepods</td>
<td>Calanoida Copepods (CALCOP)</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Harpacticoid Copepods (HARCOP)</td>
<td>3</td>
</tr>
<tr>
<td>Decapoda (DPOD)</td>
<td>Callinectes sapidus</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Carcinus maenas</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Palaemon sp. (GSHRIMP)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Xanthidae</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Zoea</td>
<td>1</td>
</tr>
<tr>
<td>Isopoda (ISOPOD)</td>
<td>Erichsonella sp. (ERICH)</td>
<td>6</td>
</tr>
</tbody>
</table>
The samples were wrapped in the tin capsules and weighed using a microbalance. An Elemental Analyzer coupled to an Isotope Ratio Mass Spectrometer (EA-IRMS) (Model: Thermo Fisher Flash EA and Delta V IRMS). The IRMS evaluates the ratio of stable isotopes for carbon ($^{13}$C/$^{12}$C) and nitrogen ($^{15}$N/$^{14}$N) of the samples, and the accuracy was assessed against standards with known $\delta^{13}$C and $\delta^{15}$N values. Standards include Caffeine, Glycine, L-Glutamic Acid and B2159-Sorghum Flour with known certified $\delta^{13}$C and $\delta^{15}$N values were run in between unknown samples to calibrate the $\delta^{13}$C and $\delta^{15}$N values of the samples. These standards were run every ten samples and were used for two-point calibration following the approaches outlined in Coplen et al. (2006). The analytical precision for $\delta^{13}$C is reported as ± 1 SD as 0.18‰ and $\delta^{15}$N is ± 1 SD as 0.10‰.

**Trophic Level Evaluation**

Cross plots of $\delta^{13}$C and $\delta^{15}$N values for individual taxa are shown in Figs 3 to 6 to evaluate their potential trophic relationships. In addition to 53 samples from 23 taxa collected in 2022, previously analyzed data from Barnegat Bay (Bologna unpubl. data) are also plotted for comparison. Because *G. vertens* is found in submerged aquatic vegetation, two potential carbon sources from primary producers included the vascular plant *Zostera marina* and a common
macroalgae, *Ulva lactuca*. To compare differences in isotope values, an analysis of variance was conducted on the three *G. vertens* populations comparing carbon and nitrogen values. A trophic mixing model was used to assess potential trophic pathways among the population of *G. vertens* using the following equations: \( F_1(\delta^{13}C) + F_2(\delta^{13}C) = \delta^{13}C (G. vertens) \) and \( F_1 + F_2 = 1 \); where \( F_1 \) represents the proportion of diet from food source 1 and \( F_2 \) represents the proportion of diet from food source 2. Food sources were assessed as the average \( \delta^{13}C \) of the two bracketing food items identified in the stable isotope analyses on either side of the average *G. vertens* \( \delta^{13}C \) value for each population. For all populations, \( F_1 \) was designated as the average value of planktonic zooplankton analyzed (i.e., copepods, crab larvae, shrimp larvae), while \( F_2 \) varied among populations with \( F_2 \) for New Jersey designated as amphipods and \( F_2 \) for CT and RI designated as isopods. These brackets represent potential night feeding in the water column and benthic food resources within the submerged vegetation.

Additionally, diatoms are potential primary producers and were used from literature sources (Fry, 1991; Burkhardt et al., 1999; Tunēns et al., 2022). Faunal sample results were then plotted to evaluate possible organic carbon sources from primary producers, while \( \delta^{15}N \) values were used to identify trophic levels of consumers under the assumption that an increase in 2‰ in \( \delta^{15}N \) values represents an increase in one trophic step (Table 3 and Figure 7; Kristensen et al., 2019). Finally, *G. vertens* were plotted to assess both their likely carbon pathways as well as trophic status and their relevance to the potential prey pool.

**Results**

In total, 142 organisms were assessed for \( \delta^{13}C \) and \( \delta^{15}N \). Specifically, 37 *G. vertens*, 46 Amphipoda, 10 Isopoda, 16 Decapod, 6 Copepoda, 13 Osteichthyes, 8 *U. lactuca*, and 6 Z.
samples were used in the trophic analysis (Table 1). Based on the δ¹³C and δ¹⁵N cross plot, a visualization of trophic pathways is possible. When assessing the differences among the three population of G. vertens from NJ, RI, and CT, we see three different patterns of δ¹³C and δ¹⁵N values (Fig. 4). First, RI samples had significantly higher values of δ¹³C (-13‰) compared to the other 2 populations (F₂,₃₄=17.17; P < 0.0001). CT and NJ populations show overlap for δ¹³C values, but there was a larger range of δ¹³C values for NJ (-16 to -23‰), likely due to the substantially greater numbers of individuals analyzed. These results suggest that these populations may differ in the trophic pathways, and the source of which their carbon reserves assimilates from. With RI looking like these individuals are receiving their carbon sources from the vascular plant Z. marina and algae (Fig. 5), while samples collected from both CT and NJ are likely dominated by carbon sources from algae, but possibly phytoplankton (Fig. 5). The δ¹³C for Z. marina is approximately -11‰ (Bird et al., 1995; Canuel et al., 1997; Kennedy et al., 2010; as cited in Sonshine, 2012). The approximate value of δ¹³C in U. lactuca is 4.9 to 21.5‰ δ¹³C, (Schwinghamer et al., 1983; Chanton and Lewis, 2002; as cited in Wozniak et al., 2006). The average range for δ¹³C values of phytoplankton, diatoms and other cyanobacteria are -15 to -20‰ (Fry, 1991), but could be as low as -21 to -23‰ (Tunēns et al., 2022), even as low -25‰ in some locations (Bird et al., 1995; Canuel et al., 1997; Kennedy et al., 2010; as cited in Sonshine, 2012). These δ¹³C values are far lower than the values of Z. marina, but within similar ranges for algae U. lactuca (-16.35 ‰ δ¹³C) in Barnegat Bay (Fig. 5). In terms of δ¹⁵N, similarities exist between CT (10.25‰ δ¹⁵N) and RI (9.22‰ δ¹⁵N) populations (Fig. 4), but NJ individuals have significantly higher δ¹⁵N values (F₂,₃₄=11.08; P < 0.0002). Based on the trophic mixing models, the NJ population showed 37.2% of trophic resources coming from the zooplankton with 62.8% coming from amphipods. CT populations showed approximately equal proportions of food
resources with 54.7% from zooplankton and 45.3% from benthic sources, while RI had only 9.1% coming from pelagic resources and 90.9% coming from benthic sources. All of these populations demonstrate substantial energy resources coming from benthic sources. This demonstrates that NJ populations are receiving their food sources from more complex food web interactions and feeding at a higher trophic level.

When other organisms are introduced to the $\delta^{13}C$ and $\delta^{15}N$ cross plot, several patterns emerge. When comparisons are made with the dominant micro-crustacean taxa (e.g., amphipods and isopods), the majority of these organisms lie within the $\delta^{13}C$ regions of algae and Z. marina, however copepods show carbon values with much lower $\delta^{13}C$ indicating food sources are coming from phytoplankton production (Fig. 6). When comparing the $\delta^{13}C$ values of G. vertens with macro-fauna (e.g., grass shrimp, fish), we see overlapping distributions of these organisms and G. vertens from NJ (Fig. 7), but their $\delta^{15}N$ values are generally higher than CT and RI populations, suggesting that these larger organisms that live within the vegetated beds are likely contributing to food sources of G. vertens in NJ, but not necessarily in other populations in CT and RI? (see Fig. 1).

In aggregate, when the average values for $\delta^{13}C$ and $\delta^{15}N$ per taxa are used, it provides a clearer picture on the trophic structure of these systems. For RI, their $\delta^{13}C$ values indicate a mixed relationship with Z. marina and algal pathways supplying the base nutrition (Fig. 8). This differs from both CT and NJ, whose $\delta^{13}C$ signals are dominated by macroalgae with possible contribution from phytoplankton. In looking at their $\delta^{15}N$ values, clearly NJ populations are feeding at 1-1.5 trophic levels above CT and RI (Fig. 8), with overlapping distributions with fish and decapods (i.e., grass shrimp). Given the three G. vertens populations differ with respects to both $\delta^{13}C$ and $\delta^{15}N$, this suggests that different trophic pathways are operating within each
region, with variations associated with the source of primary production and the length and complexity of secondary consumers contributing to the food sources of *G. vertens*.

**Figure 4.** The $\delta^{13}C$ and $\delta^{15}N$ values for all *G. vertens* collected. They are color-coded by their regional location, NJ: New Jersey, CT: Connecticut, RI: Rhode Island.
Figure 5. The δ^{13}C and δ^{15}N values for *G. vertens* from the specific locations to the main primary producers: *Ulva lactuca* (Green Algae) and *Zostera marina* (Eelgrass).
Figure 6. The $\delta^{13}$C and $\delta^{15}$N values for all *G. vertens* collected compared to Amphipods and Copepods as whole groups. In addition to comparing the primary producers.
Figure 7. The C:N isotope values for all *G. vertens* collected compared to larger macrofauna, Fish and Grass Shrimp; in addition to comparing the primary producers.
Figure 8. Cross plot of $\delta^{13}C$ and $\delta^{15}N$ values for all organisms collected.

Discussion

Whenever a non-native species enters a new habitat, critical evaluations regarding the role they play is important in determining whether the species poses a significant threat to the other members of the community. *G. vertens* has shown a wide invasive trajectory over the last century with a global distribution (Edwards, 1977; Bakker, 1980). However, their abundance can vary among years and few locations have been studied to determine whether it merely poses a threat to humans from stings (e.g., Marchessaux et al., 2023) or whether it could impact food webs through direct consumption or competition for food resources. Previous studies have
described *G. vertens* as a planktivore with a passive feeding style (Mills, 1983; Houghton, 2010). As such, the convention is to assume that this hydrozoan should gain its food sources from primarily planktonic pathways. This assumption is based on laboratory feeding studies where this was observed. However, this night-time feeding behavior suggests that they have a very limited amount of time to feed, given their temperate to sub-arctic distribution. Since medusae blooms occur in the spring and summer months, greater day-length would suggest that night-time feeding is reduced, opening the doorway for different trophic pathways which might take advantage of their tendency to cling to submerged vegetation. The results from the Trophic Mixing Model help start to piece the crucial missing trophic links in the diet assessment of *G. vertens*. When using pelagic versus benthic food sources within the model, each *G. vertens* population is being supported through benthic food webs dynamic to varying degrees, with RI individuals showing less than 10% of trophic resources coming from common pelagic prey. Both NJ and CT populations had a greater mixed diet of pelagic and benthic resources, but these results support the argument of a much more complex food web based on habitat and behavior of *G. vertens*.

With vegetative areas being highly productive and containing high densities of various organisms such as fish and macro-invertebrates (Heck et al., 1995; Snickars et al., 2014), this proximity to alternate food resources may play a bigger role in their diet. Expanding the palate of *G. vertens* from copepods to a much wider selection of prey would fit better into their habitat requirements. When we look at the relationship of $\delta^{13}$C and $\delta^{15}$N among *G. vertens* populations and potential primary producers, we see that RI population lies between *Z. marina* and *U. lactuca*, while CT and NJ are directly above algae (Fig. 5). This corresponds to the *G. vertens* habitats found within each region with RI dominated by *Z. marina*, NJ dominated by macroalgae.
and CT being a mix. However, does that sync with the potential food resources within these vegetated habitats?

Using the data collected on major groups of organisms found with the habitats of *G. vertens*, we can see linkages between *G. vertens* and their possible prey items. When looking at the Amphipods and Copepods $\delta^{13}C$ and $\delta^{15}N$ values allowed us to view the trophic hierarchy occurring within this system. With the copepod’s value (Fig. 6), we can see they are directly one trophic placement below the NJ *G. vertens* ($\delta^{15}N$) values. With all *G. vertens* and the various amphipod species found amongst the seagrass beds, we see similar trophic placement as copepods. Specifically, for the NJ *G. vertens* samples, we can see better link between the amphipod families Aoridae and Melitidae, where the RI *G. vertens* have values closer to the Isopods *Idotea balthica* and *Erichsonella* sp. Overall, we can see a lower trophic placement for Copepods as well as Amphipods and Isopods compared to *G. vertens* (Fig. 6). Based on the $\delta^{13}C$ and $\delta^{15}N$ values generated from these organisms, we have the supportive data to the observations occurring within the field collections of consumption of fish and macroinvertebrates (Fig. 1). This allows us to demonstrate the likelihood that *G. vertens* are actively a higher-level consumer within benthic food webs, consuming both micro-crustaceans (e.g., copepods, amphipods, and isopods) and macro-fauna (e.g., fish, Fig. 1).

Other species of hydrozoa have been described to have other feeding behaviors and choices. Species such as *Obelia* spp., monitored within laboratory settings, were noted to adjust water currents in the direction of their mouth, which is unlike the typical behavior of free-floating medusae (Boero et al., 2007). *Blackfordia virginica*, another hydrozoan, is an invasive species in Portugal and was monitored by Marques et al. (2015). They noted that *B. virginica* exhibited feeding mechanisms in a similar manner to the *G. vertens* found in NJ with higher
activity during darker hours. After examining gut contents of collected jellyfish, they concluded that Copepods were most prevalent (specifically *Acartia* spp.), based on various prey items presented. With NJ having longer periods of day during medusae blooms, we can deduce that *G. vertens* would have greater feeding opportunities during the day while they cling to vegetation, compared to swimming among the plankton, similar to what was expressed in Marques et al. (2015). Size of the medusae also has an impact on jellyfish feeding habits, as an organism grows the diet choices may change to maximize food intake. In Wang et al. (2020), they used $\delta^{13}C$ and $\delta^{15}N$ values to determine the trophic placement of food choices in *Cyanea nozakii*. The typical $\delta^{13}C$ values are between -18.81 to -16.88‰ and from 8.23 to 10.46‰ for $\delta^{15}N$. They concluded that the diet changes with growth based on the significant difference in $\delta^{15}N$ (Wang et al., 2020).

Surprisingly, the $\delta^{15}N$ for this study ranged between 8.5 to 16‰ (Fig. 4), which suggests a similar or higher trophic status as the larger scyphozoan from Wang et al. (2020). It is possible that benthic food webs would have. However, the higher carbon values -12 to -20‰ in $\delta^{13}C$ of *G. vertens* would suggest greater trophic alignment with benthic vegetation rather than pelagic primary producers (Fig. 5). In Fleming et al. (2015), they compared three different species of jellyfish (*Aurelia aurita*, *Cyanea lamarckii* and *C. capillata*) and various baseline feeding choices. Their results suggested the three co-occurring species consume different prey and the $\delta^{13}C$ and $\delta^{15}N$ differences infer species body size result in trophic differences. *Aurelia* sp. are similar to *G. vertens* in that they consume various copepod species, but in Pereira et al. (2014) the authors noted that various fish are contributing to the diet of *Aurelia*, broadening the dietary niche. In summary, jellyfish have expanded their feeding habits from the traditional view of passive feeding to an active approach of consuming what is in their surrounding habitat. Food choices depend on the primary producers within an area and the various prey items available to
predators. Consequently, the results presented promote the expanding diet of hydrozoans like *G. vertens*, in a similar manner as scyphozoans (Meredith et al. 2016).

**Conclusion**

Overall the data collected within this research study provides an outlook on how *G. vertens* is developing within the United States Coast specifically within Barnegat Bay. Having a base understanding of the wide range of food items opens the opportunity to better access the growth and spread of the species. If food items, the various macrofauna and microcrustacean increasingly become available we can assume greater survival of the species and potential over abundance (Houde, 1978). The biggest support this study provides is the proof of consumption of copepods, amphipods, fish and other organisms expanding upon what was previously suggested.

Future work would be continuing to monitor *G. vertens* and as the population numbers increase, monitor the quantities of the prey populations. Depending on the abundance densities, it will in turn determine the population numbers of *G. vertens*. Additionally, by continuing collecting samples and analyzing $\delta^{13}$C and $\delta^{15}$N values of predator and prey, we can note any diet changes over a temporal scale. Expanding the collection from Barnegat Bay we can also start to include and elaborate the spatial scale. With knowing predator and prey trophic linkages between organisms with the food chain, higher and lower compared to *G. vertens*, we can piece together large dynamics within the ecosystem of Barnegat Bay.
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