Assessment of clinging jellyfish *Gonionemus vertens* populations in New Jersey 2016 - 2020

Final Report

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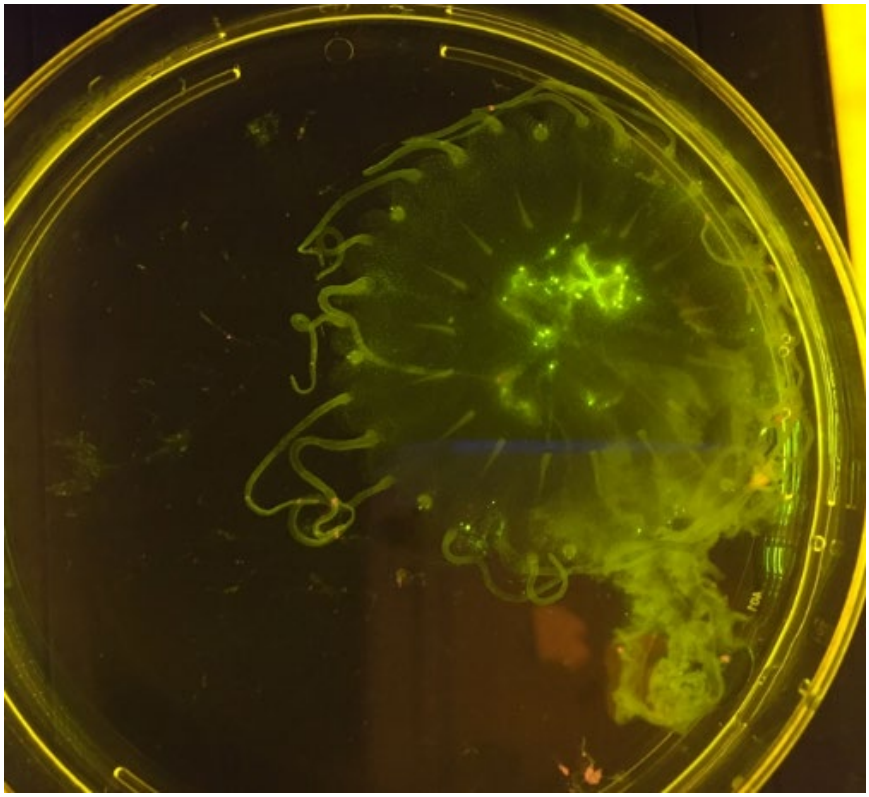

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Executive Summary

Gonionemus vertens (Clinging Jellyfish) is a species of small hydrozoan native to the Pacific Ocean. In 2016, it appeared in New Jersey with the first individual being documented from the Manasquan Canal and subsequent individuals collected in the Shrewsbury River Estuary. Research regarding the distribution of *G. vertens* was conducted during the summers of 2016 and 2017 in northern Barnegat Bay, the Manasquan River, and the Shrewsbury River Estuary. While the first individual *G. vertens* confirmed was from the Manasquan Inlet, no other individuals were ever collected from this region nor in the northern reaches of Barnegat Bay. All individuals, including recruiting polyps, were identified from the Shrewsbury River Estuary (54 individual *G. vertens* medusae were collected in 2016 and 218 collected in 2017). In both years, individual polyps were collected on JADs (Jellyfish Attracting Devices), but no large-scale larval recruitment of polyps was observed, as only one single individual polyp was on a JAD during 2016 and 2017. Subsequent laboratory observations of polyp development from larvae suggest it often takes 3 to 4 months for polyps to fully develop, so it is probable that the polyps from the field JADs likely had merely climbed onto the JAD surface and were not actively recruiting there. It is clear that a well-defined population exists in the Shrewsbury River and continued blooms in the late spring and early summer are expected in the future. Since no other individuals were collected in the Manasquan and Barnegat Bay estuaries after the first individual was observed, it is unclear as to whether that observation was anomalous or whether another population remains in this area, but is not actively blooming. During surveys of the Manasquan River a large, viable eelgrass (*Zostera marina*) bed was identified near the original collection site of the first *G. vertens*, so the preferred habitat for *G. vertens* is present, however the abundant populations in the Shrewsbury River are using macroalgae as habitat, so both algae and seagrass are viable habitat for *G. vertens*.

Continued sampling in the Shrewsbury River will determine if more populations exist. The bloom of *G. vertens* ended under different conditions in 2016 compared to 2017. Specifically, the bloom was present in 2016 until the sea nettle (*Chrysaora chesapeakei*, (formerly *C. quinquecirrha*)) population increased. Laboratory studies demonstrated that *C. chesapeakei* actively consumes *G. vertens* and we surmise that the population decline was a result of predation by *C. chesapeakei*. In 2017, an early spike in regional temperature, coupled with significant algal biomass in the region, created regions of low oxygen, potentially causing the decline of adults after this event. Samples from locations which had previously yielded *G. vertens* among the algal beds, demonstrated substantial population declines and the presence of dead algae and anoxic conditions. The most likely scenario for this early decline was excessive heat, hypoxic conditions, and the loss of usable algal biomass as habitat.

Beginning in 2018, several developments and research avenues were pursued. In 2018, abundant populations of *G. vertens* were observed in Barnegat Bay ranging from the Metedeconk River to Barnegat Inlet. This expansion likely existed earlier, but individuals were not observed during the first two years of this project. In fact, reports of people being stung and needing emergency help were reported at the beginning of July from Tices Shoals. In 2019, *G. vertens* was identified from North Wildwood in the southern part of

the State, indicating that the entire coastal region of New Jersey may support clinging jellyfish, although no other regions have been identified. Laboratory experiments carried out from 2018-2020 showed that most *G. vertens* populations have a physiological thermal tolerance limit of 28ºC, but the North Wildwood population shows some latitudinal variation with a slightly higher thermal limit of about 30ºC. For much of New Jersey's inland bays, the 28ºC thermal limit temperature coincides with late June and early July, so it is likely that water temperature plays a major role in their decline. However, this time frame is also the unofficial start of summer vacation (i.e. increased recreational usage) so continued vigilance on their presence and abundance is needed to inform the public of potentially dangerous encounters with this invasive species.

How to Cite this Report

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Introduction

Invasive species are well documented to have significant impacts on the communities that they have invaded (Thomsen et al. 2015). For recently identified species, it is unclear what potential impacts may be caused as a result of their presence or establishment. The native range of *Gonionemus vertens*, the clinging jellyfish, includes coastal regions of the North Pacific Ocean (Fofonoff et al. 2003). Its common name originates from the behavior of the medusa stage to 'cling' to vegetation using modified adhesive tentacle pads. The medusae are predators of small zooplankton and epifaunal organisms which share the same vegetated habitats. Edwards (1976) suggests that two variants of this species exist. Following inspections by Mayer (1901) of medusae collected from Woods Hole, MA, he proposed a new species – *G. murbachii* – which he believed differed morphologically from *G. vertens* (originally described by Agassiz 1862 from Puget Sound, WA). However, debate continues on whether these variants are different species, subspecies, and so on (Govindarajan et al. 2017). Further, Govindarajan and Carmen (2015, after Naumov 1960) reported that these possible alternative forms may also exhibit different levels of sting potency. Specifically, variants from the western North Pacific may be more venomous, while those from the eastern North Pacific possess less potent stings. This species has now been documented to have invaded Norway, the Baltic Sea, the Mediterranean Sea, the northwest Atlantic, and Argentina (Fofonoff et al. 2003, and references within; Schuchert 2016). Govindarajan and Carman (2016) review and document the invasions within the Cape Cod region of Massachusetts and the occurrence in other regional New England estuaries. Interestingly, the initial introduction in the late 1800s appears to be of the less venomous form and the potential re-introduction in the 1990s is of the more toxic variety. *Gonionemus vertens* has now become established in New Jersey (Gaynor et al. 2016).

Project Rationale

The appearance of the invasive clinging jellyfish, *Gonionemus vertens*, in coastal New Jersey estuaries (Fig. 1) poses a potential conflict between the use of coastal estuaries and the safety of the general public using these waters. *G. vertens* is known to deliver substantial stings, which in some cases have resulted in hospitalization. This species is encountered in shallow water during the day where it clings to various substrates including seagrass and macroalgae. Its behavior involves swimming into the water column and drifting to the bottom feeding on organisms that it encounters. Because its distribution lies within shallow coastal embayments, there exists the potential of encounter with the public using these areas for recreation. The challenge we face with this invasive species is that nothing is known about the distribution and abundance of the adult medusa in New Jersey, nor the location of the polyp stage of their life history. This research provides data to identify and quantify the distribution and abundance of *G. vertens* from the Shrewsbury Estuary and the northern Barnegat Bay/Manasquan River Estuaries. Additionally,

understanding the development of blooms and ultimate declines in New Jersey are needed to safe guard public health.

Figure 1. Image of *Gonionemus vertens* collected in New Jersey.

Phase 1 Research Agenda 2016-2017

This project was initiated in June of 2016 and continued through August 2017. During this timeframe, research was initiated to assess the biology and ecology of this invasive species with the following primary research objectives:

Research Objectives

1) Assess the distribution of adult *Gonionemus vertens* from the Shrewsbury River Estuary and from the Manasquan Estuary and Northern Barnegat Bay
2) Assess the distribution of *Gonionemus vertens* ephyrae and recruiting larvae (polyps) from the Shrewsbury River Estuary
3) Assess the distribution of *Gonionemus vertens* ephyrae and recruiting larvae (polyps) from the Manasquan Estuary and Northern Barnegat Bay

Study Locations

To assess the primary objectives, a stratified sampling protocol was established to initially determine the potential distribution of *G. vertens* in the Shrewsbury River Estuary and from the Manasquan Estuary and Northern Barnegat Bay. During 2016, nine sites were established in the Manasquan Estuary, 4 sites in Northern Barnegat Bay and 10 sites in the Shrewsbury River Estuary (Table 1). In 2017, the sampling stations were adjusted to target regions where *G. vertens* was reported or collected and narrow the research focus based upon 2016 initial results. As such, in 2017 we sampled 4 sites in the Manasquan bracketing the 2016 observation, 4 sites in the northern section of Barnegat Bay, and 8 sites in the Shrewsbury River Estuary (Table 2).

Table 1. Locations sampled in accordance with the research objectives from each of the three sampled estuaries for 2016.

Manasquan River	**North**	**West**	**Date Sampled**
Ablemare	40.112583	-074.093717	6/30/16, 7/21/16
Beverly	40.103567	-074.094233	6/30/16, 7/21/16
Treasure Island East	40.09085	-074.093717	6/30/16, 7/21/16
Treasure Island West	40.0913	-074.07405	6/30/16, 7/21/16
Golf Course	40.09805	-074.063033	6/30/16, 7/21/16
Gull Island Railroad Canal	40.101833	-074.04965	6/30/16, 7/21/16
Barnegat Bay	**North**	**West**	**Date Sampled**
Bay Head	40.06604	-074.04702	6/29/16, 7/20/16
East Drive	40.06028	-074.07797	6/29/16, 7/20/16
Carrol Fox Road	40.06422	-074.06629	6/29/16, 7/20/16
Herring Island	40.05190	-074.05157	6/29/16, 7/20/16
Shrewsbury River	**North**	**West**	**Date Sampled**
Blackberry Bay	40.324	-074.011983	7/6/16
Raccoon Island	40.335867	-073.988583	7/6/16, 8/3/16
Hook	40.3369687	-073.996283	7/6/16, 8/3/16
Gertrude Place	40.336967	-073.996167	7/6/16
Wharfside Manor Marina	40.337283	-073.982933	7/6/16, 8/3/16
Oyster Bay	40.32433	-073.982867	7/6/16, 8/3/16
Plum Island	40.40955	-073.982933	7/11/16, 8/3/16
Little Silver Creek	40.34224	-074.02180	7/11/16, 8/3/16
Oceanport Creek	40.32008	-074.02075	7/11/16, 8/3/16
Trautmans Creek	40.32521	-073.98904	7/11/16, 8/3/16
Manhasset Creek	40.31487	-073.98966	7/11/16, 8/3/16
Rumson County Club	40.34096	-074.01043	7/11/16, 8/3/16

Table 2. Locations sampled in accordance with the research objectives from each of the three sampled estuaries for 2017.

Manasquan/Barnegat Bay	**North**	**West**	**Date Sampled**
Treasure Island	40.0913	-074.07405	5/31, 6/15, 8/3/2017
Gull Island Railroad Canal	40.10183	-074.04965	5/31, 6/15, 8/3/2017
Manasquan 'Canal'	40.08966	-074.06458	5/31, 6/15, 8/3/2017
Riverside Park	40.08995	-074.09734	5/31, 6/15, 8/3/2017
Bay Head	40.06604	-074.04702	5/31, 6/15, 8/3/2017
East Drive	40.06028	-074.07797	5/31, 6/15, 8/3/2017
Carrol Fox Road	40.06422	-074.06629	5/31, 6/15, 8/3/2017
Herring Island	40.05190	-074.05157	5/31, 6/15, 8/3/2017
Shrewsbury River	**North**	**West**	
Blackberry Bay	40.324	-74.011983	5/18, 6/2, 6/14, 6/28, 7/18, 8/1/2017
Raccoon Island	40.33587	-73.988583	5/18, 6/2, 6/14, 6/28, 7/18, 8/1/2017
Hook	40.336967	-73.996283	5/18, 6/2, 6/14, 6/28, 7/18, 8/1/2017
Wharfside Manor Marina	40.337283	-73.982933	5/18, 6/2, 6/14, 6/28, 7/18, 8/1/2017
Sedge Island	40.345702	-73.985603	5/18, 6/2, 6/14, 6/28, 7/18, 8/1/2017

Oyster Bay	40.32433	-73.982867	5/18, 6/2, 6/14, 6/28, 7/18, 8/1/2017
Manhasset Creek	40.31487	-73.98966	5/18, 6/2, 6/14, 6/28, 7/18, 8/1/2017
Bartley Point Island	40.378070	-73.989565	5/18, 6/2, 6/14, 6/28, 7/18, 8/1/2017

Phase 1 Methods

Water Quality Measurements

During each sampling event at each site, water quality measurements were taken to assess water temperature, salinity, and dissolved oxygen using an NJDEP certified YSI® Model Pro 2030 multi-meter.

Field Sampling Protocol

Plankton Tow Samples

To assess the distribution and density of *G. vertens*, we used several methods to collect individuals. For collection of ephyrae and the zooplankton community, we used triplicate plankton tows at the established sites. Collection of zooplankton was completed through triplicate plankton tows using a 335 µm zooplankton net at each site as near to shore and/or above submerged aquatic vegetation or macroalgae, which are natural habitat for adults, but also could be release areas for ephyrae. Length of tow was standardized using a mechanical flow meter to assess the distance traveled. Data were then transformed into density (#/m^3 water). Each sample was pulled from the stern of the boat for one minute with the engine minimally engaged. All plankton tow samples were sieved in the field, using a 250µm sieve. Each sample was then preserved in the field with ethanol. When returned to the lab these samples were stained with rose bengal, to make animals easier to identify. The animals found in samples were identified to the lowest taxonomic level of certainty. If any gelatinous zooplankton were found they were identified and separated from the rest of the animals; if the sample was a hydrozoan or could not be identified morphologically, it was identified molecularly by DNA extraction, PCR, and subsequent DNA sequencing (see below for methodology).

Submerged Aquatic Vegetation Sampling

During 2016, macroalgal beds were sampled to assess the presence of *G. vertens* using a framed benthic sampling net (scoop). Algae scoops were taken in chest to waist deep water using a net with 2.5mm mesh with an opening of roughly 0.75m x 0.5m. Samples were taken until the net was full of submerged aquatic vegetation or until all vegetation in the area had been swept with the net. If the bottom sediment was too difficult to walk (e.g., fine muds, unconsolidated sediments) dips were taken from the boat, following the same protocol for filling the net or sampling the vegetation in the area. As these samples were quite large and difficult to process in the field, each sample was placed into a tub and rinsed to remove any sediment from the sample. Then any clinging jellyfish or other animals attached were removed for identification. Once rinsed submerged aquatic vegetation was removed and placed in a plastic zipper bag and kept on ice. Once the grass and algae were removed from the sample (in the tub), the animals were collected, and any remaining water was filtered through a 1mm sieve. The animal portion of algae scoops/dips were preserved in the field with ethanol. Once in the lab the zipper bags were frozen and the animal portion of the samples were stained with rose bengal, and processed through a sieve series (4mm, 2mm, and 1mm). As with the plankton tows species were

identified to their lowest taxonomic level and any gelatinous zooplankton were separated, and any hydrozoans were molecularly identified.

In 2017, the sampling protocol was modified using long-handled nets with 3mm mesh. Samples included sweeps of the benthos from the boat to collect algae and *G. vertens*, if present. These sampling events were used to principally collect individuals at sites to establish which specific locations had high population numbers.

Jellyfish Attracting Devices (JADs)

For collection of adults and polyps, we employed Jellyfish Attracting Devices (JADs) in regions of identified abundance. JADs are artificial seagrass mats attached to vexar mesh and then the units were cable-tied onto a brick to anchor them in place, with a marking buoy attached to allow for relocation in the field. The artificial blades (i.e., polypropylene ribbon) mimic seagrass blades and serve as structure for adults to congregate, but they can also act as recruitment substrate for settling larvae developing into polyps or for existing polyps in the field to climb and utilize. JADs were deployed at all sites in both 2016 and 2017. JADs were sampled regularly in association with site visits (see Table 1, 2).

Laboratory molecular confirmation of *G. vertens* from collected samples

We utilized standard molecular identification techniques, which we have verified in our laboratory (see Gaynor et al. 2016, 2017), for the identification of *Gonionemus vertens* in NJ waters. The first reported *G. vertens* was collected in the Manasquan River estuary with subsequent initial samples (n=5) collected from the Shrewsbury River estuary. Individuals were returned to Montclair State University where tentacles were dissected from these five samples. Tentacles were excised from each individual (4 from each sample) and DNA was extracted using a CTAB/NaCl protocol (Winnepennickx et al., 1993) with the following modifications. All extractions were carried out in 500 µL volumes in 1.7 mL microcentrifuge tubes and homogenized by grinding for 30s with a micropestle (Eppendorf). Homogenized samples were incubated @60ºC for 60 min, and RNA was digested by incubation with Rnase A (Sigma-Aldrich; 10 µg for 30 min @37C) prior to precipitation. DNA was precipitated with 2/3 volume of isopropanol, pelleted in a microfuge (16,100 x g for 10 min @4ºC), washed twice with ice-cold 70% (v/v) ethanol, briefly dried in a Speed-Vac, and resuspended in 20 µL of TE (10 mM Tris, 0.1 mM EDTA, pH 8). DNA concentrations and $OD_{260/280}$ ratios were determined in a NanoDrop ND-1000.

PCR amplifications were performed using ChoiceTaq Master Mix (Denville Scientific, Denville, NJ) according to manufacturer's directions with the exception that we used 20 µl reaction volumes. Primers used for amplification of the 16S rDNA and COI loci are listed in Appendix 1. PCR was carried out in a ProFlex Thermal Cycler (Applied Biosystems, Inc.) according to the following parameters: 95ºC for 1 min (1X); 95ºC for 20 s, 55 ºC for 20 s, and 72ºC for 30 s (30X); 72ºC for 7 min (1X); followed by a hold @4C. Positive and negative template controls were always run and typically 10 µL of each sample was run on a 1% (w/v) agarose gel to assess purity and yield of PCR product. PCR products were subject to direct Sanger dideoxy sequencing on an ABI3130 Genetic Analyzer. Sequencing reactions were carried out using BigDye Terminator Ready Reaction Mix V3.1 according to manufacturer's instructions except that we used 1/16

diluted reactions. Excess unincorporated fluorescent dideoxy nucleotides were removed by Performa Gel DTR Gel Filtration Cartridges (EdgeBio, Gaithersburg, MD). Both strands of each product were sequenced using the same primers used to generate the fragment. Raw sequences were edited and aligned using 4 Peaks (http://nucleobytes.com/4peaks/index.html) and CLUSTAL Omega (Sievers et al. 2011; http://www.ebi.ac.uk/Tools/msa/clustalo/), and searched for homology against all known genetic sequences using the BLAST algorithm (Altschul et al. 1990). DNA sequence data generated is permanently archived in GenBank (http://www.ncbi.nlm.nih.gov). Our initial sequencing data provided a match for *G. vertens* (see Fig. 2).

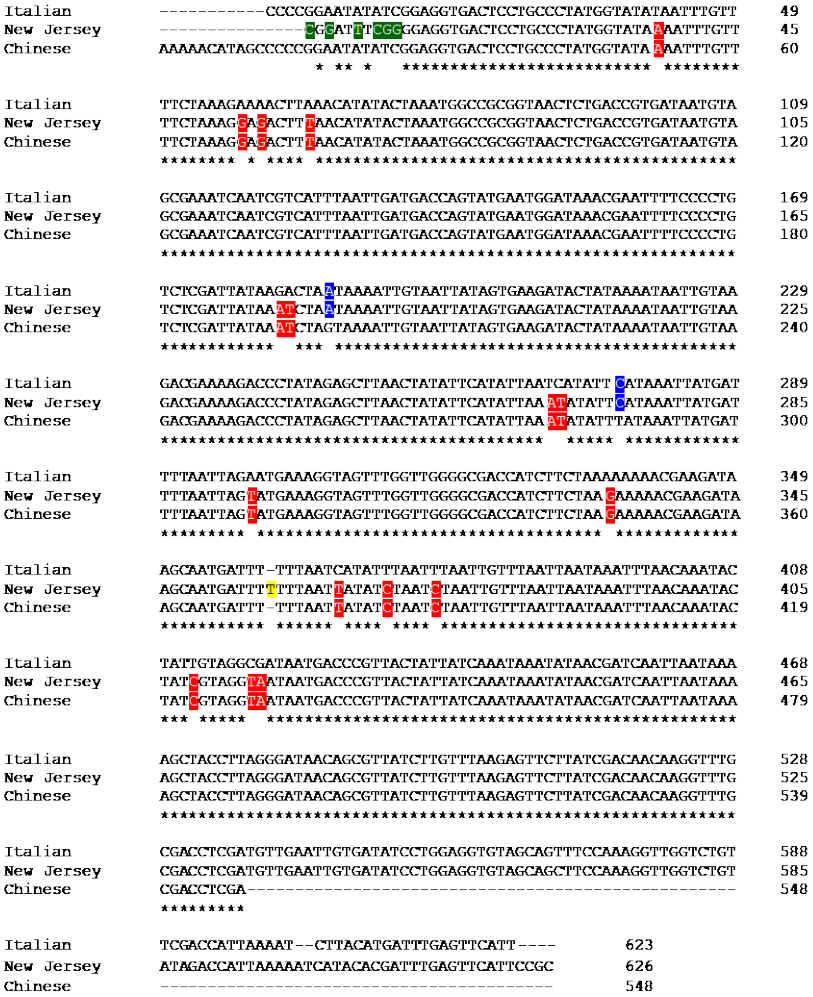

Figure 2. CLUSTAL Omega Alignment of *Gonionemus vertens* 16S rDNA Sequences. Asterisks show identity of all three aligned sequences. Red identifies unique homology between New Jersey and Chinese samples; blue identifies unique homology between New Jersey and Italian samples; green identifies bases unique only to New Jersey samples; and yellow demonstrates the point of insertion of one additional T in a run of 6 T's found in the other two samples. (GenBank Accession numbers for 16S loci are KF962471 (Chinese), EU293976 (Italian), and KX656923 (New Jersey).

Dock Swabs

The last method used was rock and dock swabbing. In this technique rocks near or along the shoreline and docks in areas where *G. vertens* had been reported, as well as in nearby areas were sampled. Sampling included using a sterile cotton swab and rubbing it along the surface of the structure; in total, an excess of 50 swabs were taken and GPS coordinates were recorded for each. Once a swab was taken in the field, it was stored in a sterile plastic tube and kept on ice until it was transported to the lab where it was stored at -80ºC until processing. The processing of these samples included boiling the swab in Chelex to extract the DNA. PCR was then performed using 16S universal cnidarian primers. Subsequent sequencing of this DNA was performed and did not reveal any *G. vertens* DNA. The most abundant animals detected by these swabs were bryozoans.

Nudibranch Collections and Molecular Diet Identification

In 2016 and 2017, nudibranchs identified from JADs deployed within the sampled waterbodies were evaluated for the presence of cnidarian DNA within them. During collection events, JADs were removed, placed in ambient sea water, and transported live to Montclair State University. Upon return, samples were processed live to identify adult and polyp stages of *G. vertens* (see above). Additionally, JADs were evaluated for the presence of nudibranchs which could be potential predators of *G. vertens* and other cnidarians.

Isolated nudibranchs were rinsed in triplicate with artificial seawater to remove any surficial trace DNA. Then, aeolid nudibranchs were placed into a sterile 1.5ml microfuge tube, covered with molecular grade EtOH, and stored at -80ºC until DNA extraction. DNA was extracted from the nudibranchs in a single tube following a CTAB/NaCl method described by Winnepenninckx, Backeljau, & Dewatcher, (1993) with modifications described by Gaynor, Bologna, Restaino, & Barry (2016). Once precipitated, DNA concentrations and $OD_{260/280}$ were measured on a NanoDrop spectrophotometer (ND-1000). DNA was amplified using modified primers targeting 16S rDNA (Restaino, 2013) which were developed as Universal Cnidarian primers by Bridge, Cunningham, DeSalle, & Buss (1995, Table 3). It should be noted that these primers do not amplify all cnidarian species and are capable of cross amplifying other invertebrate species. Additionally, *C. quinquecirrha* primers developed by Bayha & Graham (2009) and modified by Restaino (2013) were also used on the extracted samples (Table 3). PCR reactions (20 µl total volume) were run using Denville Choice Taq Master Mix (2X; Denville Scientific, Denville, New Jersey, USA) according to the manufacturer's protocol. PCR amplification was performed on an ABI Veriti 96 well thermocycler using the following parameters: 94ºC (1X for 3 minutes), 30X: 94ºC (20 seconds), 58ºC (20 seconds), 72ºC (20 seconds), 72ºC (10 minutes), and held at 4ºC. PCR amplification was checked by running samples on a 1% agarose gel. Successfully amplified samples were then sequenced (Sanger Dideoxy) using an ABI 3130 genetic analyzer, via the Big Dye Terminator Ready Reaction Mix V3.1 on diluted (1/16) reactions following the manufacturers protocols. Raw sequences were edited and aligned using 4 Peaks (http://nucleobytes.com/4peaks/index.html) and CLUSTAL Omega (Sievers et al. 2011; http://www.ebi.ac.uk/Tools/msa/clustalo/), and searched for homology against all known genetic sequences using the BLAST algorithm (Altschul, Gish, Miller, Meyers, & Lipman, 1990).

Table 3. PCR Primer Sequences used to identify taxa from pooled nudibranch samples. Reverse sequences for both the Universal Cnidarian (UC) and *Chrysaora quinquecirrha* (CQ) were modified and optimized by Restaino (2013).

Primer Name	Primer Sequence	Reference Source
UCF	5' –TGTCACCTAATTAGTGAATGGT – 3'	Bridge et al., 1995
UCR1	5'–RCGGAATGAACTCAAATCRTGTAWG – 3'	Restaino, 2013
CQF	5' – TCGACTGTTTACCAAAAACATAGC – 3'	Bayha & Graham, 2009
CQR1	5 – GCCCCAACCAAACTGTCTTA – 3'	Restaino, 2013

Unknown Jellyfish Medusae

In 2016 and 2017, numerous small jellyfish were collected during plankton tows. Because of their diminutive size (1-15 mm), they were isolated and evaluated for identification using molecular techniques. Isolated medusae were placed into sterile 1.7 ml microcentrifuge tubes with 100 ml of sterile 5% (w/v) Chelex® 100 mesh size 150-300 µM (Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547) in 50 mM Tris base (pH 11). DNA extraction was performed in accordance with the methods of Walsh, Metzger, & Higuchi (1991), modified for optimization. Samples were boiled (100ºC) in a water bath for 10 minutes, vortexed for 30 seconds, and then cooled on ice for 2 minutes. Samples were then vortexed again for 30 seconds. After the second round of vortexing, samples were centrifuged at 14,000 x g for 10 minutes and the supernatant removed and stored at -20ºC. PCR was carried out in 20 µl reactions using ChoiceTaq Master Mix (2X) (Denville Scientific, Denville, NJ, USA). Universal Cnidarian primers designed for 16S rDNA (originally described by Bridge et al. (1995) and modified by Restaino (2013); see Table 3) were used to characterize each unidentified sample. PCR was performed in a Veriti^{TM} 96 Well thermal cycler (Applied Biosystems Inc.) according to the parameters described in Gaynor et al. (2016). In addition to experimental samples, both positive and negative (no template) controls were also run during each PCR trial. Following PCR, 10 µl of PCR products were run on 1% (w/v) agarose gels to confirm reaction success and verify the size of the amplicon produced. Sanger-dideoxy sequencing was then performed in-house on successful reactions. Sequencing samples were produced for both the forward and reverse strands, using the same primers that produced the PCR amplicons. Sequencing was completed on an ABI 3130 Genetic Analyzer using BigDye Terminator Ready Reaction Mix V3.1 following the manufacturer's protocol. Forward and reverse sequence data were aligned and edited using 4Peaks (http://nucleobytes.com/4peaks/index.html). Edited sequences were searched against known sequences using BLAST (Altschul et al. 1990).

Regional Population and Phylogenetic Analysis of *G. vertens*

Collection & DNA Extraction

Whole individuals of *G. vertens* were collected from Mumford Cove, CT, Groton Long Point (Venetian Cove), CT, Farm Pond (Martha's Vineyard), MA, and Monmouth Beach (Shrewsbury River), NJ using dip-nets and seins. Once organisms were collected individual tentacles were removed using sterile scissors/scalpels/forceps and stored in

ethanol. Tentacles were rinsed individually in artificial seawater to remove any organisms or debris that may have possibly been attached. Tentacles were then placed in sterile 1.5ml tubes with 75-100ul of Chelex® 100 mesh particle size 150-300 µM (Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547) in 50 mM Tris base (pH 11). DNA extraction was performed in accordance with the methods of Walsh et al. (1991), modified for optimization. Tentacles in Chelex® were heated at 100ºC in a water bath for 10-15 minutes. Following heating tubes were vortexed for 30 seconds and then spun at 14,000 x g for 2 minutes. Afterwards, the supernatant was removed and placed in a sterile 1.5ml tube. Samples were frozen at -20ºC until PCR was performed.

Additionally, 30 individuals were collected in Berre Lagoon, France, by Guillaume Marchessaux (Marchessaux et al. 2017). Tentacles were removed in France following the above protocol and mailed individually in 1.5ml tubes with ethanol. DNA extraction of these samples was processed in accordance with the samples collected in the US.

PCR

Gonionemus vertens DNA was amplified at mitochondrial loci: 16S rDNA and COI using the following primers: Reactions containing DNA from individual tentacles were prepared for PCR (20µl reactions) using the following: 1µl Forward Primer, 1µl Reverse Primer, 10µl ChoiceTaq Master Mix (2X) (Denville Scientific, Denville, New Jersey, USA), 6.5µl sterile DIH_2O, and 1.5µl of DNA. Individual PCR reactions were performed on samples for each locus using the following primers: Universal Cnidarian primers designed for 16S rDNA (Table 3) and Metazoan COI primer designed by Folmer et al. (1994) Forward 5'-GGTCAACAAATCATAAAGATATTGG-3', Reverse 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. PCR was executed in an ABI Veriti thermal cycler using the following parameters: 94ºC for 1 minutes, 35 cycles of: 94ºC for 20 seconds, 55ºC for 20 seconds, and 72ºC for 20 seconds, 8 minutes at 72ºC, and held at 4ºC until samples were removed. PCR reactions were checked for successful amplification using agarose gel (1%) electrophoresis.

DNA Sequencing & Analysis

Samples that were successfully amplified through PCR were submitted for DNA sequencing on an ABI 3130 genetic analyzer, using BigDye Terminator Ready Reaction Mix V3.1 following the manufacturer's protocol. Sequences were edited using 4Peaks (http://nucleobytes.com/4peaks/index.html) and then run against sequences in GenBank using BLAST (Altschul et al. 1990) to check for homology against known sequences of *G. vertens* and *Gonionemus sp.* Sequences for each population were aligned using CLUSTAL Omega (Sievers et al., 2011; http://www.ebi.ac.uk/Tools/msa/clustalo/) to determine any unique SNPs that were present and to ensure that correctness of sequence data for that population.

Phylogenetic Tree Assembly

Neighbor-joining trees were constructed independently for each locus using BLAST.

Phase 2 Research Agenda, 2018-2020

Introduction

Results from the initial investigations of 2016 – 2017 demonstrated repeated large blooms in the Shrewsbury River, but sampling in the northern part of Barnegat Bay and the Manasquan River where the first identified individual was recovered (Gaynor et al. 2016) did not reveal their presence. Additionally, temporal sampling revealed that the clinging jellyfish seemed to decline in June and were not collected in sampling efforts conducted in the end of July. This gave rise to additional research questions as to why *G. vertens* declined during this time period, whereas medusae populations in other regional states were present throughout the summer (e.g., RI, CT, MA). In 2018, after being relatively absent from Barnegat Bay, numerous sightings of *G. vertens* were reported in this region. Thus, a renewed effort to identify regions of the bay where *G. vertens* was present intensified. Additionally, very little information was available regarding their life history, habitat requirements, and influence on trophic webs in many invaded coastal habitats. As such, several new research objectives were developed to help understand the ecology of *G. vertens* in New Jersey and these include:

1. **Identifying the potential causes of the *G. vertens* bloom decline**
    a. **Thermal Tolerance**
    b. **Top-Down Control**
2. **Food Web Assessment through Stable Isotopes**
3. **Assessing in-field medusa population growth rates**
4. **Assess polyp asexual growth rates**

Methods

Objective 1: Potential Causes of Bloom Declines: a. Regional Thermal Tolerance Experiments

Gonionemus vertens Collections

G. vertens were collected during the months of June-July of 2018, May-July 2019, and May-June 2020. Collection sites (Fig. 3) in both years included several areas of Barnegat Bay, NJ, including the mouth of the Metedeconk River (40.05605ºN, -74.06517ºW) and Tices Shoal (39.8118ºN, -74.1018ºW). In 2019, *G. vertens* were observed and collected for the first time in a pond at North Wildwood, Cape May (39.010426ºN, -74.819770ºW) and again in 2020. In both 2018 and 2019, *G. vertens* were collected from Potter Pond, Rhode Island (41.388195ºN, -71.532684ºW), and in 2019 *G. vertens* were collected from Mumford Cove, Connecticut (41.322533ºN, -72.018764ºW).

Collections were performed both over the side of a boat with a dip net and in the water with seine nets and dip nets. Nets were dragged through areas of 1-2 m depth that had algae and/or seagrass beds and brought out of the water to separate *G. vertens* from the plant material using a pipette to transfer them to a sample jar. When returned to the laboratory, the number of *G. vertens* were counted and each individual's bell diameter was measured (mm) and recorded before being placed in a holding tank. Tanks were equipped

with a filtrating air pump and kept at ambient room temperature (~21-22ºC). Aquarium plants and/or algae were placed in each tank to provide a substrate to which *G. vertens* could cling. Newly hatched *Artemia* were added daily to each tank Monday-Friday (~6mL per 10-gallon (37L) aquaria, and adult *Artemia* were provided every two weeks between June and August 2019 as a nutritional boost.

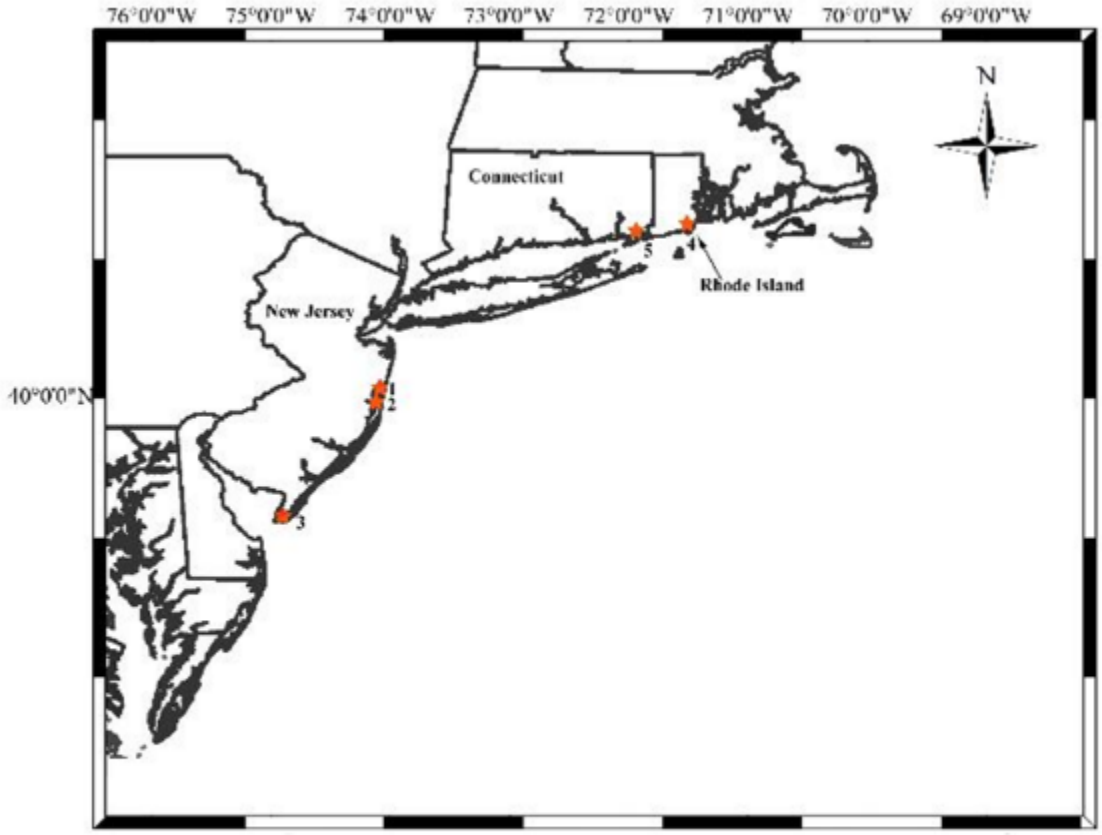

Figure 3. Map of 2019 *G. vertens* collection sites. 1= Metedeconk River, NJ; 2= Tices Shoal, NJ; 3=North Wildwood, NJ; 4=Potter Pond, RI; 5=Mumford Cove, CT.

Thermal Tolerance Experimental Design

Temperature-controlled tanks were established in the laboratory with the purpose of holding adult *G. vertens* at constant temperatures for 96 hours. Eleven liter-sized tanks were fitted with a divider to keep medusa separate from an aeration stone and a heater. The divider had six small mesh windows to allow circulation of water, and a mesh skirt which prevented medusa from being pulled under the divider. A modified jellyfish attracting device (JAD) was constructed from lengths of green ribbon tied through holes drilled through a flat piece of PVC. Alternatively, plastic aquarium plants were also used in several tanks. The purpose of the JAD or aquarium plant was to simulate the natural coastal environment in which *G. vertens* is often found and provide medusae with a substrate to cling to, as they are often found in the field clinging to seagrass or algae.

Experiments were monitored daily (24h, 48h, 72h, and 96h) to ascertain survival and health of *G. vertens*. Visual observations of the medusae's relative health were made each day, taking into account clinging behavior, bell diameter changes, and partial or complete disintegration. The salinity of the water in each tank was monitored with a refractometer throughout the trials and maintained between 20-25 ppt. Tanks were outfitted with an aquarium heating unit (either PENN-PLRX Cascade Heat® Model CH850 or EHEIM JAGER® Aquarium Heater) and a thermometer, and monitored daily to avoid temperature fluctuations. Three healthy medusae were measured (bell diameter) before being placed into each tank, and the bell diameters of surviving medusae were measured after the 96-hour experimental trial. Medusae in each tank were heavily fed before running the trials, and were also fed at the same time each day with ~1 mL freshly hatched *Artemia* solution.

2018 Stable Temperature Experiments

In the summer of 2018, temperature trials were run at 22ºC, 24ºC, 26ºC, and 28ºC with five runs of each and three *G. vertens* medusae per trial. Four of the trials used *G. vertens* from Potter Pond, RI, while one trial was conducted with *G. vertens* from Barnegat Bay. Percent survival was calculated for each temperature at each time point, both separated by location and compiled as a whole. Sample size in 2018 was limited by several factors, including the late initiation of experiments (7/31-8/20/18), at which point many *G. vertens* had already been used for feeding and predation experiments and were no longer available as novel individuals. In addition, it has been observed that *G. vertens* senescence typically occurs after 2-3 months, which certainly limited the number of trials that were conducted during this time period.

2019 Stable Temperature Experiments

The same experimental set-up and protocol was observed in 2019, although experiments were initiated as soon as adult *G. vertens* were encountered in large numbers from field collections, and the number of temperature-controlled tanks was expanded to five sets by June 24, 2019. Temperatures tested were also expanded to 22ºC, 24ºC, 26ºC, 28ºC, 30ºC, and 32ºC. The 22ºC tanks were meant as a constant control, but was only run for six trials because it was only 1-2ºC above ambient and the medusae were not under any sort of thermal stress. Instead, the tanks' temperatures were increased to 24ºC, 26ºC, 28ºC, and 30ºC, the latter of which was included because medusae from North Wildwood in Cape May County seemed to survive longer at 28ºC, especially when the medusae were younger. Trials were also run at 32ºC with Cape May (6 trials), Connecticut (1 trial), Metedeconk (2 trials) and Rhode Island medusae (2 trials) to verify upper temperature tolerance limits and to assess potential latitudinal variations to thermal stress.

A total of 21 trials was run at 24ºC (Cape May=7, CT=4, Metedeconk=5, RI=5), 26ºC (Cape May=7, CT=4, Metedeconk=5, RI=5), and 30ºC (Cape May=6, CT=4, Metedeconk=2, RI=9), while 25 trials were conducted at 28ºC (Cape May=9, CT=4, Metedeconk=5, RI=7). Three medusae were included in each 96-hour trial until the week of 8/12/19, when numbers were only sufficient to include two medusae from Connecticut in one set of trials at 24ºC, 26ºC, 28ºC, and two medusae from Rhode Island in another set of trials at 24ºC, 26ºC, 28ºC, and 30ºC. At the end of the 96-hour trial each medusa was categorized as alive or dead and the percent survival for each trial was calculated at all time

points. Data were both separated by location and compiled as a whole in order to analyze potential latitudinal differences.

In addition to the regular daily feeding of medusae with newly hatched *Artemia* solution, holding tanks were fed every two weeks with adult *Artemia* in an effort to improve medusa nutrition and health before participating in these metabolically stressful trials. Verification of dissolved oxygen content was performed with a Sper Scientific® DO Pen beginning in the trials the week of 8/5/19 and continued through the trials of 8/12/19, and verification of temperature occurred via the use of submerged Onset® temperature recorders in twelve of the trials (28ºC=3 trials, 30ºC=7 trials, 32ºC=2 trials).

2019 Oscillating Temperature Experiments

In order to take into account the decrease in temperature that would occur at nighttime in coastal waters, trials began August 5, 2019 that used a 12-hour timer. These trials plateaued at 28ºC and 30ºC in order to determine whether cooler nighttime temperature could possibly have an alleviating effect on *G. vertens* medusae. If they were to experience less physiological stress, the hypothesis was that they would survive longer than medusae held at constant temperatures above their tolerance threshold. The tank systems and three *G. vertens* medusae per trial were measured in the same way as the stable temperature experiments, except that the heaters were set to either 28ºC or 30ºC and plugged into the 12-hour timer. Daily observations of health and survivorship were made, and any surviving *G. vertens* were measured after the trial.

In total, four trials were run at 28ºC with medusae from both Connecticut (CT) and Rhode Island (RI), and four trials were run at 30ºC with medusae from both CT and RI. However, two of the RI trials set to 28ºC and two of the trials set to 30ºC daytime temperatures were run with only two medusae due to limited stock remaining in the holding tanks.

Verification of dissolved oxygen content was performed with a Sper Scientific® DO Pen, and verification of temperature occurred via the use of submerged Onset® temperature recorders in eight of the trials (28ºC=4 trials, 30ºC=4 trials).

2020 Stable Temperature Experiments

Based on the initial 2019 thermal experiments, the North Wildwood population identified in 2019 showed a higher temperature tolerance and 2020 experiments focused on identifying the thermal maximum for this southern population. The same experimental set-up and protocol was used as 2018 and 2019, although experiments were initiated as soon as adult *G. vertens* were collected from North Wildwood. Four temperatures were used to assess the survival during 96h experiments (26ºC, 28ºC, 30ºC, and 32ºC). Four experimental trials were initiated on 6/8/20, 6/17/20, 6/23/20, and 6/29/20. Three adult *G. vertens* were introduced into each tank at ambient temperatures (~22ºC) and then tank heaters were turned on. Survival was assessed at 1, 24, 48, 72, and 96 hours after initiation. Results from these data were then incorporated into our thermal tolerance analyses from previous years.

Field Temperature Assessments

During field surveys in New Jersey, water quality measurement were collected using a YSI® multimeter to assess temperature, salinity, and dissolved oxygen. Additionally, Onset® temperature recorders were placed in the field at several locations to assess seasonal temperature profiles. Specifically, probes were dropped in Tices Shoal, NJ (39.8229ºN, -74.1022ºW) on 6/12/19 and recovered on 7/2/19 and in Potter Pond, RI (41.388195ºN, -71.532684ºW) on 7/18/19 and recovered on 10/14/19. Upon recovery of Onset® temperature recorders from the field, their data were plotted to show the changes in daily temperatures over the time periods for which they were deployed.

Objective 1. Potential causes of the *G. vertens* bloom decline: b. Top-Down Control

Predation Experiments with *Chrysaora chesapeakei*

Feeding experiments were conducted with *G. vertens* (GV) and its known predator, *Chrysaora chesapeakei* (CC), with the goal of determining the minimum bell ratio at which *C. chesapeakei* will be able to kill *G. vertens* prey; as well as the ratio of bell diameters at which *C. chesapeakei* is able to bring the *G. vertens* into its gut and successfully consume this prey. 3.8-liter circular tanks were set up at ambient temperature (~21-22ºC) and 20-25 ppt salinity, to which similar size *C. chesapeakei* and *G. vertens* were added and observed for 48 hours. Prior to initiation of the trial, the bell diameters of both *G. vertens* and *C. chesapeakei* were measured, and a ratio calculated. *G. vertens* health was categorized as alive or dead at 1 hour, 24 hours, and 48 hours, with additional qualifying observations made to distinguish if the *G. vertens* had been caught at that time, but was still alive, or if the *G. vertens* had been killed and was partially or fully digested. Data were graphed with the mean bell ratios vs. the status of *G. vertens* (Alive, Dead but not consumed, Dead and partially or totally consumed) to determine the bell ratio at which *C. chesapeakei* will kill *G. vertens.*

Reciprocal Predation Experiments

To assess the potential trophic interaction between GV and CC based on size interactions, in 2017 adult *G. vertens* medusae were paired with either *C. chesapeakei* ephyrae (N= 15) or larger juveniles (N = 10) and monitored for 24 hours, and survival and consumption were recorded. *G. vertens* and CC ephyrae experimental trials were conducted using 100 ml chambers, while interactions with juvenile *C. chesapeakei* occurred in 3.8 L circular tanks. Additionally, a second series of experimental trials was conducted to ascertain the minimum size or size ratio of CC feeding on GV. Prior to these experiments between *G. vertens* and juvenile *C. chesapeakei*, bell diameter of both species were recorded to evaluate a minimum size or bell diameter ratio where juvenile *C. chesapeakei* were successful in predation attempts. Experiments used *C. chesapeakei* with bell diameters ranging from 6-22mm and *G. vertens* with bell diameters of 4-15.5mm.

Objective 2. Food Web Assessment through observations and Stable Isotope Analysis

Field Collected Observations and Laboratory Prey Choice Experiments

During field collections, observational data were collected to document various food items observed in the gastro-vascular pouch of *G. vertens* or actively caught within

the tentacle and feeding apparatus. Based on these observations, laboratory feeding trials were conducted. Experiments consisted of a single *G. vertens* being isolated in a 100 ml chamber and then providing them a single or multiple prey item(s). Prey items included 1-3 Amphipods or 1 Isopod (*Idotea balthica*). Both of these taxa had been observed in the gastro-vascular pouch of field collected individuals. Experiments were monitored at 1h and again at 24h by assessing the presence and consumption of prey. 120 experimental trials using amphipods were conducted, while 25 experimental trials were conducted with *I. balthica*. Data were recorded as prey consumed, prey dead in chamber, or prey alive to assess preference and feeding potential.

Stable Isotope Analysis

Upon returning to the lab with *G. vertens* fresh from the field, several were isolated after being measured, but before being placed in the holding tanks, for stable isotope analysis. Each individual was placed in an Eppendorf microcentrifuge tube and labeled to allow for future identification. Samples of algae, seagrass, amphipods, copepods, isopods, shrimp, crabs, and larval stages of fish were also isolated and labeled. Specifically, the following taxa were identified and analyzed: *Ulva lactuca* (sea lettuce), *Zostera marina* (eelgrass), Amphipods (Aoridae, Caprellidae, Melitidae), Isopods (*Erichsonella* sp., *Idotea balthica*), Calanoida Copepods, Harpacticoid Copepods, Ostracoda, Decapods (*Callinectes sapidus*, *Palaemonetes* sp., *Carcinus maenas*, Xanthidae), Polychaeta, and fish (*Apeltes quadracus, Brevoortia tyrannus, Fundulus heteroclitus, Menidia menidia*). Samples were then frozen at -20ºC prior to analysis and sent to the Cornell Isotope Laboratory (COIL), where samples were processed and analyzed. Data reported included the sample weight, N_2 and CO_2 amplitude, %N and %C, $\delta^{15}N$ vs. Atmospheric Air, and $\delta^{13}C$ vs. Vienna Pee Dee Belemnite (VPDB).

These data were processed to exclude samples that were below detectable limits and sorted by organismal group (*G. vertens,* amphipod, copepods, fish, isopod, shrimp, ostracod, plant, and crab). Data were separated in this way because $\delta^{15}N$ vs. Atmospheric Air and $\delta^{13}C$ vs. VPDB values are indicative of trophic level and can be used to determine possible predator-prey relationships by identifying contributions to a consumer's diet. Finally, these sorted data were plotted with $\delta^{15}N$ vs. $\delta^{13}C$ levels, in order to better understand the trophic position of *G. vertens* and the several other groups of organisms sampled.

Objective 3. Assessing in-field medusa population growth rates

During 2019, one location in Barnegat Bay (Metedeconk River, 40.05124ºN 74.04793ºW) maintained high populations of *G. vertens* throughout the summer. To assess population demographics and calculate adult *G. vertens* growth rates, samples were collected on 5/16/2019, 5/22/19, 6/4/2019, 6/19/2019, 6/26/2019, 7/2/2019, 7/10/2019, and 7/16/2019. Samples were collected using dip nets with medusa isolated and returned to MSU where all individuals were measured for bell diameter to the nearest mm. 1455 medusa were collected in total, with individual samples ranging from 25 (7/16/19) to 441 (6/19/19) individuals.

Objective 4. Assessment of polyp asexual growth rates

Substantial numbers of adult *G. vertens* were collected in 2019 and allowed to reproduce in a 150 L aquaria. *G. vertens* were fed newly hatched *Artemia* twice a week. Glass plates were placed in the aquaria to allow the settlement and collection of metamorphosed larvae. In January of 2020, plates were removed from the 150 L aquaria and inspected for the presence of polyp stages of *G. vertens*. Plates were isolated and monitored regularly from January to July of 2020 and assessed for the number of polyps and polyp stages observed on the glass plates (Fig. 4).

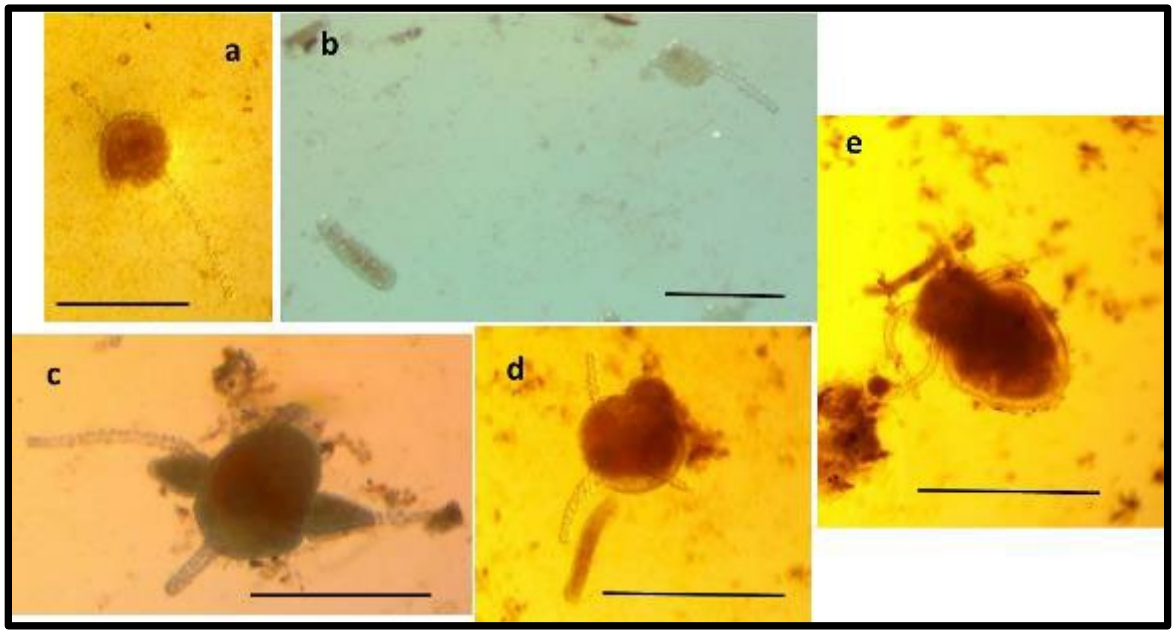

Figure 4. Images of polyps and development stages. **A.** developing polyp demonstrating only 2 tentacles emerging; **b.** developing polyp in upper right with a frustule appearing in the bottom left; **c.** mature polyp with two developing frustule buds; **d.** mature polyp with a developing frustule bud and a recently detached fully formed frustule; and **e.** mature polyp generating a medusa. Black line represents a scale bar of 0.5mm for each image.

Project Results

Water Quality

During all sampling periods water temperature, salinity, and dissolved oxygen data were collected. All parameters were collected except for one site during 2016 when the meter failed, and on one date in 2017 where the dissolved oxygen calibration could not be achieved. In general, for both years water quality followed typical summer patterns with relatively warm temperatures, estuarine salinities, and varying dissolved oxygen concentrations ranging from hypoxic to super-saturated. In 2016 (Table 4), salinity ranged from 10-28 ppt from the Manasquan and northern Barnegat Bay, with similar values measured from the Shrewsbury (Table 5). Temperature was also similarly high due to seasonality, 23-27ºC. Oxygen concentration did vary among sites, with low dissolved oxygen being reported at Raccoon Island and Oceanport Creek (Table 5), and supersaturation being recorded at the Golf Course site in the Manasquan River (Table 4) and Manhasset Creek and Rumson County Club in the Shrewsbury (Table 5). Both hypoxia and super-saturation are representative of excessive nutrients, which contributes to excessive algal growth; this consequently, was observed at these sites. For 2017, similar results were observed (Tables 6 and 7).

Table 4. Water Quality Data collected from sites in the Manasquan River and Northern Barnegat Bay from 2016. * YSI Water quality meter failure.

Salinity (ppt)	**6/30/2016**	**7/20/2016**
Ablemare	22.3	23.3
Beverly	10.4	20.1
Treasure Island East	25.9	27.5
Treasure Island West	24.1	25.7
Golf course	24.1	26.4
Gull Island Railroad Canal	27.7	27.8
Bay Head	24.7	24.9
East Drive	*	23.2
Carrol Fox Road	23	24.4
Herring Island	23	25.7
Temperature (°C)	**6/30/2016**	**7/20/2016**
Ablemare	25.1	25.6
Beverly	26.1	26.6
Treasure Island East	23.4	24.8
Treasure Island West	27.2	25.2
Golf course	26.4	26.2
Gull Island Railroad Canal	22.2	24.6
Bay Head	25.7	26
East Drive	*	27.1
Carrol Fox Road	28.1	27.3
Herring Island	25	26.6
Dissolved Oxygen (mg/l)	**6/30/2016**	**7/20/2016**
Ablemare	5.35	4.94
Beverly	6.44	7.9
Treasure Island East	6.44	7.62

Treasure Island West	6.26	5.6
Golf course	10.24	12.71
Gull Island Railroad Canal	6.25	8.71
Bay Head	8.14	6.19
East Drive	*	6.41
Carrol Fox Road	8.49	8.41
Herring Island	7.38	6.76

Table 5. Water Quality Data collected from sites in the Shrewsbury River from 2016. * YSI Water quality meter failure.

Salinity (ppt)	**7/6/2016**	**7/11/2016**	**8/3/2016**
Blackberry Bay	22.3		24.7
Raccoon Island	24	25.1	26.2
Hook	23.7		25.8
Wharfside Manor Marina	24.7		26.5
Oyster Bay	24.6		26.7
Plum Island		26.2	27.9
Little Silver Creek		22.5	22.3
Oceanport Creek		21.1	22.1
Trautmans Creek		23.5	24.8
Manhasset Creek		18.4	22.4
Rumson County Club		24.7	22.3
Temperature (^{o}C)	**7/6/2016**	**7/11/2016**	**8/3/2016**
Blackberry Bay	27.3		26.6
Raccoon Island	26.6	26.1	24.7
Hook	28.8		25.2
Wharfside Manor Marina	26.9		25
Oyster Bay	28.4		24.5
Plum Island		24	23.6
Little Silver Creek		25.5	25.4
Oceanport Creek		26.9	25
Trautmans Creek		28.2	26.5
Manhasset Creek		28.3	27.1
Rumson County Club		25.9	24.2
Dissolved Oxygen (mg/l)	**7/6/2016**	**7/11/2016**	**8/3/2016**
Blackberry Bay	5.35		7.17
Raccoon Island	3.75	5.8	7.71
Hook	5.9		7.3
Wharfside Manor Marina	5.9		8.23
Oyster Bay	6.01		5.17
Plum Island		8.01	7.06
Little Silver Creek		8.38	5.36
Oceanport Creek		*	4.5
Trautmans Creek		8.51	6.63
Manhasset Creek		13.76	12.32
Rumson County Club		12.23	5.71

Table 6. Water Quality Data collected from sites in the Manasquan River and Northern Barnegat Bay from 2017.

Temperature (°C)	**5/31/2017**	**6/15/2017**	**8/3/2017**
Herring Island	18	24.6	28
Osborn Island	16.6	22.2	26
Manasquan Canal	16.6	22.4	24
Gull Island Railroad Canal	16.3	20.7	23.8
Riverside Park	16.5	22.1	25.1
Bayhead	17.6	23.5	25.5
Carrol Fox Rd	18.4	25.4	28.6
East Drive	18.3	25.6	28.6
Salinity (ppt)	**5/31/2017**	**6/15/2017**	**8/3/2017**
Herring Island	17.9	21.5	23
Osborn Island	10.8	23.3	22.8
Manasquan Canal	22.5	23.8	26.3
Gull Island Railroad Canal	23.1	26.4	27.1
Riverside Park	21	23.5	23.8
Bayhead	17.5	21.5	24
Carrol Fox Rd	16.7	20	21.8
East Drive	11	18.9	23.7
Dissolved Oxygen (mg/l)	**5/31/2017**	**6/15/2017**	**8/3/2017**
Herring Island	9.71	9.25	8.41
Osborn Island	8.9	7.85	5.6
Manasquan Canal	8.33	8.66	4.26
Gull Island Railroad Canal	8.16	8.26	5.74
Riverside Park	8.25	7.52	5.61
Bayhead	9.88	8.66	6.67
Carrol Fox Rd	9.38	8.74	6.82
East Drive	11.26	8.62	7.5

Table 7. Water Quality Data collected from sites in the Shrewsbury River from 2017. *Dissolved Oxygen readings could not be stabilized or calibrated.

Temperature (°C)	**6/2/17**	**6/14/17**	**6/28/17**	**7/18/17**	**8/1/17**
Blackberry Bay	20.7	26.1	24.9	28.7	24.8
Oyster Bay	21.2	27.3	25	28.5	25.2
Raccoon Island	22	26.5	24.4	28.6	26.5
Hook	21.7	25.8	24.1	29.2	26.4
Manhasset Creek	21.5	26.5	24.5	28.2	25.4
Bartley Point Island	21.7	24.3	23.4	27.1	24.3
Sedge Island	19.4	25.1	23.1	26.8	24.1
Wharfside Manor Marina	21.7	26.4	24.1	28.1	26.2
Salinity (ppt)	**6/2/17**	**6/14/17**	**6/28/17**	**7/18/17**	**8/1/17**
Blackberry Bay	18.7	19.5	21.6	22.4	23.1
Oyster Bay	18.8	18.9	20.8	21.9	23.1
Raccoon Island	20.4	20.2	22.5	23.5	24

Hook	20.2	19.9	22	23.2	23.6
Manhasset Creek	17.1	16.2	18.5	21	20.8
Bartley Point Island	20.2	19.9	22.4	23.6	23.6
Sedge Island	20.4	20.1	22.5	23.1	23.9
Wharfside Manor Marina	20.7	20.2	22.9	23.6	23.9
Dissolved Oxygen (mg/l)	**6/2/17**	**6/14/17**	**6/28/17**	**7/18/17**	**8/1/17**
Blackberry Bay	*	6.03	9.33	6.43	9.57
Oyster Bay	*	5.73	8.1	6.58	6.52
Raccoon Island	*	7.48	9.45	8.77	12.41
Hook	*	7.5	9.39	8.34	11.74
Manhasset Creek	*	3.53	9.99	6.08	10.31
Bartley Point Island	*	7.04	9.25	7.42	7.71
Sedge Island	*	7.06	8.44	6.82	7.12
Wharfside Manor Marina	*	9.02	8.67	8.17	12.1

2018 and 2019 Water Quality

In 2018 and 2019, the shift in research directions away from monitoring the initial two regions to investigating the bloom in Barnegat Bay and looking at how *G. vertens* population dynamics were occurring, and what was driving blooms and decline in abundance, regular water quality sampling was limited. Several sites in the Shrewsbury where *G. vertens* abundance was noted were monitored for their presence during these years and included the Blackberry Bay (polyp settlement on JAD), Hook, and Raccoon Island sites. Results from sampling in these years showed similar seasonal trends in temperature and salinity, along with lower dissolved oxygen occurring in July and August as previously and are presented in Table 8 (2018) and Table 9 (2019).

Table 8. Water Quality Data collected from sites in the Shrewsbury River from 2018.

Temperature (°C)	**5/24/2018**	**7/11/2018**	**8/10/2018**	**8/15/2018**
Blackberry Bay	21.4	28.2		25.7
Racoon Island	22.4	26.1	25.6	
Hook	20.8	25.9		25.4
Salinity (ppt)	**5/24/2018**	**7/11/2018**	**8/10/2018**	**8/15/2018**
Blackberry Bay	16.6	23.6		20.4
Racoon Island	18.7	24.4	21.6	
Hook	18.7	24.1		20.8
Dissolved Oxygen (mg/l)	**5/24/2018**	**7/11/2018**	**8/10/2018**	**8/15/2018**
Blackberry Bay	13.0	9.20		4.57
Racoon Island	14.72	4.80	5.91	
Hook	12.82	4.80		6.27

Table 9. Water Quality Data collected from sites in the Shrewsbury River from 2019.

Temperature (°C)	5/31/2019	8/16/2019
Blackberry Bay	22.0	26.0
Racoon Island	20.3	25.7
Hook	20.5	25.8
Salinity (ppt)	**5/31/2019**	**8/16/2019**
Blackberry Bay	16.30	21.2
Racoon Island	17.7	22.4
Hook	17.7	21.8
Dissolved Oxygen (mg/l)	**5/31/2019**	**8/16/2019**
Blackberry Bay	7.35	5.25
Racoon Island	6.30	4.77
Hook	5.65	4.96

Phase 1 Results

Plankton Tow Results 2016

Shrewsbury River

After complete morphological and molecular processing of plankton tow samples, no *G. vertens* were identified. The absence of *G. vertens* in these samples may have been the result of their ability to cling to seagrass and therefore stay on the benthos even when the water column above had been disturbed. Additionally, tidal flux, day-time sampling, predation, and/or competition for food resources may have played a role in their abundance within the system and their absence in these samples. The most abundant animals (not including gelatinous zooplankton) found in plankton tow samples were: Calanoida spp., Caridea, Brachyura, Aoridae, and fish eggs; for gelatinous zooplankton the most common animals were: *Chrysaora chesapeakei (formerly quinquecirrha)* and *Mnemiopsis leidyi*.

While no *G. vertens* were recorded during plankton sampling, significant variation in the distribution and timing of pelagic organisms was recorded. Specifically, to appropriately evaluate the spatial and temporal differences, the two sites were separated because they differed in their dates of collection. When data from the Shrewsbury were evaluated, adult *C. chesapeakei*, ephyrae of *C. chesapeakei*, *M. leidyi*, Calanoida spp., Ostracoda, fish eggs, Caridea larvae, and Brachyura larvae showed significant differences in density among sites (Table 10), and *C. chesapeakei*, *M. leidyi*, and fish eggs also showed significant differences among dates of collection. A subsequent correlation analysis was conducted and showed significant correlations among taxa with extremely high relationships among several Peracarida groups and each other, as well as floating submerged aquatic vegetation (SAV) wrack (Table 11).

Table 10. Average densities (#m^{-3}) of taxa exhibiting significant differences among Shrewsbury sites in 2016. Significance convention *= 0.05, ** = 0.01, *** = 0.0001. Sites designated as: Blackberry Bay (BB), Raccoon Island (RI), Hook, Wharfside Manor Marina (WM), Oyster Bay (OB), Plum Island (PI), Little Silver Creek (LSC), Oceanport Creek (OC), Trautmans Creek (TC), Manhasset Creek (MC), Rumson County Club (RCC).

Species	BB	RI	HOOK	WM	OB	PI	LSC	OC	TC	MC	RCC
*C. chesapeakei***	0.97	0.43	0.03	0	0	0.03	.03	0.36	0.03	0.54	0
C. chesapeakei ephyrae*	0.8	0.18	0.13	0.04	0	0	0.22	0.35	0.33	0.32	0.43
*M. leidyi**	0	0.07	0.05		0.05	0.2	0.006	0	0	0.02	0
Calanoida *	1.21	41.5	2.03	8.3	27.0	40.2	2.3	0.08	1.3	6.6	1.13
Ostracoda*	0.03	1.4	0.4	0.04	0	0	0.006	0.04	0	0	0.27
Caridea larvae***	0.03	1.3	0.03	0.24	0.2	5.2	0.13	0.25	0.14	0.12	0.03
Brachyura larvae ***	4.3	5.0	3.1	0.5	13.0	38.5	2.6	4.8	1.9	2.3	0.5
Fish Eggs ***	0.03	0.13	0.17	2.1	0.12	12.9	0	0	0	0.07	1.8

Table 11. *Peracarida* correlation analysis for plankton tows in the Shrewsbury River sites in 2016. Values in table represent the Pearsons 'r' with significance indicated by ** P < 0.01, *** P < 0.001. Taxonomic abbreviations as follows: PB = Plant Biomass, AOR =Aoridae, MEL =Mellitidae, ER = *Erichsonella* spp., IB = *Idotea balthica*, GAM = *Gammarus* spp., CAP = Caprellidae.

	AOR	MEL	ER	IB	GAM	CAP
PB	0.9***	0.6***	0.9***	0.9***	0.5***	0.7***
AOR		0.5***	0.9***	0.7***	0.4**	0.5***
MEL			0.6***	0.7***	0.6***	0.5***
ER					0.4***	0.7***
IB					0.4***	0.7***
GAM						0.5***

Barnegat Bay-Manasquan System

For plankton tow samples from the Manasquan and Barnegat Bay, similar results occurred with *C. chesapeakei*, gelatinous ephyrae of *C. chesapeakei*, *M. leidyi*, Calanoida spp., Ostracoda, fish eggs, Caridea larvae, and Brachyura larvae showing significant differences in density among sites (Table 12) and *C. chesapeakei*, Calanoida spp., Ostracoda, fish eggs, and Brachyura larvae also showing significant differences in density among dates. A subsequent correlation analysis was conducted and showed significant correlations among taxa with extremely high relationships among several Peracarida groups and each other as well as floating SAV wrack (Table 13).

Table 12. Average densities (#m^{-3}) of taxa exhibiting significant differences among the Barnegat Bay-Manasquan sites in 2016. Significance convention *= 0.05, ** = 0.01, ***

= 0.0001. Site designations and abbreviations: Ablemare (AB), Beverly (BEV), Treasure Island (TIE, Golf Course (GC), Gull Island Railroad Canal (GI), Bay Head (BH), East Drive (ED), Carrol Fox Road (CFR), Herring Island (HI).

Species	AB	BEV	TI	GC	GI	BH	ED	CFR	HI
*C. chesapeakei***	0.27	0	0	0	0	0	0	0	0
C. chesapeakei ephyrae*	0	0	0	0	0	0	0.35	0.36	0
*M. leidyi**	0.2	0	0	0	0	0	0	0.06	0
Calanoida *	418.6	514.6	74.3	429.7	197.9	141.6	156.9	101.7	149.7
Ostracoda*	0.06	0	0.6	0.4	0.02	0	0	0	10.8
Caridea larvae***	2.1	3.3	6.3	8.3	2.5	2.4	1.1	0.8	2.0
Brachyura larvae ***	95.0	88.4	67.1	46.1	20.1	14.6	5.8	4.6	5.7
Fish Eggs ***	0.08	0.2	4.6	3.3	3.7	75.5	3.3	0.2	23.5

Table 13. Peracarida correlation analysis in the Barnegat Bay-Manasquan sites in 2016. Values in table represent the Pearsons 'r' with significance indicated by ** P < 0.01, *** P < 0.001. Taxonomic abbreviations as follows: PB = Plant Biomass, AOR =Aoridae, MEL =Mellitidae, COR = Corophiidae, GAM = *Gammarus* spp., CAP = Caprellidae.

	AOR	MEL	COR	GAM	CAP
PB	0.8***	ns	0.6***	0.3	ns
AOR		0.4**	0.8***	0.7***	0.5***
MEL			0.6***	0.6***	0.9***
COR				0.6***	0.5***
GAM					0.8***

2017 Results

Shrewsbury River

After complete morphological and molecular processing of plankton tow samples, one *G. vertens* was identified. The absence of *G. vertens* in the majority of samples may have been the result of their ability to cling to seagrass and therefore stay on the benthos even when the water column above had been disturbed. Additionally, tidal flux, day time sampling, predation, and/or competition for food resources may have played a role in their abundance within the system and their absence in these samples. The most abundant animals (not including gelatinous zooplankton) found in plankton tow samples were: Calanoida spp., Caridea, Brachyura, Aoridae, and fish eggs; for gelatinous zooplankton the most common animals were: *Chrysaora chesapeakei (formerly quinquecirrha)* and *Mnemiopsis leidyi*.

During plankton sampling, significant variation in the distribution and timing of pelagic organisms was recorded. When data from the Shrewsbury were evaluated, gelatinous ephyrae of *C. chesapeakei*, *M. leidyi*, Calanoida spp., Caridea larvae, and Brachyura larvae showed significant differences in density among sites (Table 14). A subsequent correlation analysis was conducted and showed significant correlations among

taxa with extremely high relationships among several Peracarida groups and each other as well as floating SAV wrack (Table 15).

Table 14. Average densities (#m^{-3}) of taxa exhibiting significant differences among Shrewsbury sites in 2017. Significance convention *= 0.05, ** = 0.01, *** = 0.0001. Sites designated as: Blackberry Bay (BB), Raccoon Island (RI), Hook, Wharfside Manor Marina (WM), Oyster Bay (OB), Bartley Point Island (BP), Sedge Island (SI), Manhasset Creek (MC).

Species	BB	RI	HOOK	WM	OB	BP	SI	MC
C. chesapeakei ephyrae**	1.8	0.034	0.06	0	0.07	0	0.008	0.35
*M. leidyi***	1.54	0.8	1.17	1.13	0.29	0.09	1.65	0.31
Calanoida *	14.5	19.4	17.5	12.06	4.6	28.7	63.8	5.7
Caridea larvae*	4.2	0.66	1.49	0.31	1.42	0.14	5.93	0.51
Brachyura larvae ***	6.01	0.35	3.22	0.3	3.1	0.42	9.83	5.56

Table 15. *Peracarida* correlation analysis for plankton tows in the Shrewsbury River sites in 2017. Values in table represent the Pearsons 'r' with significance indicated by * P < 0.05, ** P < 0.01, *** P < 0.001. Taxonomic abbreviations as follows: PB = Plant Biomass, AOR =Aoridae, AMP = Ampelescidae, MEL =Mellitidae, LJ = Lilljeborgiidae, COR = Corophiidae, CAP = Caprellidae, ER = *Erichsonella* spp., IP = *Idotea phosphorea*, IB = *Idotea balthica*.

	AOR	**AMP**	**MEL**	**LJ**	**COR**	**CAP**	**ER**	**IP**	**IB**
PB	0.2*	ns	0.2**	ns	ns	0.4***	0.2*	Ns	ns
AOR		ns	0.7***	ns	ns	ns	ns	Ns	ns
AMP			0.2**	ns	ns	ns	0.2*	0.3***	ns
MEL				0.5***	0.5***	0.3***	ns	0.4***	ns
LJ					0.8***	ns	0.3***	0.4***	ns
COR						ns	ns	0.5***	ns
CAP							0.7***	0.2**	0.7***
ER								Ns	0.8***
IP									ns

Barnegat Bay-Manasquan System

For plankton tow samples from the Manasquan and Barnegat Bay, similar results occurred with *C. chesapeakei*, gelatinous ephyrae of *C. chesapeakei*, Calanoida spp., and Brachyura larvae showing significant differences in density among sites (Table 16). A subsequent correlation analysis was conducted and showed significant correlations among taxa with extremely high relationships among several Peracarida groups and each other as well as floating SAV wrack (Table 17).

Table 16. Average densities (#m^{-3}) of taxa exhibiting significant differences among the Barnegat Bay-Manasquan sites in 2017. Significance convention *= 0.05, ** = 0.01, *** = 0.0001. Site designations and abbreviations: Riverside Park (RP), Treasure Island (TI),

Gull Island Railroad Canal (GI), Manasquan Canal (MC), Bay Head (BH), East Drive (ED), Carrol Fox Road (CFR), Herring Island (HI).

Species	RP	TI	GI	MC	BH	ED	CFR	HI
*C. chesapeakei***	0	0	0	0	0	0.12	0.47	0
C. chesapeakei ephyrae*	0.03	0	0	0	0.02	0.37	0.43	0.09
Brachyura larvae ***	0.68	0.55	2.54	2.2	0.45	0.54	0.16	1.31
Calanoida *	4.5	1.3	23.3	97.6	11.3	1.5	0.12	1.7

Table 17. *Peracarida* correlation analysis for plankton tows in the Barnegat Bay-Manasquan sites in 2017. Values in table represent the Pearsons 'r' with significance indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Taxonomic abbreviations as follows: PB = Plant Biomass, AOR =Aoridae, MEL =Mellitidae, LJ = Lilljeborgiidae, LYS = Lysianassidae, COR = Corophiidae, GAM = *Gammarus* spp., CAP = Caprellidae, ER = *Erichsonella* spp., IB = *Idotea balthica*.

	AOR	**MEL**	**LJ**	**LYS**	**COR**	**GAM**	**CAP**	**ER**	**IB**
PB	0.6***	0.7***	0.7***	0.7***	0.6***	0.8***	0.5***	0.4**	0.7***
AOR		0.7***	0.9***	0.8***	0.2*	0.3*	0.2	-0.03	0.9***
MEL			0.8***	0.8***	0.7***	0.7***	0.8***	0.5***	0.8***
LJ				0.9***	0.3*	0.3**	0.2*	-0.02	0.9***
LYS					0.3*	0.3**	0.3*	-0.02	0.9***
COR						0.9***	0.7***	0.9***	0.3*
GAM							0.8***	0.8***	0.3**
CAP								0.7***	0.3*
ER									-0.02

Field Algal Sampling

As mentioned above, the behavior of *G. vertens* includes its ability to firmly attach itself to seagrass and remain well below the surface. In order to assess the benthic distribution and density of *G. vertens,* bottom and subsurface grass and macroalgae samples were taken. No *G. vertens* were identified in any of these samples. Currently, the most abundant animals found in these were: *Chrysaora chesapeakei, Gemma gemma,* Aoridae*,* Palaemonidae, Gammaridae, *Illyanassa* spp., Caprellidae, and Melitidae. In subsequent years (2018 and 2019), standardized algal samples were not collected, but *G. vertens* were frequently encountered in macroalgae and in eelgrass (*Zostera marina*) beds in Barnegat Bay.

JADs

In addition to field algal sampling, we used JADs to assess *G. vertens* presence. These devices were deployed at all of the proposed sites during the first sampling of the area; unfortunately many of the JADs were not able to be located at the end of the study as a result of people tampering with them when the research team was not on site. When the JADs were assessed in the field, a sample of several grass blades were taken on the second

sampling event. These grass blades were stored in sterile plastic tubes and kept on ice, and then frozen once returned to the lab. Each blade was observed under a dissecting scope to look for the presence of clinging jellyfish polyps. Once visually examined, the blades were sent for molecular identification to determine if any *G. vertens* DNA could be detected. During these initial sampling events, no clinging jellyfish were observed attached to any of the JAD blades and no clinging jellyfish DNA was detected. Upon the second visit to each site, JADs were collected as whole units and transported back to the lab in 5-gallon buckets containing water from each site. Once returned to the lab each bucket was placed on ice until the sample was processed. During processing each blade of grass and each oyster shell were visually examined for jellyfish polyps. During sample processing, many *C. chesapeakei* polyps were found as well as many nudibranchs, known predators of cnidarian polyps (see Bologna et al. 2020). Additionally, one polyp that did not appear to belong to a native species was identified on a JAD blade mimic collected from the Blackberry Bay site on the Shrewsbury River (Fig. 5). After molecular identification, this polyp was confirmed to be that of *G. vertens* suggesting that polyp recruitment has occurred within this system. While Blackberry Bay was near sites where *G. vertens* adults were located, this site itself had no reported sightings or collection of adult *G. vertens* individuals, suggesting that polyp recruitment occurred in areas that had low or no adult population densities. Examination of oyster shells contained in JAD bags showed no polyp recruitment for *G. vertens,* and only one polyp from the Treasure Island JAD was identified, and this polyp did not belong to *G. vertens* or *C. chesapeakei*. Unfortunately, this polyp was not recovered for molecular identification, so the exact species is unknown. The lack of polyps found on JADs was likely a result of the shells being buried in the sediment as opposed to sitting on top of the sediment; additionally, the number of artificial seagrass blades attached to the vexar bags was quite high and the density of these blades may have contributed to the lack of polyp sediment on the shells below.

The last method used was rock and dock swabbing. In total, an excess of 50 swabs were taken and GPS coordinates were recorded for each. Once a swab was taken in the field, it was stored in a sterile plastic tube and kept on ice until it was transported to the lab where it was stored at -80ºC until processing. The processing of these samples included boiling the swab in Chelex to extract the DNA. PCR was then performed using 16S universal cnidarian primers. Subsequent sequencing of this DNA was performed and did not reveal any *G. vertens* DNA. The most abundant animals detected by these swabs were bryozoans.

For the Barnegat Bay/Manasquan locations, the sample methods were exactly the same as those described for Objectives 1 and 2. While there were two sighting of *G. vertens* near the Point Pleasant canal, which leads into Barnegat Bay, there were no confirmed sightings in these waters. Additionally, our sampling did not collect any *G. vertens* and there was no confirmation of *G. vertens* DNA through molecular techniques. While this suggests that there has been no transport into other water bodies, this can realistically neither be confirmed nor rejected as we only sampled four locations in this region. *Gonionemus vertens* may be present in this or other nearby systems, but recruitment may be limited, and thus densities are below the limit of detection or populations may be located in areas other than those sampled. However, given the collection of numerous adults by citizens and our lab (outside of the sampling for this study), as well as the detection of polyps, it is likely that recruitment has occurred to new areas within and outside of the

affected systems; and more sampling will be needed in order to detect this phenomenon. Additionally, the presence and identification of *G. vertens* polyps within the Shrewsbury River estuary suggests that this species will return each spring or early summer, presenting the same human health risks and concerns for recreational use of this system.

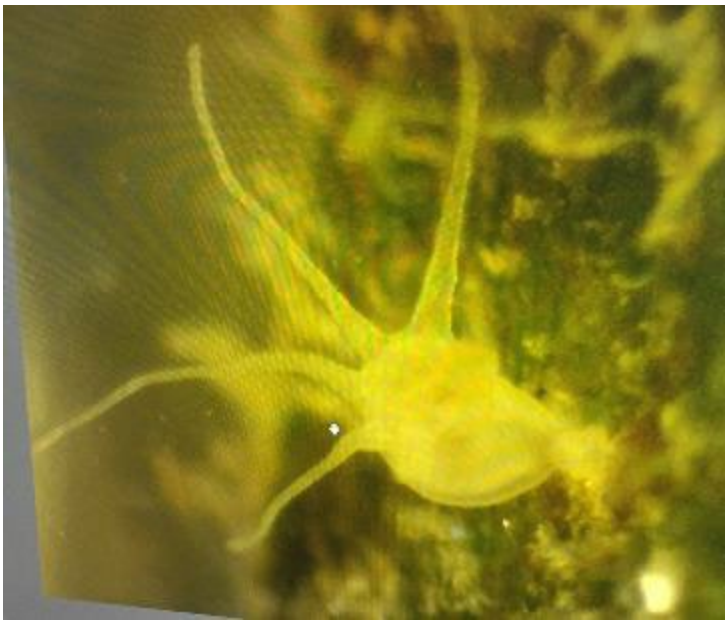

Figure 5. *Gonionemus vertens* polyp found on JAD deployed in Shrewsbury River.

Unknown Jellyfish Collected during Sampling

Aequorea sp.

There were 22 *Aequorea* unknowns collected in Summer 2017. All samples were stored in 70% ethanol and were extracted using 5% Chelex and PCR amplified with both UCF/UCR1 (16S) and COIF/COIR (COI) specific primers (Table 3). A few samples failed to amplify after repeated attempts. Of those that amplified and produced a visible band, they were subjected to automated Sanger dideoxy sequencing. Sequence data extracted from those electropherograms were compared to known sequences in GenBank using the BLAST algorithm (N or X). All samples, at both loci, forward and reverse sequences matched *Aequorea australis* as the top match (Table 18). Although we did not include GenBank accession numbers for these matches, in almost all cases the match was to a population collection of *Aequorea australis* from the South China Sea (usually Isolate #7). The only sample that matched a different *Aequorea* was our Sample 29 (coded as PB80), which matched Polyp 9 (and 8), collected by D. Restaino in Barnegat Bay. This match was near perfect (99.8%) and only differed by 1 nt out of 504. Unfortunately, PB80 DNA did not amplify with the COI primers so we do not have this locus available for comparison. Polyp 9 was identified from a settling plate from 2014, which suggests that some descendants of this clone still exist and are presently generating medusae.

Table 18. Molecular Identification of *Aequorea* Specimens collected in 2017 using 16S and COI loci. ID #'s relate to individuals isolated from plankton samples and referenced

from the notebook of D. Restaino (1/22/2018). N/X reflects the search methodology of either N = BLASTn or X = BLASTx searches. F = forward sequence; R = reverse sequence. All of the *A. australis* matched samples from China (usually Isolate 7).

Sample ID#	Genus/species	N/X	F/R	Locus	Coverage %	E value	% Identity
PB103	*Aequorea australis*	N	F	16S	99	2 e^{-168}	95
PB103	*Aequorea australis*	N	R	16S	96	0	96
PB103	*Aequorea australis*	N	F	COI	97	0	92
PB103	*Aequorea australis*	X	F	COI	97	3 e^{-104}	96
PB103	*Aequorea australis*	N	R	COI	97	0	93
PB63	*Aequorea australis*	N	F	16S	85	0	95
PB63	*Aequorea australis*	N	R	16S	100	0	96
PB63	RXN FAILED	N	F	COI			
PB63	*Aequorea australis*	N	R	COI	95	0	93
PB64	*Aequorea australis*	N	F	16S	83	1 e^{-170}	95
PB64	*Aequorea australis*	N	R	16S	100	0	95
PB64	*Aequorea australis*	N	F	COI	98	0	91
PB64	*Aequorea australis*	N	R	COI	97	0	93
PB67	*Aequorea australis*	N	F	16S	85	0	94
PB67	*Aequorea australis*	N	R	16S	99	0	94
PB67	*Aequorea australis*	N	F	COI	98	0	91
PB67	*Aequorea australis*	N	R	COI	95	0	92
PB68	*Aequorea australis*	N	F	16S	82	4 e^{-48}	79
PB68	*Aequorea australis*	N	R	16S	99	0	95
PB68	RXN FAILED	N	F	COI			
PB68	*Aequorea australis*	N	R	COI	93	0	93
PB69	*Aequorea australis*	N	F	16S	86	0	95
PB69	*Aequorea australis*	N	R	16S	96	0	95
PB69	*Aequorea australis*	N	F	COI	95	0	90
PB69	RXN FAILED	N	R	COI			
PB79	*Aequorea australis*	N	F	16S	86	0	95
PB79	*Aequorea australis*	N	R	16S	96	0	95
PB79	*Aequorea australis*	N	F	COI	98	0	91
PB79	RXN FAILED	N	R	COI			
PB80	*Aequorea sp.,Polyp 9*	N	F	16S	86	0	99
PB80	*Aequorea australis*	N	R	16S	77	7 e^{-136}	89
PB94	*Aequorea australis*	N	F	16S	86	0	95
PB94	*Aequorea australis*	N	R	16S	95	0	95
PB94	*Aequorea australis*	N	F	COI	97	0	92
PB94	*Aequorea australis*	N	R	COI	95	0	93
PB95	*Aequorea australis*	N	F	16S	86	0	96
PB95	*Aequorea australis*	N	R	16S	98	0	96
PB95	*Aequorea australis*	N	F	COI	98	0	91

PB95	*Aequorea australis*	X	F	COI	96	6 e^{-110}	94
PB95	*Aequorea australis*	N	R	COI	97	0	92
PB96	*Aequorea australis*	N	F	16S	86	0	95
PB96	*Aequorea australis*	N	R	16S	95	0	96
PB97	*Aequorea australis*	N	F	16S	86	0	96
PB97	*Aequorea australis*	N	R	16S	96	0	96
PB97	RXN FAILED	N	F	COI			
PB97	*Aequorea australis*	N	R	COI	97	0	93
PB99	*Aequorea australis*	N	F	16S	86	0	96
PB99	*Aequorea australis*	N	R	16S	95	0	96
PB99	*Aequorea australis*	N	F	COI	98	0	92
PB99	*Aequorea australis*	N	R	COI	97	0	92

***Bougainvillia* sp.**

A diminutive jellyfish initially identified as *Bougainvillia* sp., which bloomed in late spring/early summer of 2017, was sent for molecular identification following our standard protocols and assessed using the 16S locus. Results indicate that the sequences did not match any of the known species in the region and that the highest matches in the database were for *Bougainvillia triestina* (Table 19). This species is poorly understood and was only recognized recently as a unique species and not a growth stage of larger *Bougainvillia* species (Batistić and Garić 2016).

Table 19. Comparative molecular identification for unknown *Bougainvillia* sp. based on 16S locus.

Species in GenBank	Accession #	Location	Locus	% Match	Homology
Bougainvillia triestina	KJ660344	Croatia	16S	96%	545/570
Bougainvillia triestina	KJ660345	Croatia	16S	96%	494/515
Bougainvillia fulva	EU305470	Eastern Pacific, USA	16S	90%	514/568
Bougainvillia britannica	AM183127	United Kingdom	16S	88%	497/567
Bougainvillia britannica	MF000551	Norway	16S	88%	497/567

Regional Population and Phylogenetic Analyses of *G. vertens*

All populations tested showed unique molecular variations mostly in the form of single nucleotide polymorphisms (SNPs). While the Connecticut, Massachusetts, and French populations show intra-population variations at both the 16S and COI loci, New Jersey populations from the individual collected in the Manasquan Canal and the Shrewsbury River are 100% homologous showing no intra-population variation. The New Jersey population has two unique variations that differentiate it from other North Eastern

populations in the United States, one at the 16S locus and one at the COI locus that are not observed elsewhere (Fig. 6). At the 16S locus there is a stretch of 7 Thymines at approximately 350 BP and in all other United States populations there are only 6 Thymines. CLUSTAL Omega alignment of New Jersey 16S sequences and those from the New England populations suggest that the additional Thymine is an insertion (Fig. 6). Interestingly, there are two individuals from the French population which also share this 7 Thymine repeat. These are the only two known/available sequences that share this same insertion, and consequently, this may reflect multiple invasion pathways for *G. vertens* in the United States.

At the COI locus there are two adjacent SNPs that are not observed in any other population tested in this study or those whose sequences are publicly available in GenBank. These SNPs occur at approximately 400 BP and involve the replacement of the first 2 Thymines in a triplicate Thymine repeat with two Cystosines (Fig. 7). Additionally, there is another SNP at roughly 60 BP where a Cytosine has been replaced with a Thymine. Again, this change in base pairs only occurs in the New Jersey Population (Fig. 7). Based on the CLUSTAL Omega alignment of the COI locus, the New Jersey population has some variability, but the polymorphisms seen at all other locations are the same as at least one other tested population (Fig. 8). Therefore, it is the occurrence of the polymorphisms, which are unique to the New Jersey *G. vertens* population that direct the formation of neighbor joining trees at both of these individual loci (Fig. 9 and Fig. 10).

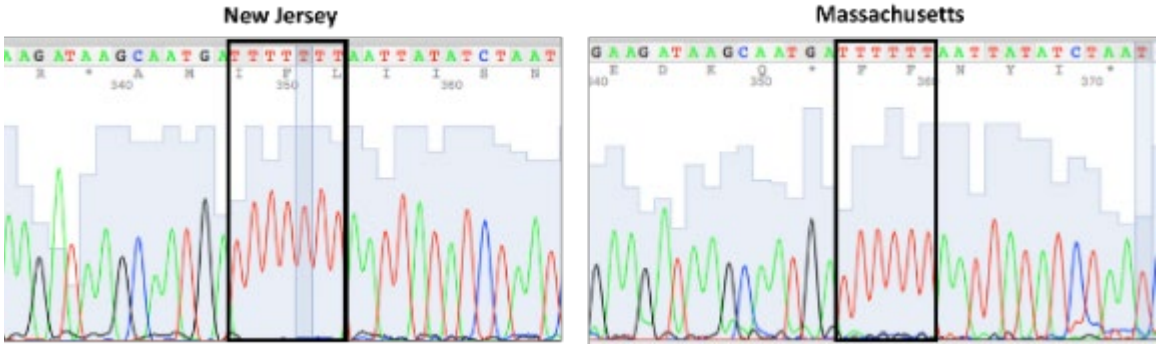

Figure 6. Electropherogram showing the insertion of a Thymine indicated by a box.

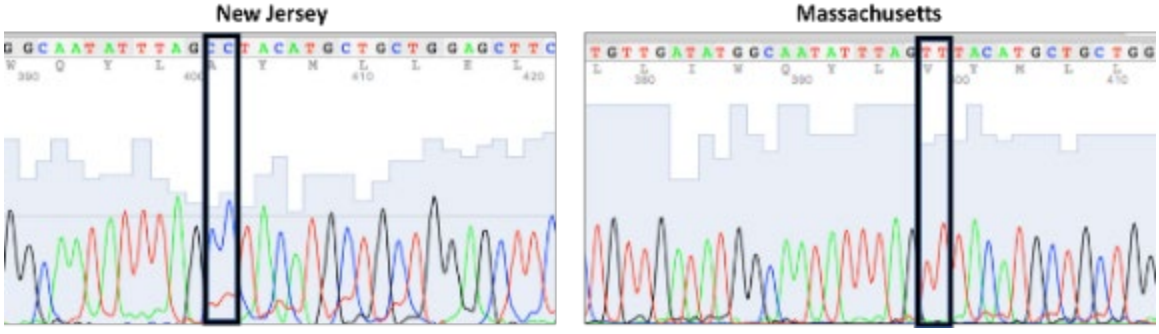

Figure 7. Electropherogram showing the two downstream SNPs at the COI locus indicated by a box.

```
France      AAAGATATTGGAACATTATATTTAGTATTTGGTGTATTTTCAGCGATGGTTGGAACCGCT  60
MC,CT       ----------------TATATTTAGTATTTGGTGTATTTTCAGCAATGGTTGGAACCGCT  44
VC,CT       ------------------TATTTAGTATTTGGTGTATTTTCAGCAATGGTTGGAACCGCT  42
NJ          AAAGATATTGGAACATTATATTTAGTATTTGGTGTATTTTCAGCAATGGTTGGAACAGCT  60
MV,MA       ----------------------------------------TCAGCAATGGTTGGAACCGCT  21
                                                     ***** ********** ***

France      TCAGGAGTTCAAGCACATTCAGGGCCCTCTGTTGATATGGCAATATTTAGTTTACATGCT  420
MC,CT       TCAGGAGTTCAAGCACATTCAGGGCCCTCTGTTGATATGGCAATATTTAGTTTACATGCT  404
VC,CT       TCAGGAGTTCAAGCACATTCAGGGCCCTCTGTTGATATGGCAATATTTAGTTTACATGCT  402
NJ          TCAGGAGTTCAAGCACATTCAGGGCCCTCCGTTGATATGGCAATATTTAGCCTACATGCT  420
MV,MA       TCAGGAGTTCAAGCACATTCAGGGCCCTCCGTTGATATGGCAATATTTAGTTTACATGCT  381
            ***************************** ******************  ********
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Figure 8. CLUSTAL Omega alignment of the COI Locus highlighting the major differences among populations. Population abbreviations: MC,CT = Mumford Cove, CT' VC,CT = Venetian Cove, CT; MV,MA = Martha's Vineyard, MA. SNPs highlighted among populations: Red = Upstream Cytosine to Adenine SNP and Blue = Downstream double Thymine to Cytosine SNPs.

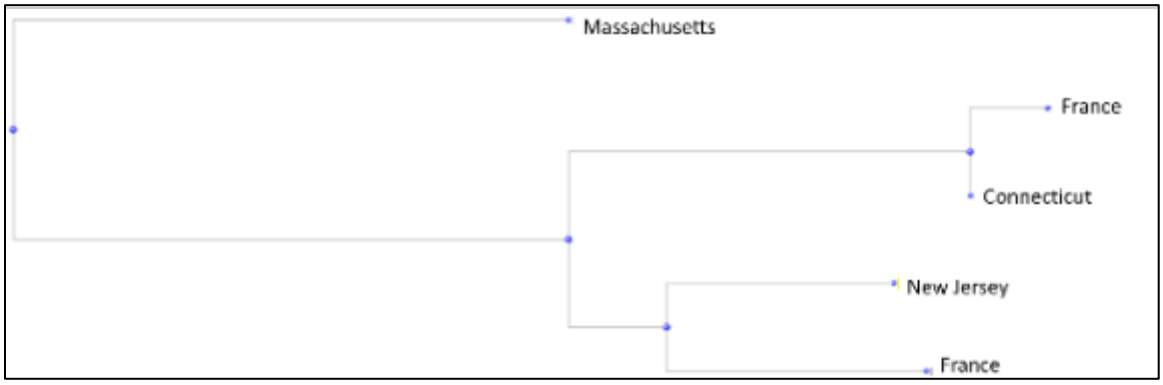

Figure 9. 16S neighbor joining tree. Showing the New Jersey population grouped with a French population containing the same stretch of 7 Thymines.

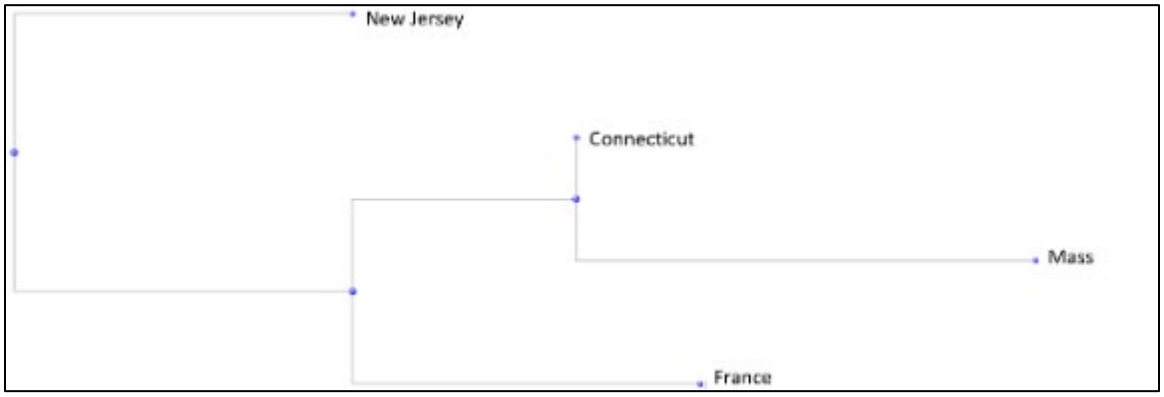

Figure 10. COI neighbor joining tree. Showing the New Jersey population as being the mostly distantly related to those populations tested.

Phase 2 Results

Continuous Water Temperature Monitoring 2018 – 2019

During 2018, continuous water temperature meters were deployed in New Jersey to evaluate whether field water temperatures corresponded to the timing of the disappearance of *G. vertens* from field sampling events coupled with laboratory temperature experiments. In 2018, field recorders were deployed and retrieved from three sites in the Shrewsbury River including: Blackberry Bay, NJ (Fig. 11), Raccoon Island, NJ (Fig. 12), Hook, NJ (Fig. 13) and one site in Barnegat Bay: Ham Island, NJ (Fig. 14). All four locations showed seasonal increases in water temperature. On or about July 1, 2018, all these sites also recorded temperatures exceeding 28ºC, which our laboratory experiments (see below) have shown is at the thermal maximum for *G. vertens* in these regions. During 2019, a continuous monitoring temperature probe was deployed near the mouth of the Metedeconk River, NJ in Barnegat Bay, our Hook site in the Shrewsbury River, NJ, and one probe was deployed in Potter Pond, RI with cooperation of the Rhode Island Department of Environmental Protection. Data collected from the Metedeconk River showed that this region did not reach the 28ºC thermal limit until July 16, 2019 (Fig. 15), which corresponds to the field observations and final collection of *G. vertens* in this region on that date, but at our Hook site, 28ºC was observed on July 4, 2019 (Fig. 16) indicating an earlier time for achieving thermal maximum tolerance. For the probe located in Potter Pond, RI (Fig. 17), this thermal threshold was never achieved throughout the summer and anecdotally, *G. vertens* were observed into August of 2019.

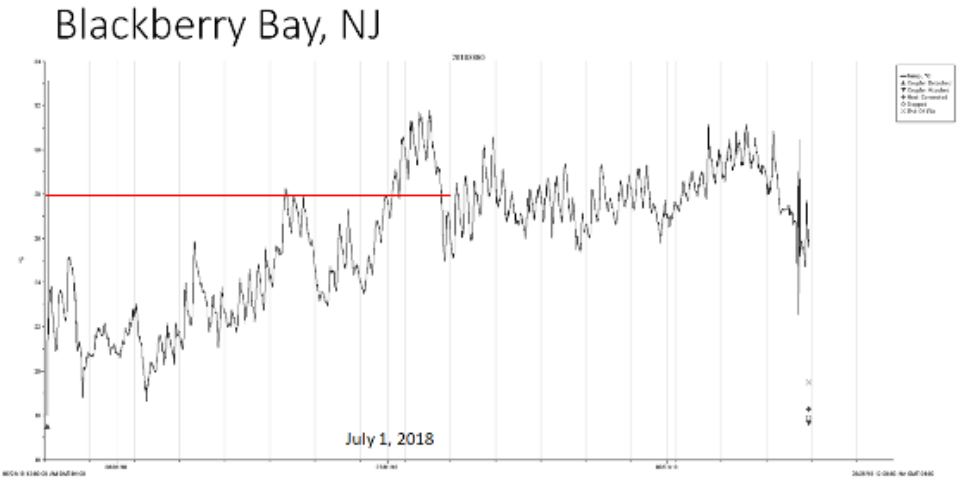

Figure 11. Continuous temperature recording device results for the summer of 2018 at Blackberry Bay, NJ. Red line reflects 28ºC, the thermal maximum for *G. vertens* in this region.

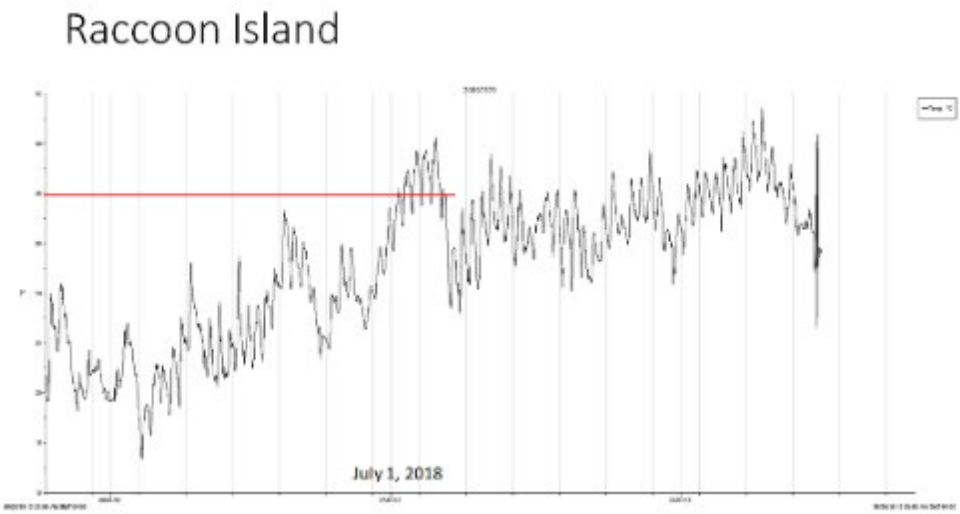

Figure 12. Continuous temperature recording device results for the summer of 2018 at Raccoon Island, NJ. Red line reflects 28ºC, the thermal maximum for *G. vertens* in this region.

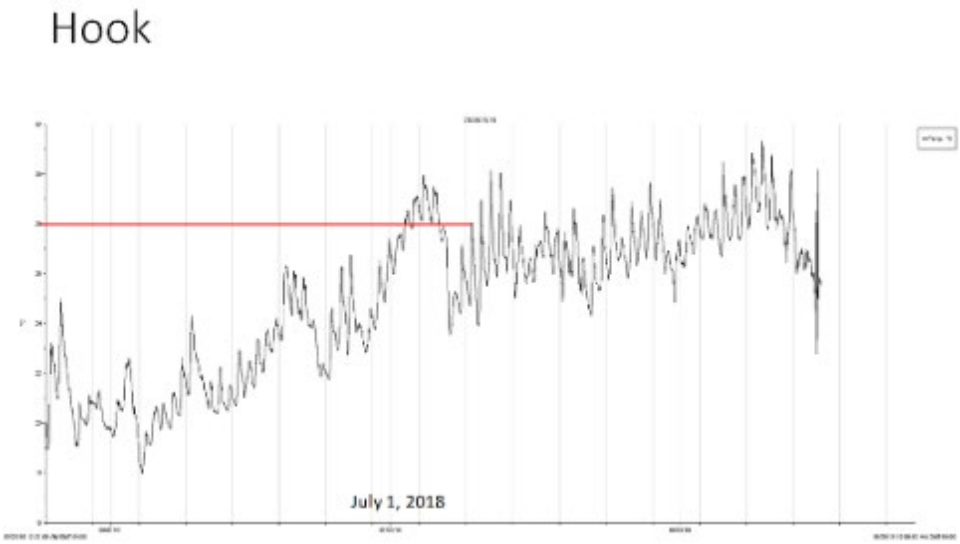

Figure 13. Continuous temperature recording device results for the summer of 2018 at our Hook site in the Shrewsbury River, NJ. Red line reflects 28ºC, the thermal maximum for *G. vertens* in this region.

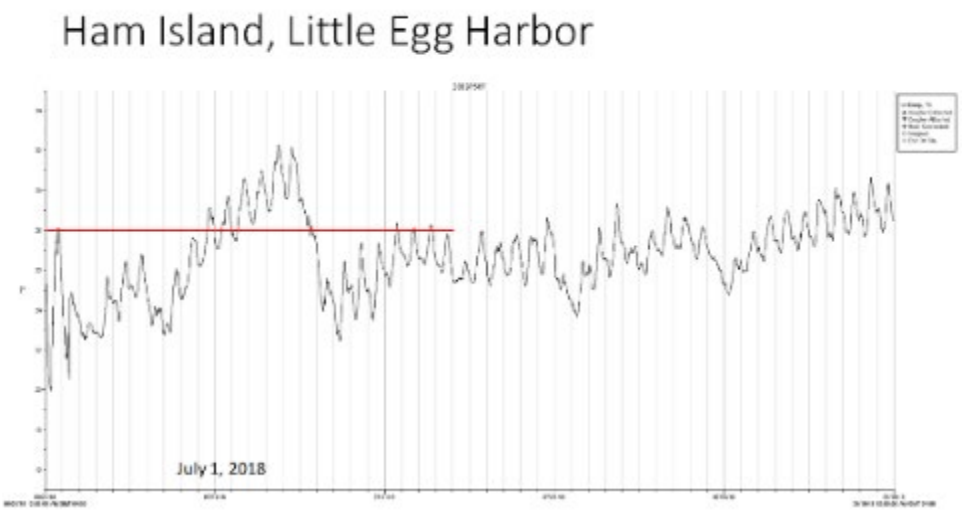

Figure 14. Continuous temperature recording device results for the summer of 2018 at Ham Island, Little Egg Harbor, NJ. Red line reflects 28ºC, the thermal maximum for *G. vertens* in this region.

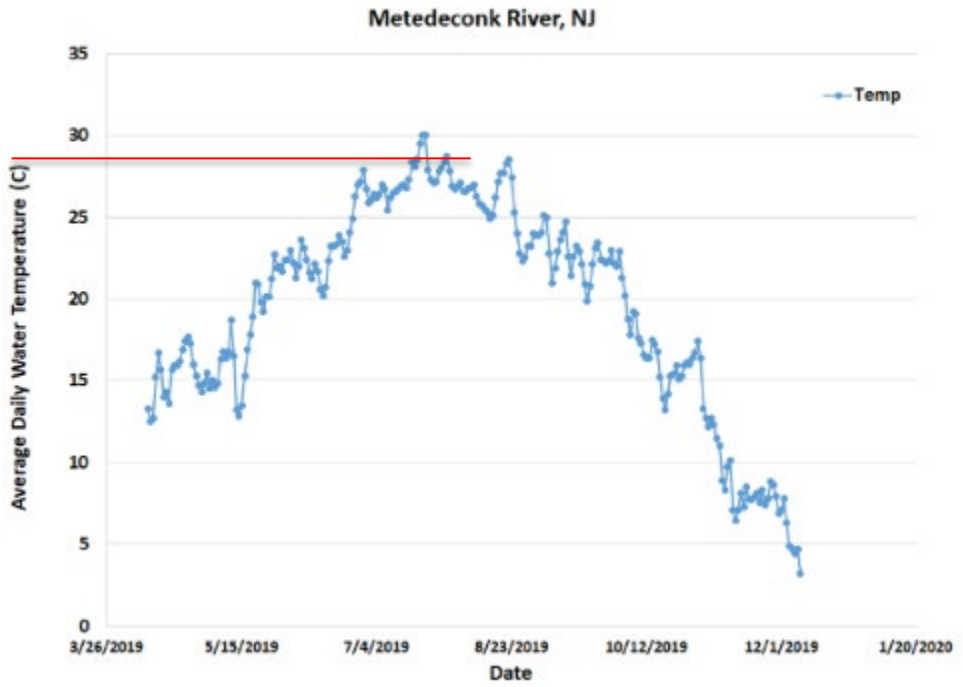

Figure 15. Continuous temperature recording device results for the summer of 2019 from the Metedeconk River, NJ. Red line reflects 28ºC, the thermal maximum for *G. vertens* in this region.

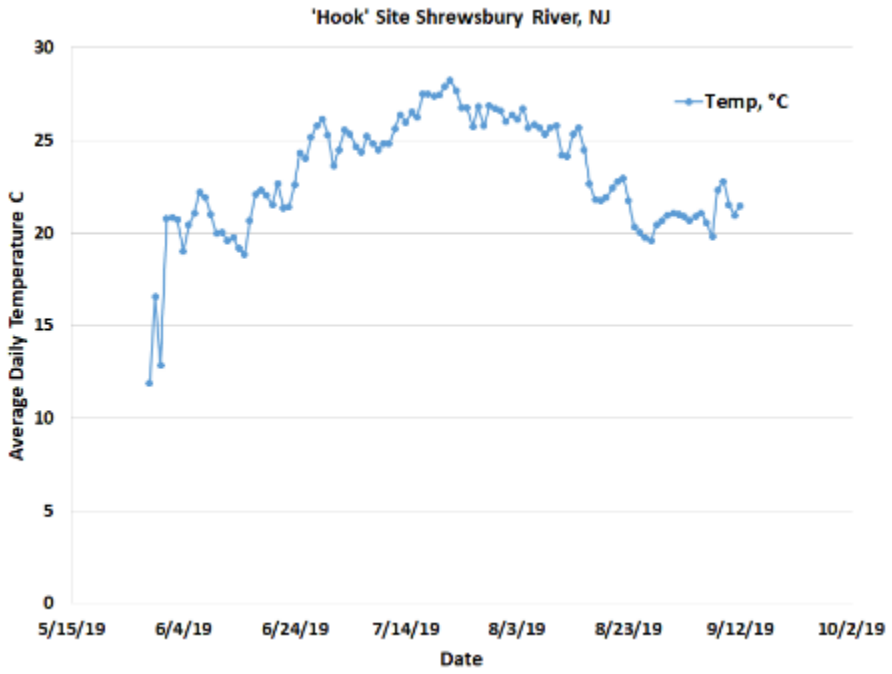

Figure 16. Continuous temperature recording device results for the summer of 2019 at our Hook site in the Shrewsbury River, NJ. Temperatures did not exceed 28ºC.

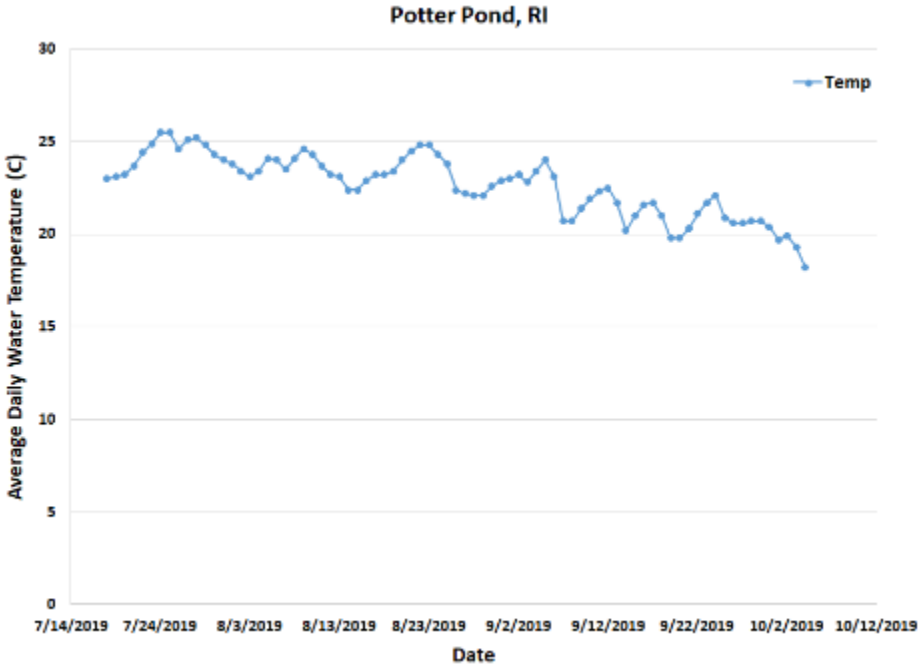

Figure 17. Daily average water temperatures I for Potter Pond, RI collected during the summer of 2019. Temperatures did not exceed 28ºC

2019 New Population Identification, North Wildwood, NJ

One intriguing facet of 2019's research was the identification of a population from North Wildwood in Cape May County. A resident in the region noticed an unusual jellyfish and contacted us for identification. Our initial evaluation was that it was *G. vertens* and subsequent sampling confirmed its identification through molecular sequence analysis. At the 16S locus, the Wildwood clinging jellyfish population is most similar to the population in the Metedeconk River. The majority of individuals in the Wildwood population, represented by Wildwood_30_16S in Figure 18, show high (>98%) sequence homology to many individuals collected from the Metedeconk River, represented by Metedeconk_16_16S in Figure 18. However, there are several individuals in the Wildwood population, represented by Wildwood_28_16S that show some of the same polymorphisms present in the Metedeconk population, but also display changes in their sequences not seen in individuals from any population present in New Jersey.

Some individuals in the Wildwood population, represented by Wildwood_28_16S in Figure 18, show a dinucleotide polymorphism at base pair: 212-213, and SNPs at base pairs: 267, 328, 415, and 462. These changes in sequence are unique to this population and do not show homology to any publicly available *G. vertens* sequences. This suggests that these individuals are different from other populations in New Jersey and those sampled in New England. It is possible that this haplotype is less frequent in populations but is more abundant in Wildwood, due to founder effect. The absence of shared haplotypes between Tices Shoal and Wildwood populations suggest that the Wildwood population does not originate from the closest GV population in New Jersey (Tices Shoal). However, the Metedeconk population shares haplotypes with both southern populations of GV suggesting that recruitment of individuals in Tices Shoal and Wildwood may have come from the Metedeconk River population in Northern Barnegat Bay. There is also haplotypic overlap between the Metedeconk population and the Shrewsbury River population. In summary, New Jersey populations show strong similarities among themselves which are not shared with other New England populations. This suggests that the New Jersey population may be an isolated or independent invasion, and this may have originated in the Metedeconk River region given the similarities it shares independently with the Shrewsbury, but also the similarities it shares with both Tices Shoals and the North Wildwood population (Fig. 18).

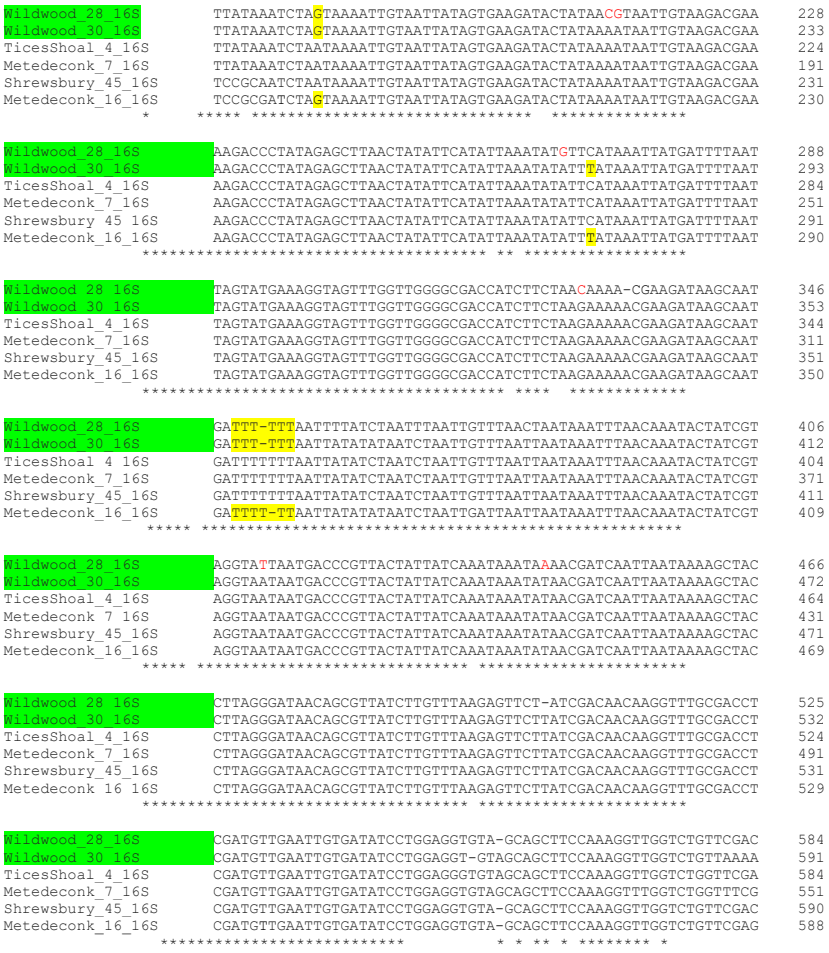

Figure 18. Clustal Alignment of unique 16S haplotypes for *G. vertens* in New Jersey waters. Polymorphisms unique to the Wildwood population are indicated by red text. Polymorphisms homologous to the Metedeconk population are highlighted in Yellow.

Identifying the potential causes of the *G. vertens* bloom decline

Regional *G. vertens* Thermal Tolerance Experiments

Gonionemus vertens adult medusae begin to bloom in May and continue to persist through the middle of July and into the fall in more northern New England regions, as well as elsewhere on the globe. Laboratory experiments and field observations demonstrate that several factors seem to contribute to the cessation of bloom conditions such as top-down pressure by sea nettles (where they occur), water temperatures exceeding 28˚C, or possibly a combination of both. Medusae were collected from various locations in New Jersey and Potter Pond during several field surveys in the summers of 2018 and 2019, as well as from North Wildwood, NJ and Mumford Cove, CT in 2019. Medusae were maintained in holding tanks in the laboratory under stable temperature (~21-22ºC) and salinity (20-25 ppt) before being allocated to experimental treatments. The majority of medusae were used in heat tolerance experiments, which ran for 96 hours at temperatures between 22ºC-32ºC. Significant mortality of *G. vertens* from all locations occurred when temperatures met or exceeded 28ºC for 72h ($F_{3,74} = 36.09$, $P < 0.0001$; Figs. 19-22), suggesting that *G. vertens'* thermal tolerance is approximately 28ºC and that populations in the field may be partially controlled by water temperatures.

In New Jersey, for instance, water temperatures may exceed 28ºC by the beginning of July, which was confirmed by deploying temperature recorders in Barnegat Bay and the Shrewsbury River (Figs. 11-16). When we contrast this with data collected from Potter Pond, RI (Fig. 17), temperatures never reached the 28ºC thermal maximum indicating that their survival was not diminished by excessive heat in 2019.

Experiments conducted in 2018, show a clear reduction in survival at 72h at 28ºC, but almost no mortality in water temperatures below that (Fig. 19). In 2019, thermal experiments demonstrated that only individuals exposed to temperatures below 28ºC demonstrated survival exceeding 50% for the Metedeconk River, NJ population (Fig. 20), Mumford Cove, CT (Fig. 21) and Potter Pond, RI (Fig. 22). However, individuals collected from North Wildwood, NJ, the southern-most identified population, showed >50% at 96h at 28ºC, suggesting their thermal tolerance may be greater than 28ºC (Fig. 23).

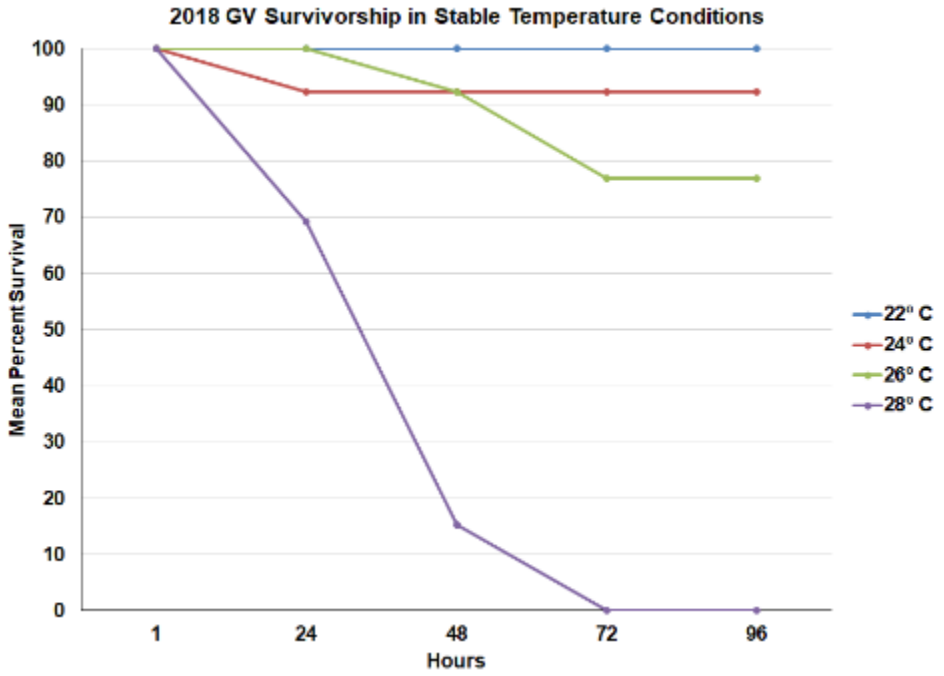

Figure 19. Survival rates of *G. vertens* collected from Potters Pond, RI (2018) at multiple temperatures across 96h constant thermal exposure experiments.

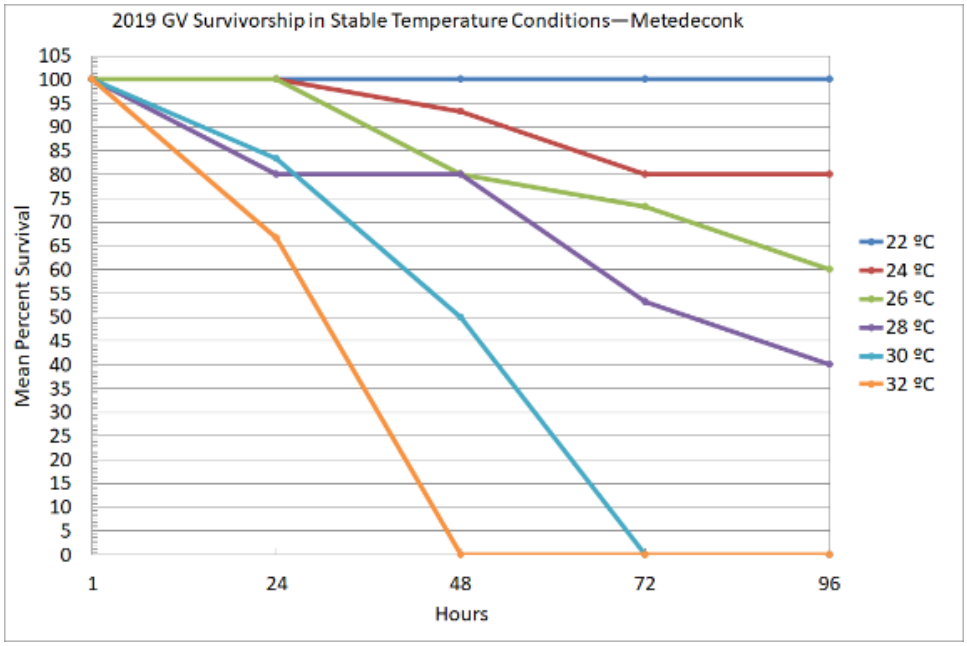

Figure 20. Survival rates of *G. vertens* collected from Metedeconk River, NJ (2019) at multiple temperatures across 96h constant thermal exposure experiments.

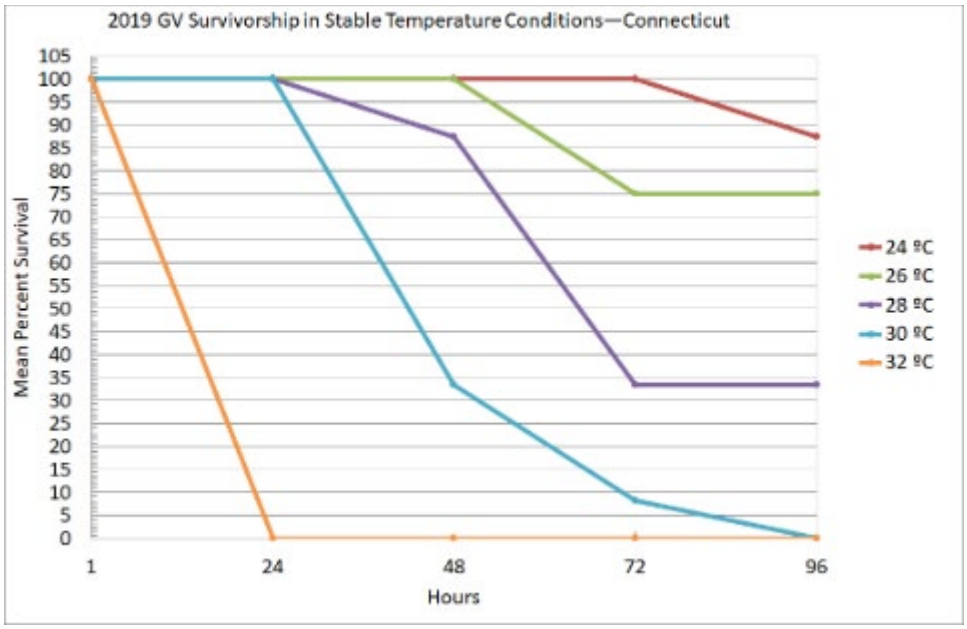

Figure 21. Survival rates of *G. vertens* collected from Mumford Cove, CT (2019) at multiple temperatures across 96h constant thermal exposure experiments.

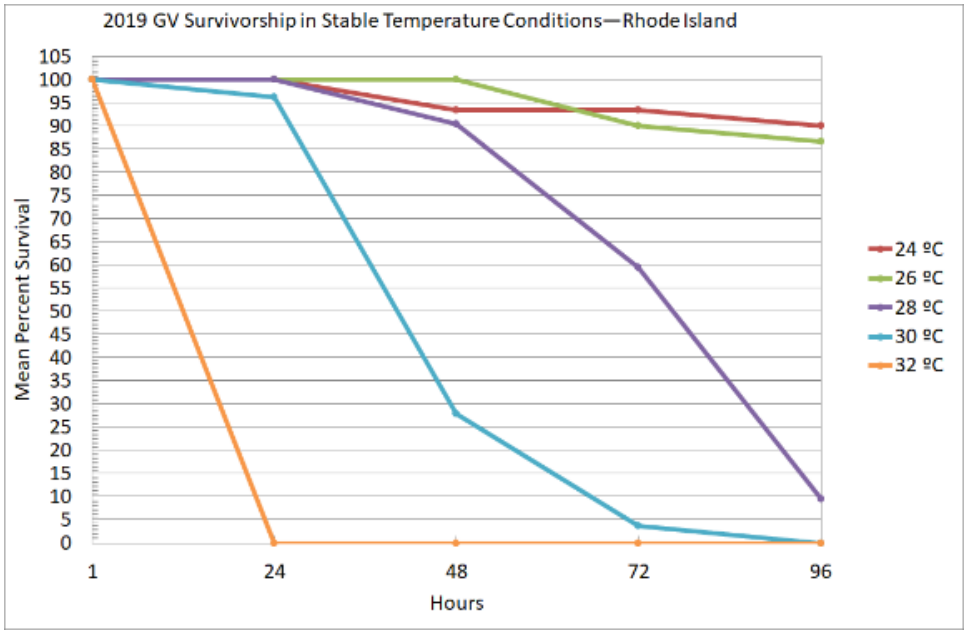

Figure 22. Survival rates of *G. vertens* collected from Potter Pond, RI (2019) at multiple temperatures across 96h constant thermal exposure experiments.

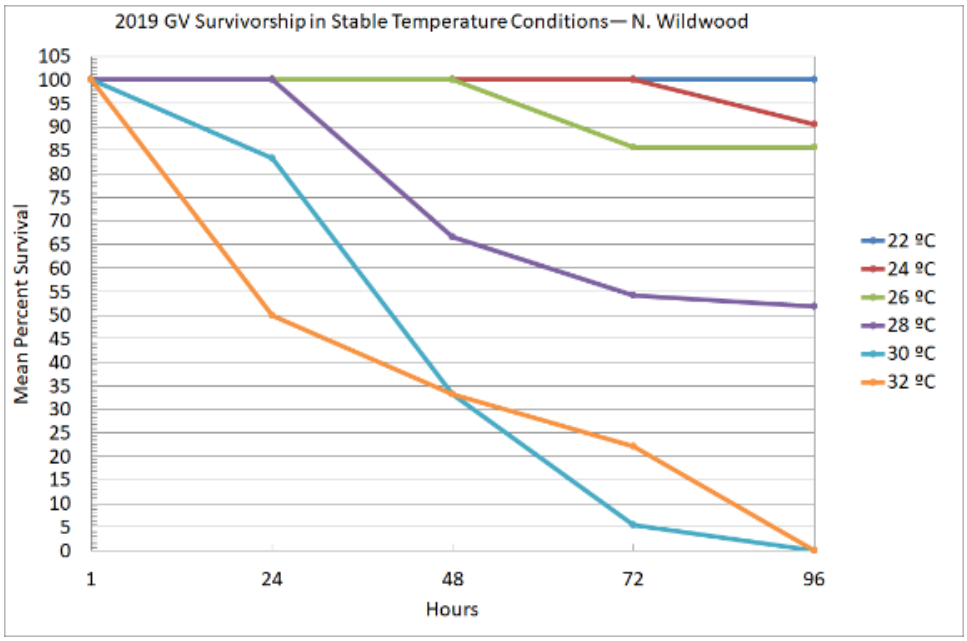

Figure 23. Survival rates of *G. vertens* collected from North Wildwood, NJ (2019) at multiple temperatures across 96h constant thermal exposure experiments.

Latitudinal Differences in Maximum Heat Tolerance

Several patterns emerged from thermal experiments. First, both populations from New Jersey had greater than 40% survival at 28ºC compared to the CT and RI populations, with RI only showing about 10% survival after 96 hours (Fig. 22). Second, the North Wildwood, NJ population showed >50% survival at 28ºC after 96h and showed relatively higher survival at 30ºC and 32ºC suggesting that there may be some latitudinal variation in thermal tolerance (Fig. 23). As such, in 2020, additional thermal experiments using only North Wildwood, NJ individuals were carried out to assess whether their thermal limits demonstrate latitudinal variation. Significant declines in survival began to occur at 32ºC after 48 hours ($F_{3,12} = 7.6$, $P < 0.005$) and continued through 72 hours ($F = 40.3$, $P < 0.0001$) and 96 hours ($F = 10.91$, $P < 0.001$). Specifically, mortality was significantly higher at 32ºC compared to 26º, 28º, and 30ºC, which demonstrated no difference in mortality among these lower temperatures (Fig. 24). As such, these results demonstrate that the North Wildwood, NJ population has a higher thermal limit compared to the other New Jersey, Connecticut, and Rhode Island populations.

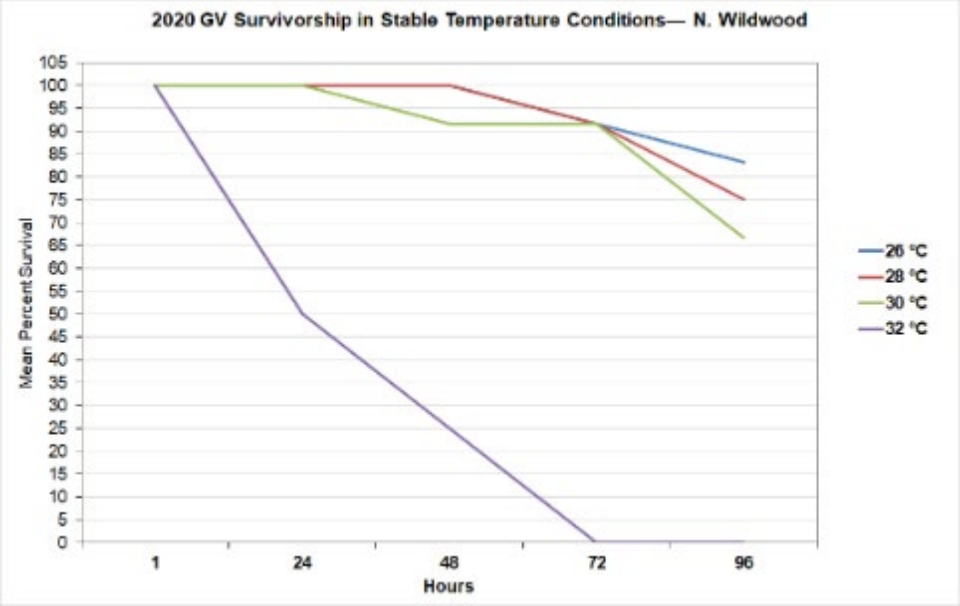

Figure 24. Survival rates of *G. vertens* collected from North Wildwood, NJ (2020) at multiple temperatures across 96h constant thermal exposure experiments.

Trophic Interactions and Potential Top-Down Control

Predation Experiments

Predation experiments were conducted to confirm previous anecdotal data that *Chrysaora chesapeakei* is a predator of *G. vertens* and to determine the bell diameter ratio at which they could be killed or consumed. Results indicate that *C. chesapeakei* actively consumes *G. vertens* in laboratory settings, confirming prior work in 2017 and 2018. In fact, almost all predator-prey settings showed active and rapid consumption of *G. vertens*. To parse whether emerging *C. chesapeakei* ephyrae may be prey for adult *G. vertens*, a reciprocal experiment using juvenile and ephyrae of *C. chesapeakei* and adult *G vertens* was conducted. Results from these experiments showed that no ephyrae were ever consumed by adult *G. vertens*, but almost all encounters between small *C. chesapeakei* medusae and *G. vertens* resulted in death and consumption (Fig. 25).

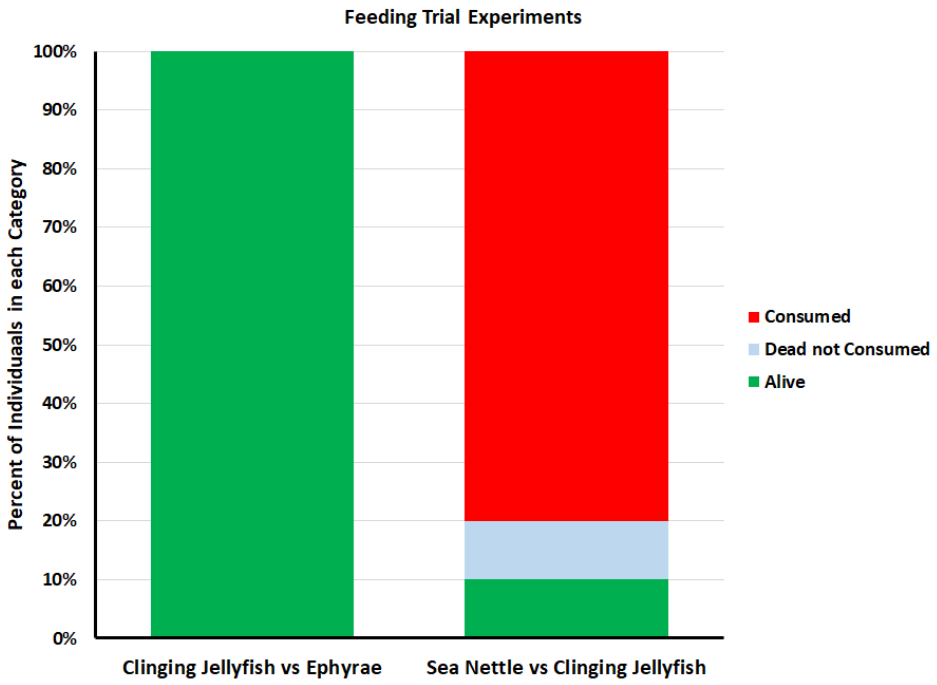

Figure 25. Reciprocal Predation experiments. Adult *G. vertens* medusae were paired with either *C. chesapeakei* ephyrae (N= 15) or larger juveniles (N = 10) and monitored for 24 hours and survival and consumption were recorded.

To further investigate the potential that juvenile *C. chesapeakei* are active predators of *G. vertens*, a second series of experiments was conducted using small *C. chesapeakei* as predators and looking at the bell diameter ratio between predator and prey (N=35). Visual records of encounters showed that *C. chesapeakei* actively swam within the experimental chambers and when they encountered *G. vertens*, they rapidly employed their feeding palps to capture *G. vertens* medusae. After capture, rapid ingestion occurred (Fig. 26), based on the relative size ratio of predator and prey. As such, *C. chesapeakei* is indeed capable of killing *G. vertens,* even at bell diameter ratios of 1.19:1 (*C. chesapeakei* only slightly larger than *G. vertens*), and partially or completely consumed *G. vertens* when bell ratios approached 1.67:1 (Fig. 27). Therefore, it seems that *C. chesapeakei* likely play a role in the decline of *G. vertens* populations in Barnegat Bay and the Shrewsbury River where they co-occur, since their populations begin to also rise in mid-June. This corresponds to the broad decline of *G. vertens* throughout the month of June and into July.

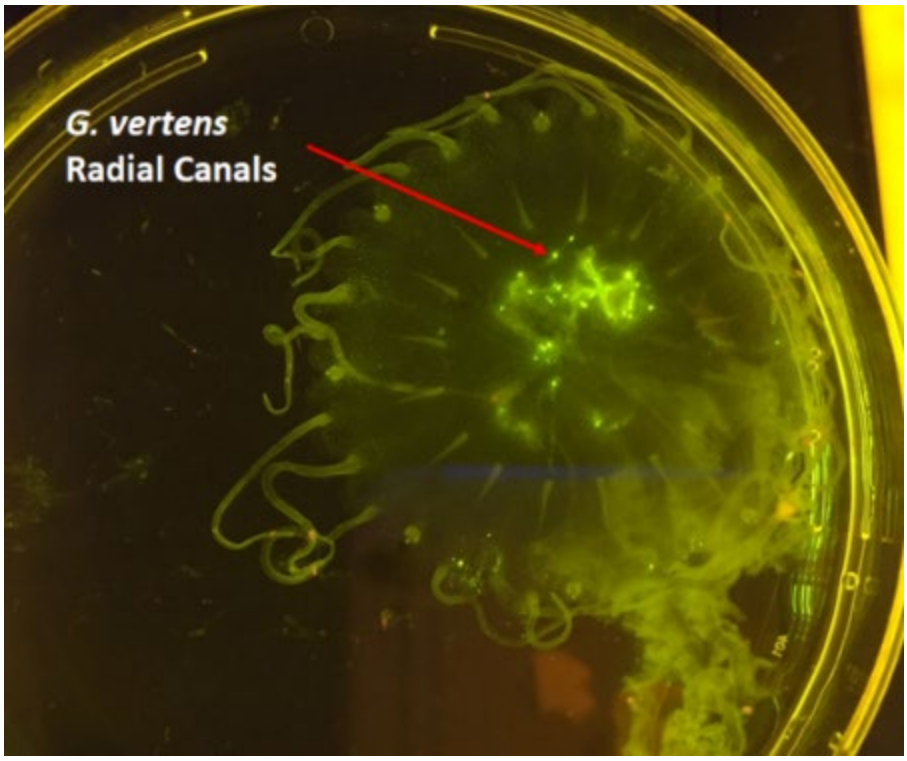

Figure 26. Image of a feeding trial experiment between *C. chesapeakei* and *G. vertens*. The image was taken under blue light illumination (~ 470 nm) and a yellow filter to demonstrate the fluorescence of *G. vertens*. Within the image, the X-shaped radial canal of *G. vertens* is clearly visible.

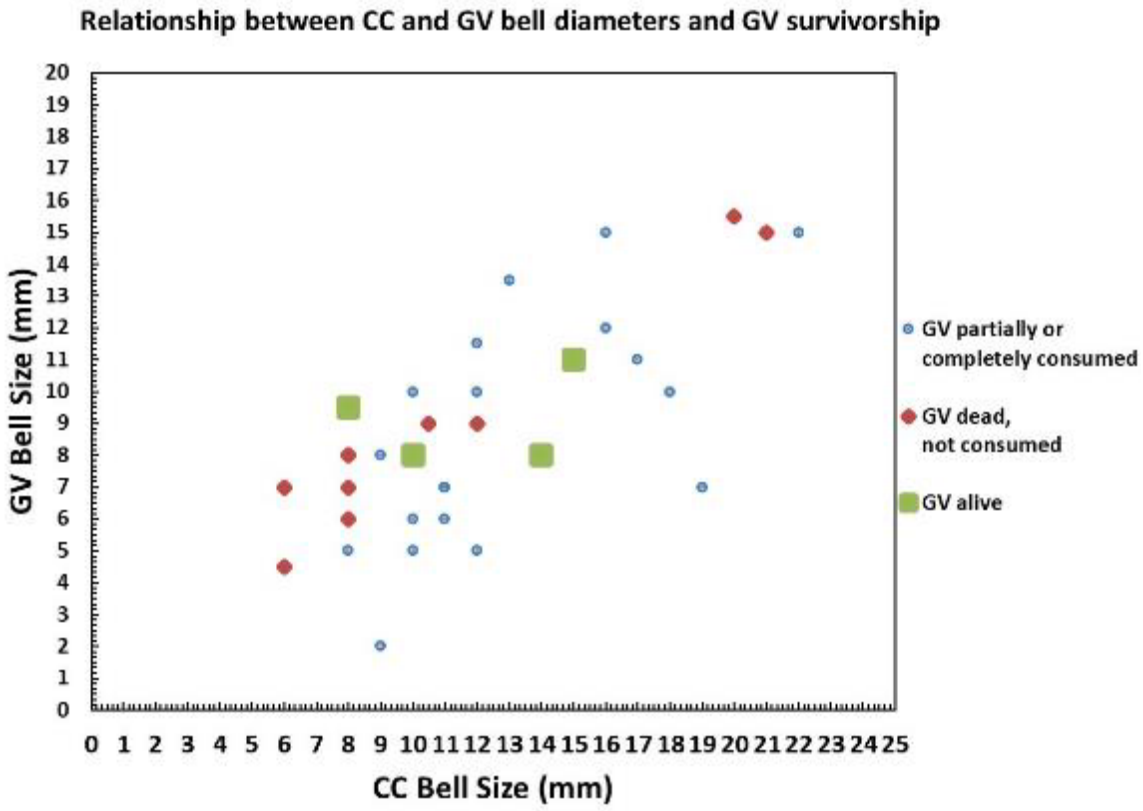

Figure 27. Relationship between the bell diameter of *Chrysaora chesapeakei* (CC) and *Gonionemus vertens* (GV) for experimental feeding trials evaluating the potential minimum size of *C. chesapeakei* to kill and consume *G. vertens*.

Nudibranch Predation of *G. vertens*

In 2020, while sampling the North Wildwood population of *G. vertens*, several large nudibranchs were collected among the algae as well. The nudibranchs were identified as *Cuthona* sp., which are known to prey upon cnidarians and harvest stinging cells which are translocated to their cerata for use as protection. During the summer of 2020, several experimental 24-hour trials were conducted to evaluate whether these nudibranchs could act as a predatory control of *G. vertens*. 24-hour experiments were initiated with evaluations at 1 hour and 24 hours. Seven trials were completed using two *G. vertens* medusae and one *Cuthona* sp. Additionally, some trials included algae as substrate/protection. While these experiments are limited in scope, a few pieces of information emerged from these trials. First, *Cuthona* actively hunted *G. vertens*, but focused on consuming tentacles (Fig. 28), possibly to obtain nematocysts. Secondly, smaller *G. vertens* were more vulnerable to attack and after 24 hours, they were often fully consumed (Fig. 29). Lastly, while the algae act as substrate and habitat for both *G. vertens* and *Cuthona* sp., the addition of algae into the experiments increased the rate of capture of *G. vertens*. Perhaps the behavior of 'clinging' to the substrate actually allowed *Cuthona* sp. to attack the *G. vertens* with greater efficiency as the clinging jellyfish was encumbered

in the structure and attacks were more successful. However, greater experimental work needs to be done to confirm these observations.

Figure 28. Predation experiment showing a nudibranch attacking a *G. vertens* medusa after consuming numerous tentacles from the other individual in the trial.

Figure 29. Experimental feeding trials demonstrating capture of *G. vertens* by *Cuthona* sp. as both use branching filamentous algae as structure. The smaller *G. vertens* medusa from the right panel was fully consumed after 24h.

Food Web and Stable Isotope Analysis

During field collections of *G. vertens*, it was common to encounter individuals in the process of capturing and consuming prey (Fig. 30).

Figure 30. Field collected *G. vertens* with successful capture of unknown fish (left) and *Apeltes quadratus* (Stickleback, right).

After these observations and literature review, laboratory studies were conducted to evaluate feeding preferences and consumption of potential crustacean prey. *G. vertens*

were offered either amphipods or the isopod, *Idotea balthica*, as potential prey in 24-hour trials. Results indicated that *I. balthica* were rarely fed upon (1 individual consumed out of 25 experimental trials), although isopods have been observed in the gastrovascular pouch of *G. vertens* collected in the field. Experiments using amphipods to evaluate the consumption and predation potential of *G. vertens* showed a dramatically different outcome. Results demonstrated *G. vertens* active predation and consumption of amphipods, but they showed a preference for smaller amphipods (Fig. 31) and had a more difficult time-consuming larger amphipods (Fig. 32).

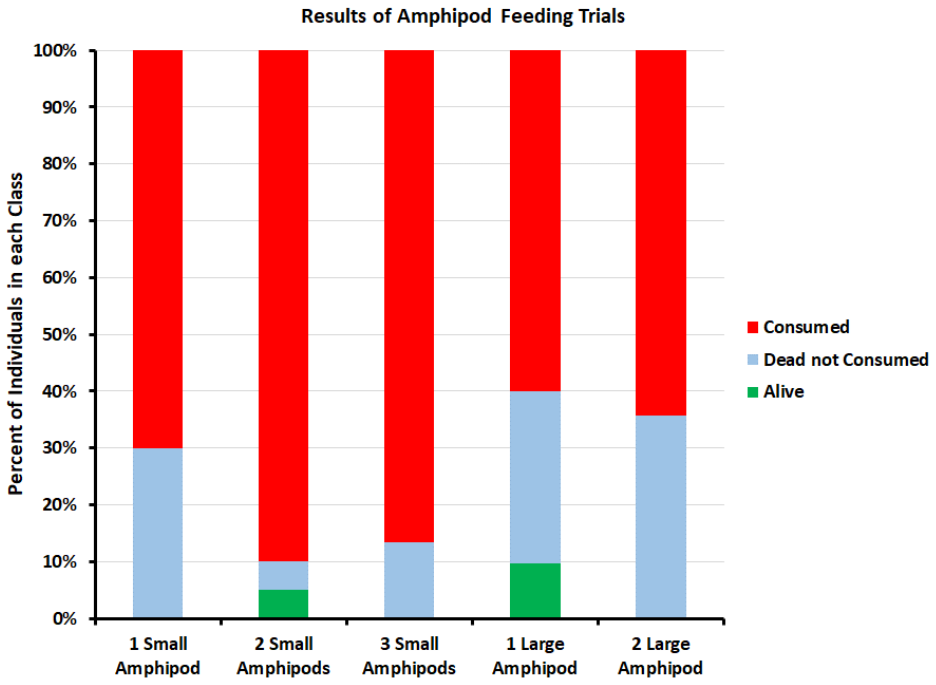

Figure 31. Summary results from 24h experimental feeding trials (N = 120) between one *G. vertens* medusa and single or multiple amphipod prey offered.

Figure 32. Image of a *G. vertens* medusa attempting to capture and consume a large amphipod (*Gammarus* sp.) during experimental predation experiments.

Stable Isotope (SI) Analysis

The graphical analysis of SI data obtained from these samples provides a broad overview of the food web in the coastal systems where the samples were collected and can be used to understand the general trophic position of organismal groups. For instance, $\delta^{15}N$ values of fishes were generally the highest, which suggests that they occupy higher trophic levels in these communities (Fig. 33). Since their values generally exceeded *G. vertens*, it is unlikely that the collected individuals used for the analysis received substantial trophic resources from fish, although *G. vertens* have been collected in the field with fish in their gastrovascular cavity (Bologna unpublished data).

To obtain a clearer picture of *G. vertens'* specific role, *G. vertens* data were plotted along with the isotopic data of suspected prey groups, based on field observations and laboratory tests (Figs. 30-31, Bologna unpublished data). These groups include the amphipods, copepods, isopods, and fish. General patterns emerge from these analyses that elucidate trophic interactions. For instance, the NJ and CT clinging jellyfish's ^{13}C signatures correlate with those of amphipods and copepods, and its ^{15}N signature is slightly more enriched (2-4‰) than that of amphipods and copepods, which suggests that amphipods and copepods are indeed important food sources for *G. vertens*, but there may be mixing of diets from organisms that were not sampled. For samples from Rhode Island, their carbon and nitrogen signatures are more similar to the isopods sampled (e.g., *Idotea balthica*, *Erichsonella sp.*) suggesting potential differences in trophic pathways, but this needs greater refinement.

Interestingly, there are differences in ^{15}N and ^{13}C fractionation rates of *G. vertens* from different locations. The $\delta^{15}N$ values of Rhode Island medusae are the lowest (mean=9.22‰), while they increase in the Connecticut samples (mean=10.25‰) and are even higher in the New Jersey samples (mean=12.45‰) (Fig. 32). The $\delta^{13}C$ values of Rhode Island medusae (mean=-13.28‰) are more enriched than the Connecticut or New Jersey samples (means=-17.1‰ and -17.87‰, respectively). Our analyses also included two dominant primary producers including the vascular plant *Z. marina* (eelgrass) and the green algae *Ulva* sp. Based on these data, both NJ and CT populations show strong carbon similarities with Ulva, while RI signatures are in between *Ulva* and *Z. marina*. Both of these primary producers are present in each of the three locations, but *Ulva* has been identified as a critical habitat for *G. vertens* in New Jersey when *Z. marina* is not present (e.g., Shrewsbury River, North Wildwood). As such, the observed differences in $\delta^{13}C$ among populations may indicate the primary trophic pathways of potential prey among sites differ and that RI *G. vertens* receive greater trophic energy from *Z. marina* pathways.

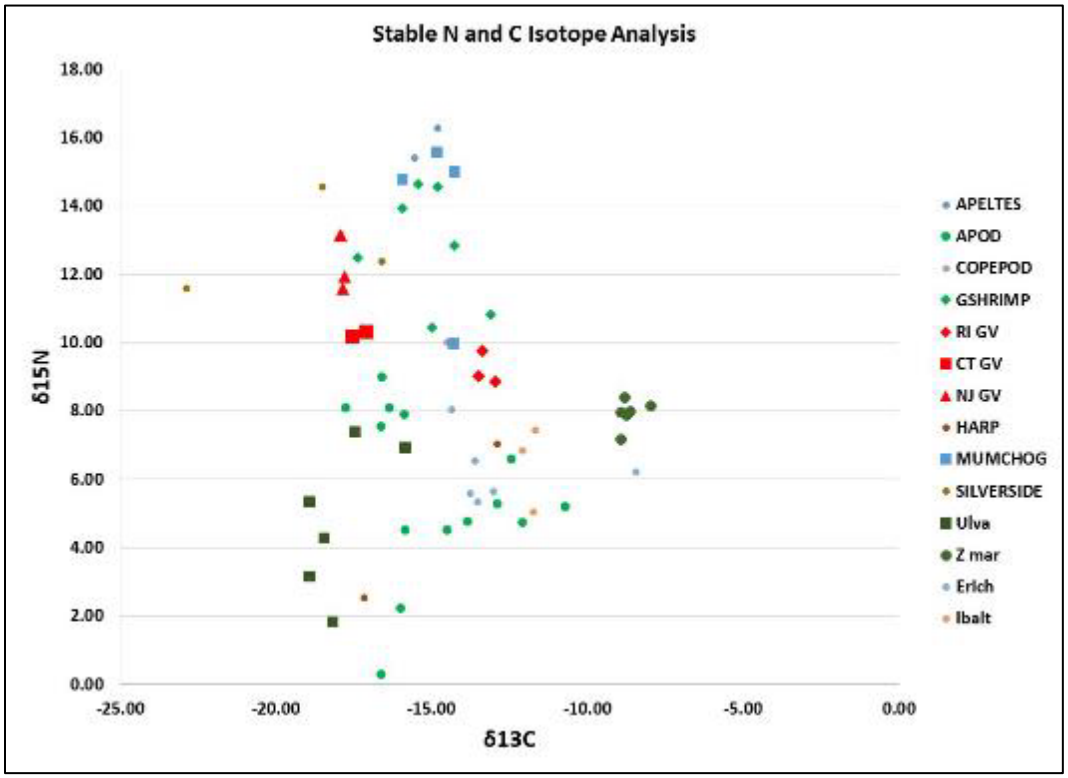

Figure 33. Stable Carbon and Nitrogen Isotope ratios of estuarine organisms collected during the collection of *G. vertens*. *G. vertens* is identified in red symbols and is separated by the three populations where samples were collected including Barnegat Bay (NJ GV), Mumford Cove (CT GV) and Potter Pond (RI GV). Figure legend abbreviations: APOD = Amphipods, GSHRIMP = Grass Shrimp, HARP = Harpactacoid Copepods, MUMCHOG = Mummichog, Z mar = *Zostera marina* (eelgrass), Erich = *Erichsonella* sp., Ibalt = *Idotea balthica*.

Assessing in-field medusa population growth rates

In 2019, collection of medusae began 5/16/19 in the Metedeconk River, Barnegat Bay, which was the site that was sampled consistently. Collections continued at this site until 7/16/19 and included a total of 1455 medusae (Table 20). The average sizes of medusae were indicative of the timing of the bloom and the relative age of the medusae sampled. For instance, the 296 medusae collected on 5/16/19 had an average bell diameter of 3.61 mm, indicating recent emergence, and subsequent samples were larger, indicating growth and a diversifying age distribution throughout the summer until populations began to decline in abundance after 6/19/19 (Table 20). While relative abundance showed declines during this time frame, the presence of very small medusa (<3mm) indicates that medusa were still being produced by polyps into July, even as the population was in severe decline. This suggests that thermal tolerance of polyps may differ than those of the medusa.

Table 20. Population demographics of repeated *G. vertens* collections from the Metedeconk River site in 2019. Abbreviations: BD = Bell Diameter, N = Number of individuals collected and measured.

Collection Date	Minimum BD (mm)	Maximum BD (mm)	Average BD (mm)	STDEV	N
5/16/2019	0.7	13	3.61	2.21	296
5/22/2019	1.5	14.5	6.1	2.64	92
6/4/2019	1	15	6.67	2.79	203
6/19/2019	2	18	9.46	2.68	441
6/26/2019	4	16	9.29	2.61	203
7/2/2019	3	16	9.49	2.94	144
7/10/2019	3	16	8.67	2.54	51
7/16/2019	5	14	9.66	2.28	25

Assessing polyp asexual growth rates

Results from the field and laboratory research demonstrate that the bloom of clinging jellyfish is limited in scope for New Jersey ranging from May to mid-July. For any of the identified populations throughout the state to maintain themselves, a large and viable population of polyps is needed. While JADs had revealed their presence in the Shrewsbury River (Fig. 5), sampling of various natural substrates (e.g., rocks, shells) in 2016 -2018 resulted in a lack of polyp presence. In April of 2019, algae was collected from the mouth of the Metedeconk River where dense populations of *G. vertens* were observed in 2018. Algae were evaluated in the lab, and several polyps were identified attached to branching algae (Fig. 34).

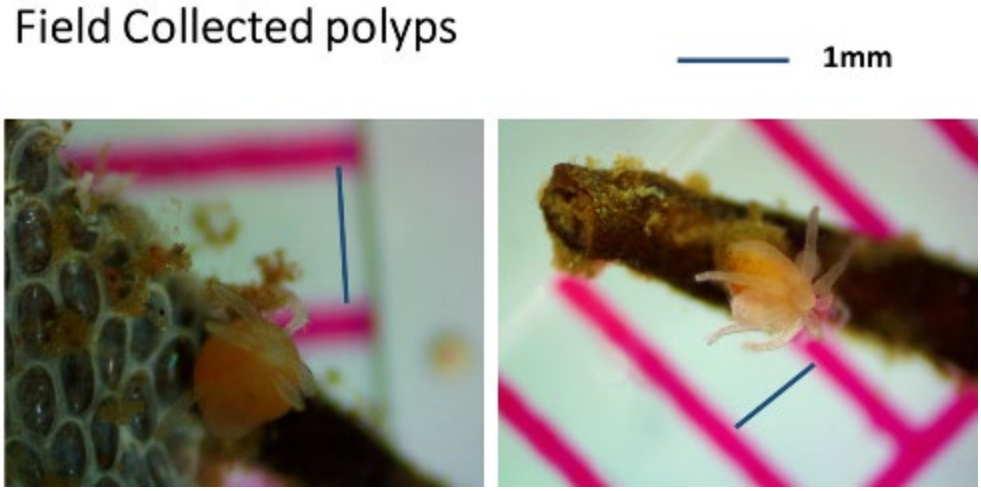

Figure 34. Identified *G. vertens* polyps from field collected algae in April 2019.

Initial attempts to induce polyp reproduction were unsuccessful, but the large number of adults collected during the following months ultimately yielded sufficient polyps from adult spawning in the tanks, which settled on glass plates (3.5" x 4" = 90.32cm^2). The first observations of polyps occurred in December of 2019, suggesting a relatively slow development of larvae transforming into fully developed polyps. During January and February of 2020, a series of growth chambers were established (20ºC, 20 ppt) to assess the asexual reproductive capacity of polyps and to evaluate general polyp life history in the laboratory. Observation of polyps led to the creation of a standardized developmental stage criteria to evaluate growth and asexual reproductive capacity of polyps (see Fig. 4).

These criteria include:

1) '**Adult'** defined as larger and robust individuals possessing at least 4 tentacles;

2) **Polyps SB** (Stolon Bud) defined as polyps demonstrating the development of one or more stolon/frustule buds on their side;

3) **Polyps LS** (Long Stolon) defined as adult polyps demonstrating the development of one or more elongated stolon/frustule buds on their side;

4) **Polyps MB** (Medusa Bud) defined as polyps generating medusa for release to complete the sexual life cycle of *G. vertens*; and

5) **D Stage** (Developing or Diminutive) defined as very small polyps generally possessing fewer than 4 tentacles. This category is a mix of polyps developing/transitioning from the asexual frustule stage generated from mobile frustules generated by other polyps, but also includes very small individuals potentially budded asexually from adults or seemingly are regenerated from former polyp tissue masses.

Plates were sampled weekly and all polyps were identified and categorized based on these criteria. Results indicate that there was a substantial decline in polyps from March to April, but this drop was likely a COVID-19 artifact because feeding and sampling were

limited during this time (Fig. 35). However, after this period, rapid population growth began and a larger proportion of the population demonstrated high levels of asexual reproduction. In fact, medusa buds were being generated consistently during this time, despite only a few individuals choosing this reproductive pathway (Fig. 35). When we converted average plate density to polyps/m^2, polyp densities ranged from about 2,000 – 9,000 m^{-2}. Given these results in the laboratory, *G. vertens* demonstrates substantial asexual reproduction through the development of budding frustules, but only a few individual actively produce medusa. Additionally, those polyps producing medusae generally only generate a single medusa, unlike scyphozoans, which create multiple medusae from this asexual reproductive event. When the data are standardized, this translates to a range of 0-47 polyps m^{-2} surface area generating medusae. These values probably underestimate field conditions and there is substantial surface area associated with the eelgrass and the branching algae adults and polyps seem to be using. Regardless, the greater reproductive efforts appear to favor more polyps than adults, meaning that areas where adult presence is high, there must be a correspondingly substantial polyp population. Unfortunately, this has been difficult or impossible to document in the field.

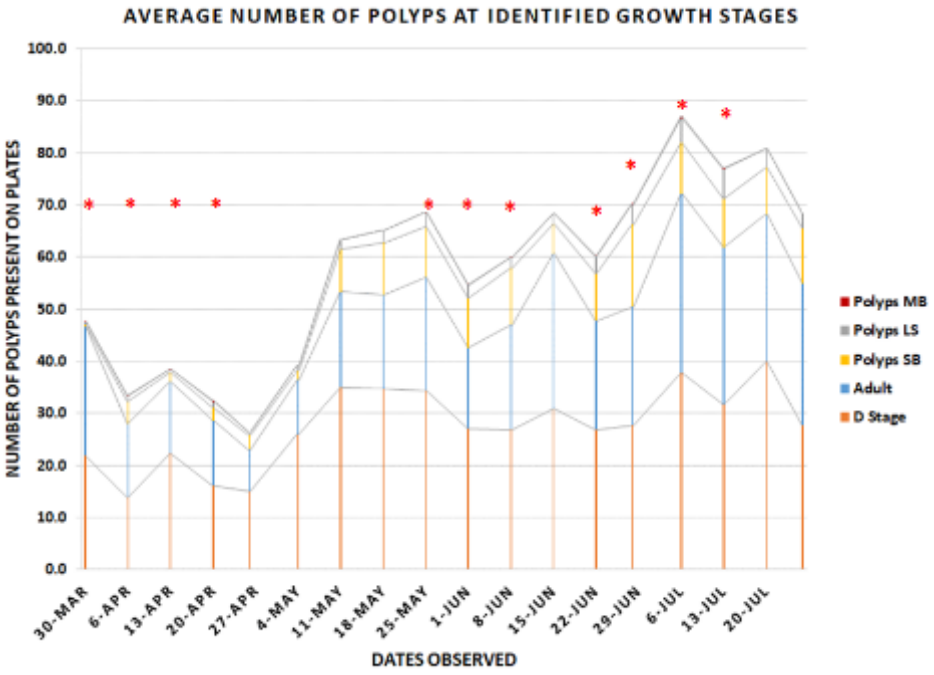

Figure 35. Average number of polyps identified from large growing plates from laboratory experiments based on identification categories (Figure 4). Red * above dates indicate that some polyps were identified as producing medusae (Polyps MB).

Conclusions

After five years of active research on trying to understand this invasive species, several key points are evident. Based on the genetic analysis, the population which has invaded New Jersey shares strong similarities to Mediterranean populations and not those in Massachusetts, where the initial observations of this non-native species were made over a century ago. Therefore, it appears that a second invasion occurred which introduced *G. vertens* to New Jersey. It remains to be known when and how this invasion occurred, as well as the regional spread of this invasion. Second, the seasonal decline in the populations within the region is strongly mediated by temperature. In general, populations from NJ, CT, RI, and MA show a thermal tolerance to water temperatures of 26ºC, but when temperatures exceed 28ºC, adults become stressed and die within 96 hours under laboratory conditions (?). In New Jersey, the back bay waters usually reach this threshold in late June and early July, so the bloom of *G. vertens* declines and recreational waters become safer for humans. However, in other more northerly populations, these thermal thresholds may not be met during the summer and possess a potential human threat throughout the summer. Unfortunately, the North Wildwood population in New Jersey seems to have adapted to greater temperatures and shows a higher thermal limit of 30ºC. It is unknown whether other NJ populations will show this greater thermal acclimation, but if they do then a greater risk of human interactions may occur, and continued monitoring and research will be needed to ensure public safety.

Several other key findings of this report point to the need to continue to monitor and investigate this species. Like many invasive species, we are just beginning to understand how this hydrozoan may fit into local food webs. Traditionally, it was accepted that *G. vertens* was predominantly a micro-zooplankton feeder. However, our field observations, laboratory experiments, and stable isotope work have shown that their diet is far more complicated. If their trophic status includes larger invertebrates like amphipods, as well as fish, then they may impact fish species through ingestion of fish larvae and act as competitors for both zooplankton and other food resources. We saw differences in stable isotope ratios among populations from RI, CT, and NJ, with New Jersey showing a much higher trophic placement (e.g., feeding on higher trophic organisms) than either of the other two locations. This might suggest that *G. vertens* in New Jersey could have an impact on species using the coastal bays in late spring to early summer as nursery grounds. Additionally, the diminutive size and cryptic nature of the polyp stage of *G. vertens* leave numerous unanswered questions. Where do polyps exist in the field and how do they survive across the winter? Do polyps have a thermal limit like the adult medusae do, or are they capable of continuous asexual reproduction throughout the summer? How might polyps be redistributed in an area, given that they occur in drift algae? What are the key triggers for population blooms to develop from polyps? It is our intention to continue to evaluate these questions in the future and to work with the New Jersey Department of Environmental Protection to monitor and assess populations.

Recommendations for Future Monitoring and Research

Several key findings from this research identify critical monitoring needs. Specifically, the maximum thermal limit for the adult medusae who pose a human health hazard appears to be between 28-30ºC for 96 hours (4 days). Continuous monitoring of water temperature during the summer when medusae are present can be an easy and cost-efficient method to ascertain risk to the general public. However, since there may be latitudinal variations, we would recommend that the benchmark be placed at 30ºC to ensure public safety. Additionally, physical sampling through dip-nets, beam trawls, or seine nets should also be employed to assess the relative abundance of *G. vertens* among the submerged aquatic vegetation (eelgrass and macroalgae) in known locations in late May and early June. If the relative population abundance is high, expanded surveys of the region would be warranted to monitor the potential spread of this invasive species. Lastly, use of eDNA techniques could prove useful for water samples collected in early June when adult populations are beginning to peak. Sufficiently large water samples or large volumes of filtered water could serve to collect sloughed tissue, gametes, and larvae of *G. vertens* from coastal estuaries. Pairing these samples with either large scale sequencing analyses or targeted gene amplification (e.g., 16S, CO1) with primers designed specifically for cnidarians and/or *G. vertens* specific could provide valuable information on potential spreading vectors before the first adult medusae are captured and identified. Since the State of New Jersey routinely collects water quality samples through the Bureau of Marine Water Monitoring, it may be possible to add specific collections designated for eDNA analysis to evaluate them for the presence of *G. vertens*. These samples could be analyzed by the NJDEP or partner Universities equipped and experienced to evaluate them.

The results from this project also point to some critical research needs which should continue to be evaluated. These research topics include identifying and evaluating the venoms produced by *G. vertens* to truly evaluate their threat to public health. While no confirmed deaths from *G. vertens* stings are known from the United States, patients seeking medical treatment display several symptoms, which are often localized and described as extremely painful. Some symptoms could include respiratory and/or neurological symptoms, however in more sensitive individuals, these can be as severe (e.g., liver and/or kidney failure). If these individuals did not receive medical treatment, then it is possible that their conditions could become worse, which would then place a greater emphasis on public safety if populations continue to grow and spread. Several other areas of research should continue to be explored and include an evaluation of where *G. vertens* fits into the food web and whether they pose a risk to commercially or recreationally important species. Since they reside in SAV, that puts them in direct contact with many larval fish and crustaceans, which are critical for coastal fisheries. However, our understanding of trophic interactions is very limited currently. Lastly, locating and identifying the polyp stage of *G. vertens* in the field has been incredibly difficult to their diminutive size. Just like many other jellyfish species, the polyp phase is the life stage that survives the winter and creates future blooms. To truly understand and potentially control outbreaks of this species, we must understand where they occur in the field. Additionally, this information, coupled with laboratory investigations into growth, asexual reproduction, and production of adult medusa, would allow us to evaluate the potential strength of future blooms based on field polyp abundance and predicted medusa production rates. This would subsequently allow

the NJDEP and partner institutions to be proactive in sampling to inform the public of any potential threats.

Production Accomplishments from Research

Results from the research funding on this project has resulted in numerous scientific presentations, publications, graduate thesis research support, and student research opportunities. Additionally, numerous news and public education avenues occurred during this timeframe discussing the presence and prevalence of *G. vertens* in coastal waters.

Publications

Gaynor, J.J., Bologna, P.A., Restaino, D.J. and Barry, C., 2016. First occurrence of the invasive hydrozoan *Gonionemus vertens* A. Agassiz, 1862 (Cnidaria: Hydrozoa) in New Jersey, USA.

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Bliese, A.D., 2020. Transcriptomic Identification of Venom Candidates in the Clinging Jellyfish *Gonionemus vertens*. M.S. Thesis, Montclair State University.

Graduate Thesis Students Conducting Research Under this Project:

Current Thesis Students

Booker, V.	MS	Marine Biology & Coastal Science	

Completed Thesis Advisees

Bleise, A.	MS	Marine Biology & Coastal Science	2020
Rigby, M.	MS	Marine Biology & Coastal Science	2020
Orologas, V.A.,	MS	Biology	2020
Restaino, D.	Ph.D.	Environmental Management	2018
Cropley, Z.	MS	Biology	2017
Khanal, A.	MS	Biology	2017

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Appendix 1

Table A-1. List of PCR Primers

Locus	Primer Sequence (5' to 3')	Reference
16S	TCGACTGTTTACCAAAAACATAGC ACGGAATGAACTCAAATCATGTAAG	Bridge et al. 1995
COI	GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994