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Environmental and Trophic Factors that Influence the Prevalence of *Gonionemus vertens* in the Northeastern United States

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Abstract

Gonionemus vertens, often referred to as the clinging jellyfish, is an invasive hydrozoan first documented in 2016 in New Jersey. Populations of the adult medusa stage bloom in May and continue to persist through the middle of July, during which time it is of particular concern from a public health standpoint. *G. vertens* ‘clings’ to sea grasses and other submerged aquatic vegetation in shallow coastal waters, which are highly utilized for recreational activities, and its sting is highly toxic, which can hospitalize victims for days with both physical and psychiatric symptoms. Encounters with highly toxic *G. vertens* are, unfortunately, becoming more frequent in areas such as the estuaries of Barnegat Bay, New Jersey and Potter Pond, Rhode Island; which could be a deterrent to those visiting popular beaches and shoals for recreational purposes.

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, water temperatures exceeding 28°C, or a combination of both. Medusae were collected from various locations in Barnegat Bay and Potter Pond during several field surveys in the summers of 2018 and 2019, as well as from Cape May, NJ and Mumford Cove, CT in 2019. Medusae were maintained in holding tanks in the laboratory under stable temperature (~21-22°C) 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 22°C-32°C. Significant mortality of *G. vertens* from all locations occurred when temperatures met or exceeded 28°C for 72h, suggesting that *G. vertens*’ thermal tolerance is 28°C and that populations in the field are at least partially controlled by water temperatures. Understanding the thermal decline in areas where predatory species are not present is critical for managing public awareness

of this hazard. Monitoring of high human use areas for thermal minimums can reduce potential encounters with this highly venomous species. In Barnegat Bay, for instance, water temperatures may exceed 28°C by the end of June into the beginning of July, which was confirmed by deploying temperature recorders.

Predation experiments were also conducted to confirm that *Chrysaora chesapeakei* is a predator of *G. vertens* and to determine the bell diameter ratio at which they could be killed or consumed. *C. chesapeakei* is indeed capable of killing *G. vertens*, even at ratios of 1.19:1, and partially or completely consumed *G. vertens* when bell ratios approached 1.67:1. Therefore, it seems that *C. chesapeakei* likely also plays a role in the decline of *G. vertens* populations in Barnegat Bay, which decline throughout the month of July.

Lastly, *G. vertens* medusae were sent to the Cornell Isotope Laboratory along with samples of potential prey items and plants from the same areas in which the medusae were collected for Stable Isotope Analysis (SIA). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ data obtained from SIA were plotted to determine the trophic position of these organismal groups, which confirmed that amphipods and copepods are likely very important prey items for *G. vertens*.

MONTCLAIR STATE UNIVERSITY

Environmental and trophic factors that influence the prevalence of *Gónionemus vertens* in the northeastern United States

By

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A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

January 2020

College of Science and Mathematics

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**ENVIRONMENTAL AND TROPHIC FACTORS THAT
INFLUENCE THE PREVALENCE OF *Gonionemus vertens* IN
THE NORTHEASTERN UNITED STATES**

A THESIS

Submitted in partial fulfillment of the requirements
of Master of Science

by

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Montclair, NJ

January 2020

Acknowledgements

I would like to thank the New Jersey Department of Environmental Protection for funding support and the Rhode Island Department of Environmental Management for assistance with Rhode Island *G. vertens* collections and temperature data collection.

This work would not have been possible without the help of numerous colleagues, undergraduate students, and Weston scholars who assisted with field collections in New Jersey and helped monitor laboratory experiments. I am deeply grateful for all of their help.

Travel funding and scholarship funds provided by Paramus Catholic High School helped me fund this work and complete my studies.

Finally, a very special thank you to Dr. Bologna for all of his support, guidance, revisions, and help with this project and its data analysis, as well as to my committee members, Dr. Gaynor and Dr. Hazard, for their review and support throughout this process. I greatly appreciate the help and support of these dedicated Montclair State University faculty members.

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Life History of *Gonionemus vertens*

Life Cycle of *Gonionemus vertens*

Encounters with *G. vertens* are likely due to its life-cycle and preferred habitat. Medusae live in shallow coastal waters and are considered semi-benthic, since they are mostly sedentary and cling to algae and seagrass near the floor throughout the day (Mills, 1993). These shallow waters put them in the same location as recreational boaters, swimmers, and those working in aquaculture. The medusae are dioecious (Govindarajan et al., 2016), and once mature, prolifically shed eggs or sperm daily until the end of their lives (Mills, 1993). Female *G. vertens* have been observed shedding 50-75,000 eggs per year (Bakker, 1980). About 12 hours after an egg is fertilized, it hatches to become a ciliated planula, which is the larval stage (Todd et al., 1966).

The larval stage persists for approximately one-three weeks, during which time the planula moves across the bottom until it stands upright and attaches itself to the substrate. At this point, the cilia are lost, the larva elongates, and develops endoderm cells in its innermost layer (Uchida, 1976). The newly formed polyp is around 0.5 mm tall and initially has two tentacles, but it soon enlarges and grows up to 2 mm and also produces more tentacles. These polyps often attach to seaweed, seagrasses, oyster shells or mussel shells and are surrounded by a gelatinous sheath (Bakker, 1980), which can become covered in detritus and other algae (Edwards, 1976), which makes their identification extremely difficult. The polyp often feeds on nematodes, but not copepods, amphipods, mites or ciliates, which are common food for the medusae (Bakker, 1980).

Polyps may persist for years until conditions are appropriate for medusa formation. For example, *G. vertens* medusae were found in 1917 in an aquarium in Vienna that was filled with water from the Adriatic in 1910, even though the water had not been changed in the intervening years (Edwards, 1976). Medusa production seems to be favored when water temperatures exceed 17-18 °C, at which point they form, detach, and grow until they are sexually mature (Bakker, 1980, Marchessaux et al., 2017). In the Northern Hemisphere, the medusae often appear at the end of April or the beginning of May and typically persist through late July, although sometimes they survive slightly longer depending on environmental conditions (Mills, 1993).

Temperature may not be the only determining factor in medusa production, since medusa in the lab have been produced at temperatures as low as 6°C, even though they were unhealthy and did not survive long (Edwards, 1976). When produced at 9-11°C, medusae were slow to develop and never attained a healthy size. In addition to temperature cues, regular availability of food may be an important trigger for medusa feeding, since medusa production at these lower temperatures was correlated to increased feeding (Edwards, 1976). In the field, synchronous population blooms are most likely adaptive since this may increase the chance of successful fertilization (Bakker, 1980).

Fascinatingly, the sexual life cycle of *G. vertens* is only half of the story of its reproduction. In addition to medusa, polyps are capable of producing frustules, which are simply planulae without cilia. These frustules detach from the main polyp and move, eventually settling and becoming newly cloned polyps. Alternatively, polyps can reduce to an immobile form with stunted tentacles, essentially becoming a jelly cyst. Medusa can develop from these cysts,

although they have fewer tentacles and they do not have statocysts, which are present in medusa produced from the typical asexual reproductive cycle. However, they soon grow more tentacles and statocysts, becoming functional cloned medusae. In addition, frustules can also develop from these cysts, which can liberate themselves, migrate, and develop into polyps. Cysts, therefore, can be used to produce both the sexual and asexual forms of *G. vertens*, which is rare among cyst-forming organisms (Uchida, 1976). It is unclear exactly how long *G. vertens* cysts and polyps can survive, or whether they are able to survive conditions that would cause mortality in medusa. However, it is likely that they are able to persist for much longer periods of time, as shown by the historical records of *G. vertens* appearances and their introduction across arctic and tropical regions, which are natural barriers to medusa range expansion (Edwards, 1976). Clearly, the reproductive cycles of *G. vertens* are complex and mysterious enough to play a role in cryptic invasions of new locations.

Anatomy, Morphology, and Behavior

When *G. vertens* is found, it is almost always discovered in its medusa form. The typical lifespan of medusae is approximately 3 months, during which time they may reach a bell size of 25-30 mm in some regions like Lake Grevelingen, Netherlands (Bakker, 1980), while topping out at 20 mm in other areas, such as the northeastern Pacific off the San Juan Archipelago (Mills, 1993). Typically, four radial canals are present in a cross pattern underneath the hemispherical bell, although three and five canals have been found in a small proportion of medusae (Marchessaux et al., 2017). Each medusa has approximately 60 tentacles evenly distributed around the bell, between which the aforementioned statocysts, or otocysts, may occur singly or doubly, although occasionally tentacles exist without statocysts alongside them (Thomas, 1921).

Statocysts are adaptations present in many aquatic organisms, which help them to maintain equilibrium by sensing the location of statoliths inside them. The statocysts of *G. vertens* are unique and complex, and are most likely important for their typical mode of feeding.

G. vertens statocysts consist of a primary vesicle, which is filled with fluid, to protect a “spheroid,” which is suspended by a pedicel inside the primary vesicle. Inside the spheroid lies a secondary vesicle, which encloses the statolith, a round calcium deposit that moves freely within the vesicle in order to help sense the *G. vertens*’ position. However, unlike other animals with statocysts, there are no sensory hairs or ridges within the secondary vesicle. *G. vertens* whose primary vesicle has been damaged display no changes in behavior, which is likely due to the fact that it may simply be a protective organ for the more important secondary vesicle (Thomas, 1921). Since statocysts are typically used for equilibrium and sensing position, they may be important for the feeding behavior displayed by *G. vertens*.

During the day, *G. vertens* typically remain sedentary, clinging to seagrass or algae in shallow waters. This clinging behavior is the root of their colloquial name, and is performed by utilizing adhesive pads, which are simply modified cells located along the length of their tentacles. When light is low, such as at nighttime or when the weather is overcast, medusae swim up through the water column and flip over, extending their tentacles in order to ensnare zooplankton (Govindarajan et al., 2016). Typical prey include copepods (Mills, 1993), gastrotrichs, ciliates, amphipods like *Gammarus sp.* and the occasional isopod like *Idotea sp.* (Bakker, 1980). Observations in the field have also noted fish larvae (Todd et al., 1966) and even larger fish, such as a three-spined stickleback, in the *G. vertens* gut (Carman et al., 2019,

Bologna unpublished data). The latter seems to be an improbable food source, however, and may have been brought into close proximity to the medusa during the collecting process.

Prey and Predators

G. vertens blooms and population increases may be correlated with increases in its prey populations. A 1992 study of copepod communities in the Navesink and Shrewsbury estuaries, which also analyzed data from the same locations in 1962, 1966, 1972, and 1976, found that copepod populations increase through the late spring and early summer, peaking in mid June (Shaheen and Steimle, 1995), just like *G. vertens* populations. The majority of copepods were calanoid, especially in the genus *Acartia*, and the community composition maintained relatively the same level of diversity and abundance over the 30 year period. There were, however, significant differences following stochastic events such as tropical storm Agnes in 1972, which severely depleted *Acartia* populations and also caused numbers of other genera to decline. As a result of Agnes, large changes to salinity and sedimentation occurred which likely affected copepod populations directly (Shaheen and Steimle, 1995).

The authors focused on copepod populations because they provide an important food source for juvenile winter flounder and striped bass, both of which are important species in the Sandy Hook Bay fisheries. However, as previously mentioned, seasonal zooplankton population blooms are also of special importance for *G. vertens*, as the polyps may time the release of medusae to match the availability of food (Edwards, 1976, Bakker, 1980). Therefore, knowledge of the timing of zooplankton population increases, along with identifiable environmental cues, may help to predict *G. vertens* blooms.

The timing of blooms and behavior of medusae are important information for public health officials, but of equal importance is when and why *G. vertens* populations start to decline and disappear. In order to describe the timing of population declines, one study in the northeastern Pacific observed predation, wound healing, and senescence in various hydromedusae species, including the clinging jellyfish. Just as medusa blooms are correlated with plankton blooms and warming temperatures, population declines and termination are associated with seasonal or ecological changes, in addition to natural senescence. However, *G. vertens* populations in the study area (San Juan Archipelago, Washington) were found to be controlled by senescence alone. They were the only species not severely affected by “associates that damage individual medusa” through predation or parasitism such as hyperiid amphipods, *Peachia anemone* larvae, or other macroscopic predators and parasites (Mills, 1993). Therefore, as *G. vertens* nears the end of its approximately three month lifespan, the population starts to decline. In other words, if blooms in the Northern Hemisphere occur in April, by mid-late July the population should be on the decline and starting to die off. This timeline has been supported frequently by field observations in both its native and invasive ranges (Bakker, 1980, Mills, 1993, Gaynor *et al.*, 2016, Marchessaux *et al.*, 2017, Carman *et al.*, 2019).

Recently, various predators of *G. vertens* including the sea nettle (*Chrysaora chesapeakei*), spider crabs (*Libinia dubia*), and aeolid nudibranchs have been identified, each of which may play a role in *G. vertens* population control. For instance, sea nettles are voracious predators of other gelatinous zooplankton and, with increased population density due to anthropogenic changes, became an apex predator in coastal estuaries that exerted top-down control of other medusa populations (Bologna *et al.*, 2017). However, Superstorm Sandy (2012)

caused drastic ecosystem changes that also caused a significant reduction in sea nettle populations. Removal of sea nettles relieved other gelatinous species from predatory pressures, and a 64% increase in gelatinous zooplankton species richness occurred (Bologna et al., 2018). It is likely that *G. vertens* medusae were one of the species that benefitted from the decline in sea nettles and that *C. chesapeakei* historically played a larger role in the control of *G. vertens* populations in New Jersey.

Other predators may play a role in the control of *G. vertens* medusa blooms, albeit to a lesser degree. For example, *L. dubia* (spider crab), which is native to the same eelgrass meadows in Massachusetts and Rhode Island that *G. vertens* inhabits, is an enthusiastic predator of the clinging jellyfish. However, laboratory trials demonstrated that spider crab mortality significantly increased when crabs consumed large amounts of *G. vertens* in 24 hours. For example, crab mortality was 100% when crabs consumed 30 clinging jellyfish within 24 hours (Carman et al., 2017). The researchers estimate that this experimental density of *G. vertens* is lower than natural densities, which means that increased *G. vertens* abundance could lead to crab declines in their native habitats.

If *G. vertens*/*L. dubia* encounters increase in these areas, there may be significant ecological impacts in these ecosystems. If spider crab populations decline, invasive green crab populations (*Carcinus sp.*) may be able to expand, especially since the green crab avoids *G. vertens* and will not consume them. Green crab population expansion may disrupt the native food web even further, since green crabs pull up and eat eelgrass shoots while foraging (Carman et al., 2017).

Alternative potential predators of cnidarians include various fish species, shrimp, scyphozoan jellyfish, lobster larvae, and nudibranchs (Sentz-Braconnot and Carre, 1966, Seapy, 1980, Heffernan and Hopkins, 1981, Brodeur et al., 1987, Purcell, 1991, Wakabayashi et al., 2012, Restaino, 2018), some of which may also prey on *G. vertens*. The only verified predatory relationship is by aeolid nudibranchs, which have the unique ability to consume cnidarian polyps and then transport their cnidoblasts to the cerata, where cnidocysts (stinging cells) are incorporated for the nudibranch's own defense. Molecular studies from nudibranchs in Barnegat Bay have identified general cnidarian DNA, as well as *C. chesapeakei* DNA in their cerata (Restaino, 2018) and laboratory experiments have confirmed that nudibranchs do in fact consume *G. vertens* medusa (Bologna unpublished data). Therefore, it is possible that nudibranchs could play a role in controlling *G. vertens* polyp abundance and subsequent medusa blooms, although to an unknown scale. Additionally, if nudibranchs consume *G. vertens* polyps and the DNA signature is identifiable, these data may help to identify where polyps have been introduced and have settled by analyzing nudibranch genetic data (Restaino, 2018). Polyps are extremely difficult to identify in the field, but nudibranchs are slightly easier to find, which may provide researchers with a tool to track *G. vertens* populations before medusa blooms occur, potentially years in advance.

Native and invasive ranges

Gonionemus vertens is currently distributed in many temperate coastal waters (Edwards, 1976) across the world, including the western Pacific, the northeastern Pacific, the northwestern and northeastern Atlantic, the Mediterranean Sea, and off the coast of Argentina (Bakker, 1980; Mills 1993; Rodriguez et al., 2014; Govindarajan et al., 2016; Gaynor et al., 2016; Marchessaux

et al., 2017). First identified in Woods Hole, Massachusetts, in 1894, *G. vertens* was frequently studied until the 1930s, when their population nearly disappeared due to a die-off of *Zostera marina* beds caused by an eelgrass wasting disease. During all of this time and until 1990, no stings were reported in the northwest Atlantic. However, the sting of the soon to be identified western Pacific form caused excruciating pain, respiratory problems, and paralysis (Govindarajan et al., 2016).

Although well known for its public health risk, the mode of introduction for *G. vertens* is a biogeographical puzzle, made even more complex by the possible existence of an alternate, less toxic form of the species. Since severe sting symptoms were not reported in the northwestern Atlantic until 1990 from Waquoit Bay, MA, but were reported in the western Pacific before this time, scientists originally classified the less venomous Atlantic form as *Gonionemus vertens murbachii* and the more venomous form from the western Pacific as *Gonionemus vertens vertens* (Govindarajan et al., 2016). Today, there is no absolute consensus on the taxonomy of *G. vertens* and its two forms; they could be two separate species, or they could be a single species with different phenotypes.

What is almost certain, however, is that *G. vertens* has been introduced to its invasive ranges by anthropogenic activities. For instance, the populations in Woods Hole (1894), Hadley Harbor, MA (1910), and Avery Point, CT (1915) all appeared suddenly, although each area had previous faunal surveys conducted (Edwards, 1976; Bakker, 1980; Govindarajan et al., 2016). These localized, sudden appearances are consistent with anthropogenic introduction, and at this point most likely came from Europe, not the Pacific, since the *G. vertens* were not extremely venomous (Edwards, 1976). One possible method for long distance invasions is that polyps

hitched a ride on bivalve shells that were attached to ship hulls or carried in ballast water, or even transported for aquaculture (Govindarajan et al., 2016). Its first introduction to an area may be difficult to detect since its asexual life stages are cryptic and near-impossible to spot (Rodriguez et al., 2014; Govindarajan et al., 2016). The natural spread of *G. vertens* between disparate regions is highly unlikely, due to the limited swimming ability and semi-benthic nature of medusae, although it is possible that *G. vertens* could be spread within a region via rafting (Thiel and Gutow, 2005).

In fact, hydrozoans are the most common groups of species that have been observed rafting. Both medusae and polyps could attach themselves to eelgrass, macroalgae, and plastics, and be carried by currents from one area to another. Some species are obligate rafters, while other species like *G. vertens* are considered facultative rafters since they are usually benthic in their normal range. Typically, rafting is an important method for maintaining coastal diversity, but it is undoubtedly also important for the distribution of invasive species after an initial anthropogenic introduction (Thiel and Gutow, 2005).

G. vertens is now well-documented and studied in the areas where it has been introduced, such as Martha's Vineyard, MA, Barnegat Bay, NJ, and southern California, USA, the Berre Lagoon, France, the coast of Argentina, Lake Grevelingen, Netherlands, and various other locations in Europe. All of these temperate locations are characterized by water temperature ranges of approximately 13-27°C, which varies by season, and a salinity of 18-29 ppt (Shaheen and Steimle, 1995; Marchessaux et al., 2017; Carman et al., 2019; World Sea Temperature, 2019). However, each introduction occurred at a different time and not all clinging jellyfish introduced are of the same venomous form.

Despite the fact that the initial discovery of *G. vertens* in Woods Hole was the relatively benign form, today the clinging jellyfish around Martha's Vineyard seems to be the highly venomous form, due to the severity of several stings reported in the area. *G. vertens* also seems to be expanding its range because, until recently, only one pond on the island had a population of *G. vertens*. In 2018, five out of eight ponds surveyed around Martha's Vineyard had populations, each of which was associated with eelgrass meadows (Carman et al., 2019). Fortunately, medusae were confined to eelgrass and were not found in areas with other vegetation such as macroalgae, and were also completely absent from ponds that had lower salinities. Salinity fluctuations are common in these coastal ponds, and should be investigated further as a potential limiting factor for future range expansion of *G. vertens*. Likewise, the viability of cysts and their resistance to desiccation and boat cleaning should be investigated, since boats are routinely transported over land from pond to pond, and could be a potential vector of transmission between ponds (Carman et al., 2019).

In New Jersey the first medusae were observed and collected in 2016, four years after the events of Superstorm Sandy and the subsequent diversification of gelatinous species in Barnegat Bay. DNA analysis of the 16S ribosomal subunit and the Cytochrome Oxidase I (COI) loci showed that NJ clinging jellyfish are very similar to those collected from the China Sea. These data, coupled with accounts of sting victims suffering excruciating pain and undergoing hospitalization for multiple days (unpublished data), show that the invasion in NJ is of the more toxic form of the species found in the western Pacific. In addition, the five samples analyzed were identical to each other, although collection sites were 20 miles apart, which means that they

were introduced at the same time to an area in Barnegat Bay, likely from the hull of a ship or ballast water, and then spread from there (Gaynor et al., 2016).

Similarly, *G. vertens* was first identified in the Berre Lagoon, France in 2016, and saw a population increase during 2017, which is likely due in part to the redevelopment of *Zostera* meadows and the stabilization of salinity levels after legislative attempts to rehabilitate the anthropogenically altered environment in the lagoon. Victims of the first stings in 2017 also suffered from extreme pain and one went into anaphylactic shock. It is hypothesized that this population of *G. vertens* was introduced through the transportation of shellfish from Japan, although they could have also been carried in ballast water (Marchessaux et al., 2017).

Ballast water is most often the culpable route of marine introductions (Carlton and Geller, 1993), which is why this method is considered most likely for the introduction of *G. vertens* to Argentina in September, 2008. 30 medusae were discovered in an aquarium containing samples from intertidal and subtidal benthic zones near an area close to one of Argentina's most important harbors, which receive shipments of both national and international cargo and is also a base for fishing vessels. Studies must continue in order to determine whether the clinging jellyfish has become established in this area, especially since the Buenos Aires coast has some of the most used recreational beaches in Argentina and beachgoers already have to worry about two other species of stinging hydromedusae. The presence of *G. vertens* in this region is thus of special importance from both a public health and an economic perspective (Rodriguez et al., 2014).

The introduction of *G. vertens* to Lake Grevelingen, Netherlands, presents an interesting story regarding the transport of *G. vertens* polyps. Lake Grevelingen is a shallow lake that was formed from the closure of a sea arm in 1971, which allowed *Zostera* meadows to increase their

coverage. Subsequently, amphipods, isopods, and other small animals such as fishes increased in abundance and in 1976, *G. vertens* medusae were first discovered. These clinging jellyfish were not distributed by ocean currents, since medusae are only found clinging to plant habitat in shallow areas of the lake that are disconnected from ocean input. However, it is hypothesized that polyps associated with shellfish could have been transported to the lake, specifically with Japanese oysters delivered via Portugal. Even if shells or shell gravel are deposited at greater depths, it is likely that attached polyps could survive and that frustules could be transported by ocean currents, allowing polyps in deeper waters to colonize nearby shallower areas (Bakker, 1980).

Polyp transport on oyster shells has also been proposed as the mechanism of dispersal responsible for *G. vertens* introductions across Europe. Oyster shells are a possible mechanism because they provide a hard substrate for polyp settling, in addition to diverse communities of epifauna on which polyps can feed. Also, oysters have been widely transported commercially for aquaculture and for research over the course of the past two centuries. For instance, historical records indicate that oyster studies were conducted at the Marine Station at Millport in the British Isles in the early 20th century, just before *G. vertens* popped up in the same location (Edwards, 1976).

Likewise, links can be drawn all across Europe to extensive oyster research and aquaculture during the late 19th to the early 20th century and the subsequent appearance of *G. vertens*. Some of these studies even managed to find polyps on oyster shells that had been transported to the location. Finally, ponds in Woods Hole were used for oyster research in the 1880s with oysters from Europe, just a decade before the less toxic form of *G. vertens* appeared

(Edwards, 1976). This theory lends credibility to the idea that the original clinging jellyfish described in Woods Hole came from the northeastern Atlantic and that the more recent and more toxic invasions to the northwestern Atlantic came from the western Pacific. At the very least, polyp transport is a likely method of dispersal and should be further investigated.

Polyp transport and subsequent dispersal and invasion of a new area is a likely mechanism for *G. vertens* range expansion, even if polyps are not attached to oyster shells. Occasionally, researchers balk at the idea that ships could carry a shallow water temperate species such as *G. vertens* medusa long distances, especially between oceans and across tropical or arctic barriers (Edwards, 1976). However, if polyps can survive for longer periods of time and at greater depths than the medusae, dispersal via shipping is possible. Additionally, polyps could have been attached to seaweed or eelgrass used for packing imported shellfish, or even to live oysters that Portuguese mariners brought back from journeys to the Pacific (Edwards, 1976). With the help of ocean currents and eelgrass rafts that carry frustules and polyps, *G. vertens* may be able to spread throughout a region, despite its relatively sedentary nature.

The history of *G. vertens* introductions is a tangled web with many possible pathways. Part of the difficulty lies in the uncertainty surrounding its taxonomy and the classification of the more toxic and less toxic forms; it is still unclear whether the two are separate species, or whether they are simply two different phenotypes whose toxicity is influenced by environmental conditions. One study compared COI sequences from mitochondria of *G. vertens* in the Atlantic to *G. vertens* from the Pacific with the goal of determining what, if any, genetic differences exist between regions in each ocean and between oceans. Results showed that haplotypes from the northwest Atlantic and northwest Pacific *G. vertens* are different from the northeast Pacific and

northeast Atlantic *G. vertens*, but further genetic studies are required to determine whether these divergences are deep enough to have caused speciation (Govindarajan et al., 2017).

Several invasion pathways have been proposed with these data, such as the traditional hypothesis that multiple introductions from the Pacific to the Atlantic occurred, first with the less toxic form, and later with the more toxic form. Alternatively, it is possible that the *Gonionemus* first found and described in the northwest Atlantic is what was originally described as *G. murbachii*, and is native to the northwest Atlantic, since it was not described in the Pacific for another two decades. In this scenario the northeast Pacific/Atlantic form is separate, which seems to be supported by the haplotype differences observed in COI loci (Govindarajan et al., 2017). However, the northwest Pacific was the first location for severe stings, which were not reported in the northwest Atlantic for several decades, which hints at a Pacific to Atlantic introduction.

Another theory views the original northwest Atlantic *G. vertens* as a different lineage of the same species found in the northwest Pacific and that there are more than two native but cryptic, lineages in both locations. Only one of the northwest Pacific lineages is highly toxic, which is the lineage that was likely introduced to the northwest Atlantic in the 1980s (Govindarajan et al., 2017). Current expansions and variations seen in populations along the northwest Atlantic are due to interbreeding between native and invasive lineages. The fact that there are unique haplotypes in both the northwest Atlantic and northwest Pacific, however, could be due to a Pacific to Atlantic trans-Arctic migration of *G. vertens* 3.5 million years ago (Vermeij, 1991). In order to better understand the invasion pathways of *G. vertens*, future studies must look more closely at various SNPs, especially between an expanded number of sampling

locations. Also, the genotypic and environmental factors that determine *G. vertens* toxicity should be examined, as should the environmental triggers of blooms. Since cysts of some species are known to last for decades, *G. vertens* populations may go undetected due to relatively dormant asexual populations, which could lead to misinterpretation of the origin of the species (Bouillon et al., 2004).

Materials and Methods

Gonionemus vertens Collections

G. vertens were collected during the months of June-July of 2018 and May-July 2019. Collection sites (Figure 1) in both years included several areas of Barnegat Bay, NJ, including the mouth of the Metedeconk River (40.05605°N, -74.06517°W) and Tices Shoal (39.8118°N, -74.1018°W). In 2019, *G. vertens* were observed and collected for the first time in a pond at North Wildwood, Cape May (39.010426°N, -74.819770°W). In both 2018 and 2019, *G. vertens* were collected from Potter Pond, Rhode Island (41.388195°N, -71.532684°W), and in 2019 *G. vertens* were collected from Mumford Cove, Connecticut (41.322533°N, -72.018764°W).

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 1-2m 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 (~21-22°C). 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 to each tank Monday-Friday, and adult *Artemia* were provided every two weeks between June and August 2019.

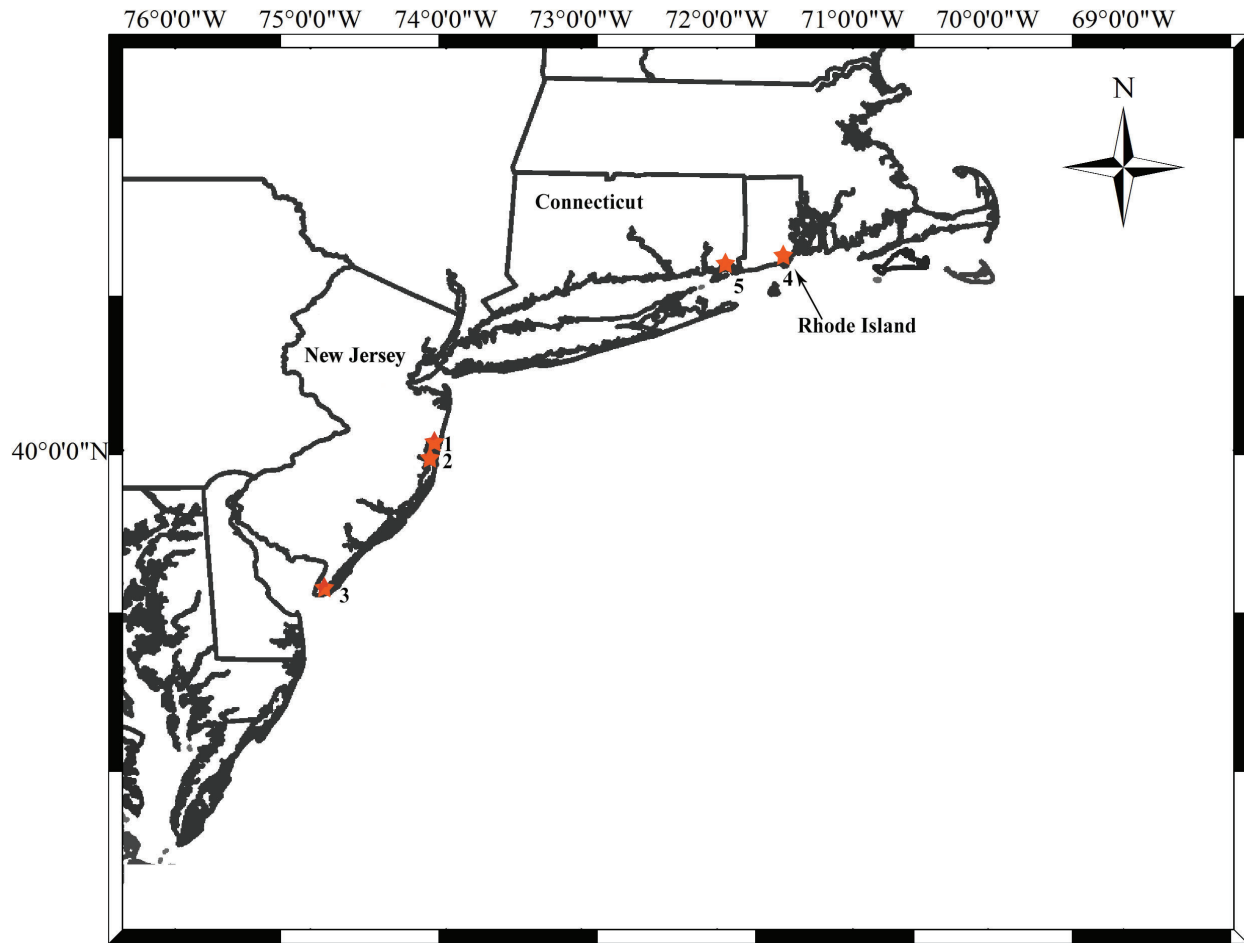


Figure 1. Map of 2019 *G. vertens* collection sites. 1=Metedeconk, 2=Tices Shoal, 3=Cape May, 4=Potter Pond, 5=Mumford Cove.

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. 11 liter 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 medusa with a substrate to cling to, as they are often found in the field clinging to sea grass or algae.

Experiments were monitored daily (24h, 48h, 72h, and 96h) to ascertain survival and health of *G. vertens*. Visual observations of the medusas' 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 fluctuations. Three healthy medusa 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. Medusa 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 22°C, 24°C, 26°C, and 28°C with five runs of each and three *G. vertens* medusa 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 22°C, 24°C, 26°C, 28°C, 30°C, and 32°C. The 22°C tanks were meant as a constant control, but was only run for six trials because it was only 1-2°C above ambient and the medusae were not under any sort of thermal stress. Instead, the tanks' temperatures were increased to 24°C, 26°C, 28°C, and 30°C, the latter of which was included because medusae from Cape May seemed to survive longer at 28°C, especially when the medusae were younger. Trials were also run at 32°C 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 24°C (Cape May=7, CT=4, Metedeconk=5, RI=5), 26°C (Cape May=7, CT=4, Metedeconk=5, RI=5), and 30°C (Cape May=6, CT=4, Metedeconk=2, RI=9), while 25 trials were conducted at 28°C (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 24°C, 26°C, 28°C, and two medusae from Rhode Island in another set of trials at 24°C, 26°C, 28°C, and 30°C. 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 (28°C=3 trials, 30°C=7 trials, 32°C=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 28°C and 30°C 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 28°C or 30°C 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 28°C with medusae from both Connecticut (CT) and

Rhode Island (RI), and four trials were run at 30°C with medusae from both CT and RI.

However, two of the RI trials set to 28°C and two of the trials set to 30°C 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 (28°C=4 trials, 30°C=4 trials).

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 (39.8229°N, -74.1022°W) on 6/12/19 and recovered on 7/2/19 and in Potter Pond, RI (41.388195°N, -71.532684°W) 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.

Predation Experiments with *Chrysaora chesapeakei*

Feeding experiments were conducted with *G. vertens* and its known predator, *Chrysaora chesapeakei*, 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 consume the prey. 3.8-liter circular tanks were set up at ambient temperature (~21-22°C) 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*.

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, sea grass, amphipods, copepods, isopods, shrimp, and larval stages of fish were also isolated and labeled. Samples were then frozen at -20°C prior to analysis and sent to the Cornell Isotope Laboratory (COIL), where samples were processed and analyzed. Data reported included the sample weight, N₂ and CO₂ amplitude, %N and %C, $\delta^{15}\text{N}$ vs. Atmospheric Air, and $\delta^{13}\text{C}$ 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, Corophiidae, copepods, fish, isopod, shrimp, ostracod, plant and crab). Data were separated in this way because $\delta^{15}\text{N}$ vs. Atmospheric Air and $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ levels, in order to better understand the trophic position of *G. vertens* and the several other groups of organisms sampled.

Results

Gonionemus vertens Collections

In 2019, collection of medusae began 5/16/19 in the Metedeconk River, Barnegat Bay, which was the site that was sampled most consistently. Collections continued at this site until 7/16/19 and included a total of 1455 medusae (Table 1). 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 after 6/19/19 (Table 1).

Collections did not occur as frequently in other locations, although collections resulted in significant sample sizes. 162 medusae were collected from Cape May, NJ over three collection dates (Table 2), 424 were collected (257 healthy) from Potter Pond, RI (Table 3) and 172 were collected from Mumford Cove, CT (Table 4).

Table 1. Average *G. vertens* bell diameters collected from the Metedeconk River in 2019.

Collection Date	Minimum Bell Diameter (mm)	Maximum Bell Diameter (mm)	Average bell diameter (mm)	STDEV	n
5/16/19	0.70	13.00	3.61	2.21	296
5/22/19	1.50	14.50	6.10	2.64	92
6/4/19	1.00	15.00	6.67	2.79	203
6/19/19	2.00	18.00	9.46	2.68	441
6/26/19	4.00	16.00	9.29	2.61	203
7/2/19	3.00	16.00	9.49	2.94	144
7/10/19	3.00	16.00	8.67	2.54	51
7/16/19	5.00	14.00	9.66	2.28	25
Total					1455

Table 2. *G. vertens* bell diameters collected from North Wildwood, Cape May in 2019.

Collection Date	Minimum Bell Diameter (mm)	Maximum Bell Diameter (mm)	Average bell diameter (mm)	STDEV	n
6/3/19	4	17	10.16	2.95	153
6/6/19	9.5	19	11.92	3.33	6
6/26/19	10	14	12.33	1.70	3
Total					162

Table 3. *G. vertens* bell diameters collected from Potter Pond, RI in 2019.

Collection Date	Minimum Bell Diameter (mm)	Maximum Bell Diameter (mm)	Average bell diameter (mm)	STDEV	n
7/11/19	1.5	25	9.44	4.04	424
Total*					424
Total healthy					257

*Overheating during transport caused several medusae to arrive in lab almost dead or dead.

Table 4. *G. vertens* bell diameters collected from Mumford Cove, CT in 2019.

Collection Date	Minimum Bell Diameter (mm)	Maximum Bell Diameter (mm)	Average bell diameter (mm)	STDEV	n
7/11/19	4	29	13.51	4.18	172
Total					172

Thermal Tolerance Experiments

2018 Stable Temperature Experiments

Despite a relatively small sample size in 2018, a pattern seemed to emerge that showed a maximum thermal tolerance of 28°C, with 100% *G. vertens* experiencing mortality after 72 hours for Rhode Island medusae and 100% mortality after 24 hours for Barnegat Bay individuals (Table 5). The large difference in survival rates may have been due to the age of *G. vertens* medusa, as RI individuals had been collected later in the summer and hence, were from a

Table 5. Percent survivorship of *G. vertens* at stable temperatures (collected July-August 2018).

Rhode Island Percent Survival						
Temperature			Hours			
	1	24	48	72	96	n*
22°C	100	100	100	100	100	4
24°C	100	100	100	100	100	4
26°C	100	100	87.5	87.5	87.5	4
28°C	100	75	25	0	0	4
Barnegat Bay Percent Survival						
Temperature			Hours			
	1	24	48	72	96	n*
22°C	100	100	100	100	100	1
24°C	100	50	50	50	50	1
26°C	100	100	100	0	0	1
28°C	100	0	0	0	0	1
Compiled Trials—2018						
Temperature			Hours			
	1	24	48	72	96	n*
22°C	100	100	100	100	100	5
24°C	100	90	90	90	90	5
26°C	100	100	90	70	70	5
28°C	100	62.5	20.83	0	0	5

*n=number of trials

“younger” population and not near senescence. Overall survival rates for these two samples dropped to 70% at 26°C by 72 hours, suggesting that prolonged exposure to elevated temperatures approaching 28°C is detrimental to *G. vertens* health. However, at lower temperatures all individuals survived, so senescence alone was not responsible for *G. vertens* mortality.

2019 Stable Temperature Experiments

In 2019, 105 experimental trials were run, greatly increasing the sample size compared to the trials in 2018. During the 2019 trials, similar patterns emerged as were observed in 2018, namely *G. vertens* from Rhode Island showed drastic decreases in survival at both 28°C and 30°C by 48 hours, to 41.67% and 16.67%, respectively. By 72 hours, medusae in the 28°C trials were all deceased, although 8.33% of the medusae in the 30°C trials were still alive. However, 0% survival was recorded for 28°C, 30°C, and 32°C trials by 96 hours (Table 6 and Figure 2). Likewise, medusae from Connecticut and the Metedeconk River experienced mortality rates upwards of 50% during the 28°C trials, and 100% mortality in the 30°C and 32°C trials. The survivorship of Cape May medusae was still 51.85% at 28°C, even after 96 hours, while the same conditions resulted in only 9.52% survivorship of Rhode Island medusae (Table 6, Figure 2A/D). The higher survival rates of Cape May medusae suggest possible latitudinal differences in populations with regards to thermal tolerance. However, a Two-Way ANOVA testing site and temperature (24-30°C) demonstrated that there were no statistically significant differences between survivorship rates among different sites ($F_{3,74}=1.15$, $P>0.1$). Therefore, all data were pooled (Figure 2E) to assess the impacts of thermal stress on the *G. vertens* populations and re-

analyzed using a One-Way ANOVA with temperature as the independent variable and survival at each experimental time frame as the dependent variable.

Results from the tests comparing survival rates at each temperature found that temperature does have a significant effect on survivorship. Differences in survival rates were not statistically significant at 24 hours ($F_{3,86}=1.78$, $P>0.1$), but there are significant differences between survival rates at increasing temperatures after 48h ($F_{3,86}=25.03$, $P<0.0001$), 72h ($F_{3,86}=40.22$, $P<0.0001$), and 96h hours ($F_{3,84}=30.17$, $P<0.0001$).

At 48 hours there is a significant decline in survivorship at 30°C compared to the other temperature trials ($p<0.0001$); and significantly greater mortality at 28°C compared to 24°C and 26°C trials. By 72 hours, medusae in the 30°C trials showed >96% mortality, significantly greater than all other temperatures ($p<0.0001$), but the 28°C trials also experienced significantly more mortality than those in the 24°C ($p<0.0001$) and 26°C trials ($p=0.0005$). By 96 hours, survivorship had continued to significantly decline. No medusae survived in the 30°C trial, which was significantly less than the 28°C trial ($p<0.007$). Additionally, the 28°C trial experienced significantly more mortality (27.7% survival) than the 24°C and 26°C trial ($p<0.0001$) at 96 hours. Throughout the course of the experiment, there were no significant differences between survivorship rates in the 24°C and 26°C trials, indicating thermal tolerance to these temperatures.

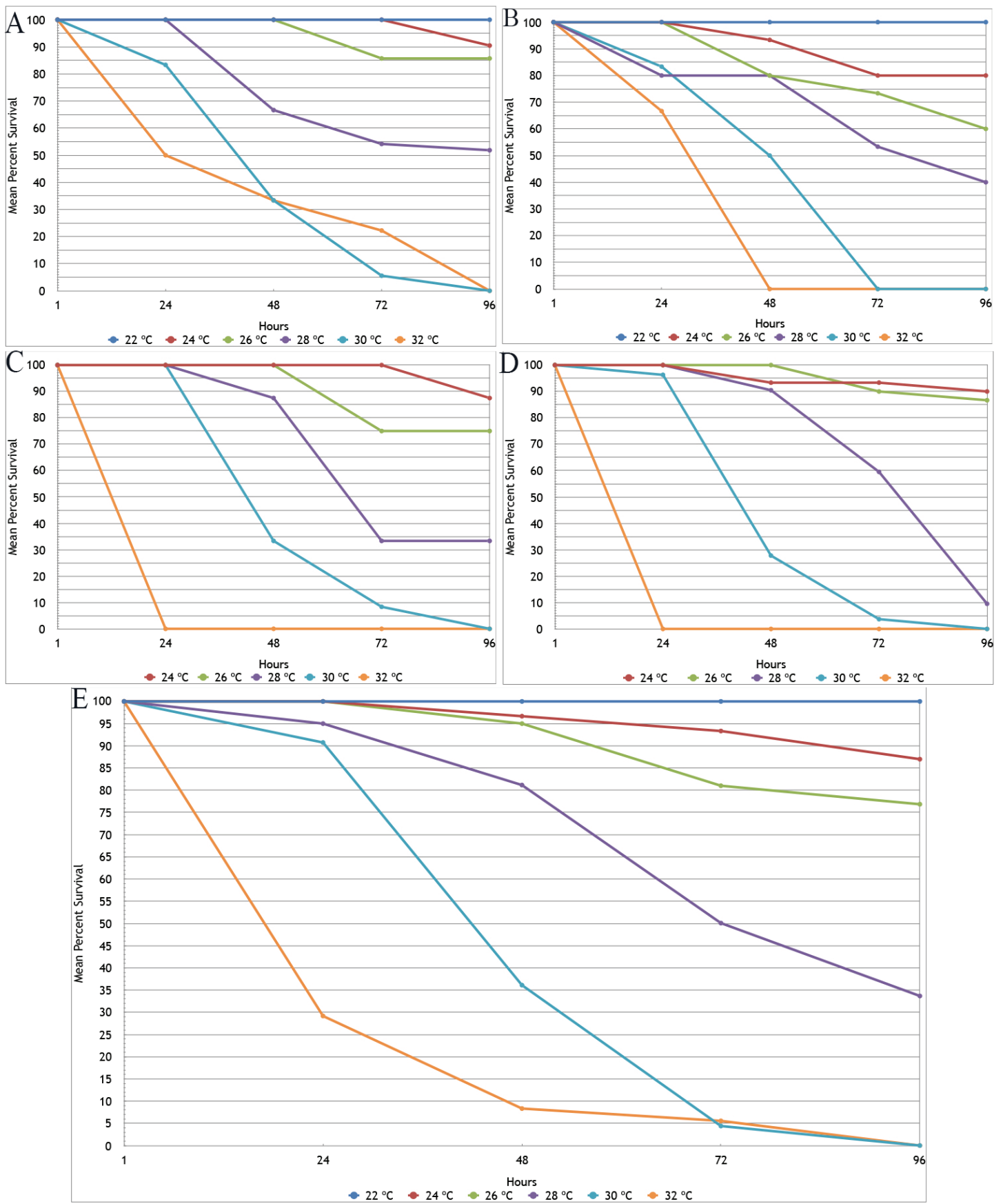


Figure 2. 2019 *Gonionemus vertens* survivorship at stable temperatures of 22°C-32°C over 96 hours.

A: Cape May, NJ; **B:** Metedeconk, NJ; **C:** Mumford Cove, CT; **D:** Potter Pond, RI; **E:** Compiled data across all locations.

Table 6. Percent survivorship at stable temperatures of 22-32°C of *Gonionemus vertens* collected June-August 2019 (separated by location).

Cape May Percent Survival						
Temperature						
	1	24	48	72	96	n*
22°C	100.00	100.00	100.00	100.00	100.00	3
24°C	100.00	100.00	100.00	100.00	90.48	7
26°C	100.00	100.00	100.00	85.71	85.71	7
28°C	100.00	100.00	66.67	54.17	51.85	9
30°C	100.00	83.33	33.33	5.56	0.00	6
32°C	100.00	50.00	33.33	22.22	0.00	6
Metedeconk Percent Survival						
Temperature						
	1	24	48	72	96	n*
22°C	100.00	100.00	100.00	100.00	100.00	3
24°C	100.00	100.00	93.33	80.00	80.00	5
26°C	100.00	100.00	80.00	73.33	60.00	5
28°C	100.00	80.00	80.00	53.33	40.00	5
30°C	100.00	83.33	50.00	0.00	0.00	2
32°C	100.00	66.67	0.00	0.00	0.00	2
Connecticut Percent Survival						
Temperature						
	1	24	48	72	96	n*
22°C						0
24°C	100.00	100.00	100.00	100.00	87.50	4
26°C	100.00	100.00	100.00	75.00	75.00	4
28°C	100.00	100.00	87.50	33.33	33.33	4
30°C	100.00	100.00	33.33	8.33	0.00	4
32°C	100.00	0.00	0.00	0.00	0.00	1
Rhode Island Percent Survival						
Temperature						
	1	24	48	72	96	n*
22°C						0
24°C	100.00	100.00	93.33	93.33	90.00	5
26°C	100.00	100.00	100.00	90.00	86.67	5
28°C	100.00	100.00	90.48	59.52	9.52	7
30°C	100.00	96.30	27.78	3.70	0.00	9
32°C	100.00	0.00	0.00	0.00	0.00	2

*n=number of trials

2019 Oscillating Temperature Experiments

Compared to the stable temperature trials, the decline in survivorship in the oscillating temperature trials was much more rapid. For instance, Rhode Island medusae survival at 48 hours in the 28°C trial was only 75% (compared to 90.48% in the stable temperature trials), and 25% at 72h (compared to 59.52% in the stable temperature trials). Similar trends of accelerated decline were observed with the Connecticut medusae at oscillating temperatures of 28°C and 30°C (see Table 7 and Figure 3). However, the sample size of these experiments was limited (4 trials at each temperature for each sample location), and trials were run in the weeks of 8/5 and 8/12, which is close to the end of the three month lifespan of *G. vertens* medusa. Indeed, the last two trials at both 28°C and 30°C with Rhode Island medusae only included two medusae each, three of which already appeared unhealthy at the start of the trials according to initial observations.

Table 7. Percent survivorship of *Gonionemus vertens* collected June-August 2019 at oscillating temperatures of 28°C and 30°C.

Connecticut Percent Survival						
Temperature						
	1	24	48	72	96	n*
28°C	100	83.33	75	25	8.33	4
30°C	100	100	75	25	8.33	4
Rhode Island Percent Survival						
Temperature						
	1	24	48	72	96	n*
28°C	100	87.5	41.67	0	0	4
30°C	100	87.5	16.67	8.33	0	4

*n=number of trials

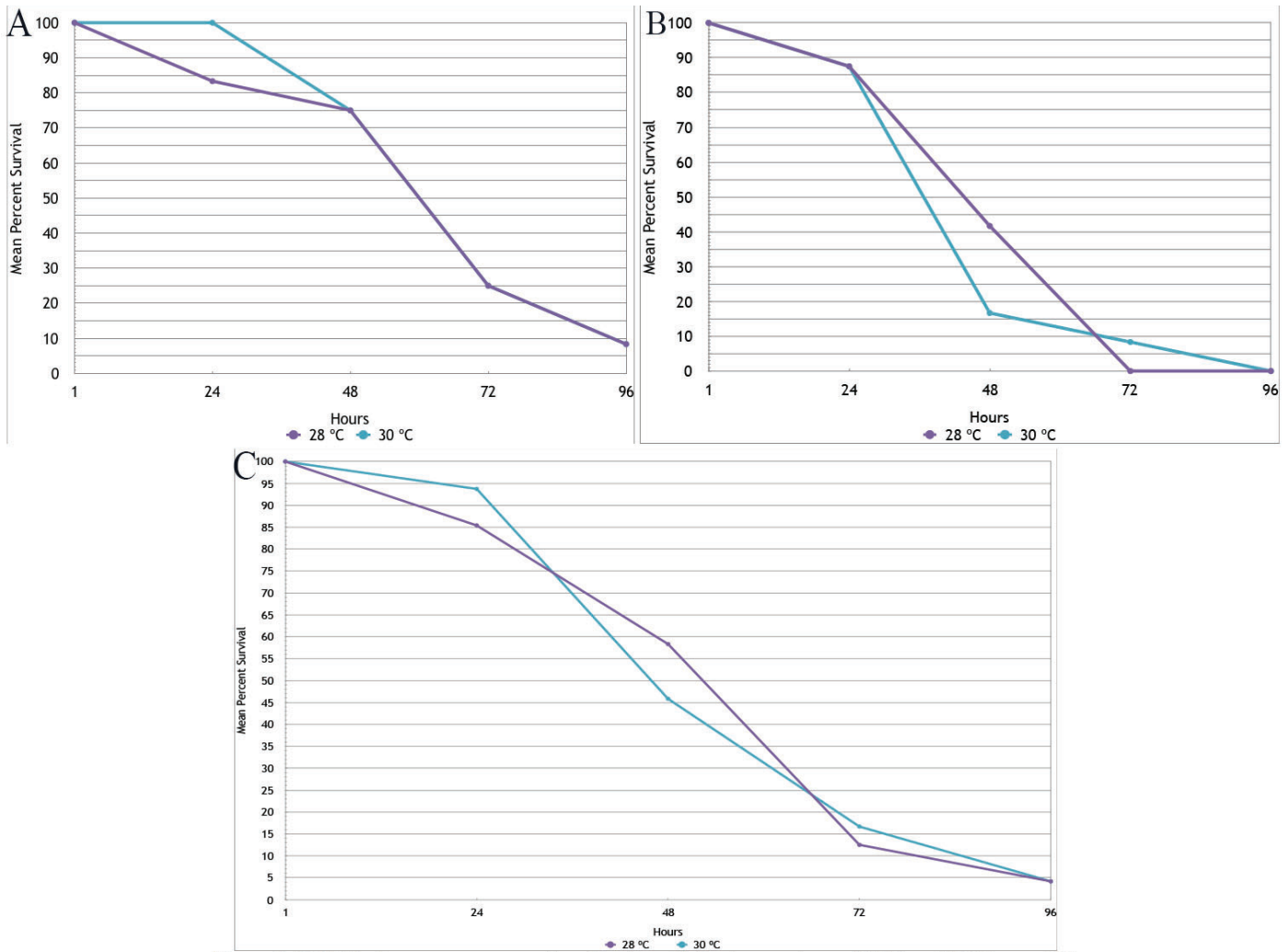


Figure 3. 2019 *Gonionemus vertens* survivorship at oscillating temperatures of 28°C and 30°C over 96 hours.

A: Mumford Cove, CT, B: Potter Pond, RI, C: Compiled data across all locations.

Field Temperature Assessments

Data from the Onset® temperature recorders were plotted to show the increase in temperatures over the summer months and fluctuations that may be observed. The data from Tices Shoal in Barnegat Bay, NJ, showed a general increase in temperatures from the middle of June to the beginning of July, with the hottest temperatures occurring 6/26-6/30. Temperatures rose above 27°C on 6/26, then hit highs of 28°C or just below on 6/27 and 6/28, and almost reached 29°C on 6/30 (Figure 4). It is important to note that this heat wave was the same time-frame as our laboratory experiments, which provides field validation to the laboratory experiments.



Figure 4. Daily temperatures from Tices Shoal, Barnegat Bay, NJ 6/12-7/2/19.

In Potter Pond, RI, the temperature recorder was in the field for three months, so daily temperatures were averaged for simplicity. However, the temperature recorder was not deployed until 7/18/19, so it is possible that warmer weather earlier in the summer was not recorded, but 424 healthy individuals were collected only seven days prior (Table 30), so it is unlikely that earlier heat waves were present. The most important trend to note is that average temperatures in the latter half of July did not rise above 26°C and that daily average temperatures during the summer stayed within the “safe” range of 22-26°C as determined by our laboratory data (Figure 5). Data from the temperature recorders show that temperatures drop at night, but often only by 2-3°C (Figure 4), unlike our oscillating temperature setup, which typically experienced a decline of 6-7°C as the tank returned to room temperature during the 12 hour “off” period (data not reported).

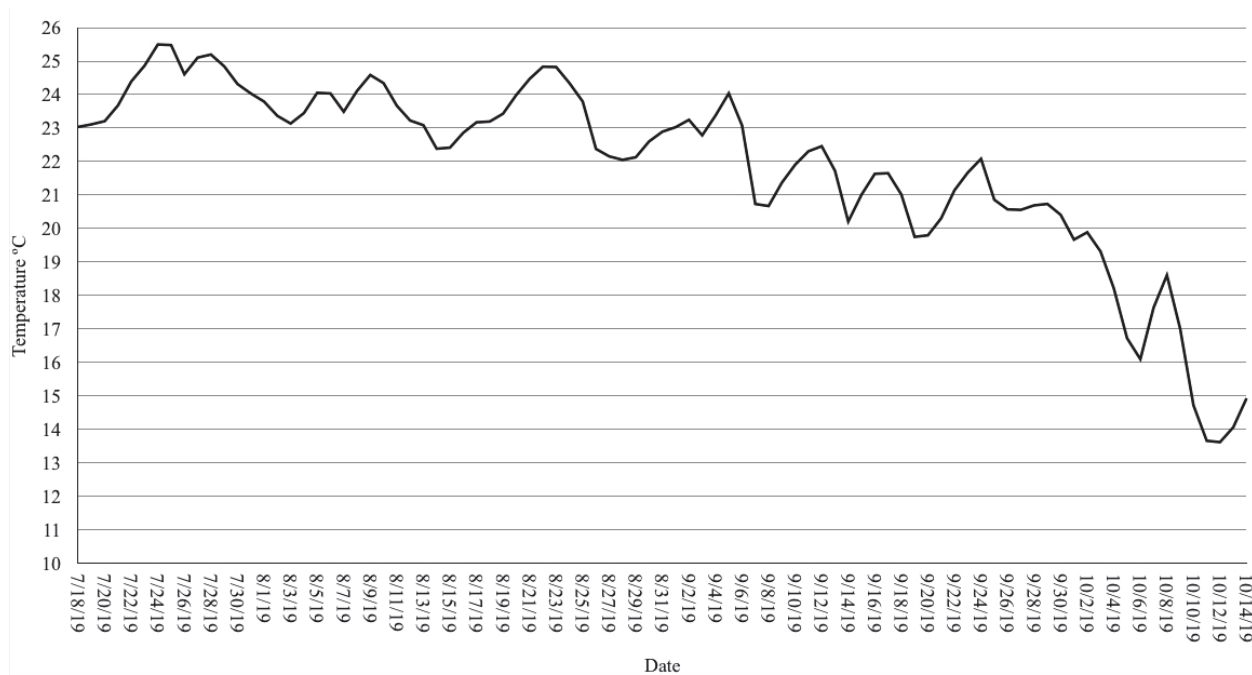


Figure 5. Average daily temperatures from Potter Pond, RI 7/18-10/14/19.

Predation Experiments with *Chrysaora chesapeakei*

The majority of feeding trials conducted with *Chrysaora chesapeakei* (CC) and *Gonionemus vertens* (GV) ended in *G. vertens* mortality (31/35 trials, see Table 8). The four trials that did not result in *G. vertens* mortality were likely due to the fact that an encounter simply did not occur between the two organisms. When bell ratios were calculated, it became evident that *G. vertens* is highly vulnerable to predation by *C. chesapeakei*. At a CC:GV bell ratio of 1.19, *G. vertens* are killed by *C. chesapeakei*, but it appears that *C. chesapeakei* medusa are not large enough to ingest *G. vertens* medusa, since the *G. vertens* medusae at this ratio were left in the bottom of the tank, uneaten. At a ratio of 1.67, however, *C. chesapeakei* are capable of partially or totally digesting *G. vertens* medusa (Figure 6). *G. vertens* were never scavenged and consumed by *C. chesapeakei* in trials which left recently deceased and partially digested *G. vertens* in a tank with *C. chesapeakei* after initial capture and *G. vertens* mortality.

Table 8. Average CC:GV bell size ratio by *G. vertens* mortality and consumption.

	Alive	Dead, not consumed	Dead and partially or totally consumed	Totals
Mean	1.30	1.19	1.67	
STDEV	0.37	0.18	0.79	
n	4	10	21	35
%	11.43	28.57	60	100.00

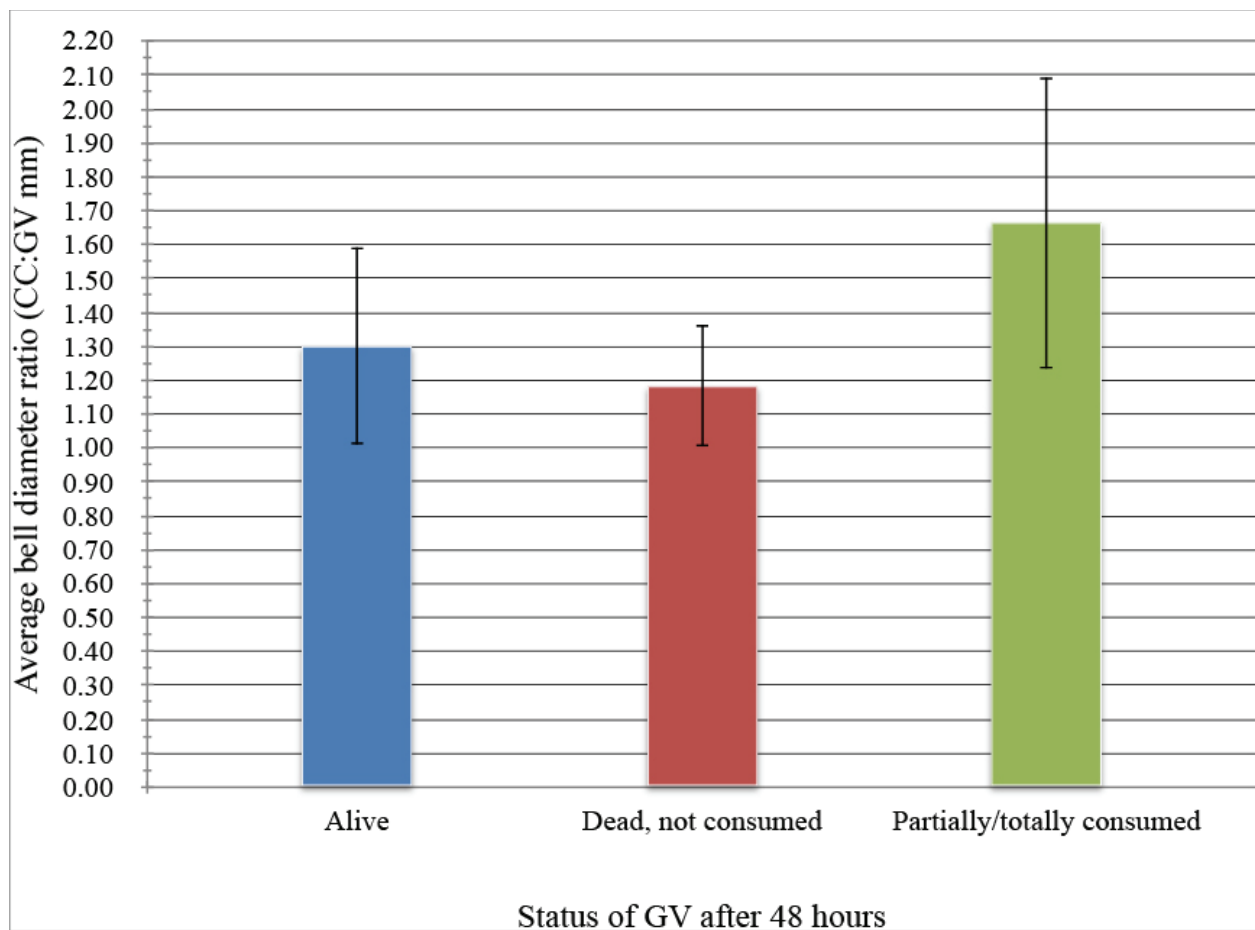


Figure 6. *Gonionemus vertens* survivorship in encounters with similarly-sized

Chrysaora chesapeakei, plotted against average CC:GV bell diameter ratio.

Stable Isotope Analysis

The graphical analysis of stable isotope 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, $\delta^{15}\text{N}$ values of fishes were generally the highest, which suggests that they occupy higher trophic levels in these communities (Figure 7). 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 (Figure 8, Bologna unpublished data). These groups include the amphipods, copepods, and isopods. General patterns emerge from these graphs that elucidate trophic interactions. For instance, the clinging jellyfish's ^{13}C signature correlates 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.

Interestingly, there are differences in ^{15}N and ^{13}C fractionation rates of *G. vertens* from different locations. The $\delta^{15}\text{N}$ 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‰) (Table 9). The $\delta^{13}\text{C}$ 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) (Table 9), which could suggest that vascular plants, (i.e., *Zostera marina*), which are greatly enriched in $\delta^{13}\text{C}$ form the base of the food web there.

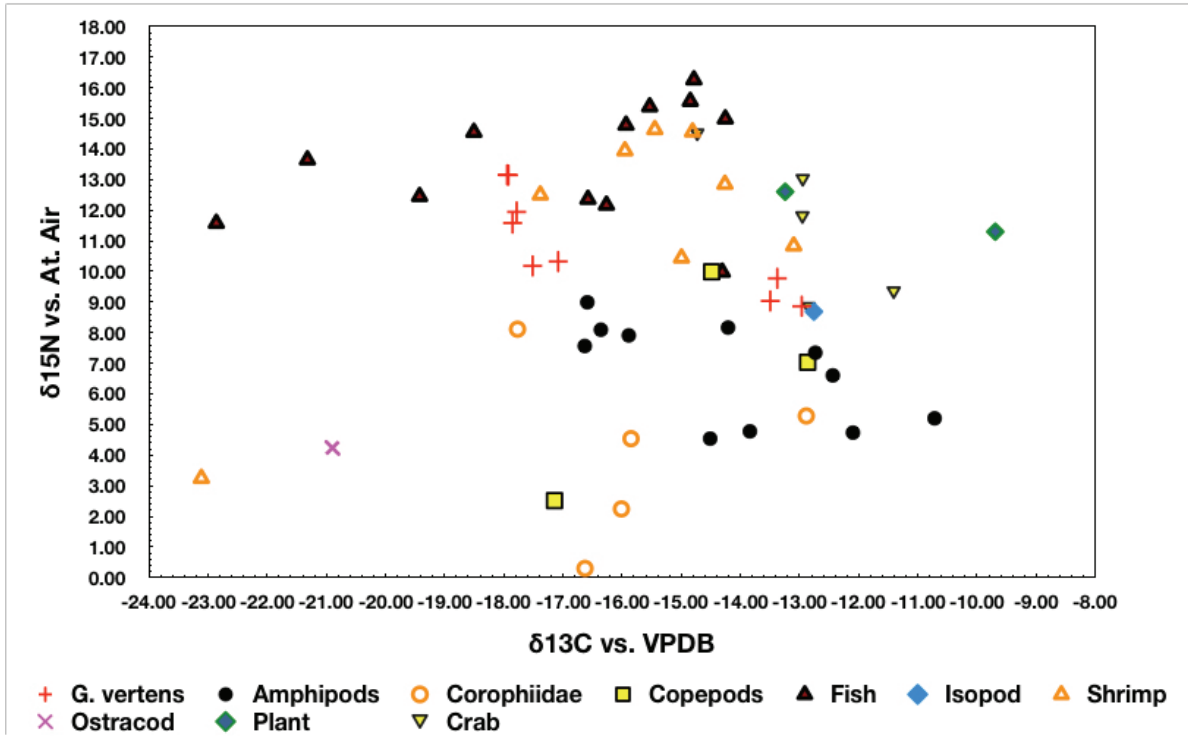


Figure 7. $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ plot of each sample collected for Stable Isotope Analysis

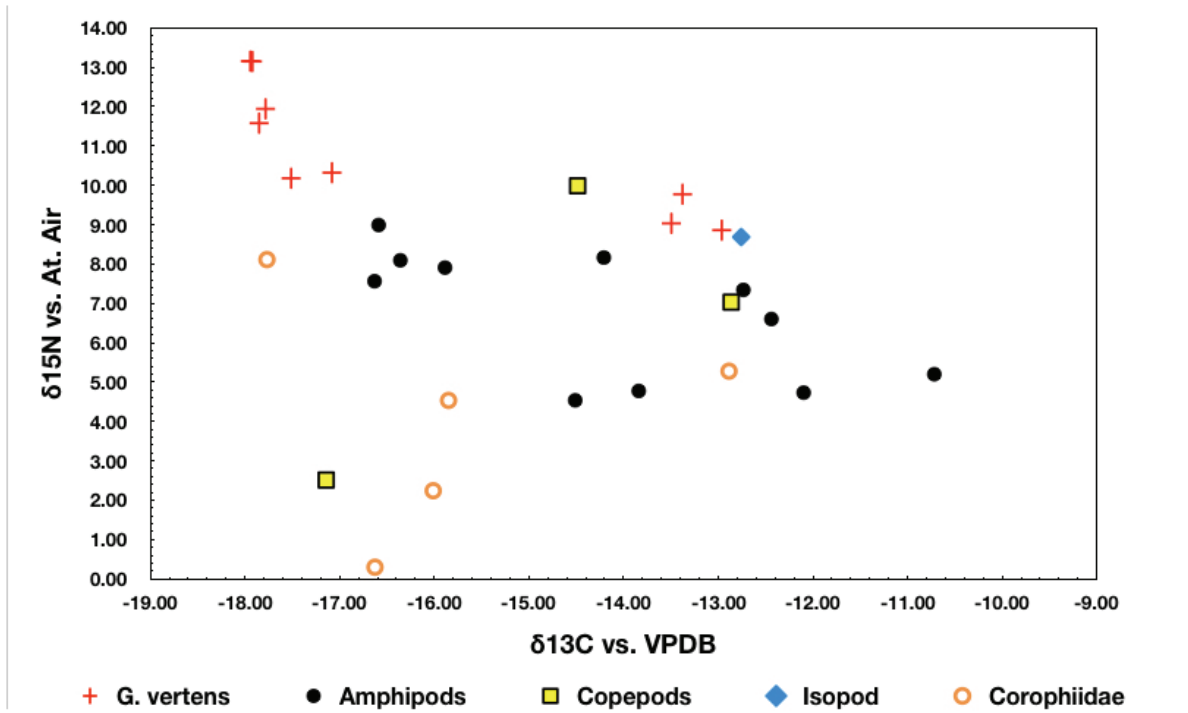


Figure 8. $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ plot of *G. vertens* samples and suspected prey groups

Table 9. SIA data from *G. vertens* samples separated by location.

Sample ID	Weight (mg)	N2 Amp	%N	$\delta^{15}\text{N}$ vs. At. Air	CO2 Amp	%C	$\delta^{13}\text{C}$ vs. VPDB
RI 1	0.995	1,259	3.04	8.86	1,328	12.67	-12.96
RI 2	0.98	1,971	4.70	9.03	2,179	21.19	-13.49
RI 3	0.948	1,874	4.63	9.77	1,912	19.11	-13.38
Mean	0.97	1,701	4.12	9.22	1,806	17.66	-13.28
NJ 1	0.289	1,150	9.16	13.15	1,164	37.15	-17.92
NJ 2	0.173	678	9.76	11.58	666	35.34	-17.85
NJ 3	0.328	1,145	8.38	11.94	1,117	31.32	-17.78
NJ 4	0.929	3,537	8.22	13.15	3,550	35.35	-17.94
Mean	0.43	1,628	8.88	12.45	1,624	34.79	-17.87
CT 14	0.99	2,039	4.43	10.18	2,048	19.05	-17.51
CT 15	0.98	1,088	2.48	10.32	1,013	9.55	-17.08
Mean	0.98	1,564	3.46	10.25	1,531	14.30	-17.30

Discussion

As an invasive species, *Gonionemus vertens* is a relative new-comer to New Jersey waters with a complex life history and an even more complex history of invasions across the globe. Encounters with highly toxic *G. vertens* are, unfortunately, becoming more frequent in areas such as the estuaries of Barnegat Bay in New Jersey and Potter Pond in Rhode Island, which are popular destinations during the summer months when *G. vertens* populations are at their peaks. In 2018, three stings occurred in Potter Pond and Point Judith Pond in Rhode Island (Kuffner, 2018), which increased to five stings the next year between July 2-July 5. All five sting victims in 2019 reported extreme pain, while two of the five also showed respiratory distress (Kuffner, 2019).

However, we lack an understanding of its position in the food web and the abiotic and biotic factors that control their population sizes. Attempts to understand its basic biology and distributions are motivated by the outsized impact that the tiny medusa can have on coastal communities. Therefore, in order to best inform public health practices regarding *G. vertens* and

describe its current and potential future ranges, this research uncovered details of the environmental controls of its populations and its trophic interactions.

Thermal Tolerance Experiments

Over the course of two summers, patterns were observed in the survival rates of *G. vertens* under different temperature regimes that point to a thermal tolerance threshold of 28°C. Heat trial experiments run at temperatures between 22-32°C over the course of four days showed that mortality significantly increased between 26°C and 28°C at 72h, and that mortality accelerates even faster above 28°C (Figures 2, Tables 5&6). These data are consistent with field collections of *G. vertens* from New Jersey across summer months. As the summer progressed and water temperatures rose (Figure 4), sites that once yielded hundreds of medusae per effort had dramatically reduced numbers of *G. vertens* by the middle of July. For instance, many fewer medusae were observed and captured in New Jersey during each survey after the population peaked on 6/19/19 (Table 1). However, no research exists on thermal tolerances in *G. vertens*, but work on other marine organisms indicates that at higher temperatures, oxidative stress may impact cellular function (Regoli and Giuliani, 2014; Aljbour et al., 2019), thereby decreasing survival.

The time-frame of the thermal experiments correlates with patterns observed in the field as well. For instance, temperatures at Tices Shoal increased above 26°C and reached daytime temperatures of 28°C for 72 hours beginning on June 27th, 2019 (Figure 4), which fits in the timeframe for which laboratory heat tolerance trials ran. Consequently, it appears that these early season heat waves result in substantial *G. vertens* mortality in the field and the majority of these hydrozoans have a thermal tolerance of 28°C. One slight deviation to this pattern is the

observation that *G. vertens* collected from North Wildwood did not show a lethal response to 28°C. In fact, at 96h at 28°C, these individuals showed >50% survival (Table 6). This suggests that there may be some latitudinal variation in thermal tolerance for this southern population.

Future work should attempt to compare young medusae sampled from Cape May, NJ to similarly aged medusae from sample sites at higher latitudes such as Potter Pond, RI. The fact that more than 50% of Cape May medusae survived 96 hours at 28°C, while less than 10% medusae from Rhode Island survived in the same conditions warrants further investigation into these latitudinal differences. The timing of trials is vital since younger medusae were more resilient, and future trials should differentiate between age groups by considering time elapsed since samples were taken. If their lifespan is only two-three months (Bakker, 1980, personal observations), it is possible that temperature increases have greater effects on aging organisms than on younger individuals. Therefore, it is extremely likely that water temperature exerts a strong control on *G. vertens* populations, but that it is an even stronger effect when temperatures exceed the threshold of aging populations.

Klein et al. (2019) demonstrated that cnidarian holobionts given a nighttime reprieve resisted bleaching for two days longer than the same holobionts exposed to a stable temperature plateau at their maximum temperature threshold. The groups with the night-time reprieve were also able to withstand temperatures 4°C warmer than those without the night-time reprieve. These data demonstrate the limitation of stable temperature experiments designed to assess temperature maxima. Therefore, oscillating temperature trials were designed with *G. vertens* to mimic the temporary reprieve from physiological stress that may be experienced in the field due to nighttime temperature decreases. Trials were run during the weeks of 8/5 and 8/12 with a 12h

on/off timer that controlled the tanks' heaters. Results from trials run with oscillating temperatures were similar to data collected from the stable temperature trials around the same time frame, with medusae experiencing high rates of mortality at both 28°C and 30°C (Table 7, Figure 3). These medusae, however, were some of the last specimens alive and were likely nearing the end of their lifespan. In fact, during the week of 8/12 the trials at both 28°C and 30°C with Rhode Island medusae only included two medusae each; three of which already appeared unhealthy at the initiation of trials. Field temperature data should also be more closely analyzed from the locations that correspond to the medusae's capture sites so that experimental design can be adjusted to more closely mimic the timing of nighttime temperature reprieves and the decline in temperature.

Putnam et al. (2011) demonstrated that coral subjected to daily fluctuations between 26-30°C in seawater temperature exhibited mortality rates greater than a 26°C stable condition and equal to populations of coral that were held at a constant 30°C. These data demonstrate that the responses to fluctuating temperatures close to a specie's thermal tolerance threshold may be as detrimental to an organism's survivorship as are stable temperatures equal to the threshold. However, later trials demonstrated that seasonal differences in responses to thermal stress and subsequent changes to population density declines occurred, possibly because of population acclimation to thermal conditions (Putnam et al., 2011). Since the effects of oscillating temperatures seem to be both species and seasonally dependent, the response of *G. vertens* to fluctuating temperature regimes must be further investigated.

G. vertens populations' temperature tolerances are significant from a public health standpoint, since these data are useful to help determine the timing of population declines during

summer months. For instance, when measured water temperatures approach and exceed 28°C, it is evident that populations of the clinging jellyfish will be on the decline, especially when these warmer temperatures come later in the summer and are coupled with natural senescence. The decline in early July (Table 1, Bologna unpublished field observations) of clinging jellyfish populations may be related to its physiological response to thermal stress. Significant increases in mortality at 28°C demonstrate that the temperature tolerance of *G. vertens* is approximately 28°C and that medusae will begin to die and populations significantly decline after 72 hours of exposure to these higher temperatures (Figure 2). This threshold is one that holds for all of the *G. vertens* populations studied, since there were no statistically significant differences between survival rates of medusae from different locations. Armed with this knowledge, officials may more accurately inform and educate the public regarding the prevalence and dangers of this invasive hydrozoan.

Littoral water temperatures in New Jersey start to exceed 28°C around the end of June in many locations (Figure 4), which is also when *C. chesapeakei* blooms are emerging and when *G. vertens* medusae are starting to become more scarce. However, the observed lifespan of medusae is about three months (Bakker, 1980), so if water temperatures were lower and sea nettle predation was limited, it could be expected that the clinging jellyfish could be observed in the field until later summer months. Correspondingly, medusae were still observed in Rhode Island until at least the middle of July (Table 3). Temperatures in Potter Pond never exceeded 26°C after July 18, 2019 (Figure 5), which would allow populations to persist until they experienced natural senescence. Likewise, medusae were still alive in our holding tanks and being used for

heat temperature experiments in August of 2018 and 2019, and small numbers of individuals held in captivity even survived into September (Bologna unpublished data).

The apparent longevity in our holding tanks is actually the normal lifespan of the clinging jellyfish medusae. Based on successful samplings, sample size, and mean population bell diameter, medusae in Barnegat Bay began to emerge in May (Table 1). For instance, 296 medusae were collected from the Metedeconk River mouth as early as 5/16/19 with a mean bell diameter of a scant 3.61mm and a minimum of 0.7mm, which denotes a young population (Table 1). Small medusae equal to or smaller than 5 mm were collected in all samples taken from the Metedeconk (Table 1), which demonstrates that medusae were still being produced throughout that time (sensu Wintzner et al., 2011). However, the minimum size began to increase after 6/19/19, denoting an aging population. If the emergence of medusae from polyps is synchronized with population blooms of their prey items as suggested in the literature (Edwards, 1976; Bakker, 1980), then further support for the emergence of medusae in May is the fact that copepod population sizes start to increase in coastal estuaries in New Jersey through the late spring and early summer (Shaheen and Steimle, 1995; Howson et al., 2017). The preliminary analysis of *G. vertens* diet through stable isotope analysis and feeding trials confirms that the clinging jellyfish $\delta^{15}\text{N}$ values are ~2-4‰ higher than that of copepods and amphipods (Figures 7 and 8). These findings, along with reported data in literature (Bakker, 1980; Mills, 1993), indicate that copepods and amphipods are most likely valuable prey for *G. vertens* medusae.

G. vertens medusae likely emerge in May from polyps that persist on hard benthic substrates. Substrates such as bivalve shells that are carried on ships or transported for aquaculture could be the initial mode of introduction for polyps to a new area since polyps are

likely able to persist at greater depths than medusa (Bakker, 1980). Then, as seawater temperatures warm in the spring, frustules could be carried by currents to shallower areas. According to the depth refugia hypothesis, marine organisms might move to deeper habitats to avoid shallow water heatwaves and temperature variability, returning to shallow waters after seasonal shifts during which conditions become more favorable. Specifically, different hydrozoan species have been documented to respond to the stresses of climate-change induced seawater warming by disappearing from shallow waters after extreme heat events and reappearing years later when recolonization occurred from populations that persisted in deeper waters (Morri et al., 2017). These hydrozoan species were from different climate regions and had very different life histories, but the response to temperature changes were generally applicable. Morri et al. (2017) also found disparate groups of the same hydrozoan species that developed thermal niches, each with its own optimal temperature at different depths. If these data are indeed a general example of hydrozoan responses to temperature changes, it is possible that *G. vertens* copes with temperature changes in similar ways. For instance, if seawater temperatures rise above the population's threshold, medusae may experience significant mortality, while polyps are able to persist at greater depths with stable lower temperatures. From there, recolonization of shallower waters (or the initial colonization of an area from newly introduced polyps) occur when environmental conditions become favorable.

Acclimation of individuals to temperature may change the thermal tolerance of a population. For instance, crabs acclimated to winter conditions had a significantly lower tolerance to temperature increases than did crabs acclimated to summer conditions (Cuculescu et al., 1998). This effect may be present in the *G. vertens* populations sampled for this work. In

order to determine the difference between acclimation effects and possible adaptation, a combination of generational temperature trials and molecular work could be performed. This work is possible because all of the *G. vertens* populations collected were separated by sample site in different holding tanks. If the medusae successfully reproduced in the each holding tank and polyps are collected and cultured, temperature trials could be performed with the F₂ generation of *G. vertens* medusae that emerge from these polyps. Data from these trials could be compared to verify the measured 28°C temperature tolerance threshold, as well as the potential for latitudinal differences and genetic adaptations. Many changes also occur on a molecular level due to temperature changes, which could be investigated to determine specific deleterious effects of temperature increases on *G. vertens*, and the potential for future adaptation.

Protein structure and function is vital for survival of an organism, but may be altered by changing temperatures (Hofmann and Todgham 2010). For instance, *Acropora sp.* coral have demonstrated physiologic responses to thermal stress such as increased production of reactive oxygen species, which outpace antioxidant enzyme production like superoxide dismutase, ascorbate peroxidase, and catalase peroxidase, decreasing the photosystem efficiency of its photosynthetic symbionts and causing bleaching (Krueger et al., 2015). These responses to temperature extremes also affect metabolic processes, stimulate pathways related to an immune response, trigger apoptosis, and increase mortality rates in the coral (Krueger et al., 2015), which may also occur and cause mortality in *G. vertens*. Other work has also demonstrated that protein structure can even be used as an indicator of adaptation to temperature changes. For example, snails that live in higher intertidal zones heat up more often and possess cytosolic malate dehydrogenase (cMDH) proteins which are more resistant to heating, due to a single amino acid

substitution (Palumbi et al., 2019). Other organisms have been shown to switch between different isoforms of proteins in order to utilize proteins that are more appropriate for their current thermal environment (Hofmann and Todgham 2010). For example, *Artemia* cysts transplanted from the San Francisco Bay to a warmer region of Vietnam demonstrated rapid temperature adaptation by increasing production of heat shock proteins (Hsp-70, artemin, p26), and *Artemia* populations collected from areas with different temperature conditions demonstrated varying degrees of thermal resistance and stress protein production (Clegg et al., 2001). In general, protein structure, integrity, and efficiency may be investigated in *G. vertens* in order to determine molecular changes that occur as temperatures rise, and also to help determine possible mechanisms of adaptation between populations found at different latitudes.

Cell membrane structure and fluidity is also vital to maintain homeostasis in an organism by serving as a physical barrier from the outside world, by controlling the movement of molecules in and out of a cell, by maintaining ion gradients between the environment and the interior of the cell, and by serving as an important part of the signaling mechanisms of a cell (Hofmann and Todgham 2010). The degree of fluidity in cells' plasma membranes is highly dependent on temperature, which is maintained at the organism's typical body temperature, but which must be adjusted at higher or lower temperatures. Various investigations with bacteria species demonstrate complex responses in the molecular composition of the plasma membrane by changing the degree of fatty acid unsaturation, changing the length of fatty acid chains, creating greater amounts of branching in fatty acids, and changing the distribution and amounts of other molecules such as glycolipids (Neidleman, 1987). Likewise, the protozoan *Tetrahymena pyriformis* monitors and manipulates fatty acid chain length and structure, in addition to polar

head group distributions, in response to temperature increases or decreases, in order to maintain membrane fluidity (Neidleman, 1987). Coral zooxanthellae resilience to temperature changes is, in large part, determined by the saturation of thylakoid membrane lipids, and is proposed as a diagnostic measure to determine the temperature tolerance of a species (Tchernov et al., 2004). Membrane composition and fluidity is an important aspect of cellular health at the molecular level, and could be useful to determine the impacts of temperature increases on *G. vertens*.

Predation Experiments with *Chrysaora chesapeakei*

Results from the predation experiments provide us with clear evidence for trophic interactions between *C. chesapeakei* and *G. vertens*. When *C. chesapeakei* medusae swim through shallow areas, their tentacles extend throughout the water column into grass beds and algae fields where *G. vertens* medusae are clinging or swimming. *C. chesapeakei* are indiscriminate predators in these littoral waters and are extremely effective in killing *G. vertens* medusae. In the lab, *G. vertens* were killed when *C. chesapeakei* were a mere 1.19 times larger, and at least partially consumed when *C. chesapeakei* were 1.67 times larger. In the field, *C. chesapeakei* bell diameters range from 17 mm to 175 mm (Bayha et al., 2017), while *G. vertens* bell diameters measured from samples that we obtained from field surveys ranged from 5 mm to 25 mm (see Tables 1-4). Thus, when mature *C. chesapeakei* medusae are present in the same system as mature *G. vertens* medusae, bell diameter ratios could exceed 7:1, accounting for heavy predation pressure from *C. chesapeakei* on *G. vertens*.

The top down pressure *C. chesapeakei* exerts on *G. vertens* is consistent with the control *C. chesapeakei* exerts on coastal communities as a non-discriminating predator. In the last 15 years, conditions in Barnegat Bay, NJ favored *C. chesapeakei* populations to grow and expand.

Eutrophication and coastal development are often correlated with increased jellyfish blooms (Mills, 2001) and many New Jersey estuaries are heavily developed and eutrophic (Kennish et al., 2007). Population studies of *C. chesapeakei* and prey items (*Mnemiopsis leidyi*, copepods, fish eggs and larvae, etc.) demonstrated that the increased populations of *C. chesapeakei* exerted a general top-down control across the entire pelagic community studied (Bologna et al., 2017). It is probable that *C. chesapeakei* was exerting top-down pressure on *G. vertens* populations within the same community, considering its effectiveness at killing *G. vertens* prey.

One possible mechanism that explains the sudden appearance of *G. vertens* in 2016 (Gaynor et al., 2016) with increased observations and abundance in 2017-2019 is predatory release. Bologna et al. (2018) showed that after Superstorm Sandy in 2012, *C. chesapeakei* densities significantly declined in Barnegat Bay, NJ during the following years. As a result, the sea nettle did not express substantial top-down control of pelagic communities and species richness of other gelatinous zooplankton, especially hydrozoans, significantly increased by 64% (Bologna et al., 2018). Since these “new” species proliferated due to relieved predatory pressure by *C. chesapeakei*, it is very likely that *G. vertens* also began to establish itself because of relieved pressure. It is possible that small *G. vertens* polyp populations were capable of producing sufficient adult medusa to sexually reproduce and expand the range and density of larvae colonizing the region. This in turn would have resulted in larger polyp populations capable of fueling the medusa blooms observed in 2016 and beyond. However, more work must be done to determine the ecological interactions and biology of *G. vertens* polyps which, like many jellyfish, are understudied compared to the medusa form (Mills, 2001).

With this work, the trophic position of *G. vertens* within its coastal New Jersey habitat is better understood. For example, the top-down control of *G. vertens* populations by *C. chesapeakei* (Figure 6) likely helped to limit and delay the emergence of *G. vertens* medusa to undetectable levels prior to Superstorm Sandy because it is an extremely effective predator of hydromedusae, including the clinging jellyfish. Results indicate that the minimum ratio of CC:GV bell diameters that will cause mortality in an encounter is 1.19:1 (Figure 6). The ratio required for consumption is a bit higher at 1.67:1, but it is important to note that the bell diameter of sea nettles in coastal waters will commonly be 5-7 times that of the clinging jellyfish. While mature *C. chesapeakei* medusae are present by the end of June-beginning of July (Cargo and Schultz, 1967, personal observations) and exert a strong predatory population control on *G. vertens*, it is unknown whether *C. chesapeakei* populations will again increase and suppress *G. vertens* in the future, or whether the *G. vertens* populations have become robust enough to resist the top-down pressure.

Stable Isotope Analysis

Jellyfish diets are difficult to quantify, since gut content analysis is not always possible with small and cryptic medusa like *G. vertens* through visual analysis alone, especially since jellyfish digestion rates are quite fast (D'Ambra et al., 2014). However, new approaches can help to identify trophic interactions between gelatinous predators and their prey. For instance, recent work that utilized next-generation sequencing with DNA fragments taken from *C. chesapeakei* gut lavage were successful in identifying 23 taxa as prey items (Meredith et al., 2016). The “shotgun” method employed in this study did not require PCR amplification and is therefore cheaper and faster than traditional methods that rely on PCR amplification. However,

this approach may be limited by the degradation of DNA in the gut of the medusa (Meredith et al., 2016) or skewed by secondary predation if the medusa had recently consumed another predator that had prey in its gut (Sheppard et al., 2005). Finally, DNA analysis of gut content only offers a snapshot of what the medusa had recently ingested, rather than a long-term picture of what the medusa has assimilated. Jellyfish diet can vary widely based on time of day, season, and medusa size, which may bias results (Meredith et al., 2017).

Stable Isotope Analysis (SIA), however, is capable of developing mixing models that represent the long term diet of a predator (Wada, 2009; D'Ambra et al., 2014). SIA of *G. vertens* provides the benefit of developing an understanding of the long term assimilated diet of medusae, rather than the brief snapshot of what it has ingested, which is inherently more valuable when attempting to develop an understanding of long term trophic interactions.

Analysis of the $\delta^{15}\text{N}$ vs. Atmospheric Air and $\delta^{13}\text{C}$ vs. VPDB values are useful to place groups of organisms into an ecological context and determine trophic interactions, since the mantra “you are what you eat (plus a few per mil)” (DeNiro and Epstein, 1976) applies to the isotopic composition of an organism’s tissues. In marine ecosystems, $\delta^{15}\text{N}$ values are relatively higher than those of atmospheric air, while $\delta^{13}\text{C}$ levels are lower than those of Vienna PeeDee Belemnite, the accepted reference standard established by the International Atomic Energy Agency (Gonfiantini et al., 1995). Therefore, general trends emerge and can be used to determine an organism’s trophic level based on the organism’s isotopic signature. For instance, the higher the values of $\delta^{15}\text{N}$ measured in an organism’s tissue samples, the higher the organism’s trophic level, since ^{15}N accumulates in organisms’ tissues in a sort of biomagnification effect. Specifically, $\delta^{15}\text{N}$ values seem to regularly increase by 3-4% (Wada,

2009) for each trophic level increase in marine food webs. Likewise, $\delta^{13}\text{C}$ values may be utilized to determine from where an animal obtains its carbon source, since the $^{13}\text{C}/^{12}\text{C}$ ratio of a consumer is similar to the ratio of the isotopes in its food, just slightly enriched.

The organisms sampled in this study were a best attempt to cast a wide net across all trophic levels in order to determine the trophic position of *G. vertens*, whose diet was relatively unquantified prior to this work. However, our data were not complete enough to fully quantify *G. vertens*' trophic position. One of the issues with these data is that we did not measure bell sizes before sending samples to be analyzed. When comparing *G. vertens* samples within the $\delta^{15}\text{N}/\delta^{13}\text{C}$ plot, it appears as if some of the medusae may have consumed prey in multiple trophic positions (i.e., copepods, fish, isopods). These data points are found above 10‰ $\delta^{15}\text{N}$ (Figures 7 and 8), and it could be that they represent larger medusae, or those from a different area, which could have consumed multi-trophic food sources.

Other species of jellyfish have been shown to assimilate isotope signatures from their diet rather quickly, which could lead to large differences across populations due to the heterogeneity of the medusae's diet (D'Ambra et al., 2014). In the future, collecting many more samples from the same area might give enough data to develop an accurate mixing model, and thus a better understanding of trophic interactions. A large variety of potential prey items should be collected, since a large variety of prey is typically consumed by jellyfish, which are mostly indiscriminate predators whose diet may differ with morphology, size, time, and behavior (Pauly et al., 2009). It will be especially important to develop and improve techniques to sample and identify certain groups such as zooplankton and copepods, which are likely an important food source (Figure 8,

D'Ambra et al., 2014). Attempts to collect copepod samples in the current study were not always successful, as several samples were below the instruments' detectable limits.

Differences in isotopic compositions between samples taken from different locations may be due to differences in feeding habits. New Jersey medusae may feed on higher-protein prey like fish than do medusae from Connecticut or Rhode Island. If medusa diets actually differ by location, greater sample sizes of *G. vertens* may help determine appropriate mixing models and trophic interactions. Alternatively, the sample sites could be isotopically different because of differences in levels of pollutants such as inorganic nitrogen fertilizers or human/livestock effluent. For instance, if a marine system like Potter Pond has large inputs of synthetic nitrogen fertilizers, the nitrogen isotope signatures of macroalgae such as *Ulva* would be greatly lowered (Orlandi et al., 2017), which would then be reflected through higher trophic levels. On the other hand, human and livestock effluent increases $\delta^{15}\text{N}$ values in seagrass (Jones et al., 2018), which would in turn raise the $\delta^{15}\text{N}$ values of consumers. Different types of nutrient inputs to highly eutrophic estuarine systems such as Barnegat Bay (Kennish et al., 2007) have different effects on $\delta^{15}\text{N}$ values of primary producers. Therefore, if differences in isotope signatures are systemic and due to the anthropogenic alteration of environments via pollutants, separation of SI data by sample site would help determine diet despite varied environmental differences.

Sample sizes of all potential prey groups should be increased and catalogued with regards to location and carefully identified. A more comprehensive mixing model for the clinging jellyfish and its trophic interactions could be developed if aspects of its preys life history and diet are accounted for after identification. For instance, corophids seem to be isotopically distinct from other genera of amphipods, which may be due to differences in diet. Another example is

the data point for the larval shrimp (Figure 7), which appears to be an outlier when compared to the general grouping of shrimp data points. Specifically, the adult shrimp $\delta^{15}\text{N}$ values are approximately 10‰ higher than the larval shrimp $\delta^{15}\text{N}$ values, which could mean that larval shrimp consume more phytoplankton, whereas adult shrimp may feed at higher trophic levels. Finally, more plants, and those more representative of *G. vertens* habitat, should be sampled (i.e., seagrass), and samples should be taken from locations where the *G. vertens* medusae are collected. If followed, these suggestions should allow for the development of more accurate and complete mixing models to describe the diet of *G. vertens*, and thus place this enigmatic species in context.

These data may also be skewed by the difference in fractionation rates between tissues. For instance, D'Ambra et al. (2014) demonstrated that certain tissues in *Aurelia* medusa, such as gonads and oral arms, have different fractionation rates, possibly due to the higher lipid content in these tissues (D'Ambra et al., 2014). Gonad and oral canal sizes relative to bell size were not determined in the *G. vertens* samples, but the simplest method that avoids bias from tissues with different isotopic ratios may be to excise a portion of the bell tissue and send that for SIA, rather than analyzing the entire medusa. Using a portion of the bell would obtain SI values that are more consistent and representative of the organism's fractionation rates without being skewed by the different fractionation rates of gonads and oral arms. Finally, excising portions of the bell should also avoid confounding data with SI values from trace amounts of food leftover in the medusa's gut or tentacles (D'Ambra et al., 2014).

Data reported here are preliminary and serve as a guide by which to develop future work.

Future research should more fully describe and categorize all samples, while also adhering to

best practices such as conducting SIA of excised bell portions and increasing numbers of medusae used for SIA. Each population of *G. vertens* in our study was slightly different isotopically, with the lowest $\delta^{15}\text{N}$ values appearing in the Rhode Island populations and the highest in the New Jersey populations (Table 9), so location differences should be taken into consideration. Many gelatinous species' trophic positions, including that of *G. vertens*, remain undefined, but methodically applying SIA techniques should help to define their place and interactions in marine systems (D'Ambra et al., 2014).

Conclusion

In summary, the life cycle and ecological role of *G. vertens* is a complex one that most likely starts with the invasion of an area such as Barnegat Bay, NJ by the polyp form. The polyp form is most likely introduced through shipping and can potentially persist and reproduce asexually for years before large numbers of the medusae emerge and establish themselves in shallow coastal waters. Polyps may time the release of medusae to coincide with an increase in temperature and in prey populations, which in New Jersey tends to happen May-June. Likely primary prey sources for *G. vertens* populations are amphipod and copepod species, as observed through various feeding trials and stable isotope analyses that include *G. vertens*.

As medusae blooms occur, the chance of contact with recreational users of coastal waters increases, which may result in toxic and painful stings. As medusae proliferate in New Jersey, they have a short window of time before waters warm above their thermal tolerance threshold of 28°C. Around the same time, *C. chesapeakei* populations are beginning to bloom, which exerts significant, broad top-down control on all of its prey groups, including *G. vertens*. While *C. chesapeakei* bell diameters may easily be 5x the bell diameter of *G. vertens*, our data show that a

ratio of 1.19:1 is sufficient to kill the clinging jellyfish. Therefore, even when the sea nettle, which typically blooms during July in New Jersey, is not large enough to consume a clinging jellyfish, an encounter is likely to end in *G. vertens* mortality. Together, temperature and sea nettle predation seem to limit *G. vertens* blooms to the early summer, and the combination of rising temperatures and increased predation may be enough to cause the decline of *G. vertens* medusa populations in mid-July before their typical three month lifespan. Future work should differentiate between age groups and location in heat tolerance trials and should try to more closely mimic the temperature changes that occur from day to night in the locations surveyed. Stable isotope sampling should be more extensive across all surveyed trophic levels, categorized by location, and performed after careful tissue preparation. These improvements will continue to develop a detailed understanding of *G. vertens*' trophic interactions and the interplay between environmental conditions and physiological processes associated with medusa age and senescence.

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