FINAL

Monitoring and Assessment of Clinging Jellyfish (*Gonionemus vertens*) Populations and Habitat in New Jersey Coastal Embayments: 2020

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EXECUTIVE SUMMARY

The NJDEP Division of Science and Research (DSR) investigated three waterbodies (Table 1) from May 26th through July 17th (2020) known from previous years as having an active, seasonal presence of *Gonionemus vertens* (commonly known as the "clinging jellyfish", *G. vertens*). Due to the unprecedented COVID-19 pandemic, monitoring was limited only to 'hot-spots' and began approximately 2-3 weeks post bloom. From these locations, sampling was conducted to verify the presence or absence of individuals, assess relative abundance through the season, collect medusae for research, as well as collect water samples for environmental DNA (eDNA) analysis. In 2019, an intensive effort was initiated to investigate new waterbodies as well as known locations for *G. vertens* ground truthing and eDNA sample collection. The Division of Science and Research initiated the development of eDNA detection methods to predict presence or absence of these cnidarians during active periods of their life cycle. During this time, *G. vertens* DNA was successfully amplified from water samples in laboratory trials, and work continues to optimize these methods. We continue to work in close collaboration with Montclair State University (MSU) to monitor known and potentially new sites, as well as continue our investigations into the life cycle, invasive history, and origins of this species. Information from both sampling efforts (approximately 71 sampled locations/three waterbodies) was used to populate data for the *NJ Clinging Jellyfish Interactive Map* (https://njdep.maps.arcgis.com/home/item.html?id=7ea0d732d8a64b0da9cc2aff7237b475), which was developed in 2016 as a tool to alert the public to areas where clinging jellyfish could potentially be encountered. Communication and outreach have also continued through public interaction, social media (MSU's "NJ Jellyspotters" on Facebook), and the *NJ Jellyfish Information* website.

Table 1. NJ coastal waterbodies sampled in 2020 for clinging jellyfish; dates in bold, red-lettering are those where medusae presence was confirmed (NJDEP-DSR sampling only).

Name of Waterbody	Dates Sampled
Metedeconk River	**6/2**, **6/9**, **6/19**, **7/2**, 7/15
Central Barnegat Bay (IBSP)	**6/26**, 7/16
North Wildwood-Hereford Inlet Salt Pond	**5/26**, **6/1**, **6/8**, **6/15**, **6/22**, 7/17

The 2020 clinging jellyfish season commenced on 5/26/20 with the discovery of immature medusae (bell diameter ranging from 4-15 mm) inhabiting a stormwater retention pond in North Wildwood that lies adjacent to Hereford Inlet (Figures 10A-B). At this time, the water temperature was about 17°C which corresponds to the time when strobilation in this species typically begins. Robust and fully formed medusae were observed by mid-June (Figure 1A). The last confirmed sighting in this waterbody was on 6/22/20, and absence confirmed on 7/17/20. Consequently, immature medusae were also observed on 6/2/20 along Wardells Neck in the Metedeconk River (Figures 8A-B), though much smaller in size (range: 2-10 mm). The last confirmed sighting occurred on 7/2/20 and absence of medusae verified on 7/15/20 upon a return visit. Island Beach State Park (IBSP) was

sampled on two occasions, with the only confirmed sighting of medusae on 6/26/20 at both Tices Shoal and Jonny Allens Cove (see Appendix A, Table A-1 for all sampled locations).

Clinging jellyfish medusae, under ideal conditions, typically have a 2.5-3 month active life cycle (Mills 1993; Rigby 2020; DSR, pers. Observation.). Mills (1993) reported that senescence was the main cause of clinging jellyfish disappearance in the wild when predation or other stressors are not acting on a population. In the laboratory, we observed that many individuals began to display signs of decline and degradation within this time frame (Figure 1B), based on observations of limited swimming capability, degeneration of gonadal structure, loss of tentacles, and often the inability to capture prey (DSR, personal observation). This decline in healthy functionality may be one of the main factors that lead to a natural die-off, although abrupt changes in water quality and other environmental variables can negatively influence the seasonal presence and duration of the cnidarian life cycle (Pitt et al. 2014). In the Metedeconk River, the disappearance of clinging jellyfish this year and in previous years appears to strongly coincide with the emergence of the Atlantic bay nettle (*Chrysaora chesapeakei*), which begins to bloom in great numbers during early July. In previous years, similar observations were made in the Shrewsbury River. Predation experiments by both MSU and DSR have confirmed that *C. chesapeakei* will readily consume *G. vertens*, which could indicate that bay nettles might have a significant influence on the seasonal duration of clinging jellyfish presence where habitats overlap. Other native predators, such as nudibranchs (e.g. *Cuthona gymnota* – Figure 2), will consume *G. vertens* polyps as well as the tentacles and bell margin of sessile or older individuals (Bologna et al. 2020; DSR, pers. Observation). The rise in water temperature (especially when consistently above 26ºC and reaching or exceeding 28ºC) will also cause the disappearance of medusae (Rigby 2020). In our laboratory, individuals from the North Wildwood and Tices Shoal populations (both collected in mid-June) were maintained until mid-September 2020, which is consistent with our 2019 observations.

Intensive monitoring of these habitats continues to yield important information on the habitat and substrate preferences that illustrate the clinging jellyfish's tolerance to a wide range of conditions, which likely contributes to its invasive capabilities. Eelgrass beds (e.g. *Zostera* spp. and other species) were once thought to be a critical habitat component for *G. vertens* establishment (Govindarajan et al. 2017); however, our research found that these jellyfish survive and are relatively abundant in areas where only macroalgae predominates. This suggests that *G. vertens* can thrive and potentially reproduce in habitats where SAV beds are minimal or absent, as long as suitable hard substrate for polyp attachment is present (Mikulich 1974). A mixed composition of macroalgae attracts a diverse assemblage of zooplankton and provides suitable habitat for fauna such as copepod and isopod species, which in turn constitute much of the preferred diet for medusae and polyps (Bakker 1976; Matias et al. 2015; Rigby 2020).

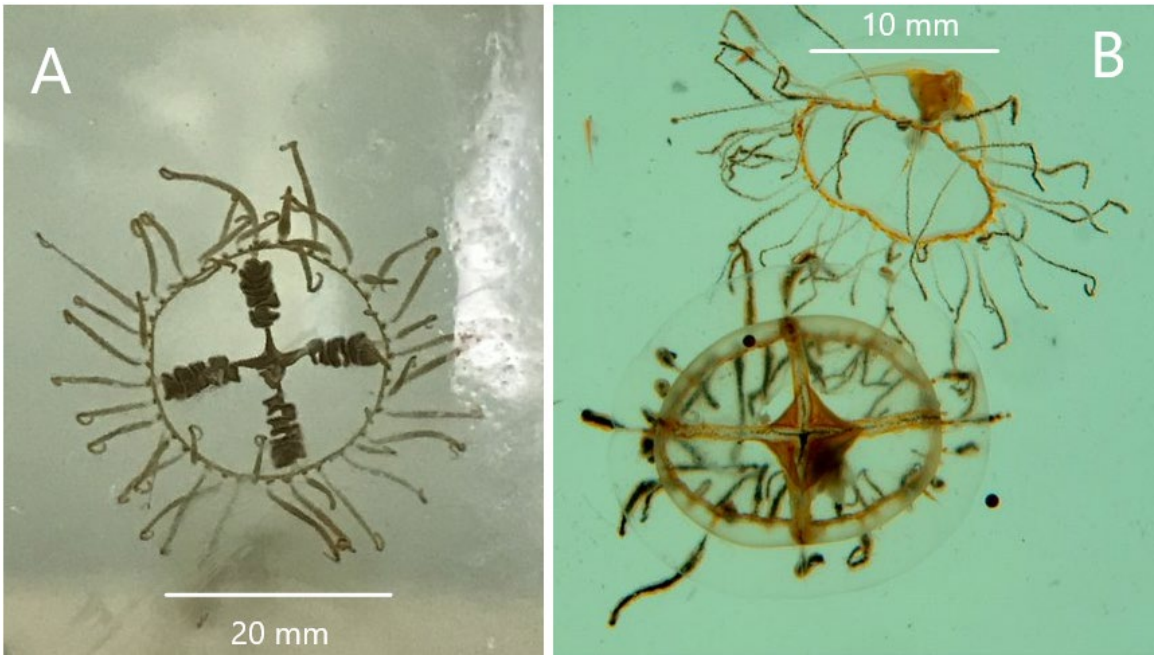


Figure 1A-B. (A.) A healthy adult *Gonionemus vertens* medusa collected from a salt pond in North Wildwood, NJ; (B) Older individuals from the same population showing signs of age and decline after 2 months held in laboratory aquaria (Photos: NJDEP-DSR).

Figure 2 A native nudibranch (*Cuthona gymnota*) collected from sites positive for the presence of *G. vertens* (Photo: NJDEP-DSR).

Given that sites in NJ such as the North Wildwood salt pond and Metedeconk River possess these conditions and *G. vertens* has been found in consecutive years, including polyps produced from these individuals in the laboratory (Figure 3), it is evident that

reproductively viable populations exist. Density and vertical structure of the substrate appears to be a major preferential feature for this species, regardless of type of SAV or algal species, as we have observed in the Metedeconk River population and for other clinging jellyfish sub-populations in New Jersey. The majority of sites sampled this year and in previous years were comprised of moderately to densely distributed branched Rhodophytes (red algae; e.g. *Polysiphonia* spp., *Gracilaria tikvahiae, Agardhiella subulata*, or *Ceramium fastigiatum*). Sea lettuce (*Ulva lactuca*), a Chlorophyte that is abundant in the Metedeconk River, was also observed to be an important component of *G. vertens* habitat (Figure 4). The North Wildwood salt pond differs dramatically in algal composition when compared to the other waterbodies (e.g. *Enteromorpha intestinalis* and filamentous forms [*Cladophora* sp. and other Rhodophyta] predominate in the shallows). Species such as *Ulothrix flacca*, coarse-branched and fine-branched Rhodophytes, and other forms comprise the habitat along the submerged boulder surfaces making up the western shoreline of the basin. However, this waterbody still illustrates the importance of structure for feeding and attachment since areas with moderate to high macroalgae, or congregations of wrack material, were areas where medusae were most often encountered. The exception to these sites were the Island Beach State Park locations, which are largely dominated by SAV (i.e. *Zostera marina*) with low to moderate densities of macroalgae (Figure 10C, 1&2). Additionally, as observed at all sites, water depth preference for *G. vertens* seems to be constrained to between 1.5’ and 5’, where 3’ to 4’ appears to be optimal.

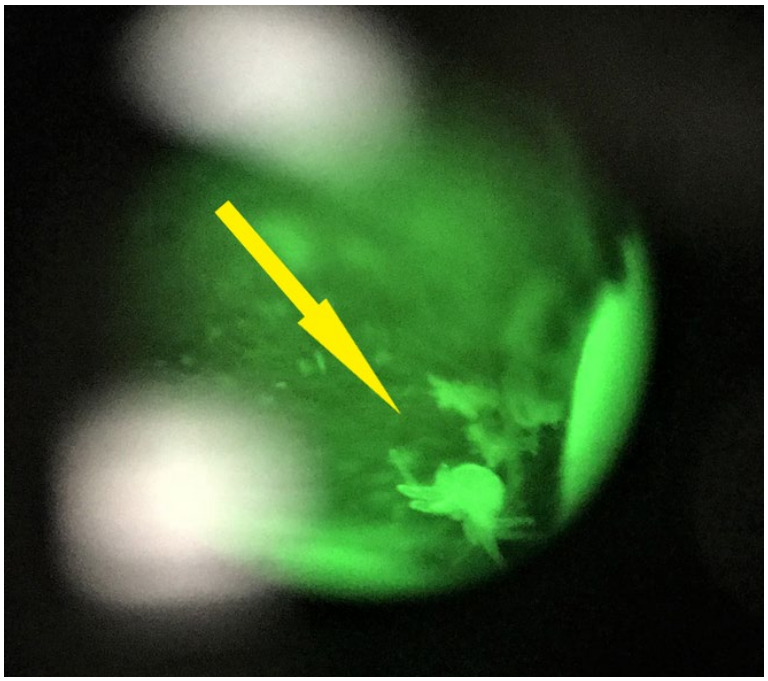


Figure 3. A laboratory cultured *G. vertens* polyp (yellow arrow) attached to the base of an artificial aquarium plant (Photo: NJDEP-DSR).

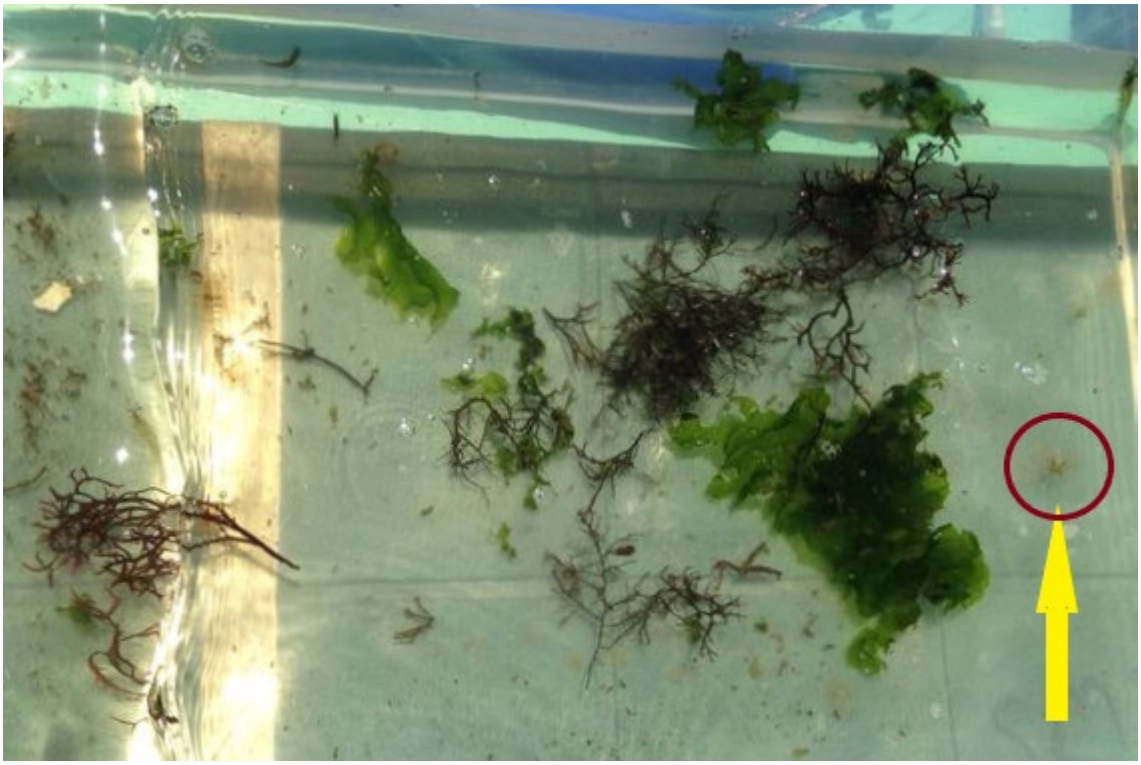


Figure 4. Typical *G. vertens* habitat comprised of mixed macroalgae (above: coarsely branched Rhodophytes and *Ulva lactuca*) in New Jersey waterbodies. Yellow arrow points to an immature medusa (circled in red), one of many present in the photograph (Photo: NJDEP-DSR).

Salinity was not found to be a limiting factor in *G. vertens* distribution, whereas this species was collected in waters with salinities ranging from 15 to 37 ppt. This is an important factor that allows for this species to be successfully translocated to other areas around the world, where typical oceanic salinities will not destroy polyps attached to ship hulls or contained in ballast. However, sub-optimal conditions related to salinity, or other changes in water chemistry can physiologically stress cnidarians such as disruption to reproduction and/or strobilation (Purcell et al. 1999; Purcell 2007; Purcell et al. 2009; Prieto et al. 2010), swimming behavior due to changes in buoyancy (Mills 1984), or negative effects on osmotic potential (Mills and Vogt 1984). Populations of the same species that live at significantly different salinity ranges may not have the same tolerance as one another, even if individuals from one salinity regime are acclimated to a new one. This was found to be true for the North Wildwood and Metedeconk populations, both of which live at very dissimilar salinity concentrations (mean salinities of approximately 32 ppt vs. 20 ppt, respectively). Experiments to test this hypothesis were conducted to compare the salinity tolerance of each group at a wide range of concentrations. Initial results show that the North Wildwood population is more tolerant of extremely high salinity concentrations (53 ppt) as opposed to the Metedeconk individuals (45 ppt) when held at these conditions over a 24-hour period. On the lower spectrum, the Metedeconk medusae were much more tolerant to extremely low salinities (7 ppt vs. 18 ppt for North Wildwood). Salinity tolerance experiments were first performed in 2019 solely on medusae collected from the Metedeconk River; similar salinity thresholds were noted compared with

2020 (range: 7-41 ppt, optimal range: 20-30 ppt). Above and below the optimal range, behavioral and physiological stress were observed. Near the minimum and maximum thresholds, morphological abnormalities were easily visible (e.g. bleaching, severe swelling/shrinking, bell margin and tentacle damage). These results suggest that the North Wildwood population may possess a genetic adaptation or phenotypic plasticity that allows these jellyfish to thrive at the high salinity conditions present in this waterbody and warrants further investigation into this phenomenon.

A total of 17 water samples were obtained from the aforementioned water bodies to investigate for the presence of *G. vertens* eDNA (in addition to 64 obtained in 2019). Although eDNA extraction from water samples is in its initial stages, preliminary work has been done to quantify DNA from *G. vertens* and from *C. chesapeakei* for means of comparison. Three DNA extraction methods were tested using whole clinging jellyfish medusae. DNA yields were highest following the Chelex extraction method, which has been used successfully in other studies (Gaynor et al. 2016). This method will also be used to extract eDNA from filtered water samples for consistency. Although genomic *G. vertens* DNA and eDNA were successfully amplified in late 2019, additional validation work on the *G. vertens* primer set still needs to be completed prior to routine application of eDNA presence/absence verifications to field samples.

It is anticipated that *G. vertens* will continue to expand its range within NJ's estuarine waters and that long-term monitoring is warranted. With this range extension, conflicts with recreational users of waters inhabited by clinging jellyfish may also increase. Detection of eDNA provides a cost-effective and powerful technique for verifying a species' presence or absence within a large geographical area, while limiting field effort. Our work continues in this regard, although once operational, other DEP programs could become involved with the sampling effort and application of this method for non-native species detection.

BACKGROUND

The recent discovery of the invasive clinging jellyfish in coastal New Jersey estuaries poses a potential safety concern to the public during normal, recreational uses of these waterways and highlights the vulnerability of NJ's coastal waters to the establishment of naturalized invasive species. Clinging jellyfish can potentially invade other coastal waterbodies along NJ's coast since suitable habitat and translocation vectors are present in great abundance. Given that boating activity is substantial in all of NJ's waterways and is suspected as being the most important vector in the spread of *G. vertens*, clinging jellyfish may soon become established in coastal embayments throughout the State. This species inhabits shallow water habitats where submerged aquatic vegetation (SAV) and macroalgae are present, and as the common name for this species suggests, clinging jellyfish 'cling' to this vegetation and other substrates (e.g. shell, rocks, and woody debris). Outdoor enthusiasts who enjoy recreational clamming and other activities in shallow areas of these waterbodies, especially with dense SAV and/or macroalgae, may be at risk for direct encounters with this species. *Gonionemus vertens* can deliver a painful and

debilitating sting, which in some cases has required the affected individual to be hospitalized. Detection of clinging jellyfish can be difficult due to their brief period of activity (in NJ, generally between May 15th and mid-July, or when water temperatures are between 15ºC and 28ºC) (Mikulich 1974; Rigby 2020) as well as their localized and limited distribution in the waters they inhabit. Information on population density and range of clinging jellyfish in NJ is limited, which can be problematic with respect to mitigating negative direct and indirect encounters with the public. Recent developments in eDNA analysis can allow for cost effective species detections even when individuals of the target organism cannot be visibly detected (Bologna et al. 2015; Minamoto et al. 2017).

INTRODUCTION

The clinging jellyfish, *Gonionemus vertens* A. Agassiz, 1862 (Olindiidae) is a small hydrozoan native to the northern Pacific Ocean. This hydrozoan is relatively diminutive in size and has a biphasic life cycle, comprised of a sessile benthic polyp phase (formed by the larvae) and a free-swimming pelagic medusa (adult) phase. Adult sizes typically range in diameters from 18-25 mm, comparable to either dime- to quarter-sized, respectively. The adults produce gametes necessary for sexual reproduction, which are shed into the water column. Once fertilized, these will typically develop into planula larvae that will then attach to a suitable substrate and form polyps (Figure 3) or become motile frustules that can become polyps once temperatures (above 15ºC) and conditions are favorable (Uchida 1976; Kayashima et al. 2019). Polyps are much smaller than medusae (approximately 1 mm), solitary, and asexually produce medusae via the process of strobilation (i.e. budding). Medusae typically possess about 60-90 tentacles surrounding the bell, which are bent at 90º angles near the distal ends. At this location, the tentacles also possess adhesive pads (Figure 5) that secrete a bonding compound that allows this species to adhere or 'cling' to various substrates (most often submerged aquatic vegetation - SAV or macroalgae) and consequently gives this species its common name (Singla 1977; Murbach and Shearer 1903).

A peculiar behavior that is unique to this species and a few others in the Family Olindiidae (Order Limnomedusae) is the way in which *G. vertens* swims through the water column while foraging. Specifically, *G. vertens* will swim vigorously toward the water surface in a serious of pulses, where once reached it will abruptly relax, turn upside-down and float straight back down with its tentacles extended (Murbach 1899; Morse 1907; Mikulich and Naumov 1974; Mills 1984; and others). Once food material is encountered, the tentacles are retracted and brought to the manubrium for ingestion of prey and repeated as necessary. This type of predatory behavior has been described as ambush predation (passive) in contrast to cruising predation (e.g. Atlantic Bay Nettle – *C. chesapeakei*) where prey are actively pursued and caught while swimming (Colin et al. 2003).

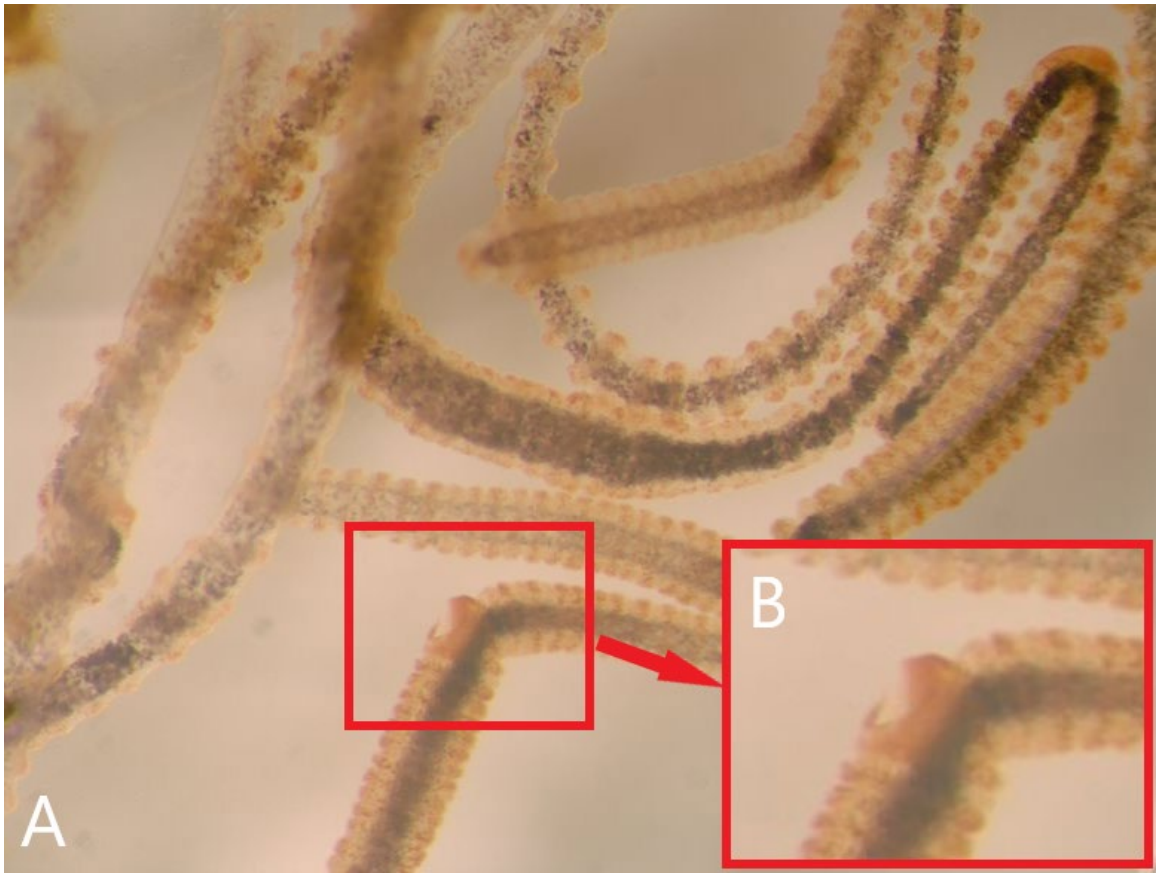


Figure 5 (A – B). (A) 4X magnification of *G. vertens* tentacles with adhesive pads (red box) and ring-like batteries of nematocysts. (B) Expanded view of adhesive pad organ (Photo: NJDEP-DSR).

As with other cnidarians, clinging jellyfish are equipped with a successive series of ring-shaped groups of stinging cells (cnidocytes) along each tentacle, and to a limited extent, along the exumbrella and manubrium as well. Each cnidocyte, in turn, contains a single intracellular organelle called a cnida or nematocyst (Figure 6). The morphology of the nematocysts in *G. vertens* is shared across and unique to the Olindiidae. Specific to *G. vertens*, these are comprised of three different types: microbasic euryteles, microbasic b-mastigophores, and basitrichous isorhizes designed to penetrate the dermis of mostly hard-bodied prey or predators, if warranted (Nagao 1973; Kabuto 1976; Purcell and Mills 1988; Govindarajan et al. 2019). When discharged, the nematocyst becomes inverted and tapered at its distal end, where it is covered by long down-turned spines and from which the distal tubule is ejected. Clinging jellyfish nematocysts contain a powerful neurotoxin composed of numerous polypeptides, many of which have not been fully characterized (Sinstova et al. 2014; Koslovskii et al. 2018). However, recent work by MSU has been successful in identifying some of the most commonly expressed venom candidates (Bliese et al. 2020, in progress). The nematocysts and venom contained therein are precisely designed to immobilize their preferred food source (i.e. copepods, mysids, and other littoral zooplankton) and facilitate ease of capture. Unfortunately, if encountered by humans, envenomation can be quite painful and require medical attention in those with weakened

immunity or allergic sensitivities; muscle cramping, respiratory difficulty, and partial paralysis are common clinical symptoms (Pigulevsky and Michaleff 1969; Fenner 2005; Govindarajan et al. 2019; Rigby 2020), and are usually followed by a host of other secondary systemic effects (Migas 1974). Studies investigating extracts of *G. vertens* venom have demonstrated that the effects are mostly neurotoxic in nature, expressly through ion channel disruption, and secondarily through other mechanisms such as manipulation of macrophage adhesion (Koslovskii et al. 2018).

Figure 6 (A-B). (A) *Gonionemus vertens* tentacles with cnidocyte batteries, each containing the venomous cnidae or nematocysts (1.5X magnification). (B) Inset (red square, upper right) shows expanded view of concentric rings that comprise a single battery of cnidocytes (Photo: NJDEP-DSR).

Clinging jellyfish were first recorded on the east coast of the United States in Massachusetts in 1894. It is speculated that the likely transplant to the western Atlantic may have been accidentally from European oyster transplants (and previously in Europe, the probable vector being Pacific oysters) or release from aquaria (Edwards 1976; Bakker 1978). Additional plausible vectors include release from ship ballast or polyps attached to ship/boat hulls from *G. vertens*-inhabited waters from either European or Pacific ports (Tambs-Lyche 1964). Once established in a location, transplants to new areas in a region can be more elusive, where either anthropogenic (e.g. local boat traffic, transfer of contaminated fishing gear between waterbodies) or natural forces (e.g. storms, water currents, waterfowl) can play a role in dispersal (Jaspers et al. 2018).

Although mostly localized to specific lagoons and back bay areas, *G. vertens* slowly spread to other locations in New England and Long Island Sound (Govindarajan et al. 2017). Clinging jellyfish have now advanced to other locations in the Northwest Atlantic (Figure 7) and are found in a number of temperate coastal embayments throughout the world (Levannter 1961; Gulliksen 1971; Edwards 1976; Bakker 1978; Rodrigez et al. 2014; Marchessaux et al. 2017; and others) including Norway, Sweden, Germany, the United Kingdom, France, the Netherlands, as well as Argentina – the first southern hemisphere record. In 2016, *G. vertens* was observed for the first time in New Jersey from both the Manasquan (Point Pleasant Canal) and the Shrewsbury River estuaries and verified by Montclair State University (MSU) (Gaynor et al. 2016). Following this discovery and a stinging incident in the Shrewsbury River, MSU was contracted by NJDEP to conduct an assessment of the distribution and reproductive potential of clinging jellyfish in NJ waters. Initially, clinging jellyfish were thought only to be restricted to localized areas within the Shrewsbury River. Samples from this population were shown to share DNA sequences similar to a sample obtained from the China Sea, indicating a potential origin for the North Barnegat Bay translocation (Gaynor et al. 2016). However, in 2018, clinging jellyfish were found to be well established in northern and central Barnegat Bay as well (i.e. Metedeconk River, Bay Head, and Island Beach State Park – IBSP, respectively). In contrast, haplotypes from this population were closely aligned with Swedish samples (Gaynor, pers. Communication; Govindarajan et al. 2019). In 2019, a new population - so far the southernmost confirmed location along the northwest Atlantic coast - was discovered in a stormwater retention pond adjacent to Hereford Inlet in North Wildwood. To date, results from a combined 5-year NJDEP-MSU monitoring effort has shown that *G. vertens* has become established in the Shrewsbury River, North Wildwood, and in the northern half of Barnegat Bay.

Eel grass beds (primarily *Z. marina* in North America) and macroalgae serve as critical habitat for *G. vertens*, which is characteristic of these and other medusae belonging to the order Limnomedusae (Kramp 1959; Tambs-Lyche 1964; Kayashima et al. 2019). In their native range, clinging jellyfish are often found among seagrasses (e.g. *Z. marina*, *Z. japonica*, *Phyllospadix iwatensis*) and seaweeds alike (Mikulich 1974; Terauchi et al. 2018). During the 1920's – 1930's, eel grass beds in Massachussetts declined significantly due to wasting disease (caused by the protist *Labyrinthula zosterae*) (Muehlstein et al. 1991), which is believed to have subsequently caused clinging jellyfish to become relatively obscure in those waters until the early 1990's (Govindarajan and Carmen, 2016). Medusae do not appear to be highly host-specific with regard to the species of seagrass or seaweed they associate with (Levannter 1961; Todd et al. 1966; Marchessaux et al. 2017), albeit SAV and macroalgae especially with a more stout, coarsely branched structuring seem to be preferred over others (DSR, pers. observation). In almost all sampling events in New Jersey, *G. vertens* and these substrates appear to be both positively and highly correlated. Unfortunately, little is known about the substrates and settling platforms preferred by planulae and polyps (Perkins 1904; van Walraven et al. 2016). Even though the adults themselves tend to be found in habitats comprised of established SAV and macroalgae, hard substrate in these areas (e.g., oyster and clam shells, rocks, or

construction aggregates) can be lacking, thus requiring further investigation of this enigmatic issue.

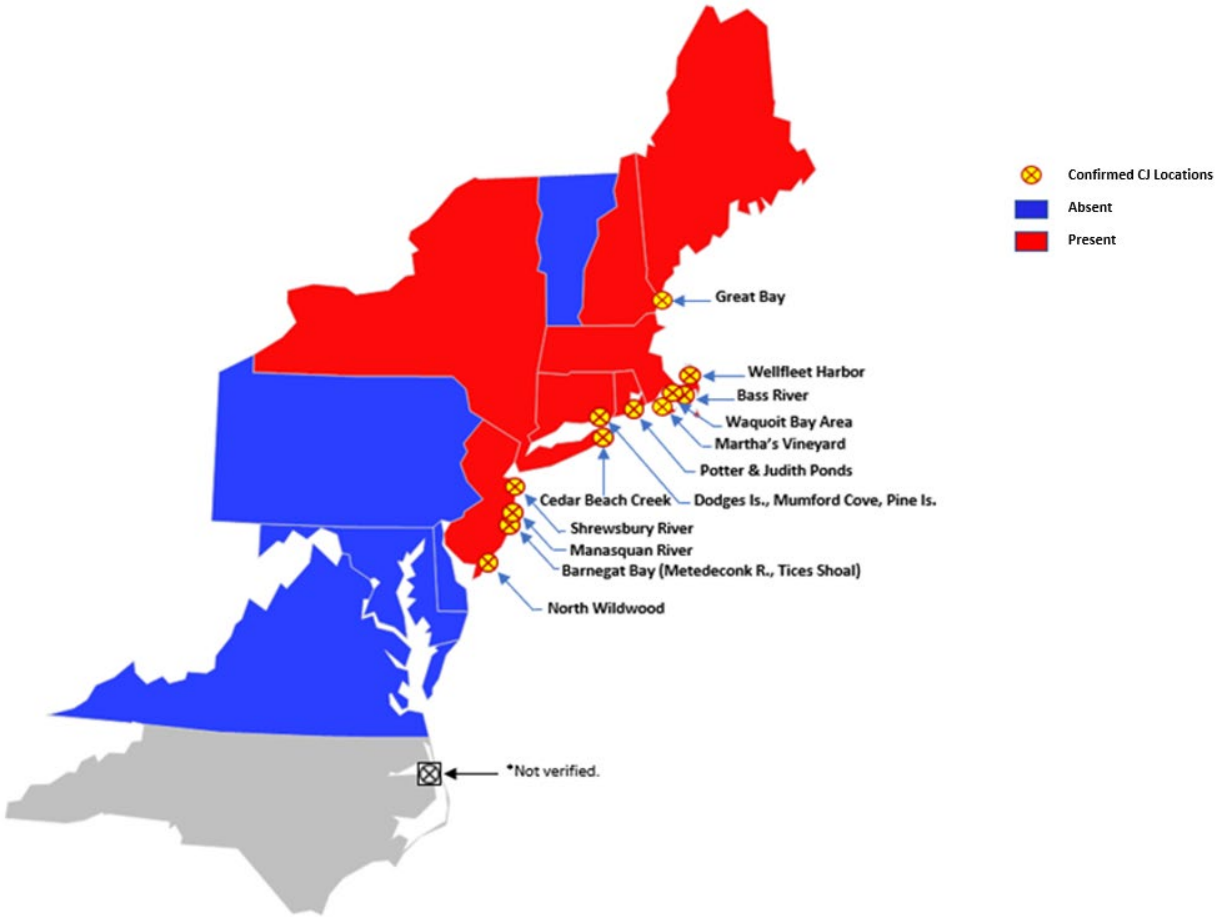


Figure 7. Clinging jellyfish distribution (known locations) in coastal waters of the Northeastern US (Source: NJDEP-DSR).

With respect to detection, eDNA is fast becoming a preferential method for its ease and rapidness in confirming presence or absence of a species. This has been noted by several studies on fish and shellfish, especially during critical periods in life history (Minamoto et al. 2017; Sansom and Sassoubre, 2017). Additional studies have successfully utilized eDNA tools to detect alien species in affected waterbodies, particularly the zebra mussel (Egan et al. 2013; Ardura et al. 2017). The presence of the target species can be detected by obtaining DNA in the environment (shed via excretion, tissue loss, or other means), although the persistence and relative concentration are limited to the periods when the species is active. In waterbodies, DNA fragments can be obtained by sampling the water and amplifying these fragments using species specific primers. These amplified regions of DNA can be fluorescently labeled and detected, indicating the presence of the target organism. The mitochondrial 16S rRNA gene is often used as an amplification target for phylogenetic studies as it is highly conserved between different species (e.g. bacteria

and hydrozoans). The COI locus is also a popular target. The 16S and COI gene loci contain highly conserved sequences between hypervariable regions, enabling the design of universal primers that can reliably produce the same sections of these sequences across different taxa (Gong et al. 2018; Moura et al. 2008), or be used to trace the invasive history of a species (Liu and Dong 2018; Govindarajan et al. 2017). Minamoto et al. (2017) demonstrated that the eDNA sampling approach is also applicable to detecting jellyfish species, as well as showing spatial and temporal distribution. Other recent research conducted on *G. vertens* (and also *G. murbachii*) have successfully produced primers that can be used for detection of the *Gonionemus* spp. (Govindarajan et al. 2017; Gaynor et al. 2016).

Objectives and Scope of Work

To assess seasonal presence and abundance of medusae in known 'hot spots' and compare site-adaptiveness of these different populations, the following objectives were pursued in 2020:

1. Assess the distribution, abundance, and habitat preferences of adult *G. vertens* from known locations identified in prior years.
2. Compare two distinct *G. vertens* populations (North Wildwood and Metedeconk River) for differences in physiological tolerance to differing salinity concentration ranges.
3. Optimize genomic and environmental DNA extraction and detection from whole *G. vertens* specimens and from water samples.
4. Develop customized *G. vertens*-specific primers to optimize detection of clinging jellyfish (eDNA) from sampled waterbodies.

METHODS

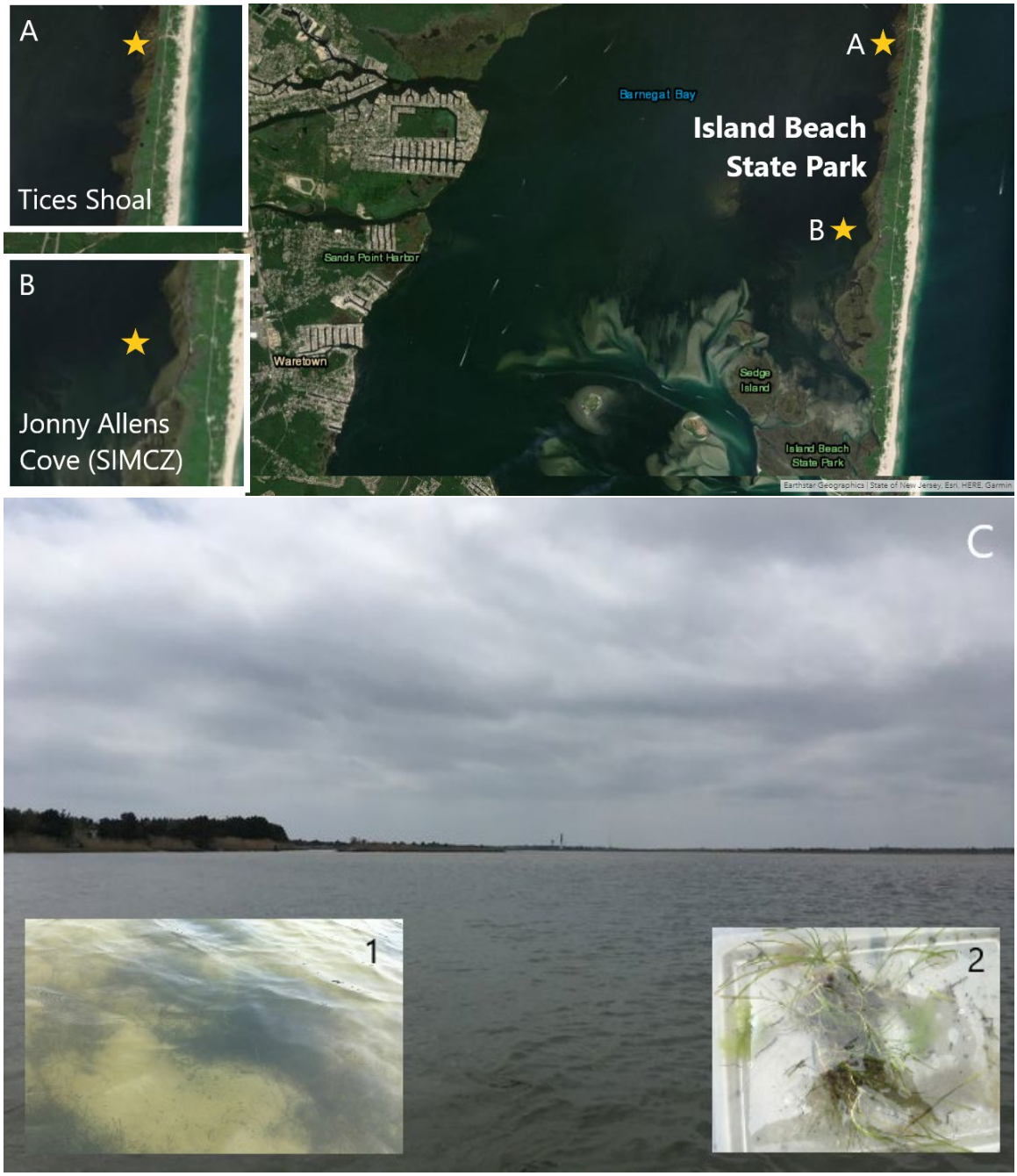
Sampling Sites and Monitoring:

Site sampling and monitoring were conducted from late May to mid-July (2020) at three locations:

- ★ Metedeconk River (MET) - North bank (Windward Beach to Wardells Neck), South Bank (Kingfisher Cove to Edwin B. Forsythe NWR), Beaver Dam Creek, and Bay Head (Figures 8A-B),
- ★ Island Beach State Park (IBSP) - Tices Shoal (TS), Jonny Allens Cove (JAC - Sedge Island Marine Conservation Zone) (Figures 9A-C),
- ★ North Wildwood (NWW) - 1st E. Avenue artificial salt pond, adjacent to Hereford Inlet (Figures 10A-B).

The only previously known, positive location excluded in this year's monitoring was the Shrewsbury River, due to equipment, staffing and traveling constraints imposed by the COVID-19 pandemic.

Figure 8A-B. (A) Metedeconk River (Monmouth County, Brick Township, NJ) (Source: NJDEP-BGIS; (B) North Bank, Wardells Neck (Photo: NJDEP-DSR).



Figures 9A-C. (A) Tices Shoal, IBSP (Ocean County, NJ) (Source: NJDEP-BGIS); (B) Jonny Allens Cove, Sedge Island Marine Conservation Zone – SIMCZ (Ocean County, NJ) (Source: NJDEP-BGIS); (C) Tices Shoal, looking south toward the SIMCZ and Barnegat Inlet; 1. Typical submerged benthic habitat at this site dominated by submerged aquatic vegetation (SAV) with lower densities of mixed macroalgae, 2. *Zostera marina* (Photos: NJDEP-DSR).

Figure 10A-B. (A) North Wildwood stormwater retention pond (Cape May County, City of North Wildwood, NJ) (Source: NJDEP-BGIS); (B) View of the pond looking south (Photo: NJDEP-DSR).

These locations (identified from monitoring in previous years) were selected based on the presence of abundant submerged aquatic vegetation (SAV) and/or macroalgae. A selective [stratified] sampling approach was employed to sample shallow areas (water

depths between 2' – 5') using adjustable-length dipnets to collect/sweep through the macroalgae and SAV. Two sweep methods were employed based on whether sampling occurred from watercraft or along the shoreline. For vessel sampling, after selection of an appropriate sampling site was made, the watercraft was first anchored then location information recorded. At each location, 3-6 net sweeps were conducted over/through observed macrophytes in waters between 0.75 m and 1.5 m depth. A net sweep, defined here as a pull of the net (2 mm mesh size) toward the vessel over approximately a 2.5 m linear distance, was taken at each site from either the port or starboard sides (Figure 11). The sweep volume, calculated as the length of the sweep multiplied by the area of the net opening (2 ft^2), was estimated to be about 16 ft^3 (0.453 m^3). Shoreline sampling was conducted along a perpendicular transect using three depths: 1.5', 3', and 4.5'. A net sweep was conducted at these depths along boulder edges/shoreline for the same linear distance as with vessel sampling (Figure 12). All material collected from nets was then placed in a 20-gallon bin for sorting and identification. Clinging jellyfish (if present) were counted and collected in a 5-gallon glass carboy; SAV/macroalgae and associated organisms were identified (to the best practical extent) and returned to the water. Jellyfish collected were later transported to the NJDEP Arctic Parkway Laboratory Facility (Ewing, NJ) for housing and research (Figure 13).

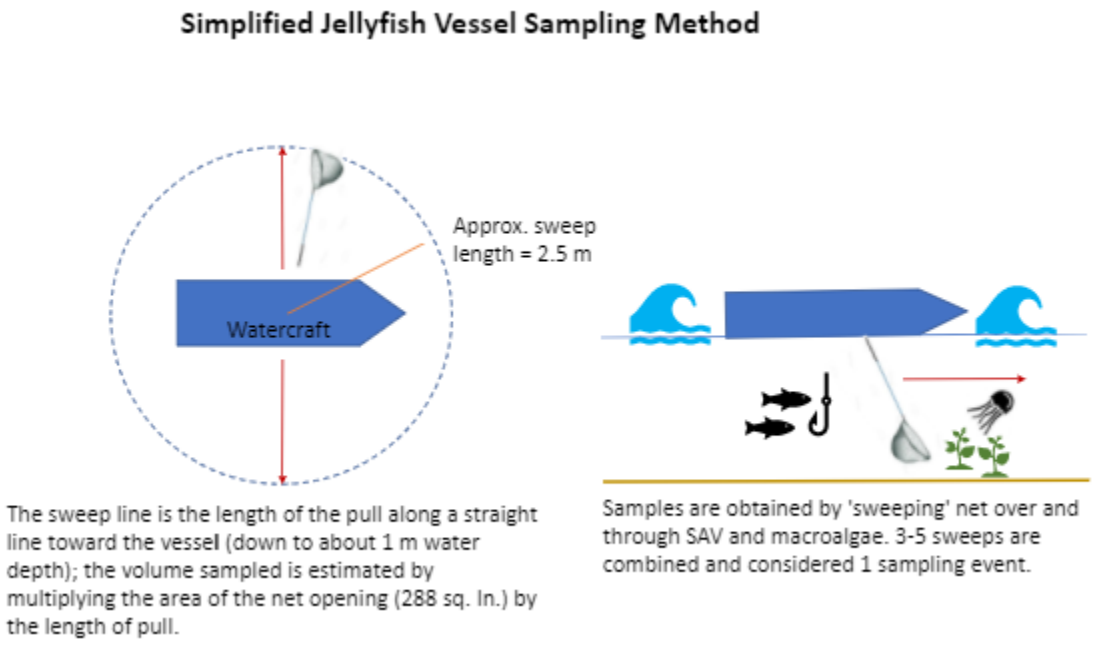


Figure 11. Schematic showing net sweep sampling method from a water vessel.

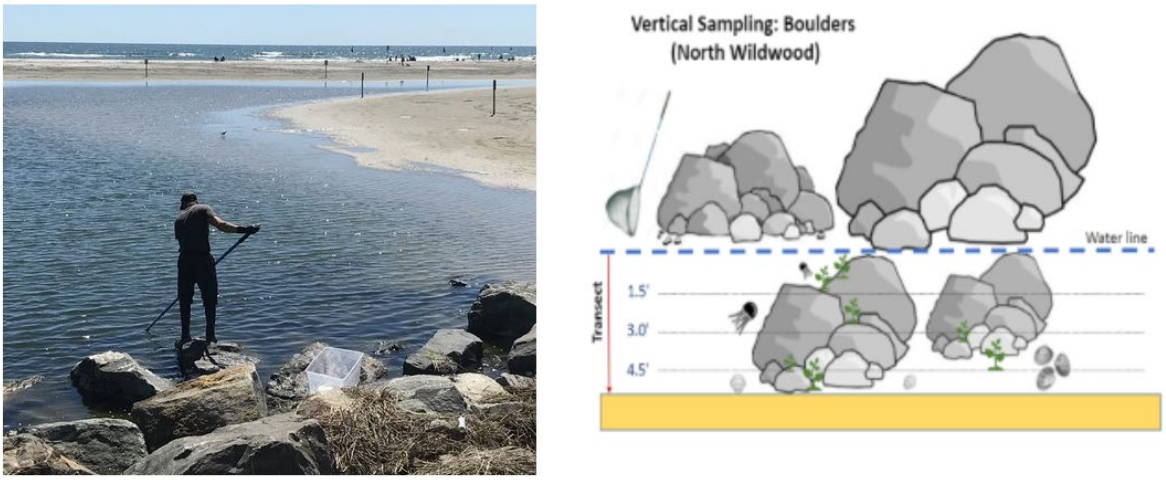


Figure 12. Schematic of perpendicular shoreline transect sampling method via sweep net employed along rock barrier at the North Wildwood salt pond (Photo: NJDEP-DSR).

Figure 13. Clinging jellyfish holding tanks fitted with artificial vegetation, NJDEP Arctic Parkway Laboratory Facility (Ewing, NJ) (Photo: NJDEP-DSR).

Water Quality and Chemistry

Physical characteristics (depth, sediment type, vegetation) and water quality/chemistry (temperature, barometric pressure, dissolved oxygen, conductivity,

salinity, dissolved solids, and turbidity) were measured using an EXO2 Multiparameter Sonde (YSI/Xylem, Inc., Yellow Springs, OH) and recorded at each site to assess habitat characteristics and for comparison to other data sources, when available. GPS location was recorded for all sites and provided to the NJDEP Bureau of GIS to plot all presence/absence occurrences for the "NJ Clinging Jellyfish Information" interactive map: https://njdep.maps.arcgis.com/home/item.htmlid=7ea0d732d8a64b0da9cc2aff7237b475

Live Culture and Housing of G. vertens

Laboratory space and protocols to maintain a seasonal culture of clinging jellyfish were established in order to conduct basic studies to determine rudimentary life-support requirements, seasonal longevity, and responses to abiotic stressors. Approximately 600 (30-50 per tank) *G. vertens* were housed in separate, aerated holding tanks (20-gallon aquaria filled with ~15 gallons of artificial seawater – Instant Ocean®) maintained at constant temperature (20ºC). Tanks were separated and maintained at different salinity concentrations dependent upon where medusae were collected (Metedeconk = 25 ppt; IBSP = 28 ppt; North Wildwood = 32 ppt). Collection dates are provided in Table 1. Salinity was checked and modified as needed twice per week. Each tank was also fed *Artemia nauplii* (brine shrimp) raised in the lab twice per week. Artificial aquarium plants (2-4) were also placed in each tank to provide vertical substrate for medusae to cling to and for polyp attachment. Native nudibranchs (*Cuthona gymnota*) were also maintained in a separate tank (28 ppt) to assess predation potential on medusae.

eDNA collection and DNA extraction

Water samples (1 L) were collected (17 total) using a grab-sample technique taken at about 5 cm below the water surface in areas above the macroalgae/SAV beds from sites where *G. vertens* sampling occurred. Water was collected in amber bottles and held in iced coolers for field holding and transport to the laboratory. Once at the laboratory, samples were refrigerated (4ºC) until processing (between 2-7 days). Environmental DNA detection from water samples was accomplished using jellyfish spatial detection methodology (with modification) following Minamoto et al. (2016).

Positive DNA controls for all detections were accomplished by extracting DNA from clinging jellyfish medusae collected from Barnegat Bay and North Wildwood following a modification of the methods reported by Gaynor et al. (2016). For the purpose of DNA extraction from *G. vertens* specimens, a select number of medusae were humanely euthanized in accordance with guidance documents from the Association of Zoos and Aquariums (2013). For ongoing and future work, tentacles were dissected from the organism and ground and homogenized in a manufacturer supplied extraction solution. DNA extraction was then carried out following the manufacturer instructions with minimal modifications. Modifications were documented and used for the final SOP generation to ensure reliability and repeatability of the extraction method.

Artificial eDNA samples have been prepared in the laboratory and were processed to determine degradation curves as well as ideal eDNA holding times for *G. vertens* DNA under different laboratory conditions (refrigerated vs. non refrigerated samples), using methods modified from those reported in Pilliod et al. (2013). All water samples were filtered and processed at NJDEP's laboratory facility (Ewing, NJ), and the remainder of eDNA extractions and qPCR runs will continue through the winter of 2020-2021.

Salinity Tolerance Experiments

Physiological tolerance to salinity was tested during two experiments conducted July (2019) and June (2020), following the Method of Direct Transfer (Filippov 1998). The 2019 experiments (Phase I) focused solely on the response of the Metedeconk River *G. vertens* population. Clinging jellyfish medusae (n = 60), collected from the Metedeconk River (5/22/19), were held at a salinity concentration close to field conditions (Control: MET = 25 ppt) and maintained at constant temperature (20ºC) prior to the experiment. Salinity response was tested during two trials, the first (Trial 1) using nine saltwater concentrations ranging from 5-45 ppt. One-liter beakers filled with artificial saltwater were prepared with deionized water and Instant Ocean salt to achieve the desired salinity. Medusae were then added and observations recorded at the following time periods: 1 hr, 4 hrs, and 24 hrs. At each time interval, stress response was evaluated by recording the following: mortality (number alive/dead), position in water column (bottom, middle, top of beaker), functional activity (e.g. frequency of bell contraction, swimming, tentacle extension), and general appearance. Once a lethal range was determined, a second trial (Trial 2) was conducted to ascertain a 24-hour LC50 to verify previous results and to determine *G. vertens*' ability to rapidly acclimate to high or low salinity environments.

In 2020, a second experiment was designed (Phase II) to compare medusae collected from North Wildwood to the Metedeconk River population. Clinging jellyfish were collected from both MET (n = 120) and NWW (n = 120) during mid-June (2020). Each population was held at different saltwater concentrations close to field conditions (NWW = 32 ppt and MET = 25 ppt) and maintained at constant temperature (20ºC) prior to the experiment. Salinity response was tested for 10 saltwater concentrations ranging from 4 – 53 ppt. Stress response was evaluated using the same criteria as in Phase I.

RESULTS AND DISCUSSION

Monitoring and Field Surveys

The 2020 field sampling effort was initiated on May 26th, beginning at the North Wildwood salt pond and concluded for all sites by mid-July (7/17/20). This is consistent with observations made in previous years, where medusae are first encountered by mid-May and disappear by mid- to late-July. The sampling effort focused on three waterbodies

verified from previous years as having a *G. vertens* presence. On the initial visit, immature medusae were encountered at the northwest end of the NWW pond with size frequencies (bell diameter) ranging from 4 - 18 mm (mean = 10.84 mm), indicating that strobilation had likely occurred at least two weeks prior. Monitoring of the MET sites began the following week (6/1/20) and concluded on 7/15/20. Immature medusae were also encountered on the first visit, though smaller in size (2 – 14 mm) than those collected from NWW. In 2019, MSU confirmed immature medusae by mid-May from the MET site. Given this, it plausible to assume that the initiation of strobilation was likely in early May since water temperatures of 15ºC and greater are needed for the production of medusae (Tambs-Lynche 1964; Mikulich 1974; Bakker 1980). The IBSP sites were visited on 6/26/20 and 7/16/20, where only seven small medusae (between 8 – 12 mm) were collected solely on the former sampling event. The highest number of medusae collected per sampling effort occurred in early and mid-June when water temperatures were between 17ºC - 25ºC. Dates of observation and medusae presence are similar to those recorded in 2019 (Table 2).

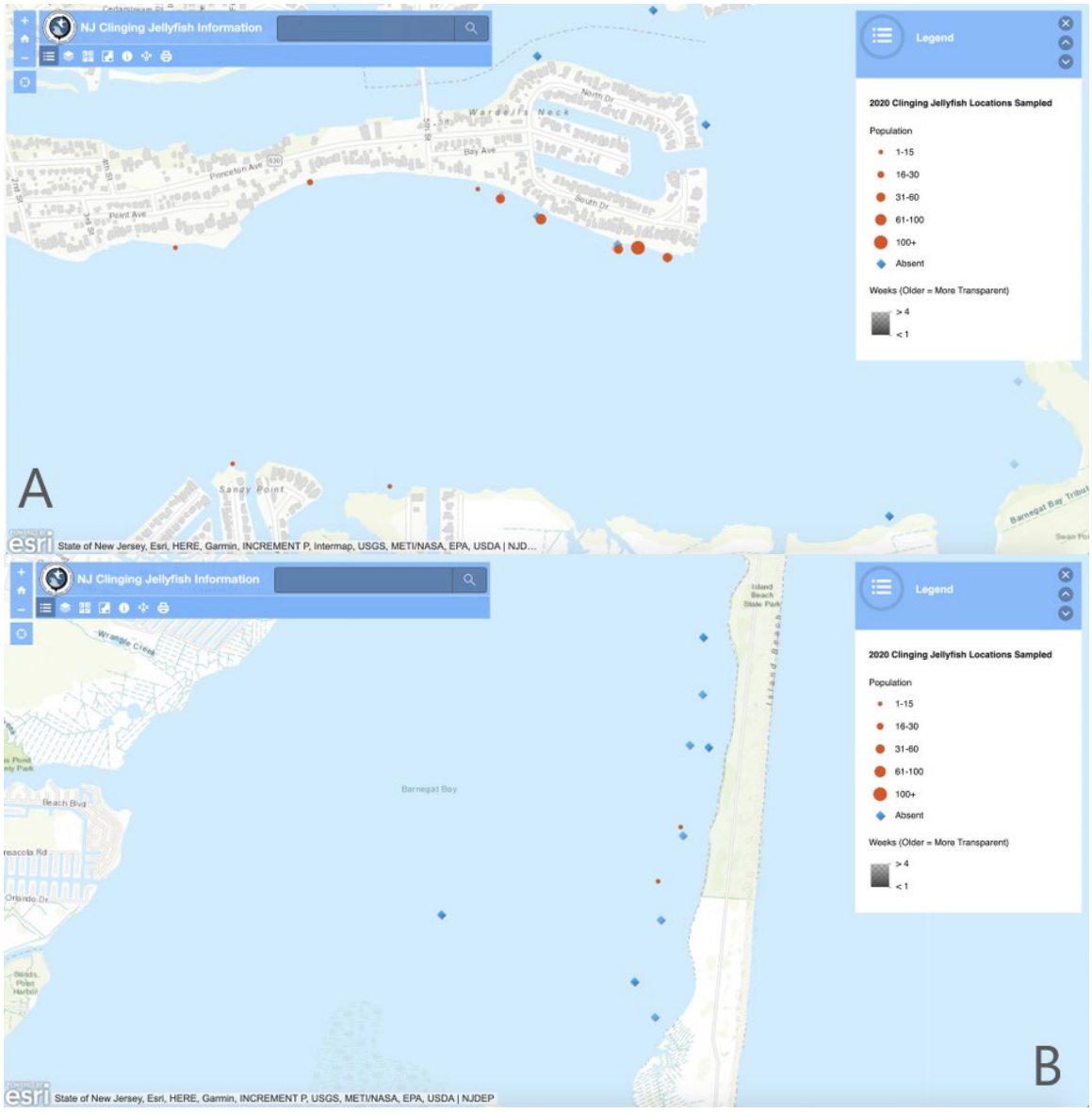
Morphologically, some interesting differences were observed in the NWW population vs. medusae from the Barnegat Bay. Specifically, NWW medusae with three radial canals (trimerous) as opposed to the common tetramerous form were often encountered during field collections (Figure 14). This phenomenon may be due to natural genetic variation in this population, or possibly other environmental, developmental, or a combination of these factors. Variable symmetry has also been observed for other cnidarians, specifically some scyphozoan species (e.g *Aurelia aurita*, and others) where strobilation can result in symmetrical anomalies ranging from 2% to as much as 10% of the time even among clonemates (Gershwin 1999). The NWW medusae also tended to be larger in size than those encountered from MET and IBSP. For example, the largest medusa collected from NWW was 30 mm in diameter, with many in the range of 26 mm and above. In contrast, MET medusae were usually under 22 mm. Additionally, color differed between both populations, where the radial canals of NWW medusae were more gray in color vs. the reddish orange typical of medusae from the Barnegat Bay. Color variation may be a byproduct of the prey type each population has access too. The NWW pond, having water chemistry that distinctly differs from the other waterbodies sampled, may host a unique assemblage of prey types that differs from the other locations. Empirically, we observed that these same medusae would change color over time in the laboratory aquaria after being fed a monotypic diet of *Artemia*.

Combined with MSU, approximately 1,300 medusae were collected during the seasonal effort. Data on number of medusae collected and locations sampled were used to populate the “NJ Clinging Jellyfish Information” interactive map. Screen captures of each of the above monitoring locations with presence/absence data points can be viewed in the Figure 15 (A-C): MET (A), IBSP (B), and NWW(C), respectively.

Figure 14. Morphological variation in the NWW *G. vertens* population (Photo: NJDEP-DSR).

Table 2. NJ coastal waterbodies sampled in 2019 for clinging jellyfish; dates in bold, red-lettering are those where medusae presence was confirmed (NJDEP-DSR sampling only).

Name of Waterbody	Dates Sampled
Metedeconk River	**5/21**, **5/22**, **7/9**, **7/19**, 7/25
Central Barnegat Bay (IBSP)	4/30, 5/7, 5/29, **6/14**, **6/27**, 7/11, 8/1
North Wildwood/Wildwood Crest	**6/11**, **6/26**


NJ Clinging Jellyfish Information
Legend
2020 Clinging Jellyfish Locations Sampled
Population
1-15
16-30
31-60
61-100
100+
Absent
Weeks (Older = More Transparent)
> 4
< 1
A
Sandy Point
State of New Jersey, Esri, HERE, Garmin, INCREMENT P, Intermap, USGS, METI/NASA, EPA, USDA | NJD...
NJ Clinging Jellyfish Information
Legend
2020 Clinging Jellyfish Locations Sampled
Population
1-15
16-30
31-60
61-100
100+
Absent
Weeks (Older = More Transparent)
> 4
< 1
Barnegat Bay
Beach Blvd
B
State of New Jersey, Esri, HERE, Garmin, INCREMENT P, USGS, METI/NASA, EPA, USDA | NJDEP

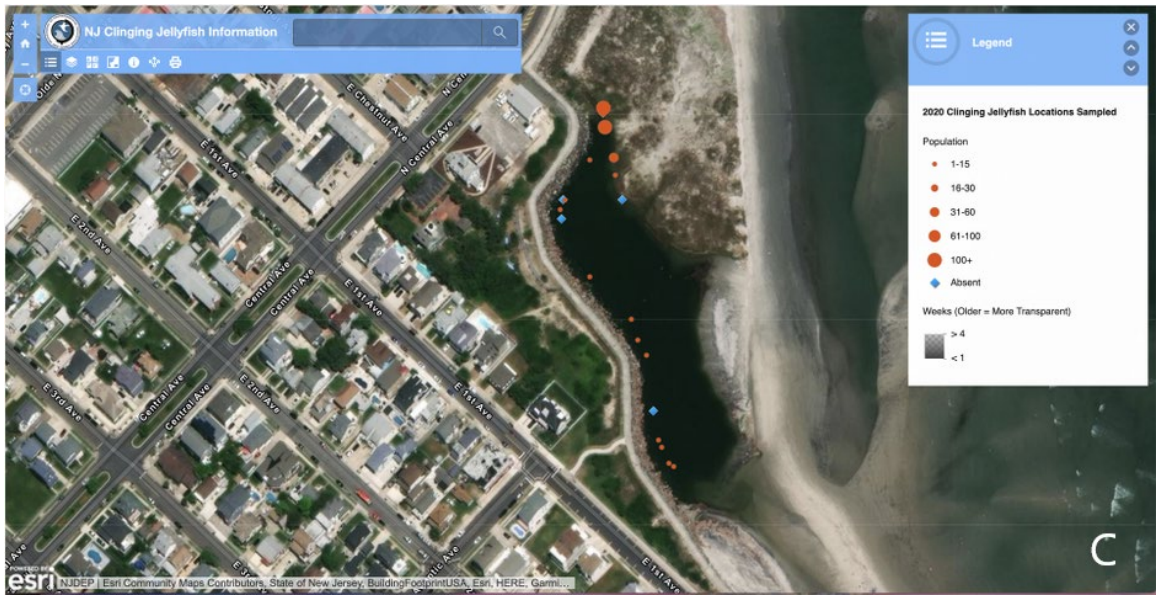


Figure 15 (A-C). Screen capture of combined NJDEP/MSU *G. vertens* 2020 sampling locations: (A) Wardells Neck (Metedeconk River, Brick, NJ); (B) ISBP (Berkeley Township, Ocean County, NJ); (C) An artificial salt pond adjacent to Hereford Inlet (North Wildwood, NJ). All screen captures taken from the "NJ Clinging Jellyfish Information" interactive map (https://njdep.maps.arcgis.com/home/item.html?id=7ea0d732d8a64b0da9cc2aff7237b475. The image shows presence/absence of clinging jellyfish at each location (represented by orange spheres and blue diamonds, respectively) along with relative abundance (Accessed 7/24/2020; Source: NJDEP-BGIS).

Habitat Characteristics and Associated Organisms

Habitat characteristics were similar across most sites where *G. vertens* was observed. In general, bottom vegetation and composition ranged between minimal coverage (~ 10% - 25%) to moderately dense (>50% to 90%+), comprised of macroalgae and or SAV (i.e. mostly *Z. marina*). The majority of sites sampled (NWW and MET) were comprised almost exclusively of macroalgae. Species compositions changed throughout the season, with the highest densities of branched and laminar algae in June and a succession to more filamentous species in July. Figure 16 (A-C) shows a representation of mean-combined SAV/macroalgae coverage for all *G. vertens* positive sites for the purpose of illustrating the common field conditions encountered. At the NWW site, jellyfish hotspots were dominated by the chlorophytes *Ulva intestinalis* (shallows) and *Ulothrix flacca* along the boulder seawall. Coarse-branched rhodophytes (e.g. graceful red weed - *Gracilaria tikhavae* and Agardh's red weed - *Agardhiella subulata*) and fine-branched rhodophytes (e.g. *Ceramium fastigiatum* and beaded weed - *Spyridia filamentosa*) were found at the 2.5' - 3' and greater depth net sweeps, though at low density. Floating wrack and the bases of exposed dune grass (*Ammophila breviligulata*) rhizomes also served as cling sites for the northeast pond shoreline area.

Into July, a greater abundance of filamentous algae was observed, especially the green alga *Cladomorpha* sp. at the same locations. The MET sites had an almost even mix

between mostly coarse-branched rhodophytes (dominated by *Gracilaria tikhavae* and possibly *Polysiphonia* spp.) and sea lettuce (*Ulva lactuca*) growing at very high density (>90% cover; 3' water depth and greater). Shallower sites (2' and less) were often mostly barren or had minimal algae presence (<20% cover). IBSP locations (Tices Shoal – TS, and Jonny Allens Cove – JAC) were slightly different from one another with respect to macroalgae types and density. Tices Shoal had a higher prevalence of fine-branched rhodophytes (e.g. *S. filamentosa* and *C. fastigatum*) compared to JAC which was more diverse (i.e. even mix of laminar chlorophytes [e.g. *U. lactuca*] and coarse-branched rhodophytes [e.g. *G. tikahavae*]). These differences between TS and JAC are likely due to the higher salinities measured at JAC and greater influence from the Barnegat Inlet. This was observed in the previous year where along the length of IBSP, salinity can differ by as much as 6.1 ppt from north to south (unpublished).

Eelgrasses, primarily *Z. marina* and occasionally *Rupia marina*, similarly appeared to have peak coverage and to be at optimal condition in early to mid-June. As we observed in 2019, epiphytic algae and other species completely cover the eelgrasses by early July, especially the filamentous Rhodophytes, causing the *Zostera* beds to deteriorate rapidly. In the Barnegat Bay, Kennish et al. (2011) reported that macroalgae blooms were more frequent in June-July, as well as August-September, which can severely limit light attenuation and contribute to eelgrass decline. Laminar species, such as *U. lactuca*, as well as filamentous species are extremely efficient at diminishing the light intensity reaching the eelgrass beds. Mentioned above, we also observed increases in algal density and succession to filamentous species by July in 2020 and in previous years. The sampling sites along IBSP were the only sites possessing a significant presence of eelgrass.

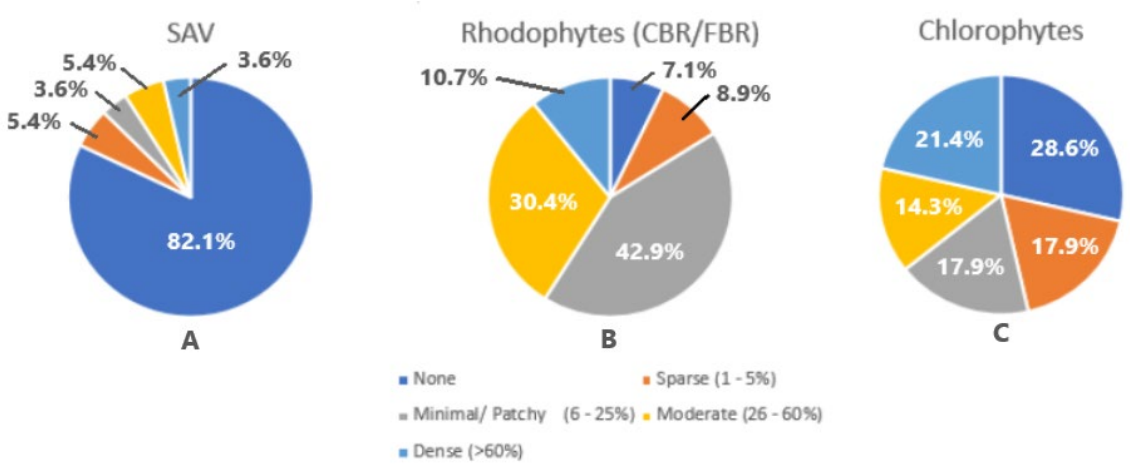


Figure 16 (A-C). Combined percent occurrence of SAV and Macroalgae composition for all *G. vertens* – positive sites. Note that the representation above combines all macrophyte data and serves only to illustrate relative composition; percent coverage and composition can differ significantly at each site. (A) Mean percent SAV coverage; (B) Mean percent, combined coarse- (CBR) and fine-branched (FBR) rhodophyte coverage; (C) Mean percent chlorophyte coverage.

Bottom substrate type appeared to be consistent across sampling sites, being comprised of either firm or loose/muddy sand with either some shell or pebble material

present. Clinging jellyfish planulae (larvae) require hard substrates for attachment as polyps, though vegetative material can substitute if these are not present (Edwards 1976). In field collections from the Amur Bay (Russia), Mikulich (1974) found that polyps were often exclusively attached to shell fragments and pebbles among *Zostera* beds rather than on the leaf blades or rhizomes. However, as in other Olindiidae species, *G. vertens* planulae can also metamorphosize into mobile frustules, which can move about unrestricted throughout the benthos (Uchida 1976; Kayashima et al. 2019). From our observations in the laboratory, polyps developed on the bases of the artificial plants provided, and none were found on either the softer plastic leaf and stem portions of these or on natural algae that was present in the aquaria. Habitats sampled at sites that were extremely muddy with substantial detritus and turbid water, on all occasions, did not yield medusae.

The positive sites sampled all supported several faunae indicative of good water quality (e.g. blue crabs – *Callinectes sapidus*, Atlantic silversides – *Menidia menidia*, northern pipefish - *Syngnathus fuscus*, common grass shrimp – *Palaemonetes* spp., and isopods such as *Idotea balthica* - an inhabitant of seaweed and seagrasses), and are known to be important foraging, breeding, and reproductive grounds for estuarine dependent species. Importantly, these littoral habitats support nematodes, copepods, and other plankton that are the preferred diet of clinging jellyfish (Mikulich 1974; Bakker 1980). Associated species and assemblages were similar between all *G. vertens*-positive sites. The most commonly encountered organisms were grass shrimp, blue crabs, isopods, and eastern mudsnails (*Tritia obsoleta*).

Cnidarian and ctenophore species most often observed at clinging jellyfish sites were *Nemopsis bachei*, *C. chesapeakei*, and *Mnemiopsis leidyi*. *Nemopsis bachei*, a more pelagic species often confused for *G. vertens*, was encountered at high densities concurrent with peak clinging jellyfish activity. During the spring in the Chesapeake Bay, *N. bachei* is the most abundant cnidarian species, and has a tremendous negative influence on the abundance of copepods (Purcell and Nemanzie 1992). In NJ waters, the early spring emergence of these holo- and meroplankton, in combination with optimal water temperatures, appears to overlap with the seasonal niche and feeding preferences of both species, which may explain their co-occurrence. *Mnemiopsis leidyi*, a ctenophore (comb jellyfish) commonly known as the "sea walnut", was active along IBSP during July sampling efforts. Similar to the clinging jellyfish, *M. leidyi*, though native to the northwest mid-Atlantic, are a very successful invasive species and thus have become globally widespread (Jaspers et al. 2019). The cnidarian predator, *C. chesapeakei,* was observed only at the MET sites beginning in June (although they are also prevalent in the Shrewsbury River Estuary, which was not sampled in 2020; personal observation). In 2018 and 2019, the decline of *G. vertens* in both the Metedeconk and Shrewsbury Rivers closely coincided with the appearance of bay nettles and warmer water temperatures ($\geq 26^{o}$C). Bologna et al. (2015) found that in the Barnegat Bay, Atlantic bay nettles had assumed the status of top predator, consuming almost any pelagic species that they are physically capable of ingesting. Purcell and Cowan (1995) likewise studied the predation of *C. chesapeakei* (formerly *C. quiquecirrha*) on *M. leidyi*, demonstrating that bay nettles had a significant top-down effect on controlling comb jelly abundance.

Carmen et al. (2017) reported that the longnose spider crab, *Libinia dubia*, was found to prey on *G. vertens* medusae, albeit with negative consequences. Whether crabs or other species can operate as efficient predators of clinging jellyfish has yet to be determined. A newly identified predator of clinging jellyfish (Bologna et al. 2020; DSR personal observation), the nudibranch *Cuthona gymnota* (collected at both the NWW and MET sites), has been shown to forage on polyps and resting medusae. Aeolid nudibranchs, in this case *Cuthona* spp., are known predators of cnidarians and many preferentially prey on only one species (Folino 1989). We coincidentally observed how effectively *C. gymnota* can remove *G. vertens* medusae from a holding tank. Following the unintentional introduction of the alga *G. tikhavae* to a MET-tank, we noticed that six nudibranchs had soon appeared and subsequently devoured the medusae (approximately 30 individuals) over a five-day period. Bologna et al. (2020) had found that in controlled experiments, nudibranchs will preferentially feed on *C. chesapeakei* polyps over sea anemones (*Diadumene lineata*) when presented with the choice, although partial consumption was often the case. Similarly, with *G. vertens*, nudibranchs will often partially consume the medusa, browsing only the tentacles and leaving the bell largely intact (we had likewise observed this phenomenon in a simple benchtop trial). Bologna et al. (2020) surmised that this species may, under ideal conditions, exert some level of population control in polyp-abundant waterbodies. In the presence of predators, aeolid nudibranchs often choose to forage on cnidarians due their ability to sequester nematocysts from them as a mode of protection, a process known as 'kleptocnidae' (Frick 2003). Future studies are needed in the Barnegat Bay and elsewhere to ascertain what level of predation pressure is being exerted on nudibranchs and if this drives their preference to consume polyps vs. other food sources. With regard to serving as agents of jellyfish population control, it is plausible that this would only occur at a small scale due to the availability of competing food sources and other extenuating factors.

Water Chemistry and Quality

Water chemistry and quality measurements were taken at all sampling sites throughout the season, with the exception of two events where only medusae collections were the sole objective. Dissolved oxygen, salinity, turbidity (TDS and FNU), and depth comparisons between the NWW and MET sites are shown in Table 3. With the exclusion of water depth and turbidity (FNU), DO, salinity, and total dissolved solids (TDS) differed significantly between sites. Salinity differences between NWW and MTT (31.6 ppt vs. 19.8 ppt, respectively) are most notable and served as the impetus for testing salinity tolerance between these populations. Similar observations for these waterbodies were made in 2019 (NWW = 30.1; MET = 20.0 ppt). Dissolved oxygen was much higher at the MET - Wardells Neck locations (mean = 9.59 mg/L), which is considered a healthy concentration that supports most fish species. IBSP was not far behind with regard to DO (mean = 8.55 mg/L), however North Wildwood was on the lower threshold at 6.94 mg/L (though during site visits, numerous small fish species and larvae were observed indicating at least sufficient water quality).

	DO (mg/L)	Salinity (ppt)	TDS	FNU	Depth (m)
NWW (n = 6)					
Mean	6.94	31.6	29.24	2.07	0.67
Standard Deviation	0.67	4.12	1.74	0.91	0.20
MET (n = 11)					
Mean	9.59	19.8	19.37	3.11	0.84
Standard Deviation	1.64	2.78	2.63	1.01	0.14
Mann Whitney U-Test (U_{crit} = 13)					
U-stat	0	1	0	18	18.5
z-stat	3.2664	3.1678	3.2664	1.4573	1.4079
p-norm	*0.0011*	*0.0015*	*0.0004*	0.1453	0.1592
p-exact	*0.0002*	*0.0007*	*0.0004*	0.1497	0.1563
p-simul	*0.0002*	*0.0003*	*0.0002*	0.1490	0.1560

Table 3. Nonparametric comparison of water quality/chemistry parameters and depth between North Wildwood (NWW) and the Metedeconk River (MET) sites ($p < 0.05$) in 2020 (n = no. of sampling events at the given location); values in red indicate statistical significance between sites for the given parameter.

Water temperatures (ºC) were recorded at the majority of sites through the 2020 *G. vertens* season and were similar across waterbodies over the sampling period. Since monitoring began at the end of May at NWW, only one temperature was recorded for this site during this time (17.1ºC). As stated earlier, immature clinging jellyfish medusae were present at this location and in high abundance. Figure 17 shows average water temperatures (June and July) at all confirmed *G. vertens* locations. For June at the NWW and MET locations, initial water temperatures were recorded at 19.4ºC and 19.1ºC, respectively. IBSP sites (TS and JAC) were not sampled until 6/26/20, though mean water temperatures recorded here (25.8 ± 0.3ºC) were similar to those measured at NWW and MET during this period (25.5ºC and 23.7ºC, respectively). By mid-July, medusae were no longer found at any of the sites, where water temperatures were close to (e.g. MET = 27.0 ± 0.4 ºC) or had credibly exceeded the physiological maximum for this species.

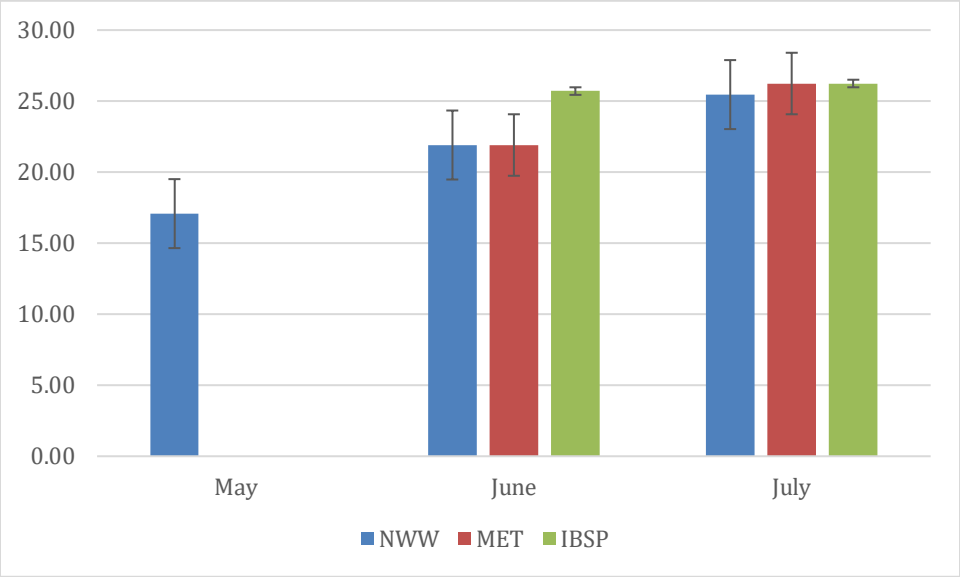


Figure 17. Mean monthly water temperatures (°C) recorded for three *G. vertens* – positive sites (NWW = North Wildwood, MET = Metedeconk River, IBSP = Island Beach State Park) during the 2020 NJDEP monitoring effort.

Environmental DNA (eDNA) Detection: (ONGOING)

Efforts to build eDNA detection capabilities are currently ongoing. The following is a brief summary of the preliminary results from this work (Note: the full extent of detail, resultant data, analyses, and conclusions will follow in a later report). Four extraction methods have been tested on whole *G. vertens* tissue and from 45 µm filters (eDNA collected from water samples):

1. Isolation of genomic DNA from tissue culture cells
2. Purification of genomic DNA from animal tissue (Promega, Inc.)
3. Chelex DNA extraction protocol for animal tissue (used by MSU)
4. CTAB Extraction protocol for genomic DNA (G-Biosciences, Inc.)

All samples (whole and partial *G. vertens*, eDNA from water samples) were compared to DNA extracted from a bay nettle sample (*C. chesapeakei*) to test for primer/probe specificity and detection ability for the 16S and COI gene loci. Amplification of both species' DNA was successful for the DSR-designed primer/probe and the MSU primer/probe sets. Initial quantification of extracted *G. vertens* genomic DNA (2019) was as follows:

- Tentacles and 1/8th body: yield = 1.34 µg/mL
- Whole body: yield = 1.84 µg/mL

However, the strength of binding to the *G. vertens* DNA was weak and a new primer/probe set needs to be redesigned. Presence/Absence assays with the probe did work, but the

detection level was very low; we expected the signal to be higher, hence the need to continue optimization. The in-house *G. vertens* samples were sent to a commercial lab (GENEWIZ, LLC, South Plainfield, NJ) for sequencing and a new primer/probe will be designed using the raw sequences provided using publicly available software, specifically the BLAST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) which compares the DNA homology against all known genetic sequences for that and related species.

Following redesign of the primer/probe set, qPCR optimization experiments will be run to create a standardization curve used for quantitation in conjunction with optimal C_t (Threshold cycle) values, and a melt curve to verify the purity of amplified product. These experiments will also determine optimal concentrations of forward (5' to 3') and reverse (3' to 5') primers for presence/absence detection. Three cohorts of filtered water samples (i.e. 45 µm filters; n =159) will be tested and analyzed during the winter of 2020-21:

- Field: 64 (2019); 17 (2020)
- eDNA degradation: 60
- eDNA dilution: 18

Field Samples

A total of 81 eDNA samples were collected from field sites in 2019 and 2020: Northern and Central Barnegat Bay, North Wildwood, Shrewsbury River, Navesink River, Grassy Sound, and Stone Harbor. These samples will be analyzed for the presence/absence of *G. vertens* DNA.

DNA Degradation

Degradation rates of eDNA using artificially prepared samples (n = 60) from lab tanks housing *G. vertens* were conducted over a period of 5-weeks (2019). This experiment was conducted in order to evaluate storage protocols of eDNA in ambient and refrigerated conditions. These samples will be compared for differences in eDNA degradation rates under both conditions.

eDNA Dilution Study

Gonionemus vertens eDNA samples (n = 18) were collected from a holding tank in 2019. Artificially prepared dilutions were created to test for consistency in detection at environmentally relevant concentrations. The results from this experiment will be used to generate a curve which should give an approximate value of how much eDNA can be collected based on proximity to adults, and to determine a minimum detection limit.

Salinity Tolerance: NWW vs. MET

The information below provides a brief summary of the preliminary results derived from experiments investigating *G. vertens*' tolerance to salinity, initiated in 2019 and

continued in the summer of 2020 (Note: full details, results and conclusions will follow in a later report).

Phase I, conducted in 2019, included medusae collected solely from the Metedeconk River. Salinity trials revealed that MET medusae can survive (at least short term) at concentrations as high as 41 ppt and as low as 7 ppt, although salinities above 35 ppt and below 15 ppt appear to severely stress these organisms, which we suspect may impact long term feeding and survival. The lower threshold 24-hour LC50 was determined to be 10.2 ppt, while the upper threshold LC50 was determined to be 41.4 ppt. In 2020, Phase II of the study compared salinity tolerance between two different *G. vertens* populations that live at markedly different ambient salinities: NWW (31.6 ppt) and MET (19.8 ppt). Preliminary results have shown that at the highest concentrations, mortality was observed for all MET medusae (above 45 ppt), where individuals from NWW were still alive yet showing signs of severe stress at 53 ppt. Consequently, these medusae recovered within 24-hours after being returned to control conditions. However, the physical condition following the 53 ppt treatment did significantly damage these individuals and they survived only for up to two weeks following the experiment. At the lower extreme, MET medusae were more tolerant than NWW jellyfish and some mortality not observed until the 7 ppt treatment (although physical damage such as distention of the radial canals was evident). Only medusae held at the 11 ppt saltwater concentrations recovered following return to control conditions. Consequently, mortality was observed for all NWW medusae at and below 11 ppt. As with other medusae returning to control conditions following extreme salinity exposure, survival following the experiments was a week at best.

In both phases, it was evident in observations that buoyancy is affected by rapid changes in salinity, which is unsurprising due to the differences in osmotic potential between the organism and its surrounding environment. At the highest salinity concentrations (≥ 40 ppt), medusae were positively buoyant (floating at the surface) and required an hour or more to acclimate to the new salinity regime. The opposite was observed at the lower salinity concentrations. For example, at 18 ppt and below, many medusae experienced difficulty swimming and were negatively buoyant (at the bottom of the beakers). Similar results were observed by Mills (1984) for *G. vertens* and other hydrozoan species, where abrupt alterations in salinity affected the organism's buoyancy and ability to swim. For many species, salinity gradients can act as barriers to movement and range expansion (Purcell et al. 1999; Nowaczyk et al. 2016). For example, movement of hydromedusae alien to the North Sea (e.g. *Nemopsis bachei*, *Blackfordia virginica*, and *Maeostis marginata*) demonstrated that salinity gradients dictated bloom dynamics and seasonal distribution (Jaspers et al. 2018). Although the interaction between organism and salinity is not always physiological, salinity gradients determine prey densities and availability which in turn can affect predator population dynamics (Purcell et al. 1999; Nowaczyk et al. 2016). Behavioral changes were also noted at the extremes, where above the optima (i.e. 20 - 35 ppt), tentacles were often partially contracted, bell contraction was either rapid (≥ 45 ppt) or happened seldomly (≤ 11 ppt) and feeding behavior non-existent.

Tissue damage (i.e. edema and distension, and distortion of portions of the radial canal and bell margin) was also observed for individual medusae at the upper and lower

limits of salinity (Figure 18). At salinities above 45 ppt, darkening of the radial canals was evident, including the mesoglea appearing cloudy and bell contracted, and the tentacles brittle and easily broken. Interestingly, at the lowest salinities, bell porosity was seemingly affected since radial canals became grossly expanded and pigments released into the mesoglea, as well as into the surrounding medium (Figure 19). Many cnidarian species can tolerate a broad range of salinities, even if their affinity is for meso- or polyhaline waters (Mills 1984; Nowaczyk et al. 2016). However, at the extreme ranges, those of which may be experienced naturally during substantial rainfall or storm events, the ability of *G. vertens* and other species to survive for short periods at the extremes may contribute to their long-term success and invasive potential. Still, future work will need to investigate the ability for *G. vertens* to acclimate to these extreme ranges in salinity slowly over time, so as not to shock organisms with drastic changes in concentration. Histology would be a useful tool to determine changes in cellular structure and function as a result of extreme salinity changes.

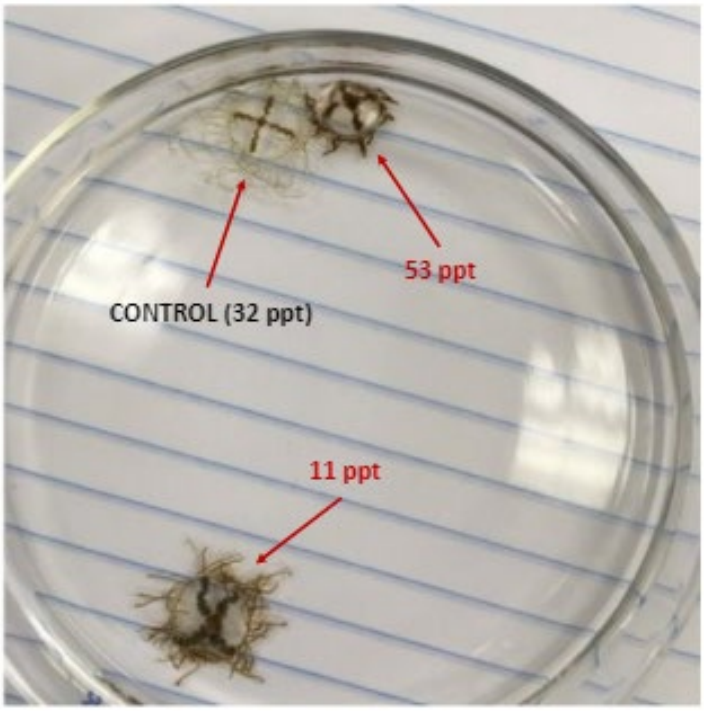


Figure 18. Visible effects on NWW *G. vertens* medusae (alive) following a 24-hour exposure to three different salinity concentrations (Control – 32 ppt vs. High – 53 ppt and Low – 11 ppt concentrations) (Photo: NJDEP-DSR, July 2020)

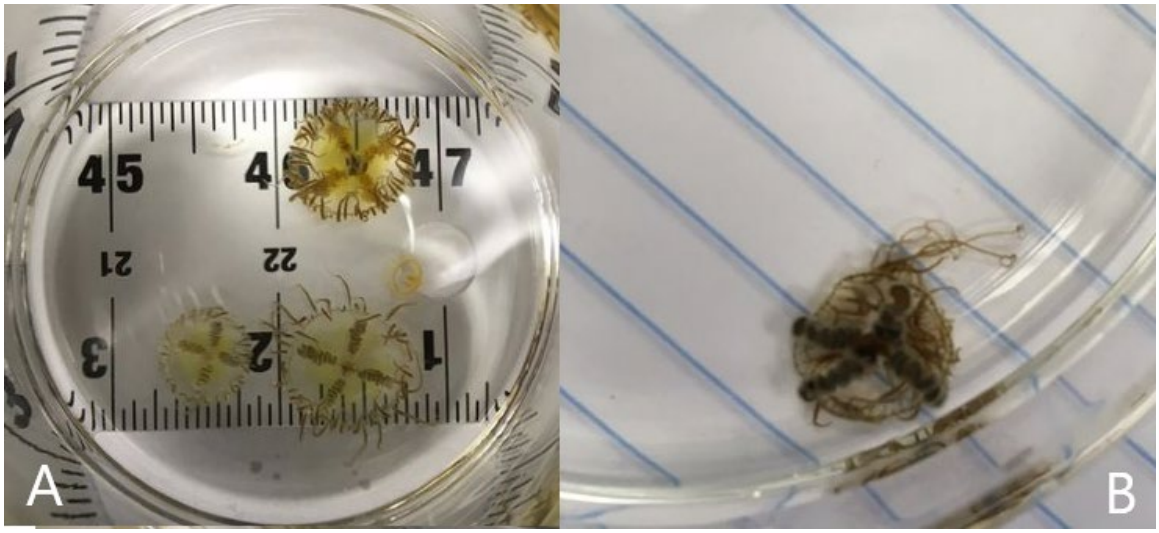


Figure 19. The visible effects of an extremely low salinity concentration (7 ppt) on NWW *G. vertens* medusae. (A) Bleaching: loss of pigments from gonadal tissue into mesoglea. (B) Gross distension of radial canals and gonadal tissues (Photo: NJDEP-DSR).

Conclusions and Recommendations for Future Study

Clinging jellyfish were first discovered in NJ in 2016, and since have been observed in subsequent years. Based on five years of monitoring, it is very likely that clinging jellyfish will continue to extend their range to other water bodies based on the availability of suitable habitat and conditions, and accidental transfer to new locations via recreational boating and or storm events. With climate change significantly affecting New Jersey's coastal environment, seasonal shifts in this species' appearance and or longevity could be experienced with unforeseen consequences (e.g. range extension, higher abundance, etc.). Consequently, the potential for increased public health conflicts is possible with magnified opportunities for contact between those using these waters and *G. vertens*.

This objective of this project is to serve as a pilot study for the Division of Science and Research to test the potential for and to optimize eDNA screening for elusive and or non-indigenous wildlife. This phase of the study is ongoing; however, positive results would indicate our ability to use eDNA to detect the presence of rare, obscure, endangered, or non-native invasive species in environmental samples. Additional studies on *G. vertens* could elucidate additional information on this cnidarian's role as an invasive species in New Jersey. Clinging jellyfish appear to be extremely tolerant of a wide range of salinities and will opportunistically feed on zooplankton and other similarly sized biota in estuarine waters. Blooms occur in highly productive estuarine habitats and it is not known if their density can impact commercially important fish or shellfish species. It is recommended that monitoring at primary locations and exploration of new waterbodies continues for this species in 2021 and beyond, given that some of the confirmed *G. vertens* locations are recreationally important (e.g. Tices Shoal), increasing the likelihood of human contact with this species. Further, to facilitate the success of monitoring efforts and to increase the power of detection (for both clinging jellyfish and other pernicious or non-

native/invasives), it is strongly recommended that the eDNA investigations continue to be supported to allow further development for application in New Jersey, as well as explore the life history requirements, physiological constraints, and invasive potential of this organism.

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APPENDIX A

Table A-1. 2020 combined NJDEP/MSU *G. vertens* sampling locations and presence/absence data (NWW = North Wildwood; MET = Metedeconk River; BDC = Beaver Dam Creek; NBB = North Barnegat Bay; TS = Tices Shoal – IBSP; JAC = Jonny Allens Cove – IBSP).

Location	Site ID	Lat	Long	Date	GV (Present-P/ Absent-A)	No. Collected (N = 1306)
NWW (rock barrier-1)	NWW0526-1	39.00571	-74.79002	5/26/20	P	5
NWW (rock barrier-2)	NWW0526-2	39.00627	-74.79018	5/26/20	P	9
NWW (rock barrier-3)	NWW0526-3	39.00721	-74.79050	5/26/20	P	1
NWW (west end/storm pipe)	NWW0526-4	39.00738	-74.79040	5/26/20	P	78
NWW (west end/storm pipe)	NWW0601-1	39.00748	-74.79041	6/1/20	P	291
NWW (rock barrier-1)	NWW0601-2	39.00700	-74.79067	6/1/20	P	4
NWW (rock barrier-2)	NWW0601-3	39.00660	-74.79050	6/1/20	P	1
NWW (rock barrier-sidewalk entr)	NWW0601-4	39.00575	-74.79004	6/1/20	P	1
MET (Wardells Neck-1)	MT0602-1	40.05728	-74.06996	6/2/20	P	50
MET (Wardells Neck-2)	MT0602-2	40.05684	-74.06880	6/2/20	P	80
MET (Wardells Neck-3)	MT0602-3	40.05618	-74.06659	6/2/20	P	60
MET: Wardells Neck-4	MT0602-4	40.05600	-74.06520	6/2/20	P	41
NWW (west end/storm pipe	NWW0608-1	39.00738	-74.79040	6/8/20	P	>100
NWW (west end/dune - shoreline)	NWW0608-2	39.00713	-74.79033	6/8/20	P	5
NWW (rock barrier-light house)	NWW0608-3	39.00695	-74.79070	6/8/20	P	1
NWW (rock barrier-W sidewalk entr)	NWW0608-4	39.00619	-74.79012	6/8/20	P	4

Location	Site ID	Lat	Long	Date	GV (Present-P/ Absent-A)	No. Collected (N = 1306)
NWW (rock barrier-E sidewalk entr)	NWW0608-5	39.00561	-74.78994	6/8/20	P	12
MET (Wardells Neck-1)	MT0609-1	40.05622	-74.07922	6/9/20	P	2
MET (Sandy Point – Beach)	MT0609-2	40.05150	-74.07760	6/9/20	P	1
MET (Wardells Neck-2)	MT0609-3	40.05765	-74.07538	6/9/20	P	19
MET (Wardells Neck-3)	MT0609-4	40.05621	-74.06659	6/9/20	P	21
Bay Head Beach (NBB)	BH0609-5	40.06268	-74.05440	6/9/20	A	0
MET (EBF- NWR)	MT0609-6	40.05037	-74.05887	6/9/20	A	0
NWW (west end-1/storm pipe)	NWW0615-1	39.00745	-74.79041	6/16/20	P	25
NWW (west end-2/dune)	NWW0615-2	39.00722	-74.79034	6/15/20	P	8
NWW (rock barrier-1/AngS sign)	NWW0615-3	39.00700	-74.79068	6/15/20	A	0
NWW (rock barrier-2/point)	NWW0615-4	39.00638	-74.79022	6/15/20	P	1
NWW (rock barrier-3/E sidewalk entr)	NWW0615-5	39.00563	-74.78997	6/15/20	P	3
NWW (west end-1/storm pipe)	NWW0617-1	39.00747	-74.79041	6/17/20	P	48
BDC-1 (north bank, west of Midstream bridge)	BDC0619-1	40.06040	-74.07600	6/19/20	A	0
BDC-2 (south bank, east of Midstream bridge)	BDC0619-2	40.06040	-74.06890	6/19/20	A	0
BDC-3 (north bank, east of Midstream bridge)	BDC0619-3	40.06140	-74.06560	6/19/20	A	0
Bay Head/NBB (east end - Wardells Neck)	BH0619-4	40.05890	-74.06410	6/19/20	A	0
MET-5 (Windward Beach)	MT0619-5	40.05610	-74.11090	6/19/20	A	0
MET-6 (south bank, Kingfisher Cove)	MT0619-6	40.04900	-74.09910	6/19/20	A	0
MET-7 (south bank, Metedeconk R. Yacht Club)	MT0619-7	40.05100	-74.07310	6/19/20	P	4
MET-8 (north bank, Wardells Neck)	MT0619-8	40.05750	-74.07060	6/19/20	P	11
NBB (EBF NWR, south of MET mouth)	MT0619-9	40.04900	-74.05740	6/19/20	A	0
MET-10 (north bank, Wardells Neck)	MT0619-10	40.05621	-74.06602	6/19/20	P	300
NWW (west end-1/storm pipe)	NWW0622-1A	39.00745	-74.79041	6/22/20	P	15

Location	Site ID	Lat	Long	Date	GV (Present-P/ Absent-A)	No. Collected (N = 1306)
NWW (west end-2/dune)	NWW0622-1B	39.00722	-74.79034	6/22/20	P	45
TS-1: North (IBSP)	TSN0626-1	39.83830	-74.09530	6/26/20	A	0
TS-2: South (IBSP)	TSS0626-2	39.82870	-74.09470	6/26/20	A	0
TS-3: South (IBSP)	TSS0626-3	39.82170	-74.09790	6/26/20	P	5
JAC-4 (IBSP)	JAC0626-4	39.81700	-74.10050	6/26/20	P	2
JAC-5 (IBSP)	JAC0626-5	39.80820	-74.10310	6/26/20	A	0
JAC-6 (IBSP)	JAC0626-6	39.81408	-74.12506	6/26/20	A	0
MET-1 (north bank, Wardells Neck)	MT0702-1	40.05600	-74.06510	7/2/20	P	26
MET-2 (north bank, Wardells Neck)	MT0702-2	40.05620	-74.06670	7/2/20	P	25
MET-3 (north bank, Wardells Neck)	MT0702-3	40.05740	-74.06990	7/2/20	P	2
NBB (Herring Island - 1)	NBB0702-4	40.05330	-74.05520	7/2/20	A	0
NBB (Herring Island - 2)	NBB0702-5	40.05150	-74.05530	7/2/20	A	0
MET-1 (north bank, Wardells Neck)	MT0715-1	40.05690	-74.06890	7/15/20	A	0
MET-2 (north bank, Wardells Neck)	MT0715-2	40.05630	-74.06660	7/15/20	A	0
MET-3 (north bank, Wardells Neck)	MT0715-3	40.05600	-74.06520	7/15/20	A	0
JAC-1 (IBSP)	JAC0716-1	39.80510	-74.10080	7/16/20	A	0
JAC-2 (IBSP)	JAC0716-2	39.81360	-74.10010	7/16/20	A	0
TS-3: South (IBSP)	TSS0716-3	39.82100	-74.09760	7/16/20	A	0
TS-4: South (IBSP)	TSS0716-4	39.82890	-74.09690	7/16/20	A	0
TS-5: South (IBSP)	TSS0716-5	39.83330	-74.09540	7/16/20	A	0
NWW (west end/storm pipe)	NWW0717-1	39.00745	-74.79041	7/17/20	A	0
NWW (west end/dune)	NWW0717-2	39.00700	-74.79028	7/17/20	A	0
NWW (rock barrier/point)	NWW0717-3	39.00690	-74.79069	7/17/20	A	0
NWW (rock barrier/sidewalk entr)	NWW0717-4	39.00590	-74.79007	7/17/20	A	0