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Assessment of the Asexual Reproductive Strategies of the Invasive Clinging Jellyfish Gonionemus vertens

Valin Jarred Booker

Abstract

Anthropogenic activities are dispersing organisms at higher rates and this dispersal leads to organisms having a higher chance at becoming invasive. The Clinging Jellyfish, *Gonionemus vertens*, was originally from the north Pacific Ocean, but was identified in New Jersey's coastal waters in 2016. *G. vertens* undergo a complex life history with an asexual polyp stage and the sexual medusa stage. Little is known about their polyp stage, which shows multiple asexual reproductive strategies that can generate high densities of medusa. Asexual reproduction of polyps was observed in 2020 to assess the rate of clonal production. Cultured polyps can have several modes of asexual reproduction including frustule production, binary fission, and budding. While frustules are a key part of asexual reproduction for this species, this is the first-time binary fission and budding were observed for *G. vertens*.

Correlations were observed between various growth stages over time, including 1) developing polyps growing to mature polyps within a week, 2) polyps generating frustule buds and the number of frustules the following week, and 3) the number of frustules produced leading to developing polyps one week later. These results indicate that clonal growth is occurring at a predictable rate, with polyp density estimates of >24,000/m^2 of surface area. This rate of production could account for the incredible numbers of medusa produced each spring in the field, but is contradictory to the lack of identification of polyp stages from field collections. There are still many questions that need to be answered about *G. vertens* polyps such as where exactly the polyps are located within the environment and the abiotic/biotic factors that influence their growth. This experiment helps to bring forth more data about the species, so that we can better understand how it's able to successfully invade foreign ecosystems.

MONTCLAIR STATE UNIVERSITY

Assessment of the asexual reproductive strategies of the invasive Clinging Jellyfish *Gonionemus vertens*

By

Valin Jarred Booker

A Master's Thesis Submitted to the Faculty of
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Introduction

Invasive species can be defined as organisms that are introduced to a habitat that eventually become disproportionately abundant and cause harm to its new environment (Ricciardi 2012). Invaders are constantly causing problems in many different ecosystems on the planet and successfully removing invasive species is almost impossible especially in aquatic environments (Thresher and Kuris 2004). From terrestrial to aquatic communities, invasive species can cause significant disruption in food web dynamics, altering chemical cycles, and even altering various habitats (Ricciardi 2012). For some species, their ability to reproduce asexually has been proposed as one reason that a species might become invasive or successfully invade an ecosystem (Havel et al. 2015). Invasive species not only affect the populations of organisms living in specific communities, but they’re also capable of causing negative effects to humans in ways that could be even dangerous to their health. Invasive species dispersal is commonly caused by unintentional anthropogenic activities, especially within marine environments. An example of an anthropogenic activity that can lead to an invasion in the ocean is the transfer of ship water ballast (Bax et al. 2003).

The dispersal of non-native oceanic species can also be caused by other pathways of introduction including aquaculture, bait production, accidental and intentional release, and floating debris transporting communities of organisms (Molnar et al. 2008). In some cases, introductions are reinforced through repeated transport pathways to the same region, such as cargo shipping where organisms are attached to the hulls of ships (Bax et al. 2003) and repeated trips from the same region allow for multiple opportunities for species to become established (Costello et al. 2022). These modes of dispersal allow for an organism to be picked up at one location and then deposited very far away, even if the organism is dispersal-limited. Due to these diverse pathways of dispersal, there are major concerns for the stability of marine habitats that suffer from invasive species. One region that is currently being affected by marine invasive species is coastal New Jersey, where industrial ports and human immigration (i.e., Ellis Island) have provided pathways for invasive species to be transported for centuries.

Invasions of Coastal New Jersey

The coasts of New Jersey are very important marine ecosystems that provide habitat for diverse marine organisms. One such area is Barnegat Bay, which is the state's largest coastal body of water and consists of estuaries, eelgrass beds, tidal flats, salt marshes, maritime forests, and also barrier islands. Barnegat Bay also suffers from anthropogenic stressors such as climate change, eutrophication, habitat loss, and pollution (e.g., sewage and agricultural waste) (Kennish et al. 2007). Issues such as these allow communities to become degraded to the point that they become vulnerable to invasive species (Mack et al. 2000).

Many species of gelatinous zooplankton (i.e., jellyfish) have recently invaded coastal areas, including Barnegat Bay in New Jersey. Invasive jellyfish such as *Chrysaora chesapeakei* (bay nettle) have successfully established themselves along the New Jersey coastal region. Bay nettles have increased in abundance in recent years and are capable of exerting strong top-down control on native communities (Bologna et al. 2017). Another invasive Cnidarian that has been identified in New Jersey is the Clinging Jellyfish, *Gonionemus vertens* (Gaynor et al. 2016). The appearance of this invasive species is concerning because of the unknown affects it will have in the region for both humans and marine organisms.

Gonionemus vertens

Gonionemus vertens is a small hydrozoan that is characterized by its ability to use tentacles with special adhesive pads to cling onto submerged vegetation including seagrasses such as eelgrass, but also macroalgae. Fully mature adults are capable of reaching 3cm in bell diameter and typically have 4 radial canals where their developing gonads are located. *Gonionemus vertens* generally have between 45-60 tentacles connected to the subumbrella rim and its stinging cells are located in spiral clusters around the tentacles (Bakker 1980). Along with the unique behavior of clinging to submerged substrates, the species also has a highly potent sting that can cause severe reactions such as muscle pain, respiratory problems, and paralysis (Carmen et al. 2017).

Gonionemus vertens in nonnative waters could potentially have negative effects on the local ecosystems' food web. Their diet consists of small zooplankton such as copepods, ciliates, fish larva, and gastrotrichs (Bakker 1980). The species typically feeds by catching prey in their tentacles, which they leave partially suspended in the water to sting prey with highly potent nematocysts. *Gonionemus vertens* themselves are fed on by relatively few predators, but organisms such as bay nettles, nudibranchs, and even spider crabs are capable of feeding on them despite records of increased mortality rate after high consumption (Carmen et al. 2017, Govindarajan et al. 2019, Rigby 2020). The lack of predators that feed on *G. vertens* in invaded waters might allow the species to reproduce in higher numbers and spread rapidly.

Gonionemus vertens is distributed in temperate coastal waters in several regions of the world, including the northwestern Atlantic, European waters, and South America (Govindarajan et al. 2017). The habitats of *G. vertens* typically consist of shallow brackish tidal areas filled with seagrass and algal beds. The recent sightings of *G. vertens* in distant global regions (Rodriguez et al. 2014, Gaynor et al. 2016, and Marchessaux et al. 2017) may be due to the species being transported by anthropogenic activities. Information on the exact vectors that have allowed for this species to appear in distant global regions is still unknown. Potential factors that may aid in the spread of this species might relate to the polyp stage being transported on shellfish that are transferred to other regions by fishermen, hull fouling, and transportation by ballast water. However, no documentation of the polyps being transported exists, so it remains a “Zoogeographic Puzzle” (Tambs-Lyche 1964). In North America, *G. vertens* was reported in waters around Massachusetts in 1894 (Perkins 1902) and New Jersey in 2016 (Gaynor et al. 2016), but actual pathways of introduction remain unknown. Regardless, once this species has successfully been established, asexual and sexual reproduction reinforce the stability of the populations.

The sexual reproduction of *G. vertens* involves adult medusae producing eggs and sperm that get released into the water. *Gonionemus vertens* medusae are dioecious and have males that eject sperm from their gonads, while females can produce around 50-75,000 eggs (Bakker 1980, Govindarajan et al. 2019). Once the sperm and the egg combine in the open water, they develop into a free-swimming ciliated planula larva. The planula swims around open waters before eventually moving to settle down onto different benthic substrates. Once they’ve settled, the planula develops into a small gelatinous polyp that becomes more active when ocean temperatures are warmer. During their polyp stage, they support various asexual pathways leading to functional clones and increasing polyp populations (Perkins 1902). To complete their life cycle, polyps produce small medusa buds which house developing medusa. As the medusa mature, they are released from the polyp and become free swimming adult medusa.

Clinging jellyfish populations continue to be identified in new regions around the world. Alongside these recordings are the increased risk of human populations being stung by their highly potent venom. The current sightings in Barnegat Bay are also concerning due to the unknown effects this species may have on the local biological community and understanding more about the population dynamics could help understand future blooms. The asexual stage of *G. vertens'* life cycle is complex with multiple stages of development that lack proper analysis. Given the critical nature of the polyp life history stage in cloning and producing new individuals, assessing the clonal growth of polyps over time could potentially help us better understand *G. vertens* as a species.

Materials and Methods

An experiment investigating the asexual reproduction of *Gonionemus vertens* was conducted over 6 months from January to July, 2020. *G. vertens* exhibits a complex polyp life history with numerous clonal pathways for individuals to be produced. *G. vertens* polyps can undergo asexual reproduction leading to the production of frustules (creeping, mobile clones), as well as producing medusae (Uchida 1976). After larval settlement or from creeping frustules, *G. vertens* polyps will emerge. Early-stage (Developing stage) polyps often are observed with only 2 tentacles (Figure 1). As these polyps grow, they will develop greater numbers of tentacles and then begin the production of asexual clones through frustule production (Figure 2), as well as medusa (Figure 3). Often, *G. vertens* polyps are capable of producing multiple frustules at once (Figure 2), which results in increasing numbers of polyps in the population.

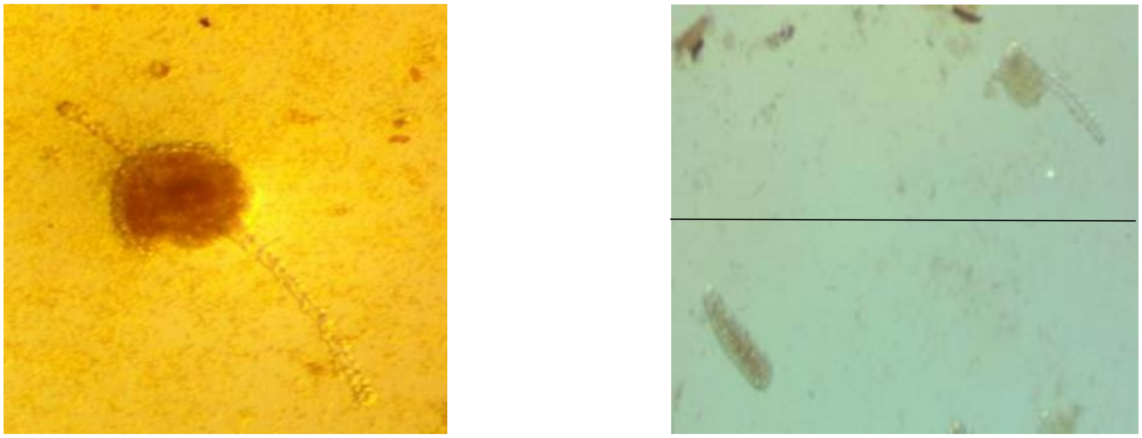

Figure 1. Early-stage *G. vertens* polyp recently transformed from a frustule. Two tentacles are present on the opposite sides of the oral cavity of the polyp (left panel). Image showing both an active frustule and a newly transformed polyp with two tentacles (right panel).

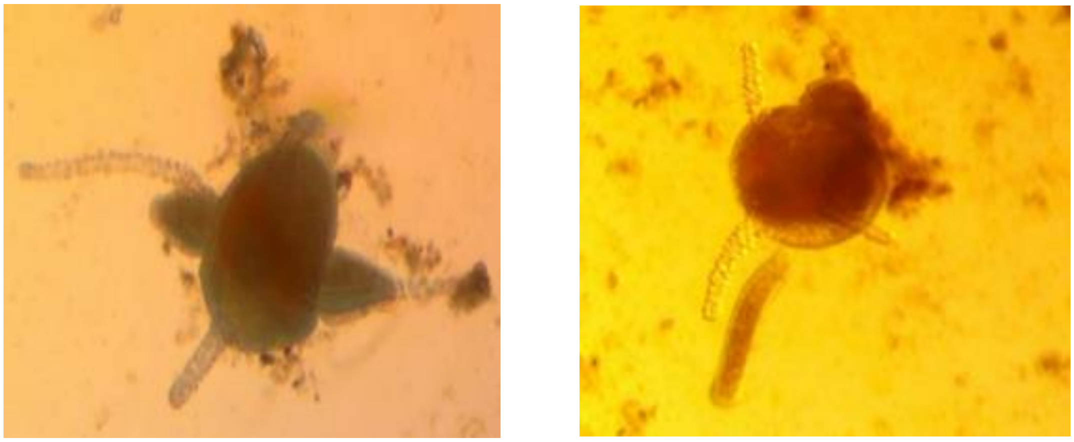

Figure 2. Images of polyps developing frustules. Left panel shows the development of two frustule buds emerging from the side of the polyp, while the Right panel shows a fully formed frustule being released from the polyp.

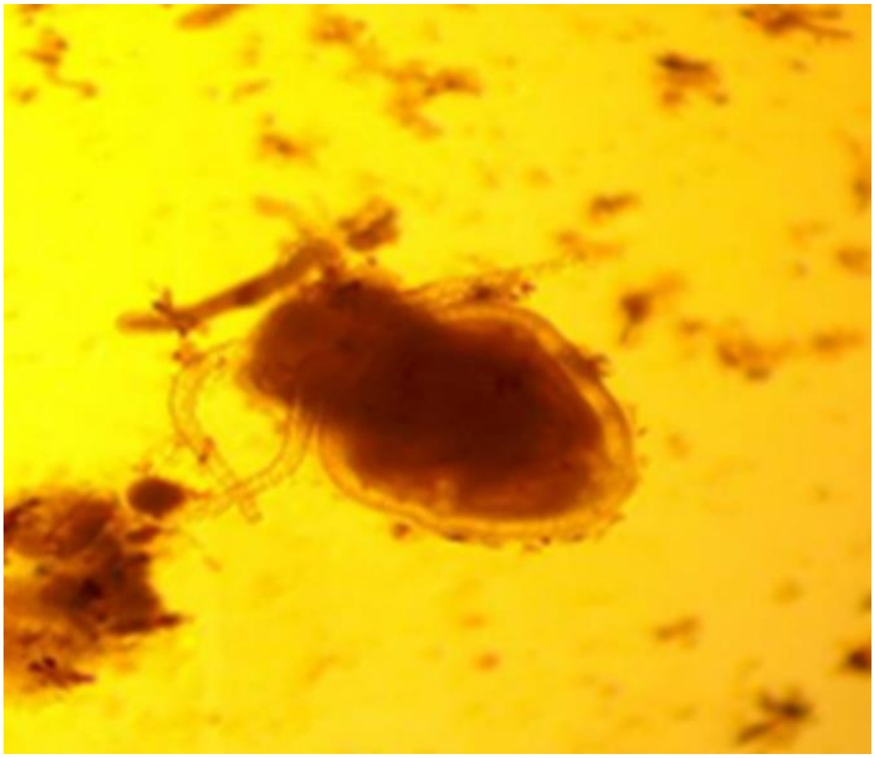

Figure 3. Polyp with a fully developed medusa bud.

Experimental Protocol

This experiment began in 2019, when adult *G. vertens* were collected from Barnegat Bay, NJ in May and June and held in a 151L aquarium. Adults underwent sexual reproduction generating larvae, which settled and developed into polyps on glass slides. Sixteen (16) large glass slides (10x8.25cm) and 8 small glass (5x5cm) were available for settlement. In January 2020, slides were isolated and placed into separate labeled 37L aquariums that were kept at 22℃ and a salinity level of 25ppt. Slides were also cleaned to remove additional marine organisms that settled onto plates, such as hydroids and tunicates, to minimize overgrowth by these species. Polyps were initially fed 3-4 times a week with newly hatched *Artemia* (brine shrimp). However, this resulted in overfeeding and fouling of the bottom of the tanks with dead *Artemia,* which led to initial mortality of polyps in February. Feeding was then reduced to 1-2 times a week to minimize tank fouling and mortality.

Data collection on polyps began on 30 March, 2020, after the feeding regime was changed and large-scale mortality was no longer occurring. However, the abundance of live polyps was substantially reduced, since routine feeding of *G. vertens* polyps and prevention of fouling were negatively impacted by SARS-CoV-2 pandemic restrictions implemented in February and March, 2020. The assessment of polyp growth commenced at this point with polyps being counted under dissecting microscopes to determine the number of individuals at different stages of their development weekly for the duration of the experiment. Polyps were distinguished among their development stages including: frustules, polyps with frustule buds, polyps with long frustules, and polyps with medusa buds (Figs. 1-3). Additional evaluations of unusual asexual reproductive methods and behaviors of polyps over the course the experiment were recorded as well.

Statistical Analyses

To assess the growth rate relationships among various asexual polyp stages, a series of correlation and regression analyses were undertaken to understand the associations among the various polyp stages, using a time-lag response of one week; corresponding to the weekly collection of data. Specifically, linked development stages were evaluated based on the data generated in one week and compared to the abundance during the following week (Week X vs. Week X+1). Initially, 1) adult stage polyps were analyzed against developing polyp stages with a 1-week lag time (e.g., developing polyp to adult polyp; Week X vs. Week X+1); 2) frustule production was compared with the number of polyps showing frustule buds and the abundance of frustules counted during the next week; and 3) the number of frustules present compared to the number of developing polyps the following week.

Results

The average number of polyps at different stages of development started to decline throughout the month of April for both large and small glass plates. Once the experiment progressed further into May, the abundance of polyps at their different developmental stages started to recover until reaching a peak total of over 80 polyps for large glass plates in early July (Fig. 4). Small glass plates experienced stable average growth until early June, when populations declined until recovering again with the total average abundance reaching a peak of over 20 in early July (Fig. 5). Relative abundance of each polyp developmental stage throughout the experiment was fairly consistent with no wide variations occurring. Average abundance of developing polyps remained consistently higher than adult polyps, even when the overall abundance of polyps declined throughout April (Figs. 4, 5). Average abundance of polyps with frustule buds also remained consistently higher than polyps with long frustules, and average abundance of polyps with medusa buds remained fairly low throughout the experiment for both large and small plates.

Summarized average values for Total Polyps, Adult Polyps, Developing Stage (D Stage) Polyps, Polyps with Medusa Buds (PMB), Polyps with Long Frustules (PLF), Polyps with Frustule Buds (PFB) and Frustules on large and small glass plates can be found in Appendix 1 and 2, respectively.

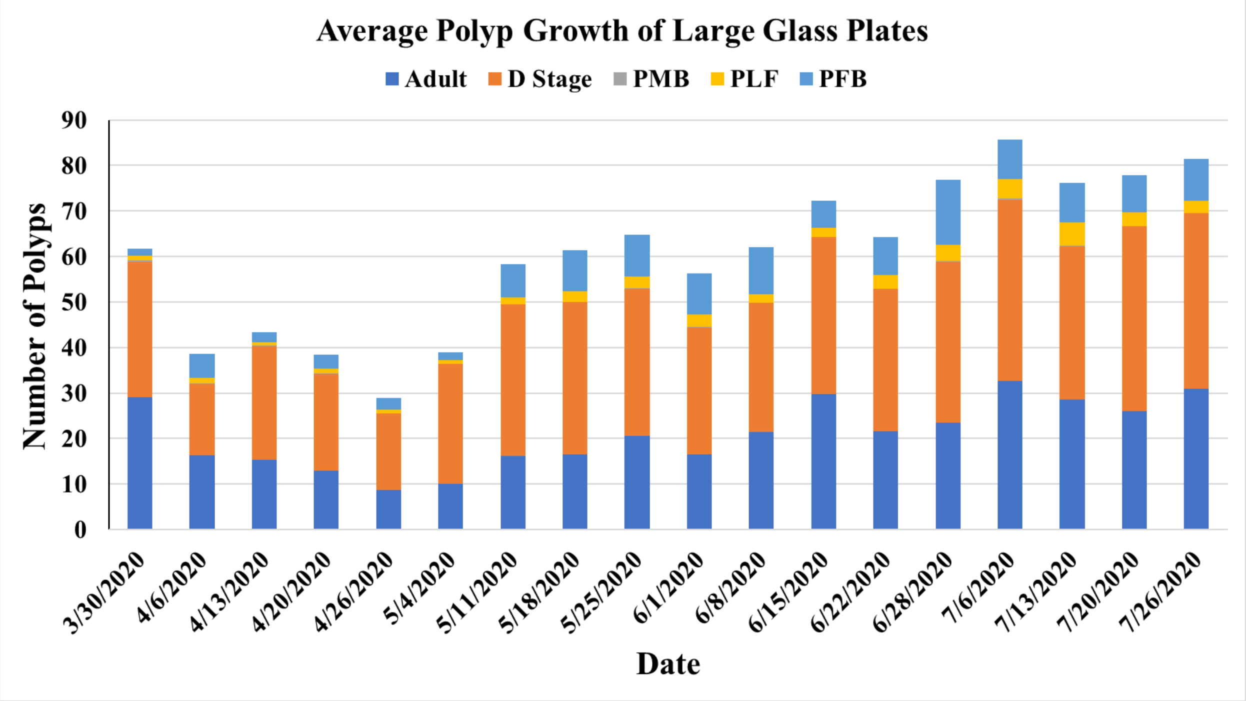

Figure 4. Average number of polyps exhibiting identified characterization stages of development on large glass plates for the duration of the experiment. The summed value of the bars represents the total number of polyps present on the glass slide. Abbreviations are included for Developing Stage (D Stage), Polyps with Medusa Buds (PMB), Polyps with Long Frustules (PLF), and Polyps with Frustule Buds (PFB).

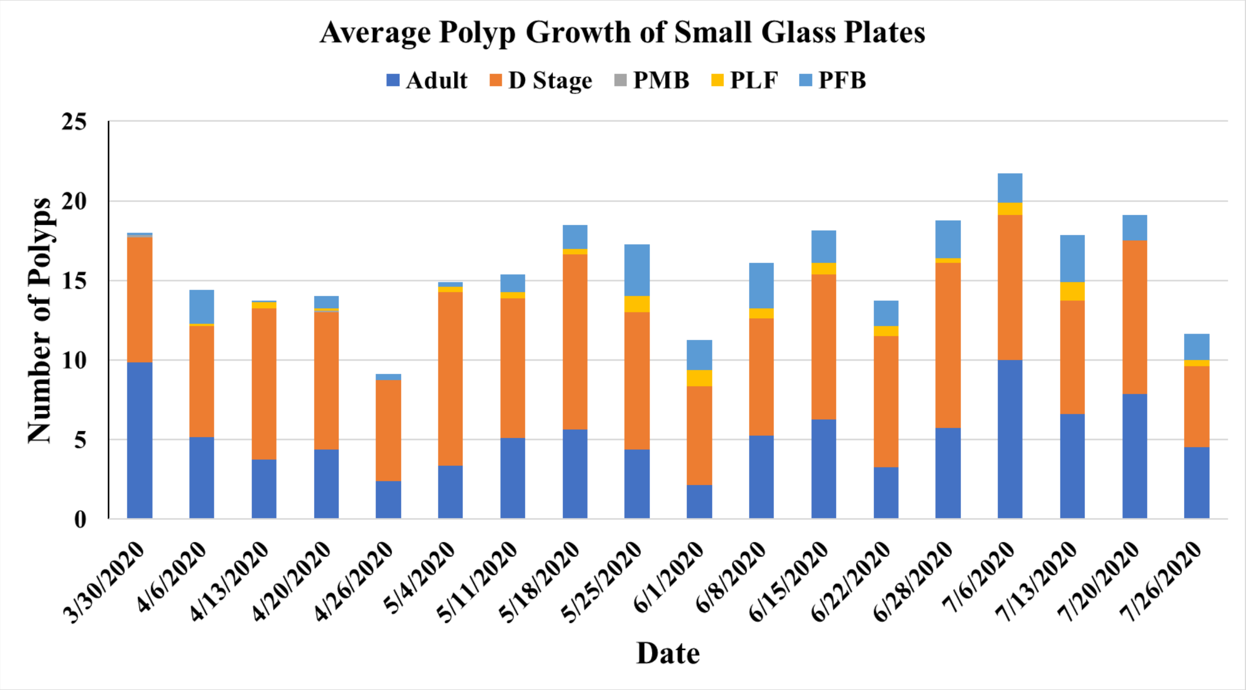

Figure 5. Average number of polyps exhibiting identified characterization stages of development on small glass plates for the duration of the experiment. The summed value of the bars represents the total number of polyps present on the glass slide.

Stage Development Comparisons

When the summary of polyps was analyzed, significant correlations were calculated between the number of D-stage polyps and the number of adult polyps the following week ($r = 0.74$, $P < 0.0007$), the number of polyps showing frustule buds with frustules the following week ($r = 0.6$, $P < 0.0001$), and the relationship between frustule abundance with the number of D-Stage polyps the following week ($r = 0.54$, $P < 0.0001$). When these relationships were investigated further, a significant regression between D-Stage polyps and the number of adult polyps the following week occurred ($F_{1,261} = 157.6$, $P < 0.0001$; Figure 6). This demonstrates the progression of polyp development stages and that it takes about one week from early-stage individuals to complete their development. This is also true for Polyps with Frustule Buds and Frustules ($F_{1,263} = 148.37$, $P < 0.0001$; Figure 7) and Frustules and D- stage individuals ($F_{1,246}$ =

90.57, P < 0.0001; Figure 8). While all these regressions are significant, these relationships are clearly more complex than presented, since they only explain about 30-35% of the variation. However, these results may be able to identify potential polyp growth patterns present in the field and contribute to our understanding of the asexual phase of this species.

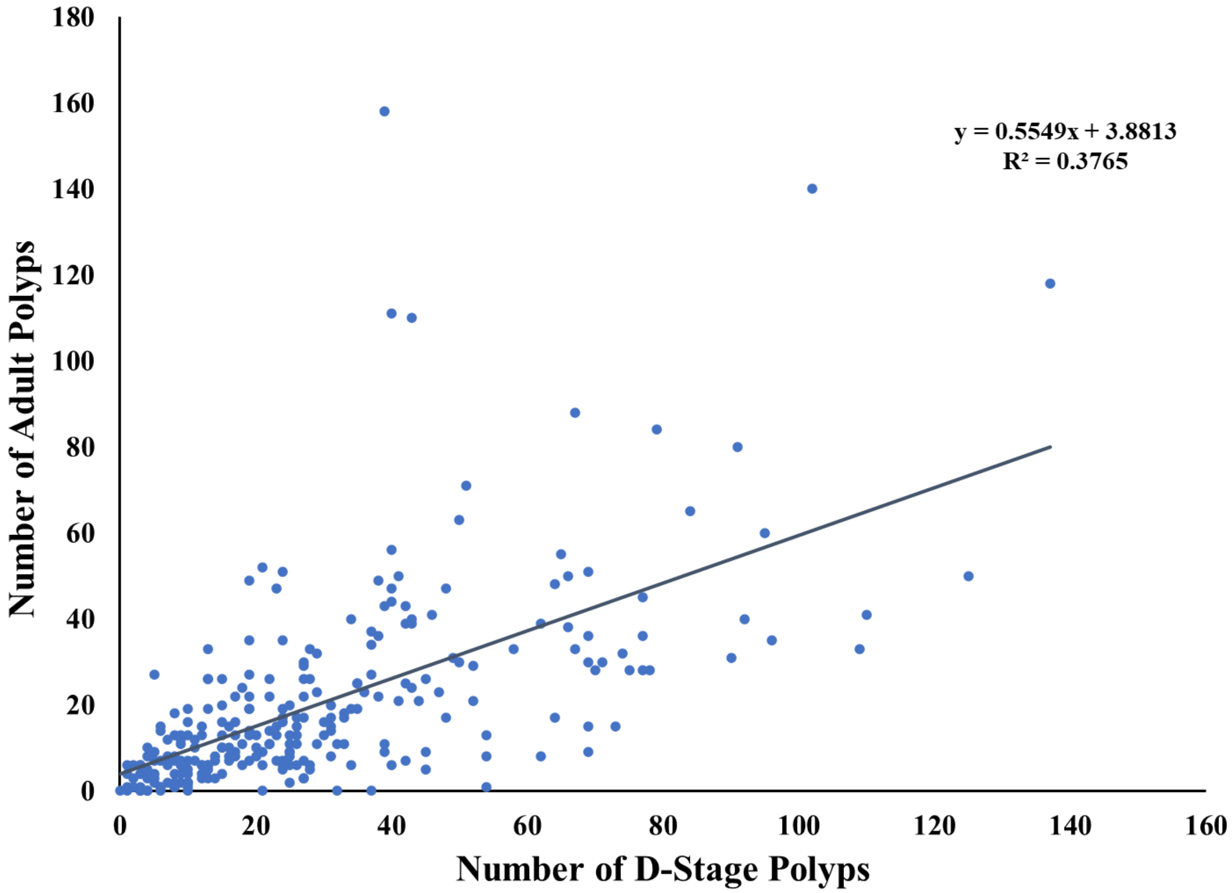

Figure 6. Regression analysis between the number of D-Stage polyps compared to the number of adult polyps identified on plates the following week.

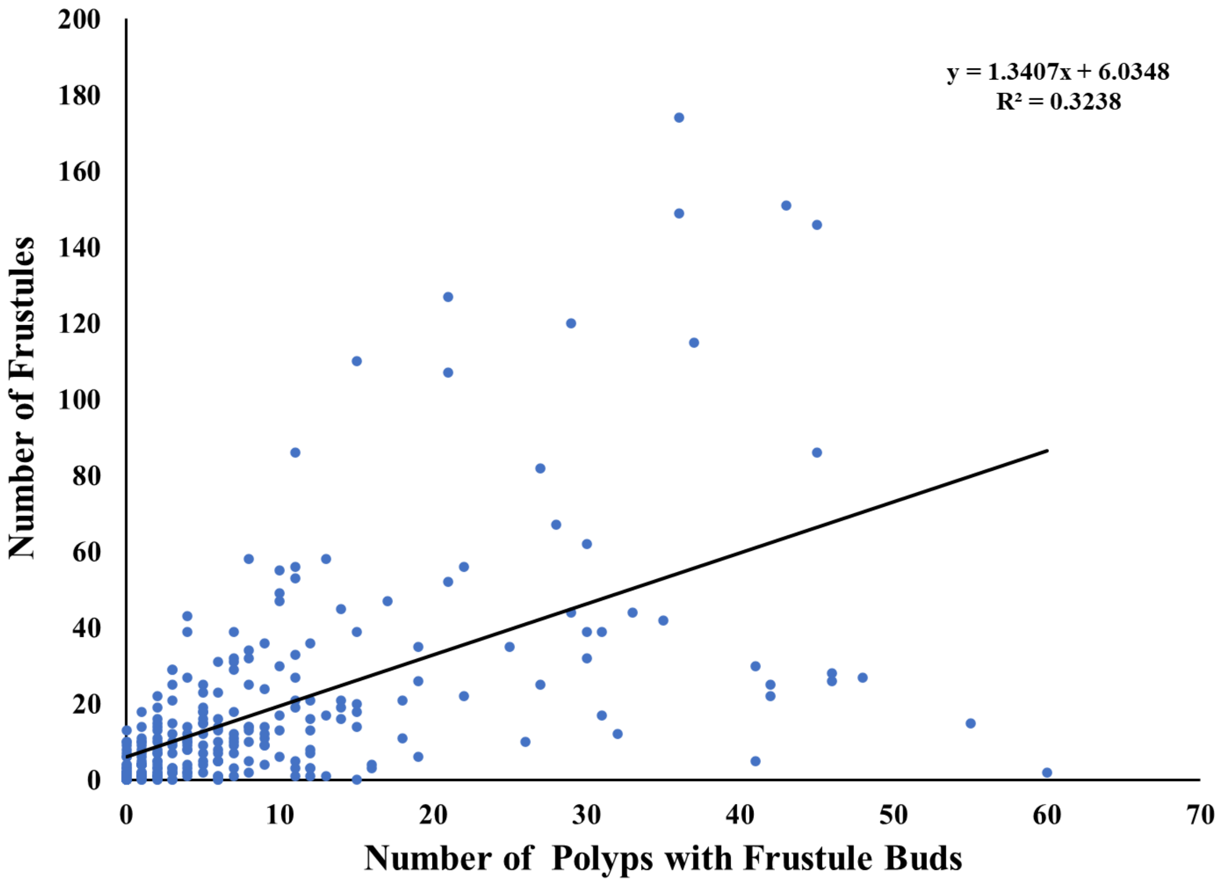

Figure 7. Regression analysis between the number of polyps with frustule buds on plates compared to the number of frustules identified on plates the following week.

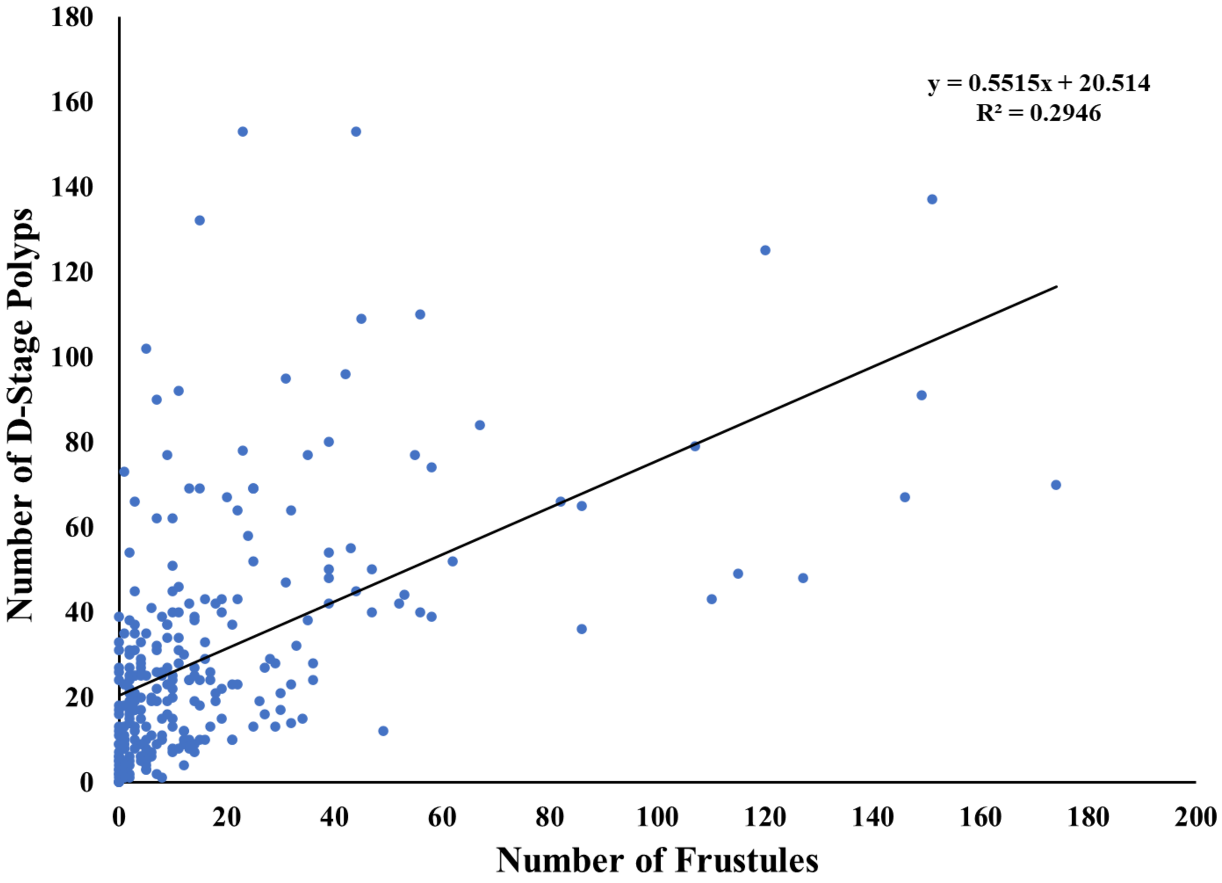

Figure 8. Regression analysis between the number of frustules on plates compared to the number of D-Stage polyps identified on plates the following week.

Extreme Difference in Polyp Growth Between Plates

Plates varied widely in the production of polyps at different stages of development during the experimental period. Polyp growth over time between two small glass plates (30 and 31) is demonstrated in Figure 9. Small Glass Plate 30 showed polyp growth that steadily increased over time from April until the end of the experimental period in July with a relatively high polyp population for the plate size (Fig. 9a). Small Glass Plate 31 however, had very little growth in polyps at different developmental stages throughout the experiment, with the populations not even reaching 5 individuals by the end (Fig. 9b). This may suggest that a minimum number of polyps is necessary to see substantial population growth.

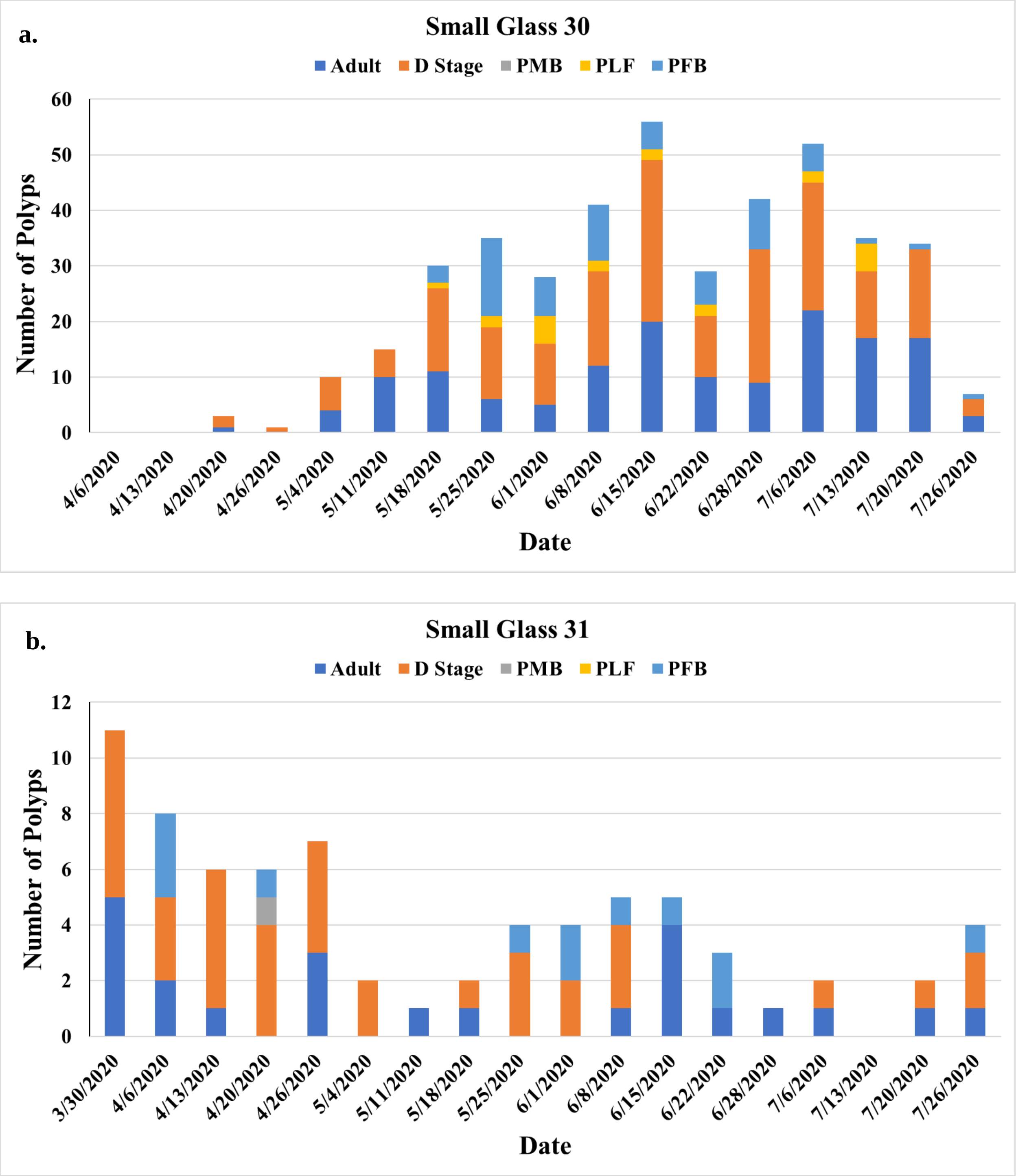

Figure 9. Comparison of the Abundance of the identified polyp stages during the experimental period between Small Glass 30 (a) and Small Glass 31 (b). Note the major differences in scale between the Y-axes.

Similar scenarios of polyp development also occurred throughout the experimental period for large glass plates. Figure 10 shows the difference in polyp growth over time between large glass plates 44 and 29. Large Glass Plate 44 started off with a minimal growth of polyps throughout the month of April that then started to decline for several weeks (Fig. 10a). Eventually, the population exhibited a large increase in individuals throughout the rest of the experiment (May-July) that reached over 200 polyps, which equates to >24,000 m^{-2}! Large Glass Plate 29 however, showed a sharp increase in individuals throughout the month of May, but then had a major decline that ultimately crashed towards the end of the experiment in July (Fig 10b).

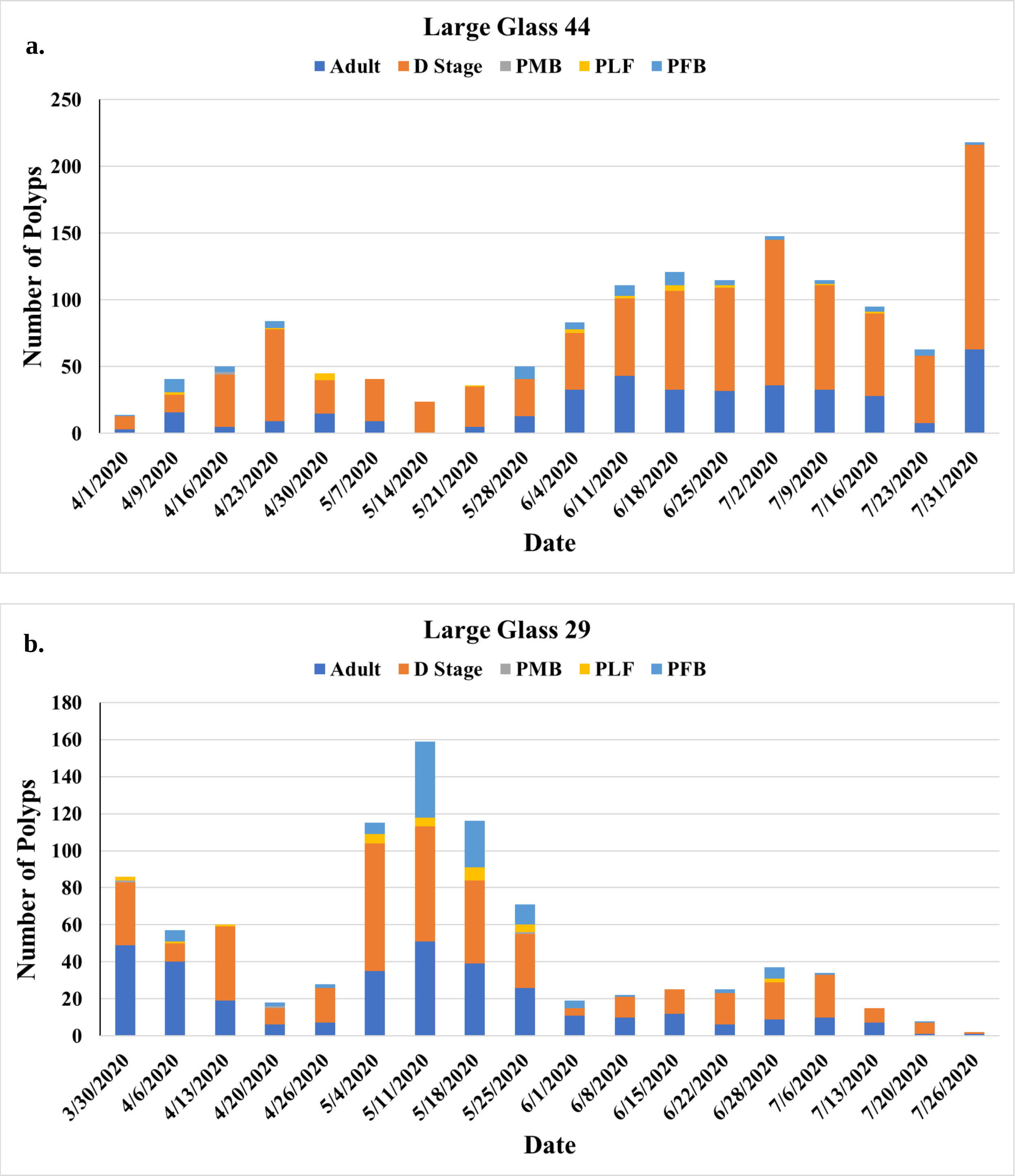

Figure 10. Comparison of the Abundance of the identified polyp stages during the experimental period between Large Glass 44 (a) and Large Glass 29 (b). Note the differences in scale between the Y-axes.

Medusa Bud Development

Throughout the experiment, polyps that were capable of producing medusa buds were observed and recorded. The number of medusa buds produced throughout the experiment was very low. The total number of Medusa buds produced on Large Glass plates was only 36 out of 8493 adult polyps, while on Small Glass plates the total was only 2 medusa buds out of 1044 adult polyps, yielding an average of only to 0.4% and 0.2% of polyps, respectively, entering the phase which produces medusa. This extremely low percentage defies what is observed in the field in terms of the substantial medusa production during May and June.

Presence of Binary Fission and Budding

During the experimental period, two modes of asexual reproduction were documented for *G. vertens* polyps for the first time, namely binary fission (Fig. 11) and budding (Fig. 12). Polyps were also observed splitting more than just once and some polyps had instances where they split multiple times from the parent (e.g., 4 identical polyps generated from one original; ‘quad’). In addition to the binary fission, *G. vertens* polyps were also recorded to be capable of asexually budding a small polyp (Figure 12). These forms of asexual reproduction were previously unrecorded for *G. vertens* polyps in the literature, but are well known for other cnidarians.

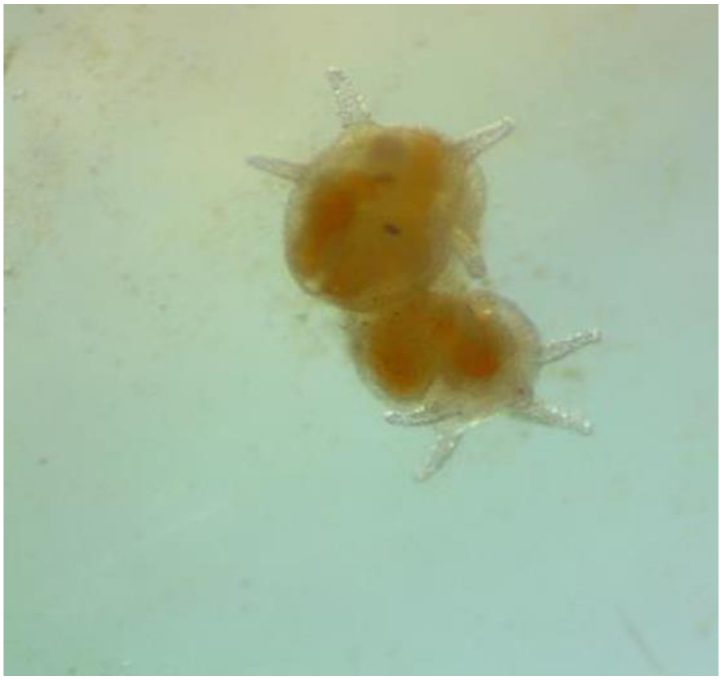

Figure 11. Binary Fission of an adult *G. vertens* polyp.

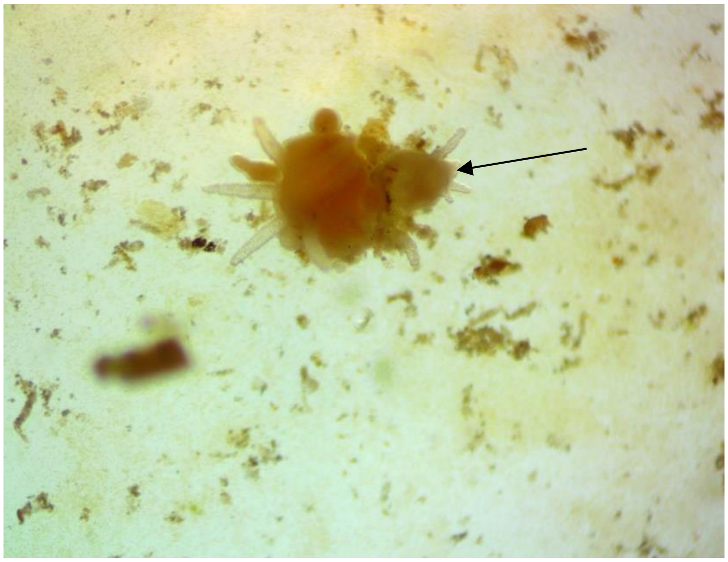

Figure 12. Asexual Budding of a Developing *G. vertens* Polyp.

Patterns of Polyp Growth

From the experiment, *G. vertens* polyps were observed to show three different generalized patterns of growth over time. Figure 13 shows the identified Pattern A for polyp development over time. This pattern of development shows a small initial polyp population that slowly increases and then demonstrates exponential growth rapidly, but ultimately crashes shortly afterward. This pattern may reflect what occurs in the field with rapid development of medusae, but the polyp crash mirrors the inability to identify polyps in the field during peak medusae season.

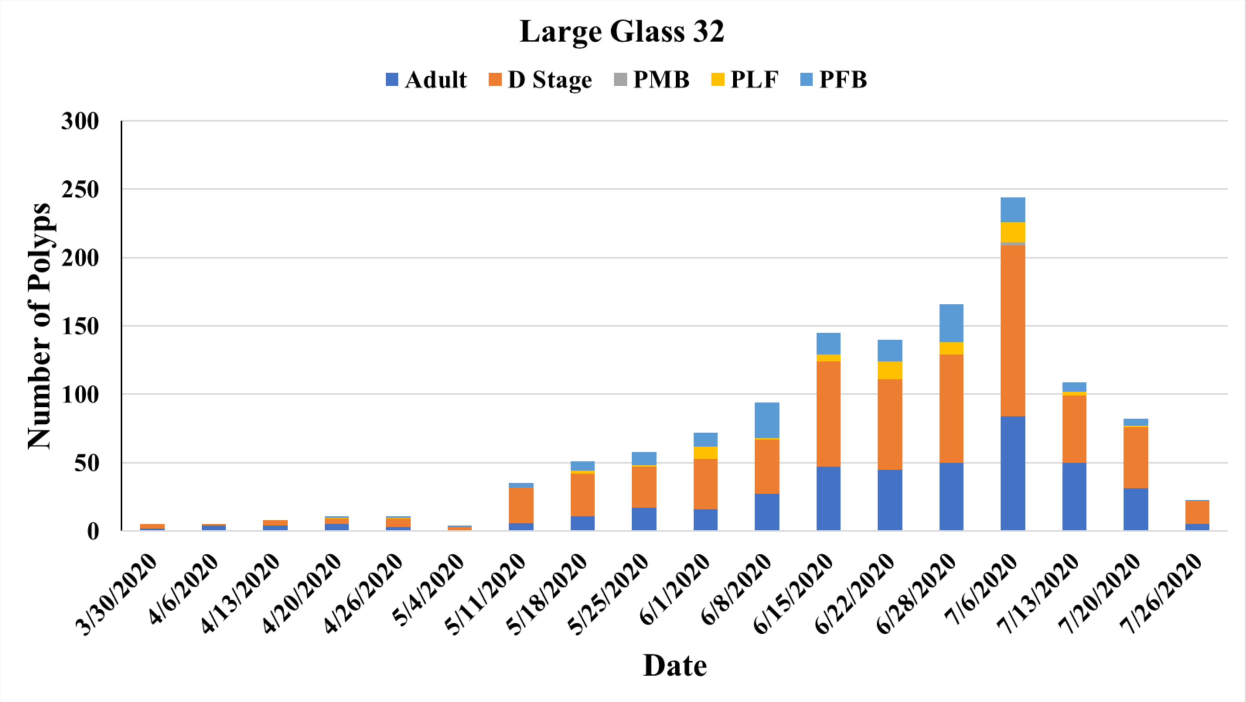

Figure 13. Polyp growth over time of the large glass plate 32 (Growth Pattern A).

Figure 14 shows the identified Pattern B for *G. vertens* polyps. This pattern of growth for *G. vertens* polyps shows an initial rapid decline in abundance during April into May, but the population rebounds and demonstrates exponential growth before crashing in July, similar to the crash observed in Pattern A. This pattern differs in the rate of exponential growth in that it only took 6 weeks to reach the peak, while Pattern A took 9 weeks (50% longer) to achieve maximum growth.

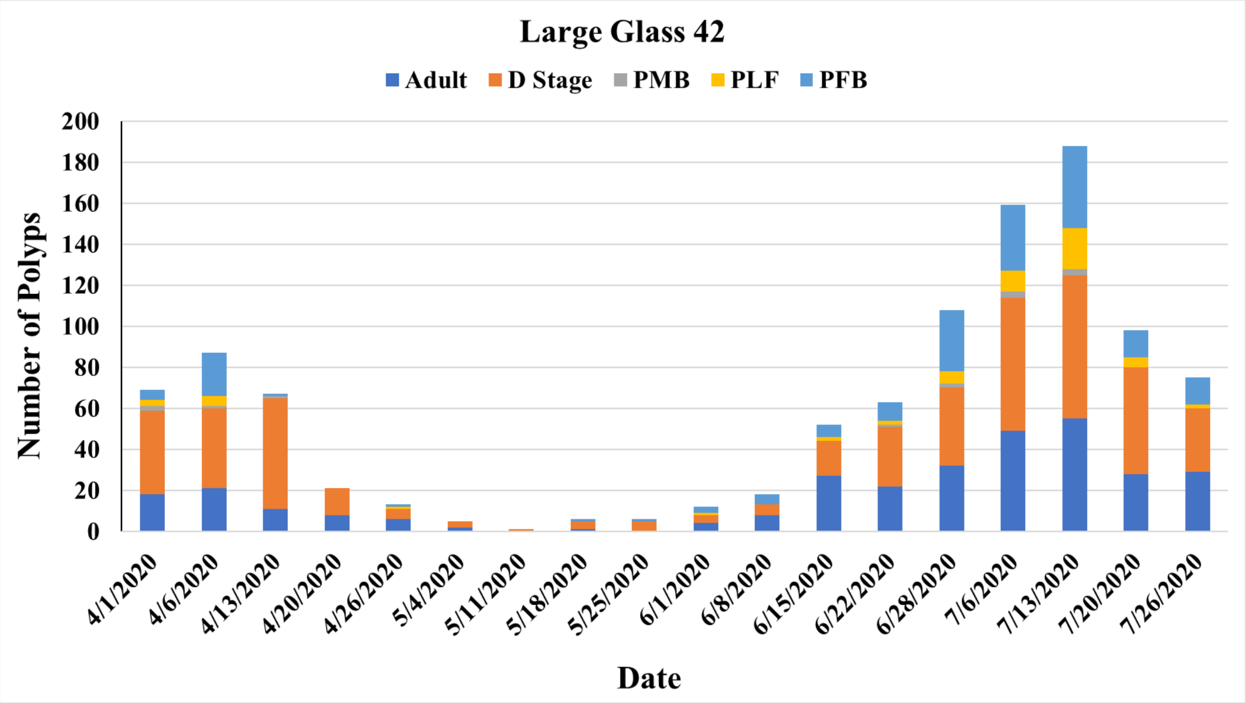

Figure 14. Polyp growth over time of the large glass plate 42 (Pattern B).

Figure 15 identifies Pattern C for polyp development over time, which shows an initial decline in April similar to Pattern B, but a rapid exponential growth in May, before a substantial decline in June which remained throughout the experiment. This differed from Pattern B in that there was not a prolonged period of limited growth, but rather the population rebounded quickly after the initial decline and reached exponential growth in only 3 weeks compared to 6 weeks in Pattern B and 9 weeks in Pattern A.

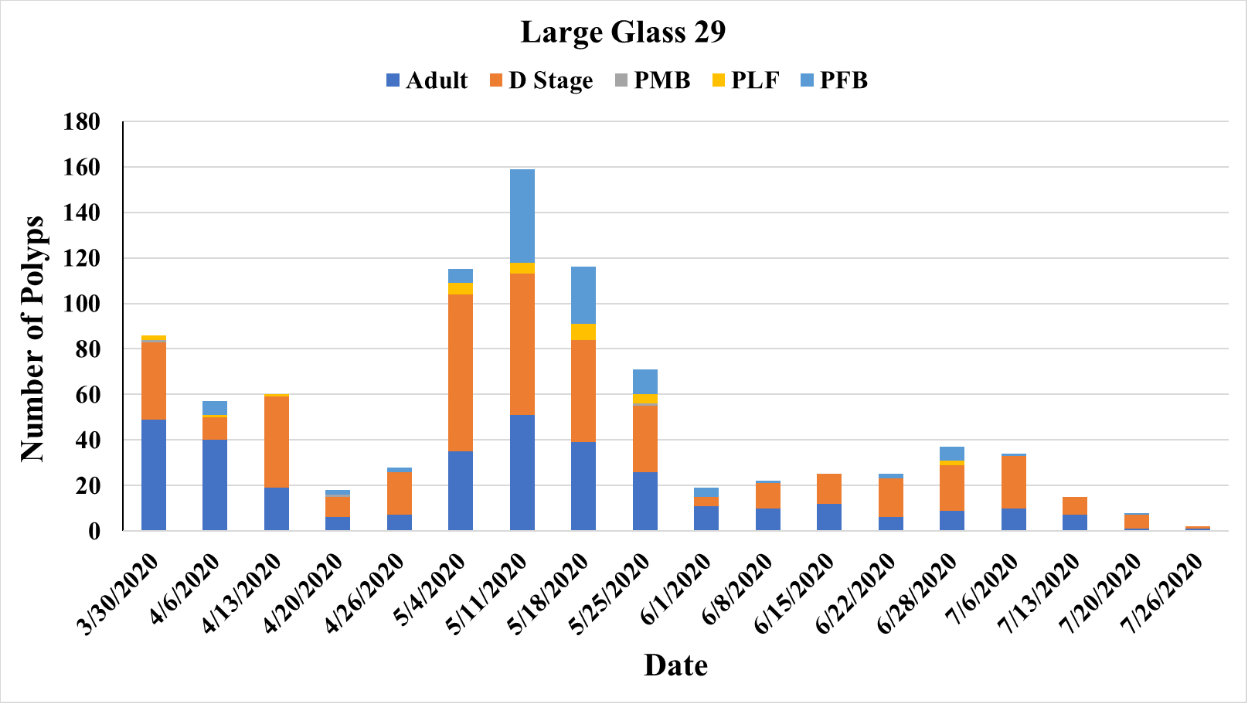

Figure 15. Polyp growth over time of the large glass plate 29 (Pattern C).

Discussion

Human-induced changes to aquatic ecosystems have the potential to support the establishment and success of invasive species. *Gonionemus vertens* has become established in many aquatic ecosystems including the Northwestern Atlantic, European waters, and even South American coastal waters (see Govindarajan et al. 2017). This is likely due to the ability of jellyfish to rapidly reproduce through asexual cloning to produce substantial numbers of individuals at multiple stages of their life cycles (Figs. 13-15). The polyp stage of *G. vertens* is an example of these individuals being capable of reproducing rapidly due to their methods of asexual reproduction involving multiple routes for polyp production.

Comparisons of Asexual Reproductive Strategies

Gonionemus vertens polyps are capable of frequent asexual reproduction events due to the production of a frustule that is released from parent polyps. Additionally, multiple frustules are generated from single polyps leading to a greater rate of overall production, although these data on the numbers of frustules per polyp were not collected during this study. However, frustules observed in this experiment were motile and capable of traveling far from the parent polyp on the glass slides. Once they moved, the frustules started developing until they matured into adult polyps themselves. Previously noted by Uchida (1976), polyps of *G. vertens* were sometimes observed with the behavior of degenerating into a cyst-like mass of tissue and similar observations occurred within this study of *G. vertens* polyps as well. This process seemed to result in polyp death and was ignored during the sampling. However, Uchida (1976) noted that some of these tissue masses eventually created frustules and on occasion, medusa. This was not observed here, as tissue masses were regarded as dead individuals and frustules on polyps nearby were not attributed to these masses. In addition to frustule development, *G. vertens* polyps were also discovered to be capable of binary fission (Fig. 11) and are also able to asexually bud from established polyps (Fig. 12). These two methods of asexual reproduction were not previously recorded for this species of hydrozoan, but have been observed for other cnidarians (Fautin 2002) and could support additional pathways for this species to establish itself in foreign ecosystems.

When compared to other types of hydrozoan jellyfish, *G. vertens* polyps share similar asexually reproductive characteristics to other taxa, but few Hydrozoa possess uniquely solitary polyps. The hydrozoan, *Scolionema suavense*, follows similar asexual reproductive strategies as *G. vertens* and also reside within similar habitats such as seagrass beds and seaweeds. *Scolionema suavense* share reproductive strategies such as having planula that metamorphose into small motile frustules that eventually become the polyps (Kayashima et al. 2019). *Scolionema suavense* was also reported to have medusae that bud from the polyp and have polyps that produce frustules similar to *G. vertens*. However, asexual reproduction in this species also extends to their planktonic stage, where small, cloned medusa asexually bud from established medusae as well (Kayashima et al. 2019), which is not known for *G. vertens*.

Compared to other hydrozoan jellyfish, the Genus *Turritopsis* shares a few asexual reproductive methods with *G. vertens*. Species such as *Turritopsis nutricula* and *Turritopsis dohrnii* are considered to be biologically immortal due to their ability to metamorphose backwards from their medusa stage to previous stages in their life cycle (Martell et al. 2016). When metamorphosing backwards, these individuals are capable of reverting to a cyst-like form similar to how *G. vertens* polyps sometimes revert to a cyst-like tissue mass. *Turritopsis* cysts however, are capable of transforming back into polyps from this stage and are then able to form stolon chains that eventually form into polyp colonies referred to as hydroids (Piraino et al.

1996). Similar to other hydrozoans like *G. vertens*, the polyps eventually form medusa buds that are liberated from the polyp and become free swimming medusae again. However, the establishment of the interconnected hydroid colony is the primary pathway of generating new polyps in this and other species. *Clytia hemispherica* is another species of hydrozoan jellyfish that shares few characteristics of asexual reproduction with *G. vertens*. The species asexually reproduces through their polyps creating large colonies that are connected through stolon chains that eventually produce medusa buds (Leclère et al. 2019). Like many other hydrozoans, many of their polyps tend to have asexual characteristics that lead them to be more colonial and connected compared to how *G. vertens* polyps can be more isolated and spread apart from each other.

This is also seen within the Genus *Moerisia*, a hydrozoan jellyfish with asexual reproductive strategies that are in some ways similar to *G. vertens*. Descriptions of the species *Moerisia lyonsi's* life history stated in Ma and Purcell (2005) show that the polyp stage of this species forms multiple colonies of individuals that are formed through long stolon chains. Polyps within these colonies are capable of budding from each other similar to our recorded *G. vertens* polyps and are also capable of forming small cysts that will eventually form into new polyps. *Moerisia lyonsi* also have individual polyps within a colony that are capable of detaching themselves from the colony, dispersing, and then forming their own colony. Alongside these characteristics of asexual reproduction, *Moerisia* are also invasive and have been reported in locations similar to those of *G. vertens*, such as the coastal waters of New Jersey (Restaino et al. 2018). Their asexual reproductive strategies that they share with *G. vertens* might also help explain how these species establish themselves within foreign aquatic regions.

Asexual Reproductive Growth of Gonionemus vertens

When it comes to asexual reproduction of *G. vertens*, the polyps are capable of producing substantial numbers of individuals within just a few weeks. Results from this experiment showed that the average number *G. vertens* polyps at various different stages of development greatly increased as the experiment ran into the warmer months. Temperatures within our laboratory did not necessarily match that of the surrounding temperatures outside, so as the seasons changed and temperature increased, our laboratory did not vary greatly. *G. vertens* medusae have been observed to spawn from late April to late July (Mills 1993), which matches with the results of our experiment showing average increases in polyp growth during these similar months. However, this may not be an accurate portrayal of what is actually occurring in the environment due to the experiment only taking place within a laboratory setting with relatively stable temperatures. Although solar cues (i.e., day length) did exist in the lab and adult medusa are negatively phototactic, so it might be possible that polyps also respond to light equating it with day length and season.

Alongside these observations, this experiment had many instances where polyp populations also did not coincide with the recorded spawning times of *G. vertens* medusae. For many glass plates, polyp populations would sometimes crash for no apparent reason. Early problems with COVID access can explain early declines, but not wholesale plummets like those seen on Plate 42 (Figure 14). For other plates, polyp populations would experience the opposite, with the highest number of total individuals on one plate reaching over 300 polyps. *Gonionemus vertens* polyp growth within this study was also found to have three different patterns of growth on several different glass slides. Some of these patterns might help with determining when exactly *G. vertens* polyps prefer to start spawning individuals. Pattern A (Figure 13) seems to show populations that closely resemble those recorded for the average growth over time results (Fig. 4), while the other two patterns differ with individuals cloning polyps and peaking either in May, or by having a small peak in April that crashes and comes back in July.

Many factors could have influenced these population crashes from improper environmental conditions within our tanks to fouling of some glass slides. Another factor that could have heavily influenced rate of polyp growth was due our inability to access our laboratory during the start of the SARS-CoV-2 pandemic until 30 March, 2020 and residual impacts. Other factors that could affect the increase/decrease of polyp populations need further investigation in order to help determine what variables might influence polyp asexual reproduction during seasonal changes. These could include assessing clonal growth at various temperatures or mimicking seasonal water temperatures in the field, as well as varying salinity to better match field conditions spatially where known populations of adults occur and seasonal reductions in salinity associated with spring rainfall. These experimental extensions of the research presented here could better identify and characterize polyp growth and production of reproductive medusae.

The Future of Gonionemus vertens in New Jersey

The effects of the recent invasion of *Gonionemus vertens* in the coastal waters of New Jersey and other foreign habitats is for the most part still unknown. The species will most likely remain established within these habitats because of its asexual reproductive strategies. The observations presented showed that *G. vertens* polyps are capable of rapid asexual reproduction through a number of different pathways, with some being previously undiscovered. Polyp development is also fairly quick, with our regression analysis showing that individual developing polyps reach their adult stage within one week under laboratory conditions (Fig. 6). Along with developing fairly quickly, polyps were also capable of producing multiple frustule buds that eventually elongated and then released to metamorphose into their own polyps. The motile frustules of these polyps are also capable of traveling further from the parent polyp, which shows the potential for how *G. vertens* might be able to disperse itself from one location to the other. When it comes to dispersal, the location of *G. vertens* polyps is still largely unknown within the natural environment. The results showed that a maximum estimated abundance of polyps per square meter was >24,000. If this is potentially similar to the amount found within the natural environment, then it opens up even more questions as to exactly where the polyps of this species are located, since few have been collected from field activities (Bologna pers. comm.). Coastal habitats have many different structures that polyps could potentially attach themselves to such as seagrasses and macroalgae. Further research into the location of these polyps is needed in order to better understand their population growth and dispersal.

Alongside polyp generation, observations were also made on the production of *G. vertens* medusa buds to try and further understand how the species is capable of generating substantial medusae populations. It is interesting to note that medusa production was fairly low throughout the duration of the experiment, with only 36 medusa buds forming out of the 8,493 fully mature polyps on large glass plates and small glass plates producing only 2 medusa buds out of 1,044 fully mature polyps. This minute fraction of polyps engaging in medusa production do not correlate with field observations of high abundances of medusa in some locations, but could reflect cycles of clonal polyp growth and occasional blooms of medusa. This could explain why many invasive hydrozoans go unnoticed until major blooms occur. Despite the low medusa generation seen in this experiment, *G. vertens* polyps within this study were observed to have two previously unidentified asexual reproductive strategies. *Gonionemus vertens* polyps are capable of asexually budding off of each other and are also able to undergo binary fission (Figs. 11 and 12). These newly identified asexual strategies help to alleviate some of the lack of information as to how the species is able to establish itself in foreign ecosystems. Other observations from this study found that *G. vertens* polyps were capable of asexually budding multiple individuals as well. The same also occurred with binary fission as polyps were capable of dividing into several individuals at the same time. These newly identified asexual reproductive strategies can help us better understand how *G. vertens* polyps are able to reproduce in such a high abundance and potentially produce so many medusae during their bloom events in the warmer months. Along with those reproductive strategies, *G. vertens* polyps within this study were also identified to have undergone degeneration into a cyst-like mass of tissue similar to those identified by Uchida (1976). While tissue masses in this study were considered a mortality, tissue masses in Uchida (1976) were recorded to be able to produce both medusa and frustules even after degeneration. These observations show that even after degeneration, *G. vertens* polyps still have the potential to continue asexually reproducing even more individuals and understanding this pathway of production is almost non-existent.

Conclusion

This research focused on the various asexual reproductive strategies that *Gonionemus vertens* is able to utilize, including discovering two previously undocumented asexual strategies. It was found that the polyps of *G. vertens* were capable of producing clones at an exponential rate, which can help to explain how this invasive Hydrozoan is able to successfully invade foreign habitats. However, there are still a few questions that are left to be answered about this species. *Gonionemus vertens* medusae are found to bloom during the late spring in New Jersey, but the location of where their polyps are is still unknown. The polyps are also capable of dispersing their frustules further from the parent polyp, so it leaves the possibility that polyps producing medusae in one area could also contribute to the expansion of the species through this secondary mechanism. Additionally, studies on what biotic and abiotic factors change asexual reproduction rates of *G. vertens* polyps need to be completed, as well as needing to assess seasonal water quality patterns in the field to determine how bloom formation occurs. This experiment was in a laboratory setting under controlled environmental settings, so the discoveries do not fully portray what might be occurring in the natural environment for these polyps. Further studies will be needed in order to truly understand how *G. vertens* population increases may impact their invaded communities and the risk they pose to human health.

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Appendix A.

Average Polyp Abundance for each development stage on Large Glass Plates collected during sampling. Data presented are Mean and Standard Deviation. "PMD" stands for Polyps with Medusa Buds, "PLF" stands for Polyps with Long Frustules, and "PFB" stands for Polyps with Frustule Buds.

Average Polyp Growth of Large Glass Plates							
Date	**Total**	**Adult**	**D Stage**	**PMB**	**PLF**	**PFB**	**Frustules**
3//30/2020	61.7 ± 47.8	29.1 ± 24.2	29.7 ± 23.4	0.4 ± 0.6	1.1 ± 2.1	1.4 ± 1.7	3.1 ± 4.7
4/6/2020	38.6 ± 27.3	16.3 ± 11.2	15.7 ± 19.1	0.25 ± 0.4	1.2 ± 1.5	5.2 ± 5.7	3.9 ± 5.1
4/13/2020	43.4 ± 31.6	15.4 ± 14.0	24.9 ± 19.1	0.2 ± 0.5	0.6 ± 1.4	2.4 ± 3.2	7.8 ± 6.7
4/20/2020	38.5 ± 27.1	12.9 ± 9.7	21.3 ± 17.6	0.2 ± 0.5	1.1 ± 1.9	3.1 ± 3.0	5.8 ± 4.7
4/26/2020	28.9 ± 20.7	8.6 ± 9.1	16.9 ± 10.1	0 ± 0	0.8 ± 1.6	2.5 ± 3.1	6.1 ± 6.2
5/4/2020	38.9 ± 33.5	10.1 ± 10.6	26.3 ± 20.5	0 ± 0	0.9 ± 1.6	1.6 ± 2.0	5.1 ± 6.1
5/11/2020	58.4 ± 52.0	16.2 ± 16.8	33.3 ± 26.5	0 ± 0	1.6 ± 2.1	7.3 ± 10.5	7.4 ± 7.3
5/18/2020	61.3 ± 48.2	16.4 ± 15.1	33.6 ± 24.0	0 ± 0	2.3 ± 3.1	9.0 ± 10.6	11.6 ± 13.9
5/25/2020	64.8 ± 54.6	20.5 ± 20.0	32.4 ± 28.3	0.1 ± 0.3	2.6 ± 3.0	9.2 ± 5.9	12.6 ± 10.8
6/1/2020	56.3 ± 41.3	16.5 ± 12.1	27.8 ± 24.3	0.3 ± 0.8	2.7 ± 2.5	9.0 ± 6.7	20.8 ± 19.0
6/8/2020	62.1 ± 44.6	21.4 ± 13.5	28.3 ± 23.0	0.1 ± 0.3	1.9 ± 1.9	10.4 ± 10.9	20.5 ± 20.0
6/15/2020	72.3 ± 40.8	29.8 ± 16.5	34.4 ± 23.0	0 ± 0	2.1 ± 1.7	6.1 ± 4.8	24.0 ± 21.8
6/22/2020	64.3 ± 39.7	21.6 ± 12.6	31.3 ± 20.6	0.1 ± 0.3	3.0 ± 3.8	8.4 ± 8.6	33.5 ± 33.1
6/28/2020	76.9 ± 58.2	23.5 ± 18.9	35.4 ± 30.9	0.2 ± 0.5	3.6 ± 3.9	14.3 ± 14.3	48.2 ± 48.1
7/6/2020	85.7 ± 80.3	32.7 ± 37.1	39.8 ± 35.3	0.4 ± 0.9	4.1 ± 5.3	8.8 ± 10.3	51.3 ± 61.9
7/13/2020	76.1 ± 93.4	28.5 ± 41.5	33.7 ± 36.5	0.3 ± 0.8	5.1 ± 10.8	8.6 ± 10.8	17.9 ± 16.3
7/20/2020	77.9 ± 89.0	26.1 ± 34.4	40.5 ± 41.9	0 ± 0	3.1 ± 5.1	8.2 ± 10.8	17.1 ± 14.8
7/26/2020	81.4 ± 104.7	30.9 ± 40.2	38.6 ± 49.5	0 ± 0	2.7 ± 5.2	9.1 ± 17.1	13.1 ± 10.7

Appendix B.

Average Polyp Abundance for each development stage on Large Glass Plates collected during sampling. Data presented are Mean and Standard Deviation. "PMD" stands for Polyps with Medusa Buds, "PLF" stands for Polyps with Long Frustules, and "PFB" stands for Polyps with Frustule Buds.

Average Polyp Growth of Small Glass Plates							
Date	**Total**	**Adult**	**D Stage**	**PMB**	**PLF**	**PFB**	**Frustules**
3//30/2020	18.0±11.9	9.9 ± 7.1	7.9 ± 5.0	0.1 ± 0.4	0 ± 0	0.1 ± 0.4	1.3 ± 1.3
4/6/2020	14.4± 2.5	5.1 ± 5.4	7.0 ± 6.2	0 ± 0	0.1 ± 0.4	2.1 ± 2.3	2.4 ± 5.2
4/13/2020	13.8 ± 8.9	3.8 ± 3.1	9.5 ± 6.2	0 ± 0	0.4 ± 1.1	0.1 ± 0.4	2.1 ± 2.2
4/20/2020	14.0 ± 9.3	4.4 ± 3.3	8.6 ± 6.0	0.1 ± 0.4	0.1 ± 0.4	0.8 ± 0.9	1.4 ± 2.0
4/26/2020	9.1 ± 5.2	2.4 ± 1.9	6.4 ± 3.1	0 ± 0	0 ± 0	0.4 ± 0.7	0.9 ± 1.1
5/4/2020	14.9 ± 14.0	3.4 ± 3.4	10.9 ± 10.3	0 ± 0	0.4 ± 0.5	0.3 ± 0.7	1.5 ± 1.6
5/11/2020	15.4 ± 15.0	5.1 ± 6.1	8.8 ± 8.1	0 ± 0	0.4 ± 0.7	1.1 ± 1.6	1.4 ± 1.4
5/18/2020	18.5 ± 16.3	5.6 ± 7.3	11.0 ± 7.9	0 ± 0	0.4 ± 0.5	1.5 ± 2.1	1.8 ± 2.3
5/25/2020	17.3 ± 11.5	4.4 ± 2.7	8.6 ± 5.2	0 ± 0	1.0 ± 1.2	3.3 ± 4.9	3.8 ± 3.8
6/1/2020	11.3 ± 7.8	2.1 ± 2.0	6.3 ± 4.4	0 ± 0	1.0 ± 1.8	1.9 ± 2.4	5.0 ± 5.6
6/8/2020	16.1 ± 12.5	5.3 ± 4.0	7.4 ± 5.1	0 ± 0	0.6 ± 0.9	2.9 ± 3.6	4.3 ± 6.2
6/15/2020	18.1 ± 16.6	6.3 ± 6.2	9.1 ± 9.2	0 ± 0	0.8 ± 0.9	2.0 ± 1.9	9.4 ± 8.0
6/22/2020	13.8 ± 13.9	3.3 ± 3.5	8.3 ± 9.5	0 ± 0	0.6 ± 0.9	1.6 ± 2.3	6.0 ± 6.7
6/28/2020	18.8 ± 16.5	5.8 ± 4.7	10.4 ± 9.6	0 ± 0	0.3 ± 0.5	2.4 ± 3.2	8.9 ± 8.0
7/6/2020	21.8 ± 17.6	10.0 ± 8.4	9.1 ± 7.5	0 ± 0	0.8 ± 0.9	1.9 ± 2.4	7.5 ± 7.5
7/13/2020	17.9 ± 13.5	6.6 ± 7.2	7.1 ± 5.3	0 ± 0	1.1 ± 1.7	3.0 ± 2.7	10.0 ± 8.5
7/20/2020	19.1 ± 13.4	7.9 ± 7.2	9.6 ± 6.0	0 ± 0	0 ± 0	1.6 ± 2.2	6.5 ± 4.4
7/26/2020	11.6 ± 9.0	4.5 ± 3.4	5.1 ± 5.8	0 ± 0	0.4 ± 0.5	1.6 ± 1.7	5.8 ± 4.7