

# Division of Science and Research

## Research Project Summary

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### *Assessing the Biological Control of Atlantic Bay Nettles (*Chrysaora chesapeakei*) by Nudibranchs*

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#### *Abstract*

A multi-year study was conducted to assess nudibranch predation as a means of controlling Atlantic Bay Nettle (*Chrysaora chesapeakei*) and other cnidarian polyps, which can successively mitigate the production and abundance of adult medusae in coastal waters. Nudibranchs collected from Barnegat Bay and the Shrewsbury River demonstrated selective predation and consumption of *C. chesapeakei* polyps in laboratory and field settings, though several factors may influence their effectiveness in controlling populations. Laboratory studies indicate that while complete predation of polyps did occur, incomplete or partial predation of polyps was also common. Under the latter conditions, nudibranchs consumed polyp tentacles, but failed to consume whole individuals. Another extenuating factor in nudibranch control of polyps is predation by nudibranchs on sea anemones observed in choice experiments. In the laboratory, predation potential was investigated using bay nettle polyps and the non-native sea anemone *Diadumene lineata*, a co-inhabitant of the man-made structures preferred for settlement by *C. chesapeakei*. The results showed a significant predation preference for *C. chesapeakei*. However, nudibranchs may be limited in their ability to act as efficient predators on bay nettle polyps if *D. lineata* are present in high numbers. The presence of other cnidarian species comprised in the diet of wild aeolid nudibranchs was also assessed. Nudibranch cerata were collected and 16S rDNA sequences of cnidarian DNA amplified from grouped individuals. Results showed that cnidarian DNA was present in over half of the samples with positive identification of *Obelia bidentata*, *Moerisia spp.*, and *C. chesapeakei*. While nudibranchs possess the potential to control *C. chesapeakei* polyps, substantial aquaculture of individuals would be needed to seed coastal communities sufficiently to act as an effective biological means of managing cnidarian populations.

#### **Introduction**

The Atlantic bay nettle (*Chrysaora chesapeakei*, formerly *C. quinquecirrha*) has recently become established in several estuaries in New Jersey, with extensive populations found in the Barnegat Bay and the Navesink-Shrewsbury Estuaries. As this species increases in abundance, it generates dramatic impacts to local communities related to living resources and recreation of coastal waters. Like most gelatinous zooplankton (“jellyfish”), the Atlantic bay nettle has a metagenetic or biphasic life history, meaning that it has both a free-swimming, sexually reproducing phase (the adult medusa) and a sessile, asexually reproducing phase represented by the polyp form (Figure 1). In general, the medusa phase is often the one which causes major impacts on coastal communities and food webs. However, the polyp stage, which requires hard structure to attach to following transforming from the larval form, is the most critical life history phase with regard to controlling populations. Each polyp can actively clone to produce more polyps, which ultimately produce more adult medusae.



**Figure 1.** Image of an Atlantic Bay Nettle polyp (Photo: MSU)

Barneget Bay (New Jersey) is a coastal lagoon system with significant urbanization and land use changes in its watershed (Lathrop and Bognar, 2001). While the ctenophore *Mnemiopsis leidyi* has been reported as an important component of the pelagic community for the last century, the recent invasion by the scyphozoan *C. chesapeakei* during the last few decades may be a result of the development and associated pressures imposed on this system. One important driver of the shift towards greater abundance of gelatinous zooplankton in coastal systems is the prevalence of hard structure (e.g. bulkheads, docks, and other shoreline modifications) that provide suitable habitat for scyphozoan polyps. Bologna (2011) showed that larval recruitment to settling plates was highly localized in northern portions of the Bay where development is high, and salinity is reduced by inputs from two large rivers. As these jellyfish have become established in the region, their impacts at the community level have yet to be evaluated.

Ultimate control of bay nettles can only be accomplished by addressing polyp populations including habitat requirements and water quality, which favor jellyfish over other species. As such, this research was initiated to assess the following research objectives:

- 1. Assess nudibranch potential as a biological control of bay nettle polyps**
- 2. Field assessment of nudibranch feeding**
- 3. Identify potential nudibranch predators of cnidarians from the field and assess their diet through molecular identification of cnidarian DNA**

## Methods

To be an effective biological control of bay nettles, nudibranchs need to be present and survive in the same conditions where the polyps are found. The nudibranchs also need to demonstrate active consumption of polyps and a preference over other cnidarian species.

- 1. Assess nudibranch potential as a biological control of bay nettle polyps**

Twenty trials were run to determine initial consumption of polyps by individual nudibranchs. Lab-cultured *C. chesapeakei* polyps (Montclair State University - MSU) were first isolated into individual chambers of a 6-well plate apparatus, then allowed 48-72 hours to adhere to the surface of the plate prior to feeding trials. Experiments commenced by placing a single Aeolid nudibranch into a chamber containing bay nettle polyps, then monitored at 1, 4, 24, 48, and 72 hours following introduction to assess predation. A second feeding experiment was conducted in larger culture dishes, where numerous cultured polyps were introduced and allowed to become established (approximately 1 week). Prior to introduction of the nudibranch, polyps were identified on the culture dish and counted. Nudibranchs were then introduced and observed at 1 hour and again at 24 hours to assess short-term predation rates, then again at 48 hours.

Lastly, a feeding experiment was conducted to assess nudibranch choice/preference. In glass petri dishes, several polyps were introduced and allowed to adhere in a similar manner as described above. Afterwards, a single *Diadumene lineata* (Orange-striped Green Anemone) was introduced to the chamber and allowed to adhere, then fed for two days on newly hatched *Artemia*. Feeding trial experiments (20 total) commenced by placing a single Aeolid nudibranch into a chamber, and then monitored at 1, 4, and 24 hours post introduction.

## 2. Field assessment of nudibranch feeding

Bay nettle polyps were cultured onto flat PVC plates (5 cm x 5 cm) and maintained in the laboratory following the methods described above. Plates (evaluated and numbered prior to deployment) were transported to several lagoon communities where the presence of both polyps and nudibranchs has been previously verified. Experimental plates were placed in proximity/contact with structures (e.g., bulkheads) to allow predators access to the polyps. Two field experimental trials were conducted: the first from 8/1/17 to 8/22/17 (n=16 deployed plates) and the second from 8/22/17 to 10/5/17 (n=4 deployed plates). Upon recovery, the settling plates were evaluated for the number of polyps remaining and assessed for the presence of nudibranchs. Nudibranchs encountered were preserved for analysis of cnidarian DNA.

## 3. Identify potential nudibranch predators of cnidarians from the field and assess their diet through molecular identification of cnidarian DNA

Nudibranchs (n = 35) were subjected to DNA barcoding using both the mitochondrial 16S ribosomal and COI loci. DNA was extracted using a modified CTAB method (Gaynor *et al.*, 2016). One  $\mu$ L aliquots of each sample were subjected to PCR amplification using either the Universal Cnidarian 16S primer set (UCF and UCR1) or the COI primer set (COIF and COIR) as described by Folmer *et al.* (1994). All DNA sequencing reactions were carried out at MSU using an ABI 3130 Genetic Analyzer. Both forward and reverse strands of amplicons were sequenced in all cases. Electropherograms were edited using 4Peaks or Geneious packages. BLASTn searches of all edited sequences were performed using GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

Isolated nudibranchs were rinsed in triplicate with artificial seawater to remove any surficial trace DNA, then pooled from a single set of plates regardless of species and stored for future analysis. DNA was extracted following a CTAB/NaCl method modified by Gaynor *et al.* (2016). DNA was amplified using modified primers targeting 16S rDNA (Restaino, 2013). Raw sequences were edited and aligned using 4 Peaks (<http://nucleobytes.com/4peaks/index.html>) and CLUSTAL Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) and searched for homology against all known genetic sequences using the BLAST algorithm. In addition, isolated nudibranchs collected in 2018 and 2019 were analyzed using COI and 16S nudibranch-specific primers (same extraction/analysis protocols as above).

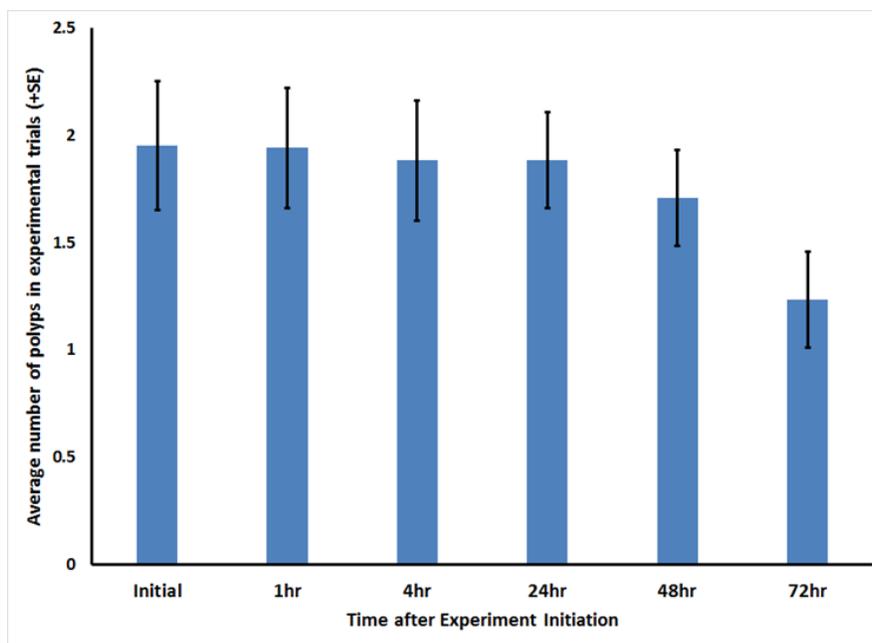
## Results

### 1. Assess nudibranch potential as a biological control of bay nettle polyps

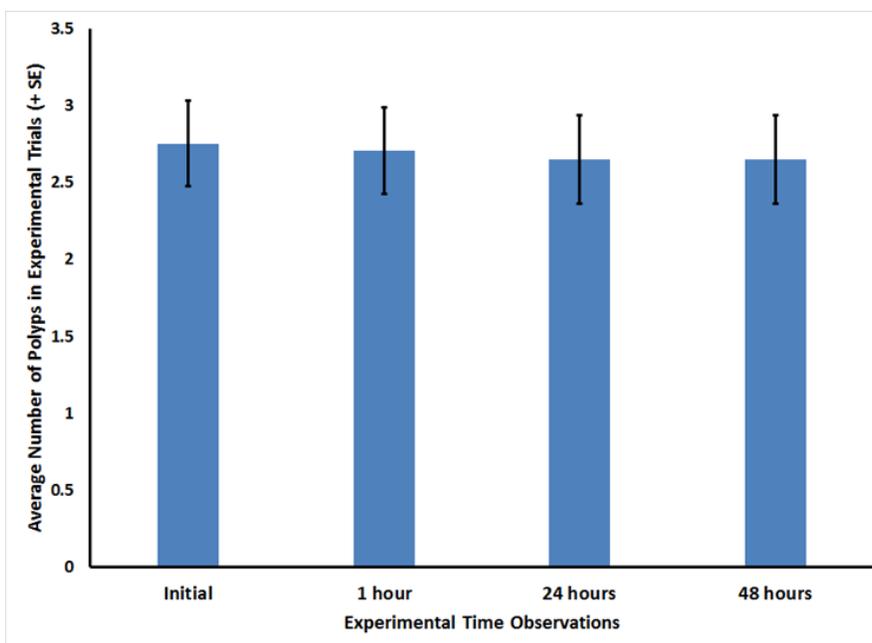
#### Laboratory Feeding Experiments

In 2016 and 2017, individual feeding trials were initiated to investigate the feeding and satiation of nudibranchs on *C. chesapeakei* polyps. In 2016, a 72-hour experiment was conducted

and in 2017, a 48-hour experiment was conducted; results of both experiments varied. For the 2016 experiment, some initial predation during the first 24 hours was observed, but larger reductions were observed at 48 and 72 hours (Figure 2). This differed with the 2017 experimental results, where some partial predation was observed, but minimal total predation occurred within the first 24 hours (mean initial # polyps/trial = 2.75; vs. 2.65 at 24h and 48h; Figure 3). This suggests that while nudibranchs are consuming *C. chesapeakei*, the rate is substantially less than one individual per day.



**Figure 2.** Results of the 2016 72-hour feeding trials assessing nudibranch predation. Values reflect the average number of *C. chesapeakei* polyps at the initiation and subsequent monitoring during the experimental trials (n=20).

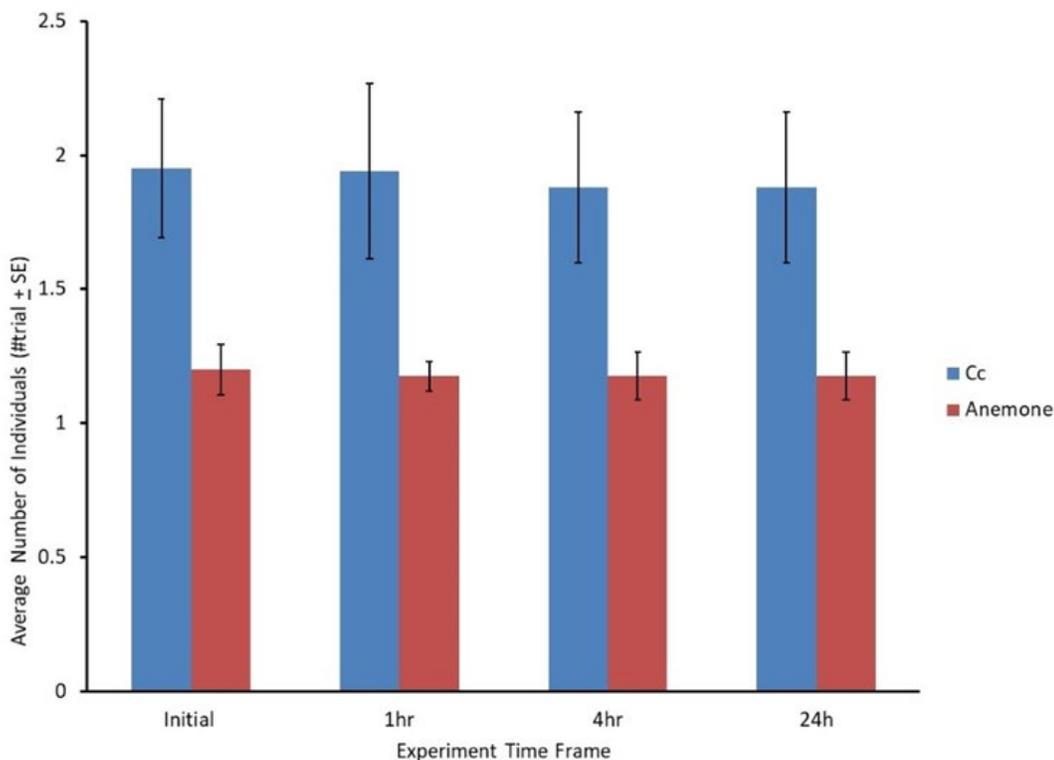


**Figure 3.** Results of the 2017 48-hour feeding trials assessing nudibranch predation. Values reflect the average number of *C. chesapeakei* polyps at the initiation and subsequent monitoring during the experimental trials (n=40).

## Laboratory Choice Experiments

In 2016, 20 laboratory trials were conducted to assess consumption potential over a 24-hour period. Each experiment contained one or two orange-striped anemones and between 1-3 *C. chesapeakei* polyps. All cnidarians were initially counted and then one nudibranch placed into the experimental trial container.

Observations occurred at 1, 4, and 24 hrs. Results indicate that *C. chesapeakei* was significantly preferred over sea anemones (Chi2 1df = 4.5,  $P < 0.05$ ). Additionally, the majority of predation occurred within 1 hour (mean initial 1.95 polyps/trial, 1h and beyond 1.88 polyps remaining; Figure 4).

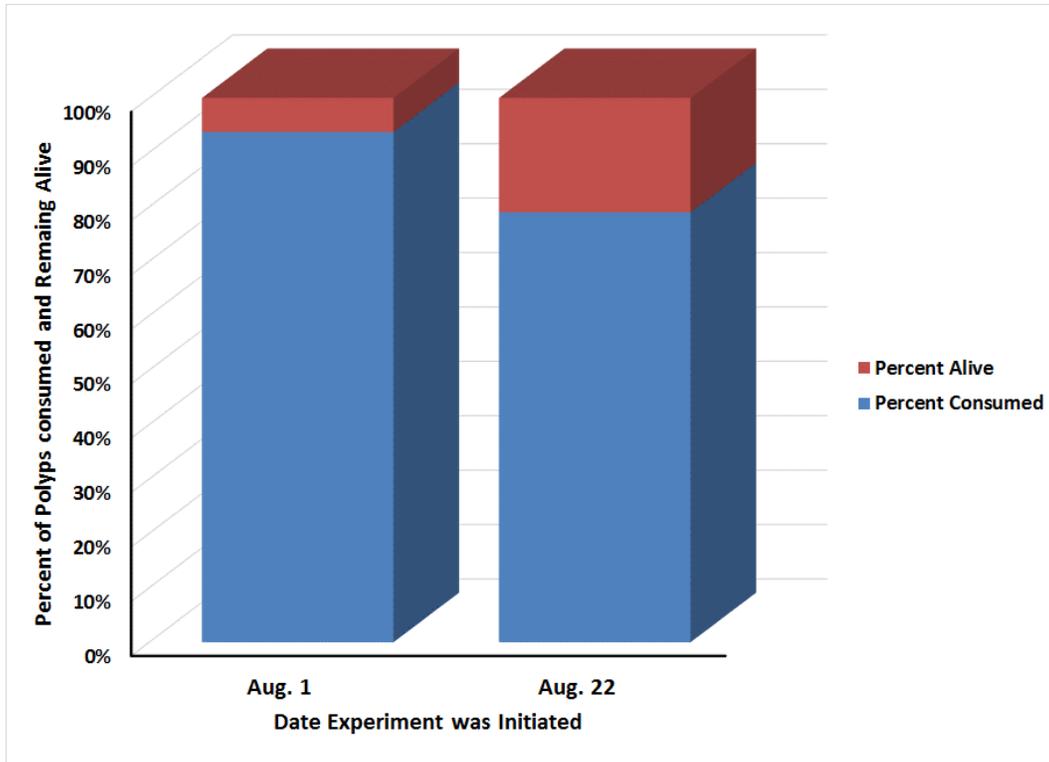


**Figure 4.** Results of the 24-hr laboratory choice experiments conducted in 2016 (n=20). Blue Bars reflect the average number of *C. chesapeakei* polyps per trial used within the study and red bars reflect the average number of the orange striped sea anemones used and subsequently counted at each timeframe of the experiments. Values represent the mean number of polyps or anemones counted alive during the experiment ( $\pm$  SE).

## 2. Field assessment of nudibranch feeding

In 2017, two experimental trials were conducted using laboratory-reared *C. chesapeakei* transported to the field. Polyps were placed into select lagoons in Barnegat Bay and then retrieved to assess predation potential. Polyp counts were recorded prior to replacement and then reassessed after field placement (Figure 5).

Experiments initiated on August 1, 2017 (n=16) showed a significant reduction in the number of polyps ( $t=3.67$ ,  $P < 0.001$ ). While the second field experiment (August 22, 2017; n=4) resulted in a 79% consumption rate (Figure 5), there was not a statistically significant reduction ( $t=1.9$ ,  $P < 0.08$ ). However, there was a highly significant reduction in polyps for both trials ( $t=4.19$ ,  $P < 0.0002$ ). Nudibranchs were observed on four of the 20 experimental arrays, indicating that nudibranchs within the system were actively feeding on *C. chesapeakei* polyps.



**Figure 5.** Results of the field feeding trials of polyps in Barnegat Bay lagoons. Results are presented based on the start date of each experiment with the relative percent consumed or remaining alive from plates.

### 3. Identify potential nudibranch predators from the field and assess their diet through molecular identification of cnidarian DNA

A total of 18 nudibranchs were collected from either the Shrewsbury River or Barnegat Bay. Additional samples (n = 16) were part of the nudibranch feeding trials from 2016. BLASTn searches were conducted for all samples (these data – 16S and COI loci – are provided in the full report). As previously demonstrated (Restaino *et al.*, 2018), the presence of cnidarian DNA were identified in some percentage of the nudibranch samples indicating that these nudibranchs had consumed polyps. Two hydrozoans, *Sarsia tubulosa* and *Rathkea octopunctata*, were positively identified as being part of the DNA ingested by the nudibranchs. The presence of both *Sarsia* and *Rathkea* DNA likely indicate that these nudibranchs were feeding on the polyps of these two genera. Both the 16S and COI analysis did identify four nudibranchs

present in these systems: *Bosellia sp.*, *Ercolania sp.*, *Ercolania fuscata*, and *Tenellia adspersa*. The hits to *Bosellia sp.* and *Ercolania sp.* were nearly identical matches to samples previously identified from Barnegat Bay.

Field collected nudibranchs were also evaluated to assess presence and identity among cnidarians in 2018 and 2019. The following genera were identified from Barnegat Bay: *Tenellia*, *Catriona*, and *Tergipies*. These identifications were accomplished through cross amplification of nudibranch DNA while using cnidarian 16S primers. Given that aeolid nudibranch species were not directly targeted in these amplifications, there are likely many species or genera that were not identified from these samples. However, by using nudibranch specific primers targeting mitochondrial DNA loci such as 16S and COI, the study was able to effectively identify aeolid species within Barnegat Bay, using DNA already extracted from known and unknown nudibranch samples. Results from these analyses confirmed taxa consisting of *Tenellia sp.*, *Cuthona sp.*, *Fiona pinatta*, and two *Aeolidia sp.*

## Conclusions and Management Implications

Based on the results from both laboratory and field experiments, it is clear that several nudibranch taxa are present in coastal New Jersey and feed on cnidarians. However, the lab results cannot confirm whether they represent a significant top-down control on cnidarian polyps. In general, nudibranchs consumed polyps or partially consumed polyps, but often showed satiation (Figures 3 and 4). However, field experiments demonstrated high rates of predation under natural conditions (Figure 5). This might imply that under field conditions nudibranchs are more efficient at feeding on polyps or that greater numbers of predators were present, leading to the substantial loss of live polyps. While nudibranchs can be abundant and are key predators of cnidarians, dietary choice of several small hydrozoan polyps (e.g., *Sarsia sp.*, *Obelia bidentata*, etc.) indicates that nudibranchs do feed on a wide variety of cnidarians. It is clear that they have a preference for *C. chesapeakei* over the invasive sea anemone (Figure 5), but field dietary choice of nudibranchs is mediated by numerous factors (e.g. prey availability, capture efficiency, digestive ease, and predation pressure).

Collectively, the generalized rise of cnidarian polyps and medusae in coastal New Jersey bays indicates that current populations of nudibranchs are insufficient to control polyp populations and act as a biological control agent. However, if nudibranch populations increase in accordance with time-lag predator-prey theory, future populations may be able to control or stabilize polyp populations. Alternately, native *Aeolidida* taxa could be bred captive and released into the environment to increase local populations; although there may be challenges to successful breeding (e.g. those species that have pelagic larval stages). Determining the scale of such activities to generate sufficient numbers of new recruits as effective predators may also be of concern, since adults are sometimes challenging to find in the field. Therefore, intensive, multi-generational culturing may be necessary to yield the required individuals for field release. Deployment to known polyp 'hot-spots' can also be most effective. For example, *C. chesapeakei*

prefer man-made structures like floating docks and bulkheads for larval settlement and polyp establishment. These structures tend to be clustered in coastal waterfront developments (e.g., lagoon communities), so they are likely candidates to host significant polyp populations. As we continue to evaluate and understand jellyfish dynamics, it is clear that addressing polyp populations is critical to management. Therefore, a combined approach to limit polyps will be the most effective strategy to manage nuisance species.

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## RESEARCH PROJECT SUMMARY

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