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

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Executive Summary

During the last 15 years, the Atlantic Bay Nettle (*Chrysaora chesapeakei*) has become established in several estuaries in New Jersey. An extensive population exists in Barnegat Bay and the Navesink-Shrewsbury Region, but its presence has been identified as far south as Cape May. As this species has increased in abundance, it has dramatic impacts to the local communities, both in regards to living resources and human enjoyment of the coastal waters. The objective of controlling adult medusae, which the public encounters in the water column, is a difficult task. However, the life history of bay nettles and other cnidarians provides an opportunity to control adult populations by focusing on the polyp stage, which generates the swimming adults. The results from this research indicate that nudibranchs do consume *C. chesapeakei* polyps in laboratory and field settings. However, several mitigating factors may influence their efficiency in being able to control populations. Laboratory studies indicate that while complete predation of polyps did occur, frequently incomplete or partial predation of polyps was common. Under these conditions, nudibranchs consumed polyp tentacles, but failed to consume the whole individuals. While this loss of tissue would cause polyps in the field to repair lost tissues, it would not curtail the growth of the polyp populations, but rather only slow them down. Another mitigating factor in nudibranch controls of polyps is predation on anemones by nudibranchs in choice experiments. In laboratory experiments, we tested predation potential with two prey: bay nettle polyps and the ubiquitous, but non-native sea anemone *Diadumene lineata*. *Diadumene lineata* was chosen because it inhabits the same man-made structures in the bay. Results from these experiments indicated nudibranch predation on *Diadumene lineata*. Consequently, in areas where *Diadumene lineata* is present in high numbers, nudibranchs may be too scarce to act as efficient predators on polyps due to competing food resource options. However, these experiments also showed a significant predation preference for *C. chesapeakei*, so if the smaller nudibranchs can avoid *D. lineata*, they can act as a potential control for polyps if present in sufficient population densities. In summary, 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 a biological control.

Additionally, nudibranchs obtain not only trophic resources from cnidarian prey, but also bio-mechanical defenses. Through the process of kleptocnidae, nudibranchs retain the cnidocysts as a defense mechanism by translocating cnidoblasts (containing cnidocysts) to their cerata. As these cells retain their DNA, they preserve a record of nudibranch diet beyond simple digestive track analyses. As part of this research, we collected aeolid nudibranchs in the field and using Cnidarian primers, we amplified sequences for 16S rDNA from grouped individuals. Our results showed that cnidarian DNA was present in over half of our samples with positive identification of *Obelia bidentata*, *Moerisia spp.*, and *Chrysaora chesapeakei*. While *O. bidentata* and *C. chesapeakei* are

common in this system, the identification of the non-native *Moerisia spp.* demonstrates that molecular techniques can be used to ascertain invasions of extremely diminutive cnidarian polyps in coastal regions. Consequently, there is great value in using amplified 16S rDNA from nudibranch kleptocnidae as a tool to identify Cnidarian polyp populations and potentially identify non-native cnidarian species invading global coastal regions.

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Jellyfish and Project Background

Gelatinous zooplankton are increasing in marine ecosystems worldwide as a result of climate change, species introductions, and a number of anthropogenic alterations to coastal food webs that favor jellyfish and ctenophores (Sullivan et al., 2001; Purcell and Decker, 2005; Hay, 2006; Kirby and Beaugrand, 2009; Kirby et al., 2009; Richardson et al., 2009). One important driver of the shift towards greater abundance of gelatinous zooplankton is the construction of hard surfaces such as bulkheads, docks, and other shoreline modifications that provide suitable habitat for scyphozoan polyps (Hoover and Purcell 2009). Another anthropogenic action that favors gelatinous zooplankton is the increase in eutrophication resulting from coastal nutrient loading, which fuels bottom hypoxia in relatively shallow systems. Jellyfish are highly tolerant of low dissolved oxygen conditions and therefore benefit from the impacts of hypoxia on their prey species which are either easier to catch in hypoxic waters or are more concentrated in the overlying normoxic waters. In either situation, jellyfish experience elevated energy intake and reproductive capacity, which ultimately contributes to population growth (Purcell et al., 2001; Grove and Breitburg, 2005; Purcell et al., 2007). Both of these drivers of gelatinous zooplankton increases are prevalent in the Barnegat Bay system. Large populations of jellyfish are detrimental to fisheries because the jellyfish are voracious feeders on zooplankton and ichthyoplankton and are therefore competitors and predators of fish (Brodeur et al., 2008).

Increasing coastal development has created environments which favor species that are tolerant of various pollutants and degraded water quality. Additionally, the hardening of shorelines and elimination of natural vegetated regions create the potential that tolerant fouling organisms can colonize and expand in these degraded systems. Many coastal estuaries are plagued by poor water quality and increasing inclusion of non-native species. As such, developed coastal estuaries are being defined by lowered species richness and diversity as invaders monopolize available space (Ruiz et al., 1997; Cohen and Carlton, 1998), quantity and toxicity of pollutants (Long et al., 1996), loss of natural habitats (Lathrop and Bognar, 2001), and simplification of food webs through redirection of energy, species introduction, and overfishing (Byrnes et al., 2007). In particular, the relative increase in gelatinous zooplankton in many regions of the ocean has led to a phase shift from 'text-book' planktonic communities dominated by zooplanktivorous fish and higher apex predators (Reid et al., 2000), to ones dominated by ctenophores, cnidarians, and pelagic tunicates (Purcell et al., 2007). While the apparent global increase in gelatinous zooplankton is actively debated (see Brotz et al., 2012; Condon et al., 2013), many specific regional locations have strong documentation of elevated abundances (Fuentes et al., 2010) often leading to food web disruption and fisheries crashes (Roohi et al., 2010).

Barnegat Bay, New Jersey is a coastal lagoon system in the mid-Atlantic region of the United States with significant urbanization and land use changes in its' watershed (Lathrop and Bognar, 2001). While *M. leidyi* has been reported as an important component of the pelagic community for the last century (Nelson, 1925; Mountford, 1980; Sandine, 1984), the recent invasion by the scyphozoan *C. chesapeakei* during the last decade may be a result of the development and eutrophication of the system (*sensu* Duarte et al., 2012) followed by a human induced introduction, but rising water temperatures may merely signal a range extension of the species (Sorte et al., 2010). 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 from two large rivers. As these jellyfish have become established in the region over the last decade, their impacts at the community level have yet to be evaluated; even though their increasing abundance has led to reduced recreational use of the bay.

Jellyfish Life History

Most jellyfish possess a 'bi-phasic' life history where the familiar medusa represents the sexually reproducing adults in the population and a benthic polyp stage which represents the asexual reproductive phase (Figure 1). In general, the medusa phase is the one which has resulted in the major impacts on coastal communities and food webs. However, after larvae are released, they need hard substrates (e.g., rocks, oyster shell, docks, bulkheads, etc.) upon which to settle and transform into the polyp stage. The polyp stage is the most critical life history phase for controlling populations, as they actively clone themselves to produce more polyps, which ultimately produce more adult medusa. In addition, they also produce podocysts (Figure 2), which are the resting stage which overwinters and/or survives during environmental changes. If we aim to gain control of adult medusa, then the focus should be on ways to control the polyp stage which would truly reduce the overall bay nettle population.

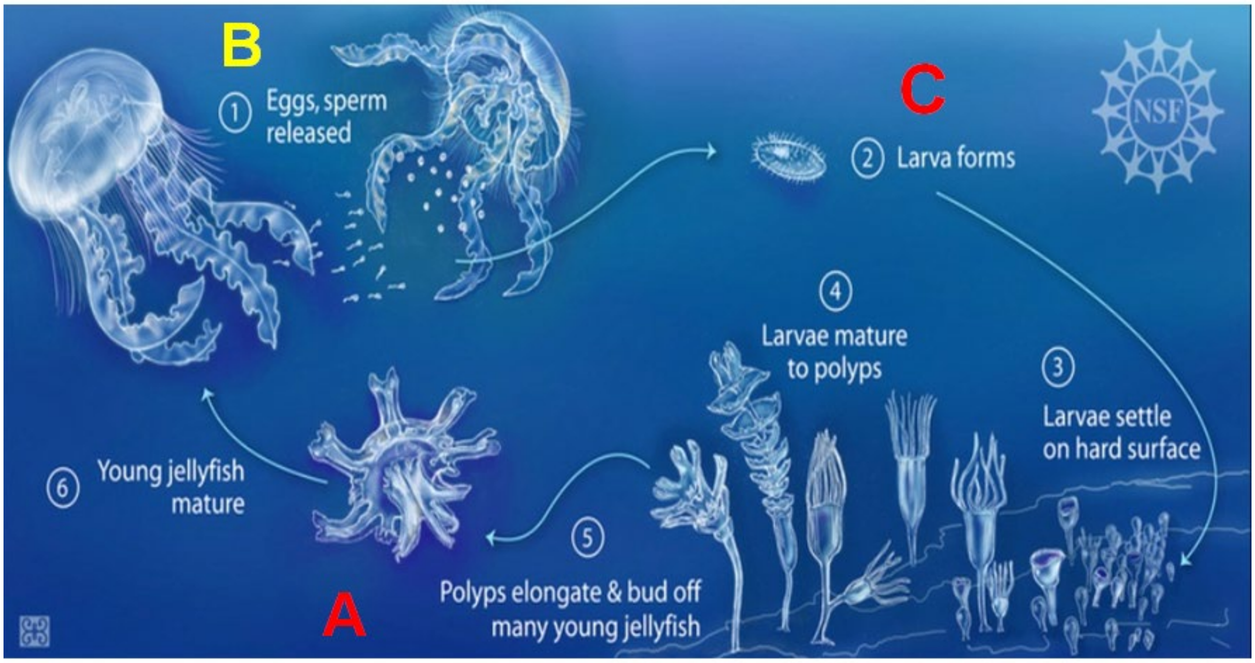

Figure 1. Life History of a typical jellyfish. A) represents the newly formed ephyra from asexually budding polyps; B) Adult stage which sexually reproduce and produce C) larvae which colonize new surfaces such as docks, piers, and bulkheads.

Figure 2. Images of an immature Atlantic Bay Nettle (polyp and podocysts). Left panel shows the polyp in focus, while right panel focuses on the podocysts produced.

Research Objectives

With increasing development and replacement of building materials which are non-toxic, *C. chesapeakei* populations will continue to have the opportunity to expand in Barnegat Bay and other coastal New Jersey lagoonal systems. Although Hurricane Sandy destroyed significant polyp and podocyst habitat, our previous data demonstrate widespread presence of *C. chesapeakei* larvae in the water and active settlement in numerous regions of the bay. 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, subsequent 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**

Methodology

To be an effective biological control, nudibranchs need to be present and survive in the same conditions as bay nettle polyps. They also need to show active consumption of polyps and a preference over other cnidarian species which produce stinging cells that they seek for predator deterrent.

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

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

To determine the effectiveness of nudibranch predators, feeding trial experiments were carried out. Specifically, 20 trials were run to determine initial consumption of polyps by individual nudibranchs. Polyps were cultured in the laboratory at Montclair State University and isolated into individual chambers of a 6-well plate apparatus. Polyps were placed in chambers and then allowed 48-72h to adhere to the surface of the plate prior to feeding trials. After adherence, polyps were fed for two days on newly hatched *Artemia* to ensure robust health. Feeding trial experiments commenced by placing a single Aeolid nudibranch into a chamber containing bay nettle polyps. Experimental trials were monitored initially after introduction and then monitored at 1, 4, 24, 48, and 72 hours 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 predator, 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. Plates were then monitored at 48 hours to assess predation potential.

Lastly, a feeding experiment was conducted to assess the choice/preference of nudibranchs. In glass petri dishes, several *C. chesapeakei* polyps were introduced and allowed to adhere in a similar manner as described above. After adherence, polyps were identified and counted, and then a single *Diadumene lineata* (Orange-striped Green Anemone) was introduced to the chamber and allowed to adhere. After adherence, polyps and anemones were fed for two days on newly hatched Artemia to ensure robust health. Feeding trial experiments commenced by placing a single Aeolid nudibranch into a chamber. Twenty experimental trials were monitored initially after introduction, and then monitored at 1, 4, and 24h to assess predation (including anemone predation of nudibranchs).

2. Field assessment of nudibranch feeding

To assess the real-world scenario of biological control, an in-situ field experiment was conducted. Bay nettle polyps were cultured onto flat PVC plates (5 cm x 5 cm) in the laboratory by the transfer and adherence method described above. Polyps were then fed 2-3 times per week with newly hatched *Artemia* until plates were transferred to the field. Prior to deployment, plates were evaluated for the number of polyps present and plates were numbered. Plates were then transported to several lagoon communities where we have established from previous work the presence of both polyps and nudibranchs. 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 one running from 8/1/17 to 8/22/17 (n=16 deployed plates) and the other running from 8/22/17 to 10/5/17 (n=4 deployed plates). Upon recovery from the field, the settling plates were evaluated for the number of polyps remaining and assessed for the presence of nudibranchs. When nudibranchs were encountered from the field experiments, they were preserved for analysis of cnidarian DNA (Objective 3).

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

A total of 35 nudibranch samples were subjected to DNA barcoding using both the mitochondrial 16S ribosomal locus and the mitochondrial Cytochrome c oxidase subunit I locus (COI). All samples were stored @-20°C in 70% (v/v) ethanol. Extraction consisted of centrifuging the tubes (16,000 x g for 10 min @4°C) to pellet all materials. Most of the ethanol was removed by pipetting and the samples dried for 5 to 10 minutes in a Savant Speed-Vac Evaporator. DNA from samples was extracted using a modified CTAB method (Gaynor *et al.*, 2016). All DNA pellets were resuspended in 20 µL of sterile TE (10 mM Tris, pH 8.0; 1 mM Na_2EDTA, pH 8.0). One µL aliquot of each sample were subjected to PCR amplification (Gaynor *et al.*, 2016) 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). Yield and quality of amplicons was assessed by agarose gel electrophoresis (Appendix A). All DNA sequencing reactions were carried out at Montclair State University 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 searchs of all edited sequences were performed using GenBank (www.ncbi.nlm.nih.gov) and the top match recorded along with % Query Coverage, E value, and % Identity.

Isolated nudibranchs were rinsed in triplicate with artificial seawater to remove any surficial trace DNA from the settling plate storage jars. Then, all aeolid nudibranchs, regardless of species, from a single set of plates were pooled and placed into a sterile 1.5 ml microfuge tube (n= 1 to 68 in a jar), covered with molecular grade EtOH, and stored at -80ºC until DNA extraction. DNA was extracted from the pooled nudibranchs in a single tube following a CTAB/NaCl method described by Winnepenninckx, Backeljau, & Dewatcher, (1993) with modifications described by Gaynor, Bologna, Restaino, & Barry, (2016). Once precipitated, DNA concentrations and $OD_{260/280}$ were measured on a NanoDrop spectrophotometer (ND-1000). DNA was amplified using modified primers targeting 16S rDNA (Restaino, 2013) which were developed as "Universal Cnidarian" primers by Bridge, Cunningham, DeSalle, & Buss (1995, Table 1). It should be noted that these primers do not amplify all cnidarian species and are capable of cross amplifying other invertebrate species. Additionally, *C. quinquecirrha* primers developed by Bayha & Graham (2009) and modified by Restaino (2013) were also used on the extracted samples (Table 1). PCR reactions (20 µl total volume) were run using Denville Choice Taq Master Mix (2X; Denville Scientific, Denville, New Jersey, USA) according to the manufacturer's protocol. PCR amplification was performed on an ABI Veriti 96 well thermocycler using the following parameters: 94ºC (1X for 3 minutes), 30X: 94ºC (20 seconds), 58ºC (20 seconds), 72ºC (20 seconds), 72ºC (10 minutes), and held at 4ºC. PCR amplification was checked by running samples on a 1% agarose gel. Successfully amplified samples were then sequenced (Sanger Dideoxy) using an ABI 3130 genetic analyzer, via the Big Dye Terminator Ready Reaction Mix V3.1 on diluted (1/16) reactions following the manufacturers protocols. Raw sequences were edited and aligned using 4 Peaks (http://nucleobytes.com/4peaks/index.html) and CLUSTAL Omega (Sievers et al. 2011; http://www.ebi.ac.uk/Tools/msa/clustalo/), and searched for homology against all known genetic sequences using the BLAST algorithm (Altschul, Gish, Miller, Meyers, & Lipman, 1990). In addition, isolated nudibranchs were analyzed using CO1 and 16S nudibranch specific primers

(Table 1) to identify additional field collected individuals in 2018 and 2019. DNA extraction and analysis procedures followed the same protocol as above.

Table 1: Primers used in Goodheart et al. 2018 to identify aeolid nudibranch species.

Primer	Direction	Sequence 5' → 3'	Reference
CO1 Partial Fragment (length 680 bp)			
C1-N-2329	Forward	ACT GTA AAT ATA TGA TGA GCT CA	Simon et al. (1994)
C1-J-1718	Reverse	GGA GGA TTT GGA AAT TGA TTA GTT C	Simon et al. (1994)
16S Partial Fragment (length 650)			
LR-J-12887 (16S1)	Forward	GGA GCT CCG GTT TGA ACT CAG ATC	Simon et al. 1994
LR-N-13398 (16S2)	Reverse	CGG CCG CCT GTT TAT CAA AAA CAT	Simon et al. 1994

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 in the laboratory to investigate the feeding and satiation of nudibranchs on *C. chesapeakei* in the laboratory. In 2016, a 72-hour experiment was conducted and in 2017, a 48-hour experiment was conducted. Results of the experiments varied. For the 2016 experiment, there was some initial predation during the first 24 hours, but larger reductions were also observed at 48 and 72 hours (Figure 3). This differed with the 2017 experimental results, where some partial predation was observed during this experiment, but minimal total predation occurred and when it did, it took place within the first 24 hours (mean initial # polyps/trial = 2.75; vs. 2.65 at 24h and 48h; Figure 4). Collectively, this suggests that while nudibranchs are consuming *C. chesapeakei*, the rate is substantially less than one individual per day. Given that polyp production is lower than creation of 1 polyp/day, nudibranchs could have an effect in controlling small populations in the field.

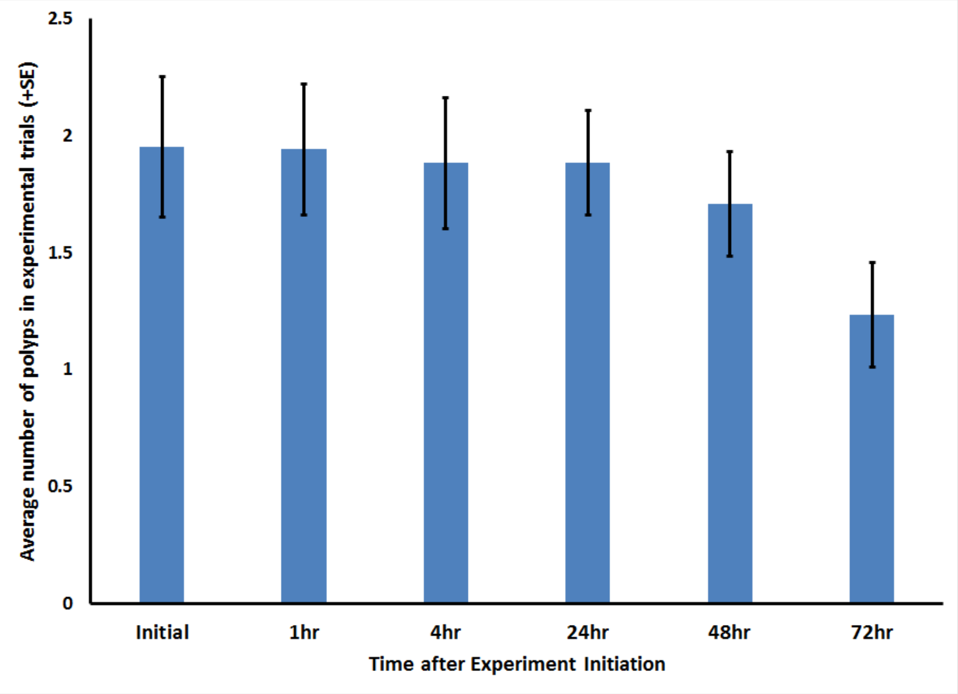

Figure 3. Results of the 2016 72-hour feeding trials assessing nudibranch predation. Values reflect the average number of *C. chesapeakei* polyps at the initiation and subsequent monitoring during the experimental trials (n=20).

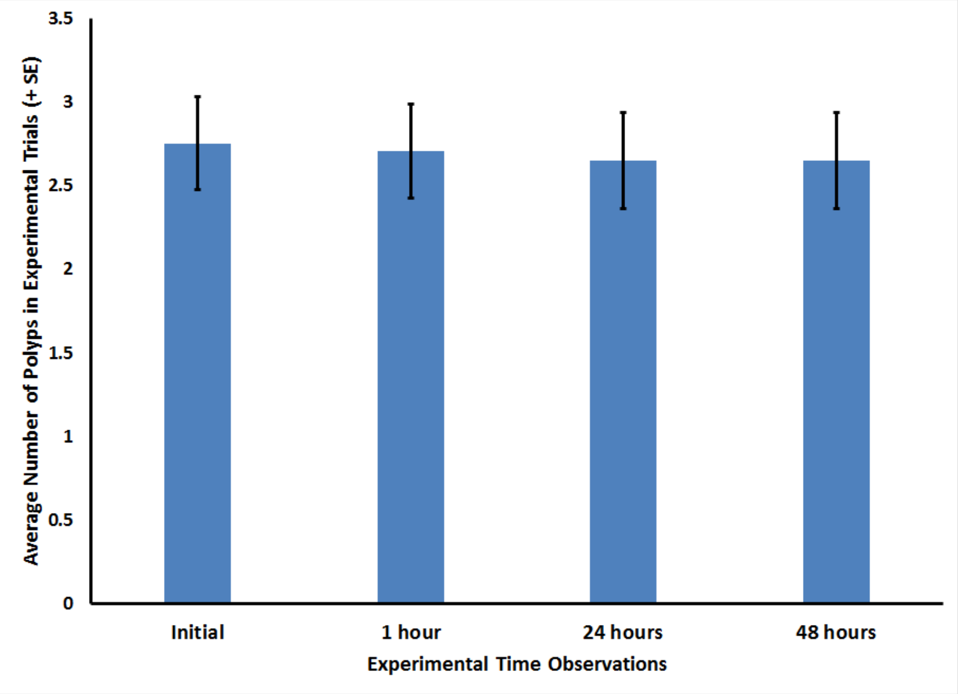

Figure 4. Results of the 2017 48-hour feeding trials assessing nudibranch predation. Values reflect the average number of *C. chesapeakei* polyps at the initiation and subsequent monitoring during the experimental trials (n=40).

Laboratory Choice Experiments

In 2016, 20 laboratory trials were conducted to assess the consumption potential of nudibranchs over a 24-hour period. 20 individual choice experiments were carried out containing one or two orange striped anemones and between 1-3 *C. chesapeakei* polyps. All cnidarians were initially counted and then one nudibranch was placed into the experimental trial container. Containers were then observed at 1 hour, 4 hr. and 24 hrs. to determine predation. Results of a Chi^2 analysis indicate that *C. chesapeakei* was significantly preferred over sea anemones (Chi^2 1df = 4.5, $P < 0.05$) and that the majority of predation occurred within 1 hour (mean initial 1.95 polyps/trial, 1h and beyond 1.88 polyps remaining; Figure 5). After that initial flurry of predation, few individuals of either species were consumed.

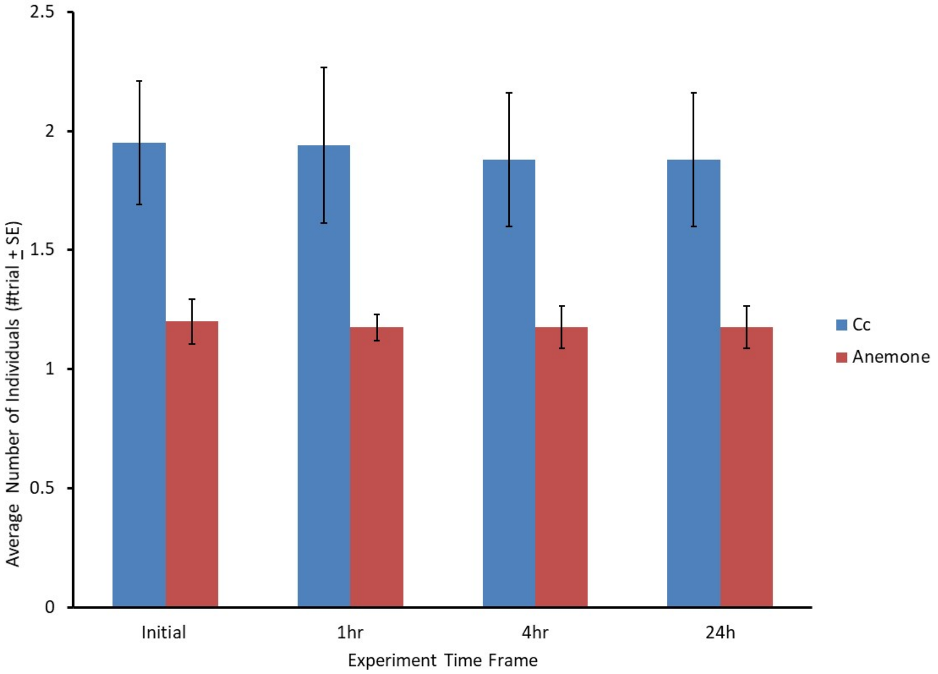

Figure 5. Results of the 24hr laboratory choice experiments conducted in 2016 (n=20). Blue Bars reflect the average number of *C. chesapeakei* polyps per trial used within the study and red bars reflect the average number of the orange striped sea anemones used and subsequently counted at each time-frame of the experiments. Values represent the mean number of polyps or anemones counted alive during the experiment (± SE).

2. Field assessment of nudibranch feeding

In 2017, two field experimental feeding trials were conducted. Both consisted of laboratory reared *C. chesapeakei* transported to the field, placed into lagoon communities in Barnegat Bay and then retrieved to assess predation potential. A total of 20 trials were conducted where the initial counts of polyps were recorded prior to replacement and then reassessed after field placement (Figure 6). For experiments initiated on August 1, 2017 (n=16), a significant reduction in the number of polyps was observed ($t=3.67$, $P < 0.001$). While the second field experiment (n=4) resulted in a 79% consumption rate (Figure 6), there was not a statistically significant reduction (t=1.9, $P < 0.08$). One factor which may have played a role is that the second experiment was later in the summer and there were fewer replicates. However, when all experimental trials were assessed, there was a highly significant reduction in polyps (t=4.19, $P < 0.0002$). Additionally, nudibranchs were located on four of the twenty experimental arrays, indicating that nudibranchs within the system were actively feeding on *C. chesapeakei* polyps.

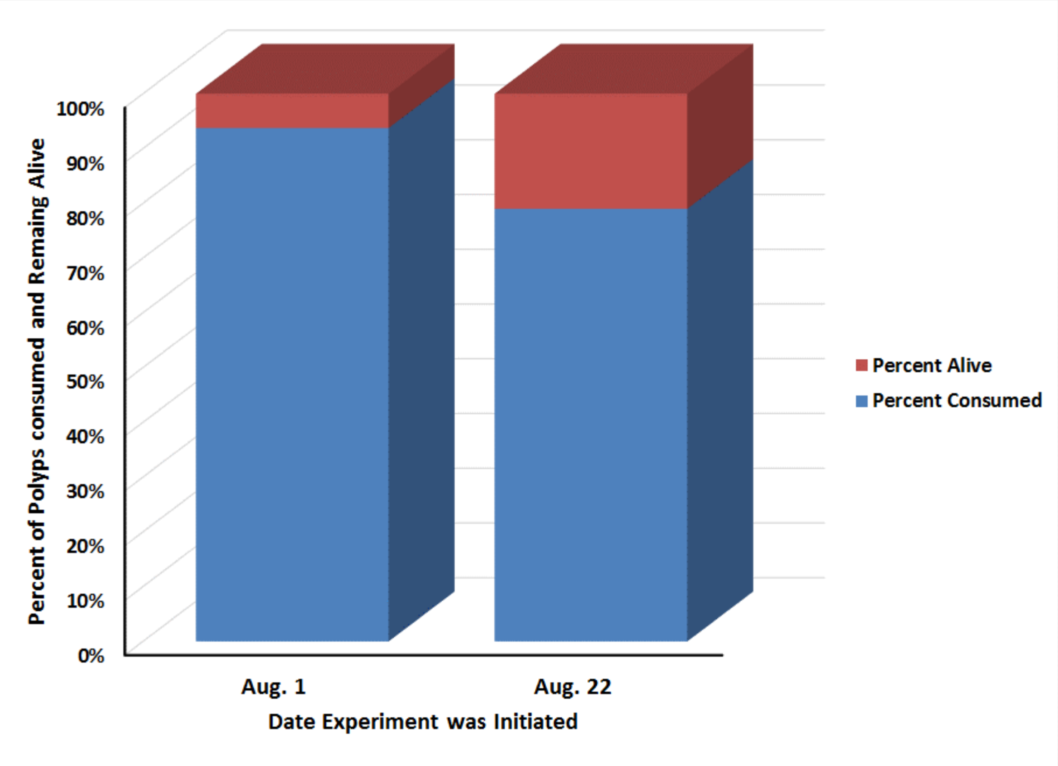

Figure 6. Results of the field feeding trials of *C. chesapeakei* polyps in Barnegat Bay lagoons. Results are presented based on the start date of each experiment with the relative percent consumed or remaining alive from plates.

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

There were a total of 18 field collected nudibranchs from either the Shrewsbury River or in Barnegat Bay, NJ. Additional samples (n = 16) were part of the nudibranch feeding trials from 2016. BLASTn searches for samples for the field collected individuals (both forward and reverse) are provided in Table 2 and Table 3. Table 2 summarizes succesfully amplified sample data based on the analysis of the 16S locus. Table 3 shows data based on the analysis of the COI locus. As we had demonstrated previously with this method (Restaino et al., 2018) we were able to identify the presence of cnidarian DNA in some percentage of nudibranch samples indicating that these nudibranchs had consumed polyps and were carrying ingested nudibranch DNA in their gut and/or cerata. As seen in Table 2 (for the 16S locus) we have positively identified two hydrozoans, *Sarsia tubulosa* and *Rathkea octopunctata*. Both of these hydrozoans had been identified by our group previously in Barnegat Bay (data not shown). However, these were seen previously only as medusa. The presence of both *Sarsia* and *Rathkea* DNA being detected in nudibranchs almost certainly means that these sea slugs were feeding on the polyps of these two genera. Thus, we now have confirmation that these two hydrozoans are resident in New Jersey waters. Table 3 (COI locus) did not identify any additional cnidarian species. Both the 16S and COI analysis did identify four nudibranchs present in our system. *Bosellia sp.*, *Ercolania sp.*, *Ercolania fuscata*, and *Tenellia adspersa* were all detected by DNA barcoding. The hits to *Bosellia sp.* and *Ercolania sp.* were nearly identical matches to samples we had previously identified on settling plates in Barnegat Bay.

Table 2. Molecular Identification of Nudibranch Specimens (16S) Collected from the Shrewsbury River and Barnegat Bay, NJ.

Sequence #	**ID#**	**Genus/species**	**F/R**	**Locus**	**Coverage %**	**E value**	**% Identity**
1	SJ	*Sarsia tubulosa**	F	16S	89	0.0	99
2	W1	*Bosellia sp.**	F	16S	79	$2\ e^{-155}$	95
3	W2	*Bosellia sp.**	F	16S	86	0.0	97
4	S1	*Bosellia sp.**	F	16S	81	$4\ e^{-169}$	97
5	S2	*Bosellia sp.**	F	16S	80	$4\ e^{-144}$	93
6	O1	*Bosellia sp.**	F	16S	86	0.0	98
7	O2	*Bosellia sp.**	F	16S	83	0.0	99
8	B1	*Bosellia sp.**	F	16S	83	$1\ e^{-176}$	98
9	B2	*Bosellia sp.**	F	16S	82	$6\ e^{-180}$	99
10	B3	*Bosellia sp.**	F	16S	82	$6\ e^{-167}$	97
11	R02	*Rathkea octopunctata*	F	16S	97	0.0	99
12	R03	*Rathkea octopunctata*	F	16S	96	0.0	97
13	SJ	*Sarsia tubulosa**	R	16S	100	0.0	96
14	W1	*Ercolania sp.* *	R	16S	87	$1\ e^{-36}$	89
15	W2	*Ercolania sp.* *	R	16S	86	0.0	99
16	S1	*Ercolania sp.* *	R	16S	97	$3\ e^{-97}$	95
17	S2	NO CALL	R	16S			
18	O1	*Ercolania sp.* *	R	16S	86	0.0	99
19	O2	*Ercolania sp.* *	R	16S	84	0.0	99
20	B1	*Ercolania sp.* *	R	16S	86	0.0	99
21	B2	*Ercolania sp.* *	R	16S	86	0.0	98
22	B3	*Ercolania sp.* *	R	16S	78	$5\ e^{-63}$	92
23	R02	*Rathkea octopunctata*	R	16S	88	$3\ e^{-58}$	92
24	R03	*Rathkea octopunctata*	R	16S	87	$7\ e^{-73}$	97

Notes: All searches were BLASTn using standard default values. Only the top hit is listed along with % Query Coverage, E value, and % Identity. Sequence direction is listed (F or R) as well as locus (16S or COI). Nudibranch genera and/or species identified are in BLACK type; Cnidarian sequences identified are in RED type. Failed sequencing reactions or failure to call organism is indicated in PURPLE. *Indicates that this organism has been identified by DNA barcoding previously in Barnegat Bay samples (Restaino, Gaynor, and Bologna, unpublished).

Table 3. Molecular Identification of Nudibranch Specimens (COI) Collected from the Shrewsbury River and Barnegat Bay, NJ.

Sequence #	ID#	Genus/species	F/R	Locus	Coverage %	E value	% Identity
25	B2	*Ercolania fuscata*	F	COI	93	0.0	93
26	B3	*Ercolania fuscata*	F	COI	90	3 e^{-57}	76
27	R02	NO CALL	F	COI			
28	P313	*Tenellia adspersa*	F	COI	90	1 e^{-174}	98
29	P344	*Tenellia adspersa*	F	COI	93	0.0	95
30	P378	*Tenellia adspersa*	F	COI	91	0.0	97
31	B2	*Ercolania fuscata*	R	COI	88	0.0	99
32	B3	REACTION FAILED	R	COI			
33	R02	NO CALL	R	COI			
34	P313	*Tenellia adspersa*	R	COI	86	0.0	98

Notes: All searches were BLASTn using standard default values. Only the top hit is listed along with % Query Coverage, E value, and % Identity. Sequence direction is listed (F or R) as well as locus (16S or COI). Nudibranch genera and/or species identified are in BLACK type; Cnidarian sequences identified are in RED type. Failed sequencing reactions or failure to call organism is indicated in PURPLE. *Indicates that this organism has been identified by DNA barcoding previously in Barnegat Bay samples (Restaino, Gaynor, and Bologna, unpublished).

We continued to evaluate field collected nudibranchs to assess presence and identity among cnidarians in 2018 and 2019. Aeolid nudibranch could play an essential role in controlling jellyfish populations, as they are the main predator of cnidarian polyps. In Barnegat Bay we have identified aeolid nudibranchs within the following genera: *Tenellia, Catriona,* and *Tergipies*. These identifications have occurred through cross amplification of nudibranch DNA while using cnidarian 16S primers. Cnidarian primers were used to determine if cnidarian DNA was present in the digestive system of these nudibranchs or in sequestered cnidocysts found in the cerata as a result of kleptocnidae. Given that aeolid nudibranch species were not directly targeted in these amplifications, there are likely many species or genera that we have not identified. However, by using nudibranch specific primers targeting mitochondrial DNA loci such as 16S and COI (see Table 1), we were 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. (sequence analysis provided in Appendix B). Specifically, field collected and identified *Cuthona* species (Figure 7) were observed among algal samples which were examined for clinging jellyfish (*Gonionemus vertens*) from Barnegat Bay. During the observation in the laboratory, *Cuthona* sp. demonstrated active predation on *G. vertens* medusa, providing another potential predatory mechanism to control an invasive species.

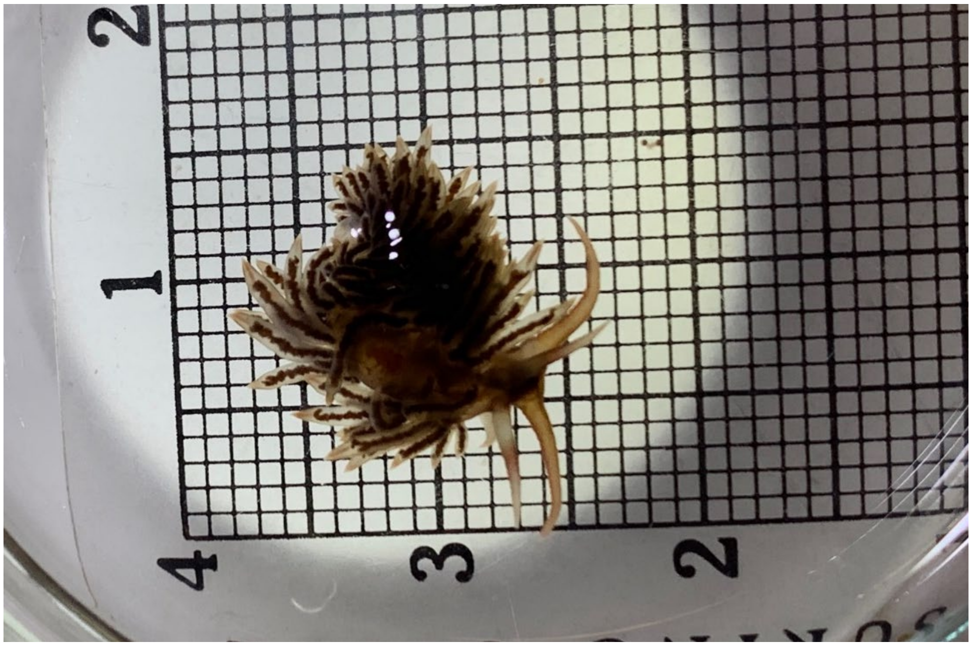

Figure 7. *Cuthona* sp. collected among algal samples from Barnegat Bay.

Conclusions and Management Implications

Based upon the research conducted and the results from both laboratory and field experiments, it is clear that several nudibranch taxa are present in coastal New Jersey and they feed on cnidarians. As to whether they represent a significant top-down control on cnidarian polyps, our laboratory results cannot confirm this. In general, nudibranchs consumed polyps or partially consumed polyps (Figure 4), but often showed satiation (Figure 5). However, our field experiments demonstrated high rates of predation under natural conditions (Figure 6). 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 under these conditions (Figure 6). While nudibranchs can be abundant at times and are key predators of cnidarians, dietary choice of several small hydrozoan polyps (e.g., *Sarsia* [Table 2], *Obelia bidentata* and *Moerisia* sp., Restaino 2018) indicates that nudibranchs 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 such as prey availability, capture efficiency, digestive ease, and predation pressure on nudibranchs, which can cause dietary shifts to more venomous taxa for protection. Collectively, the generalized rise of scyphozoan and hydrozoan polyps and medusa in coastal New Jersey bays indicate 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, critical native *Aeolidida* taxa could be bred captively and released into the environment to increase local populations; although there may be challenges to successfully breeding nudibranchs. Many species have pelagic larval stages, which may make culturing difficult. However, in a controlled environment with sufficient food resources, they can be bred. 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. If successful though, they can be isolated and bred independently and potentially released into high polyp regions. If successful, these individuals could exert top-down pressure and reduce local polyp populations. This potential implies that the location of polyp distributions are known and nudibranch release corresponds to 'hot-spots' where they can be most effective. For *C. chesapeakei*, a nuisance species in coastal New Jersey, our previous work has demonstrated that man-made structures like floating docks are critical polyp habitat. These structures are clustered in coastal waterfront developments (e.g., lagoon communities), so they are likely candidates to host polyps. Captive breeding and release into these areas could result in polyp reductions and ultimately reductions in adult medusa. Alternatively, other methods of polyp removal such as high-pressure washing or removal of floating structures in these regions could also be an effective management strategy to minimizing jellyfish populations. Lastly, improvement in water quality, especially in low turnover lagoon developments, could tip the scales in terms of competitive interactions between cnidarian polyps and other species. Cnidarians have a robust metabolic activity where they show limited stress to low dissolved oxygen concentrations. As such, they can often survive and flourish in areas where repeated hypoxia deters other species. These increases in polyp populations may be a result of depressed nudibranch populations in these same regions due to poor water quality and oxygen stress on nudibranch, limiting their survival and growth. Additionally, in terms of other space requiring species (e.g., barnacles) which could compete and outcompete cnidarian polyps, improved water quality leading to normoxic waters would favor these interspecific interactions and limit polyp populations. However, under current water conditions where hypoxia is common, cnidarian polyps are favored due to their tolerance of these conditions, a process often referred to as 'winning by default'. As we continue to evaluate and understand jellyfish dynamics, it is clear that addressing polyp populations is critical to management. There are several options which could lead to minimizing their populations including physical removal, removal of structural habitats (e.g., docks), predator mediated biological control, and improved water quality. It is likely that a combined approach to limit polyps will be the most effective strategy to limit these nuisance species.

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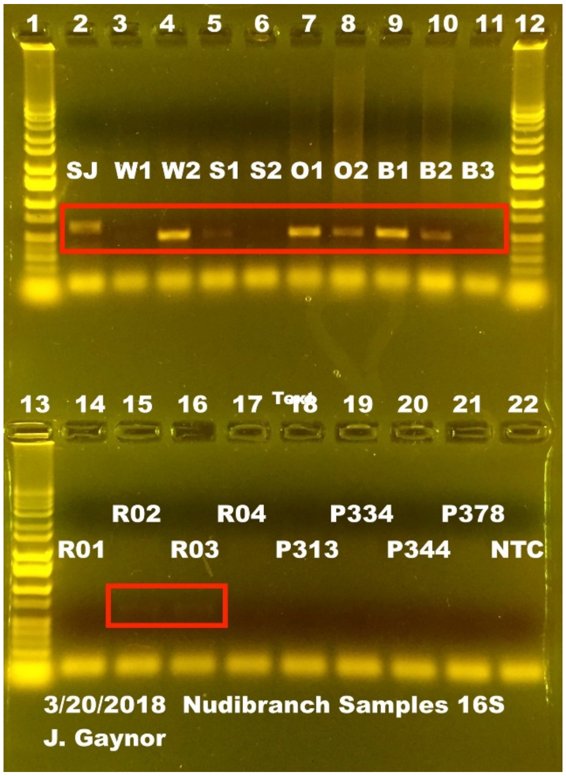
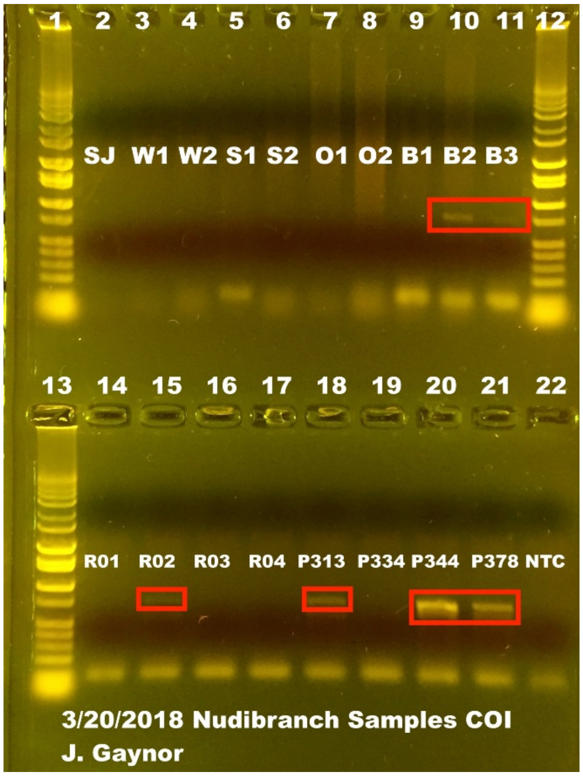
Appendix A. Gel Images from PCR products generated from nudibranchs.





Appendix B. Sequence Identification based on Nudibranch specific Primers of Field Collected Nudibranchs

>1 *Tenellia sp./Cuthona sp.*
CGAAACAGGAACAACGGGATCCCCAGTAACGGCGAGTGAAGCGGGACGAAGCCCA
GCGCCGAATCCGCCGCGGCTATTAGCCCGGCCGGAACTGTGGCGTGAGGGACGCGG
CCAGTCGACCGGAAGGACGCTCGAGTCCCCCCGAGCGGGGCCCTTCCCATAGCGGG
TGCCAGGCCTCTGCCGGCGTCCGTCCGGTCGGCCGCTGCCGTCCCCGGAGTCGGGTT
GTTTGGGAATGCAGCCCGAAGCGGGTGGTAAACTCCACCTAAGGCTAAATACCGGC
GCGAGTCCGATAGCGGACAAGTACCGCGAGGGAAAGTTGAAAAGAACTTTGAAGA
GAAGAGTTCAA

>2 *Tenellia sp./Cuthona sp.*
ATCGTTCGCGGATCTTGTCGCTATCGGACTCGCGCCGGTATTTAGCCTTAGGTGGAG
TTTACCACCCGCTTCGGGCTGCATTCCCAAACAACCCGACTCCGGGGACGGCAGCGG
CCGACCGGACGGACGCCGGCAGAGGCCTGGCACCCGCTATGGGAAGGGCCCCGCTC
GGGGGGACTCGAGCGTCCTTCCGGTCGACTGGCCGCGTCCCTCACGCCACAGTTCCG
GCCGGGCTAATAGCCCCGGCGGATTCGGCGCTGGGCTTCGTCCCGCTTCACTCGCCG
TTACTGGGGGAATCCCGGTTGGTTTCTTTTCCTCCGCTTAGTTATATGCTTAAATTCA
GCGGAT

>3 *Tenellia sp.*
CGTAAAGGACTACGGGATCGCCAGTACGGCGAGTGAAGCGGGACGAAGCCCAGCG
CCGAATCCGCCGCGGCTATTAGCCCGGCCGGAACTGTGGCGTGAGGGACGCGGCCA
GTCGACCGGAAGGACGCTCGAGTCCCCCCGAGCGGGGCCCTTCCCATAGCGGGTGC
CAGGCCTCTGCCGGCGTCCGTCCGGTCGGCCGCTGCCGTCCCCGGAGTCGGGTTGTT
TGGGAATGCAGCCCGAAGCGGGTGGTAAACTCCACCTAAGGCTAAATACCGGCGCG
AGTCCGATAGCGGACAAGTACCGCGAGGGAAAGTTGAAAAGAACTTTGAAGAGAG
AGTTCA

>4 *Fiona pinatta*
AGTTCGCGATCTGTCGCTATCGGACTCGCGCCGGTTTTAGCCTTAGGTGGAGTTTAC
CACCCGCTTCGGGCTGCATTCCCAAACAACCCGACTCCGGGGACGGCAGCGGCCGA
CCGGACGGACGCCGGCAGAGGCCTGGCACCCGCTATGGGAAGGGCCCCGCTCGGCG
GACTCGAGCGTCCTTCCGGTCGACTGGCCGCGTGCCTCACGCCACAGTTCCGGCCGG
GCTAATAGCCGCGGCGGATTCGGCGCTGGGCTTCGTCCCGCTTCACTCGCCGTTACT
GGGGGAATCCCGGATGGTTTCTTTTCCTCTGCTTAGATTAAATGCTTAAATTCAGCT

>5 *Aeolidia sp.*
CGGGAAAGGAACAACGGGGGATCCCCAGTAACGGCGAGTGAGCGGGAATAGCCCA
GCGCTGAATCCCCCTGGGCCTTGTAGGGGGAGCGGAAGCGTGCGGGTCGTTGTCTA
GCGGGATCGGGAGCGTCGCGCGAGTCTTCTCGAGCGTGGCTTGCCCAGAGCGGGTG
CCAGGCCCTTGCCGGCGATGCTCCCTTCCGCCGACGTCGACCCCGGAGTCGGGTTGC
TTGGCAGTGCAGCCCGAAGCGGGTGGTAAACTCCACCTAAGGCTAAGTACCTGCGC
GAGTCCGATAGCGAACAAGTACTGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAG
AGTTCAA

>6 *Aeolidia sp.*
ATCCTTCAGTACTTGTCGCTATCGGACTCGCGCAGGTACTTAGCCTTAGGTGGAGTTT
ACCACCCGCTTCGGGCTGCACTGCCAAGCAACCCGACTCCGGGGTCGACGTCGGCG
GAAGGGAGCATCGCCGGCAAGGGCCTGGCACCCGCTCTGGGCAAGCCACGCTCGAG
AAGACTCGCGCGACGCTCCCGATCCCGCTAGACAACGACCCGCACGCTACAGCTCC
CCGCGGACAAGGGCCCAGGGGGATTCAGCGCTGGGCTATTCCCGCTTCACTCGCCGT
TACTGGGGGAGTCCCCGTTGGTTTCTTTTCCTCCGCTTACTGAAATGCTTAAATTCAG
CGGGTA