

qPCR Detection of Early Life History Stage *Chrysaora quinquecirrha* (Sea Nettles) in Barnegat Bay, New Jersey

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ABSTRACT

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The sea nettle *Chrysaora quinquecirrha* has become abundant in the Barnegat Bay estuary and frequently blooms in warm summer months. Various factors have been attributed to the increasing localized appearance of sea nettles and other jellyfish including eutrophication, overfishing, global warming, construction, and species introduction. Despite its abundance and frequent distribution within estuarine systems, very little work has been done to detect and quantify the early life history stages of this organism. Free-swimming larval stages of *C. quinquecirrha* can be detected and quantified using a quantitative polymerase chain reaction assay specific for the *C. quinquecirrha* 16S ribosomal (r)DNA locus of the mitochondrial DNA. This assay is species specific, linear over a 9-log range, and can detect as few as 10 copies of 16S rDNA. Twenty-liter field samples were sequentially filtered through 500- and 100- μ m mesh to separate ephyra from planula larvae and gametes, respectively. Quantifiable levels of *C. quinquecirrha* 16S rDNA were detected at all eight paired locations in Barnegat Bay, with levels varying on both spatial and temporal scales. This research is apparently the first comprehensive field-based survey mapping, both spatially and temporally, the early life history stages of a scyphozoan in a major estuary using environmental DNA. Quantitative molecular data on the distribution of early stage *C. quinquecirrha* may prove useful in both understanding and managing blooms of sea nettles in Barnegat Bay.

ADDITIONAL INDEX WORDS: eDNA, ephyra, planula, 16S rDNA.

INTRODUCTION

The scyphozoan *Chrysaora quinquecirrha* (Atlantic Sea Nettle) is common to estuaries along the Mid-Atlantic coast (Shultz and Cargo, 1971). While the first documented observation of *C. quinquecirrha* in New Jersey coastal waters occurred in the early 20th century (Mayer, 1910), *C. quinquecirrha* were not reported in Barnegat Bay in subsequent surveys (Mountford, 1980; Nelson, 1925; Sandine, 1984). However, they have now become established with a large resident population (Crum *et al.*, 2014). Sea nettles are able to tolerate low dissolved oxygen and low salinity, allowing *C. quinquecirrha* to outcompete other resident species for food and nutrients (Purcell and Arai, 2001; Purcell *et al.*, 1999; Purcell, Uye, and Lo, 2007). Thus, in increasingly eutrophied conditions, sea nettles are well positioned to dominate the brackish waters (salinity range 19–25 ppt) in Barnegat Bay and other estuaries. Over the last decade, the frequency of *C. quinquecirrha* blooms has increased in the northern reaches of Barnegat Bay (Bologna, Gaynor, and Meredith, 2015) where urban development is higher (Lathrop and Bogner, 2001), resulting in higher nutrient inputs (Kennish and Fertig, 2012). However, the paucity of earlier research on *C. quinquecirrha* makes evaluating and understanding these blooms difficult. Many other marine and estuary systems have also experienced more

frequent and larger blooms of scyphozoans and other gelatinous zooplankton (Condon *et al.*, 2012; Hoover and Purcell, 2009). This makes it increasingly important to study and understand how these organisms are able to adapt and dominate in estuarine systems and the environmental factors contributing to the success of *C. quinquecirrha*.

In addition to outcompeting other organisms for food and nutrients, the *C. quinquecirrha* two-phase life history helps produce large numbers of individuals and creates permanent polyp colonies. Like many cnidarians, the *C. quinquecirrha* life history includes both a sessile and a pelagic stage. The free-swimming adults, or medusae, can range in size from a few millimeters to 25 cm in diameter (Calder, 1972). *Chrysaora quinquecirrha* medusae release their gametes into the water, where fertilization occurs, producing a free-swimming planula. Within 24 to 48 hours, planulae settle onto a hard substrate (Cargo and Schultz, 1966, 1967). Once affixed, the planula metamorphoses into the scyphistoma or polyp phase and extends tentacles into the water for feeding (Littleford, 1939). After sufficient acquisition of nutrients and proper environmental cues, the scyphistoma differentiates and reproduces asexually by producing a structure known as a strobila. These structures undergo a process known as strobilation (Littleford, 1939), which releases ephyrae into the water column. These ephyrae rely mostly on currents for their dispersal. Once an ephyra begins to develop tentacles, it is then referred to as a juvenile medusa (Calder, 1972). Once this juvenile matures, they release gametes and the cycle begins again (Calder, 1972).

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The sessile polyp of *C. quinquecirrha* is the most crucial factor in the establishment of resident populations (Condon, Decker, and Purcell, 2001; Hoover and Purcell, 2009). Polyps can also lay down podocysts, dormant forms of polyps, that can overwinter and survive until the next spring when water temperatures begin to rise (Purcell *et al.*, 1999).

The ability of *C. quinquecirrha* to reproduce both sexually and asexually has the potential to affect populations at different life history stages. While recruitment of sexually produced larvae generate expansion and colonization of polyps within regions, the asexual propagation of polyps, podocysts, and ephyrae ultimately control both the current population of adult medusae as well as future generations (Lucas, Graham, and Widmer, 2012). When blooms do occur, large conspicuous adults are observed, but rarely are the strobilated ephyrae that precede the bloom detected. Ephyrae are small and mostly translucent, making observation difficult (Cargo and Schultz, 1966), but detection of ephyrae may identify source polyp populations and environmental conditions that lead to the establishment of blooms (Decker *et al.*, 2007). Planulae are substantially smaller than ephyrae and likewise harder to observe. However, identifying their spatial and temporal presence within systems provides details about their distribution and can identify recruitment potential and expansion into new regions (Cargo and Rabenold, 1980). Consequently, detecting these early life history stages of *C. quinquecirrha* is important to understanding how and why this organism became so dominant in Barnegat Bay and other coastal estuaries and may also serve as a way to help monitor the health of the bay.

Because early life history forms of *C. quinquecirrha* are so small, assessing population size and distribution within traditional sampling methods is difficult and time consuming. However, with the advancements of molecular biology, it is possible to assess the temporal and spatial variability of *C. quinquecirrha* ephyrae and planulae through the detection of environment DNA (eDNA) (Thomsen *et al.*, 2012b). eDNA extracted from water can be used to detect the presence of *C. quinquecirrha*, and extraction with quantitative polymerase chain reaction (qPCR) has been shown to assess population abundance accurately in marine systems (Kelly *et al.*, 2014; Thomsen *et al.*, 2012a,b).

eDNA has been shown to be an invaluable asset to understanding biodiversity in both freshwater and wetland systems, because it allows for the accurate detection of rare or underrepresented organisms found in traditional sampling methods (Ficetola *et al.*, 2008). It can also be used to detect and identify early life history stages where morphological differentiation is indistinct (*e.g.*, eggs and larvae). eDNA monitoring is not only used to quantify populations of known organisms, it can also serve as a method for the early detection of invasive and potentially disruptive organisms in new systems (Jerde *et al.*, 2011). eDNA extracted from the water column has been shown to detect not only small organisms, but also large marine mammals, detected through sloughed off tissue that remains in the water column (Foote *et al.*, 2012). Detecting whole organisms or tissues of larger organisms within sizable volumes of either fresh water or salt water requires an

extremely sensitive assay and gene targets that allow for detection (Lodge *et al.*, 2012).

The mitochondrial 16S ribosomal (r)DNA marker is commonly used, along with other mitochondrial (mt)DNA markers, to identify an organism, because the locus is polyploid and present in many copies within a single cell (Ryu *et al.*, 2012; Thomsen *et al.*, 2012b). Not only does each cell have multiple copies of the 16S locus in each mitochondrion, but each cell also contains multiple mitochondria, potentially allowing us to detect 16S copy number at the single cell level. This low limit of detection is vital, because eDNA in large bodies of water can be difficult to detect (Thomsen *et al.*, 2012a). Therefore, by targeting the 16S rDNA locus, the success rate of the qPCR assay is much higher than if targeting nuclear DNA. We have sequenced this locus from hundreds of individuals of *C. quinquecirrha* harvested from Barnegat Bay over the past 6 years (Gaynor and Tare, unpublished data). This locus is highly conserved in many regions but shows sufficient divergence to permit the development of unique primer pairs for PCR and qPCR.

Using filtered water samples and a species-specific qPCR assay, this study aimed to examine the temporal and spatial variability of early life history stages (ephyrae and planula larvae) of *C. quinquecirrha* sampled biweekly from 16 sites throughout Barnegat Bay. On the basis of recent jellyfish bloom data from Barnegat Bay, it is hypothesized that ephyra eDNA will peak in late June, a month before an anticipated spike in the adult population, whereas planula eDNA levels should start to rise in early to mid-August, just weeks after the adult population peaks (Hoover and Purcell, 2009; Purcell *et al.*, 1999). This study describes (1) a species-specific qPCR assay developed and optimized to detect *C. quinquecirrha* eDNA from Barnegat Bay water samples and (2) how this assay is employed to assess the temporal and spatial variability of ephyrae and planula larvae in Barnegat Bay, New Jersey, by qPCR of 16S rDNA extracted from eDNA.

METHODS

Field Collections

Water samples were collected at 16 locations (eight paired east-west sites) in Barnegat Bay, ranging from the Metedeconk River in the north to Tuckerton Bay in the south (Figure 1). Each site was sampled in triplicate during eight collection periods, ranging from late May 2012 through mid-September 2012. At each site, three 20-L samples of bay water were collected and poured through two nested filtering units constructed of polyvinyl chloride (Restaino, 2013). Coupled to the upper unit was a 500- μ m mesh filter and to the lower a 100- μ m mesh filter (Industrial Netting Inc., Minneapolis, Minnesota). Water poured through the top filter (500 μ m) was immediately and sequentially filtered through the bottom filter (100 μ m). The filter device was rinsed at each site before assembly of fresh filters by submerging the disassembled unit into the bay. This occurred at each location to ensure no carryover of material from previous collecting sites. Once a 20-L sample was filtered, the nylon membranes were removed and the filter was inserted into a labeled, sterile 50-mL tube (BioExpress, Kaysville, Utah) and kept on ice until transported back to the laboratory. Each tube was assigned a unique code, and all samples were stored at -80°C until DNA extraction.

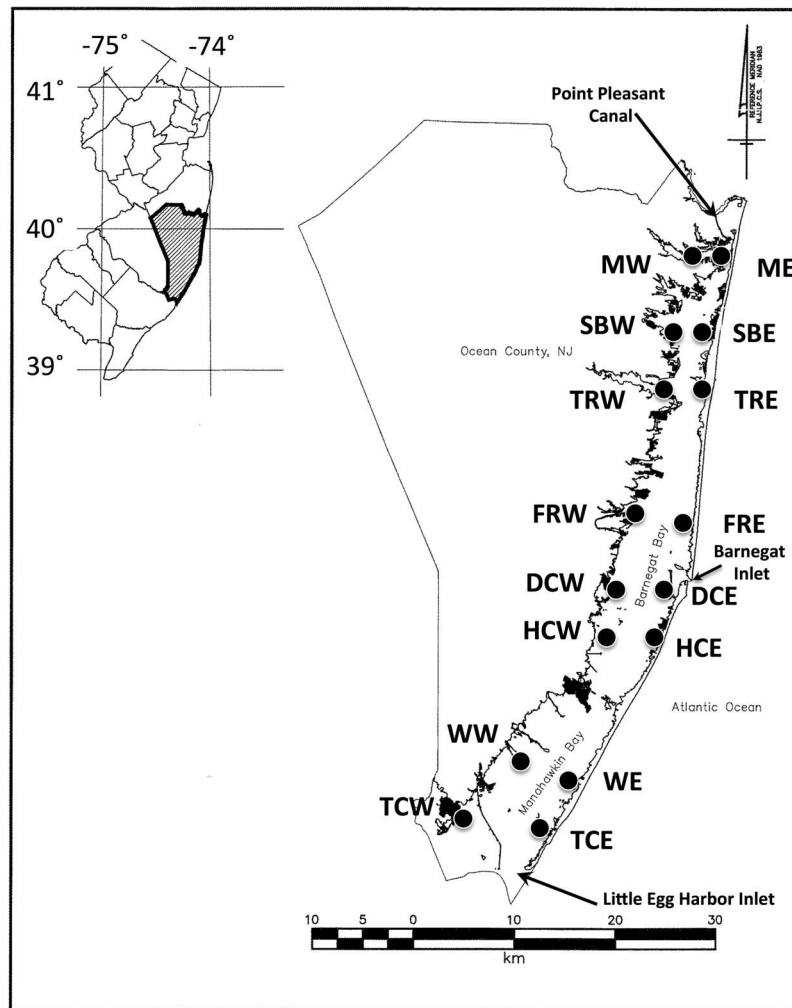


Figure 1. Map of the eight paired sampling sites in Barnegat Bay, New Jersey. Each star denotes a sampling location: Metedeconk East and West, Silver Bay East and West, Toms River East and West, Forked River East and West, Double Creek East and West, Harvey Cedars East and West, Westeconk Creek East and West, and Tuckerton East and West.

A field blank was also collected at the end of each sampling day using the same protocol, but using laboratory deionized (DI) water to assess contamination of the filtering units. Filtering units were rinsed with DI water and then assembled in the same fashion as at the sampling locations. Twenty liters of DI water were poured through the nested units. Once a 20-L sample was filtered, the nylon membranes were removed, and the filter was inserted into a sterile 50-mL tube and kept on ice in a cooler until returned to the lab.

DNA Extraction

Samples were thawed in the original 50-mL tubes and allowed to reach room temperature quickly. Once thawed, nylon mesh was pressed into the bottom of the tube, and 10 mL of sterile 5% (w/v) Chelex[®] 100-mesh size 150–300 μ m (Bio-Rad Laboratories, Hercules, California) in 50 mM Tris base (pH 11) was added to each tube (Walsh, Metzger, and Higuchi, 1991). Tubes were vortexed for 10 seconds on high to ensure the filters

were covered in the Chelex solution. Each tube was boiled for 30 minutes and then cooled on ice. Tubes were vortexed again for 10 seconds, and 1000 μ L of the Chelex solution was transferred to a sterile 1.5-mL microcentrifuge tube. Tubes were then centrifuged for 5 minutes at $16,000 \times g$ (Eppendorf 5415C microcentrifuge) at room temperature, and the supernatant was removed and transferred to a second sterile 1.5-mL microcentrifuge tube. Labeled tubes were stored by collection and kept at -80°C until qPCR analysis. From each sample, 100- μ L aliquots were removed and added to a sterile 1.5-mL microcentrifuge tube. These aliquots were used as working samples, as not to degrade the original samples with repeated freeze/thaw cycles.

qPCR of 16S rDNA

The 16S rDNA locus was used for the development of a qPCR assay used in this study. Primers were designed to be specific for *C. quinquecirrha* (see “Primer Development” and “Primer

Optimization” sections of “Results”), and the primer set was optimized according to the manufacturer’s protocol (Applied Biosystems Inc., Foster City, California). qPCR amplifications were performed in 25- μ L volumes, in 100- μ L thin-walled Fast Optical PCR tubes (part nos. 4358293 and 4323032; Life Technologies Corporation, Grand Island, New York) or in 96-well plates (part nos. 4366932 and 4311971) in an ABI StepOne Plus Real-Time PCR System. The reaction was optimized by running 10-fM (femtomolar) 16S rDNA samples with varied annealing temperatures: 54°C, 56°C, 58°C, 60°C, 62°C, and 64°C.

We used a cloned fragment of the 16S rDNA gene from *C. quinquecirrha* for the determination of the standard curve. A 208-bp fragment of the 16S rDNA gene (GenBank GU300724; NCBI, 2010) was produced by standard PCR amplification, and the fragment was gel-purified from a 1.5% (w/v) agarose gel (Sambrook and Russell, 2001). Linear ends were repaired, *Eco*RI linkers were ligated, and the modified fragment was cloned into the *Eco*RI site of the pUC19 plasmid using standard molecular biology methodologies (Sambrook and Russell, 2001). This plasmid was transformed into *Escherichia coli* strain DH5- α . Purified plasmid (isolated by density banding on CsCl gradients) carrying this insert has been restricted with *Eco*RI, and the cloned insert was isolated by gel purification and quantified by ultraviolet spectroscopy at 260 nm (Sambrook and Russell, 2001) using a NanoDrop ND-1000. This purified 16S fragment was diluted in sterile deionized water to a concentration of 1000 copies of the target gene per microliter and stored in 100- μ L aliquots at -80°C. This is the source of DNA used for all limits of detection (LOD) assays.

Using this purified 16S rDNA from *C. quinquecirrha*, a set of standards was generated to be linear across a 9-log scale: 10^9 , 10^8 , 10^7 , 10^6 , 10^5 , 10^4 , 10^3 , 10^2 , and 10^1 . Each standard (copy number of the gene) was run in triplicate on each reaction plate, along with a no template control (NTC), also run in triplicate. To verify that the standards were all functional and that primers do not bind to and amplify other species common to Barnegat Bay, a standard PCR assay was conducted, followed by visualization of amplicons on 1% (w/v) agarose gel electrophoresis (data not shown).

qPCR reactions (total reaction volume of 25.0 μ L) were prepared using 23.0 μ L of Power SYBR[®] Green 2 $\times$ Master Mix, P/N: 4367659 (Abi Warrington, Warrington, U.K.), to 2.0 μ L of eDNA extracted from filters. Each sample was run in triplicate on each plate along with the standard curve and a NTC, also run in triplicate. After each plate was loaded, it was sealed with MicroAmp[®] Optical Adhesive Film (Life Technologies, Grand Island, New York) and MicroAmp Adhesive Film Applicator (Life Technologies). The film was applied according to manufacturer protocol, and the applicator was used to score the film between rows to ensure proper sealing. Each sample was run at 94°C for 10 minutes to activate the *Taq* DNA polymerase and initially denature the DNA (1 $\times$), then 40 cycles of 95°C for 1 minute and 60°C for 1 minute. After each qPCR run, a melt curve was generated to ensure that only *C. quinquecirrha* 16S rDNA was being amplified. After qPCR quantization, standard deviations and averages were calculated for each replicate group. This qPCR assay conforms to the

MIQE Guidelines as delineated by Bustin *et al.* (2009) for amplification from genomic DNA.

DNA Sequencing of PCR Products

To ensure that the assay was targeting and amplifying the 16S rDNA locus of *C. quinquecirrha*, qPCR amplified fragments were subjected to automated Sanger dideoxy sequencing. One microliter of a PCR reaction was sequenced using either the forward (CQF) or reverse (CQR1) primer with the BigDye Terminator Ready Reaction Kit (Version 3.1) from Applied Biosystems. Reactions were performed according to manufacturer’s instructions (Applied Biosystems Ready Reaction Kit, 4337035 Rev. B) with the exception that Ready Reaction Mix was diluted to 1/16 of recommended volume, precipitated in ethanol, dried briefly in a Speed-Vac Concentrator, and resuspended in 20 μ L of Hi-Di formamide before loading onto the sequencer. Samples were heated to 90°C for 3 minutes and quickly cooled on ice before loading. DNA sequencing was performed on an ABI Prism 3130 Genetic Analyzer using a 36-cm capillary column and POP-7 polymer. Bases were called using KB Basecaller software.

Calculations

The 16S rDNA copy number present on each filter was calculated by averaging each sample replicate; standard deviations (SDs) were also calculated for each sample using the ABI StepOne Plus software. The total 16S rDNA copy number at each site was determined by averaging the mean 16S copy number of each sample previously run in triplicate. Total 16S copy number was calculated along with standard deviations in Microsoft[®] Excel.

RESULTS

Primer Development

The *C. quinquecirrha* primers used in this study were modified from *C. quinquecirrha* species-specific primers described by Bayha and Graham (2009). These primers targeted the 16S rDNA locus of the mitochondrial genome. When used together, they generate a 208-bp DNA fragment. The sequence of these primers is as follows: CQF (forward), 5'-TGTCACCTAATTAGTGAATGGT-3'; CQR (reverse), 5'-CCCCAACCAAAGTGTCTTACT-3'.

The new primer set reflects a base shift of two nucleotides downstream in the reverse primer sequence that conferred binding at the 3' end of the primer to a nucleotide that is not conserved between the three jellyfish common to Barnegat Bay. The modified reverse primer (CQR1, 5'-GCCCCAACCAAAGTGTCTTA-3') still targets the same 16S rDNA locus as the Bayha primer but has increased specificity to the study organism. This modification to the reverse primer ensures that the 16S rDNA gene of *Cyanea capillata* and *Aurelia aurita* will not be able to be amplified.

Primer Optimization

Because qPCR assays employing SYBR Green intercalating dye are very sensitive to primer artifacts and the synthesis of any unwanted double-stranded DNA fragments generated during the amplification cycle, it is highly recommended that primer pairs be optimized by examining a range of primer concentrations in combination (Gunson, Gillespie, and Car-

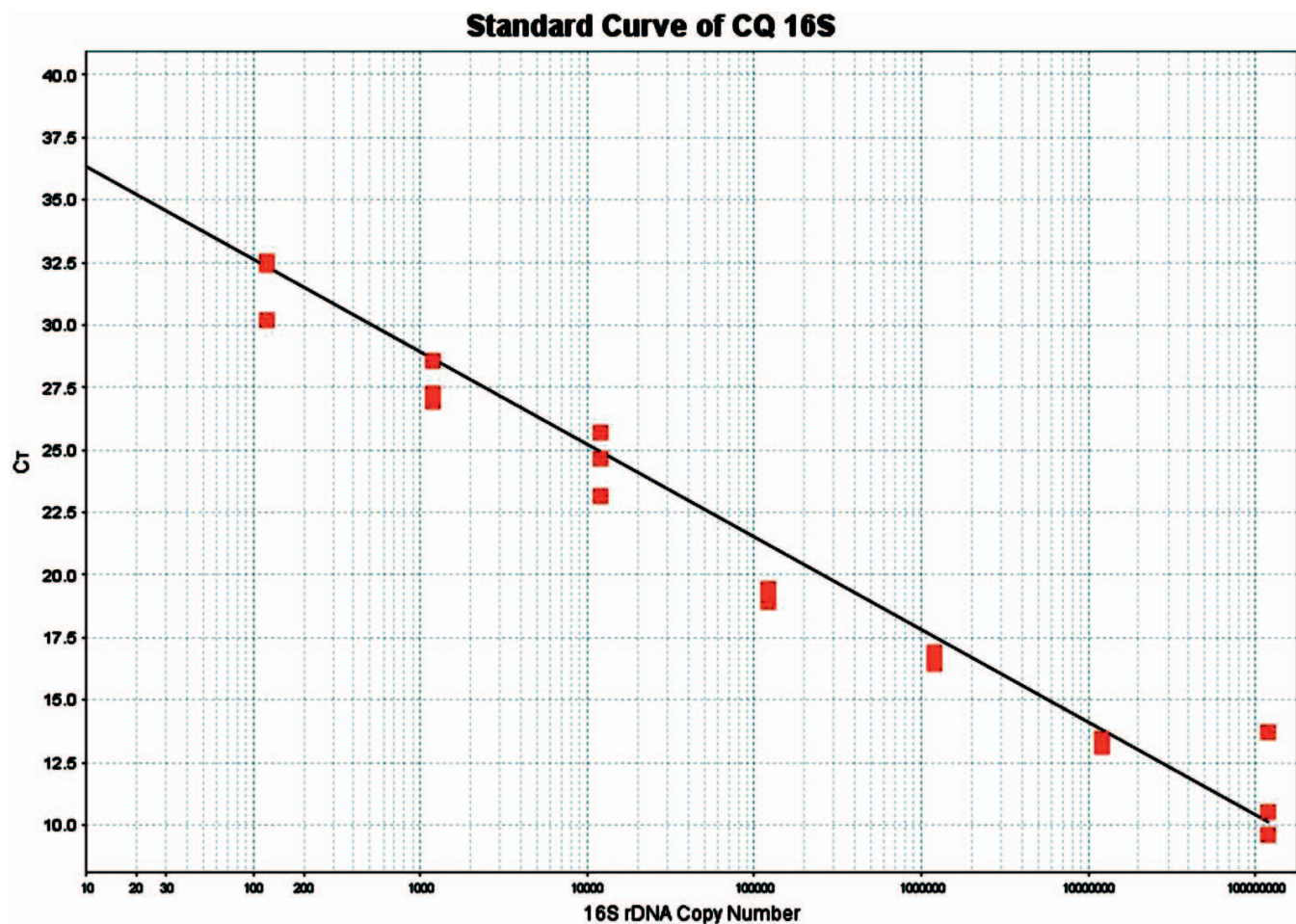


Figure 2. Standard curve generated using known quantities of *C. quinquecirrha* 16S rDNA ranging from 12 billion copies to 120 copies of this gene. The curve shows that the reaction is linear over the 9-log range and is highly reproducible ($r^2 = 0.982$).

men, 2003). Optimal amplification was determined by primer chessboarding (Applied Biosystems, 2011, 23; Gunson, Gillespie, and Carmen, 2003) with qPCR product amplification determined on the ABI StepOne Plus qPCR thermal cycler. Reaction optimization was obtained by increasing the concentration of the reverse primer to three times that of the forward primer: 900 nM *vs.* 300 nM, respectively (Restaino, 2013).

The qPCR reaction itself was optimized to determine the best annealing temperature for this assay. Amplification of qPCR products with varied annealing temperatures was confirmed using the ABI StepOne Plus qPCR machine. A temperature of 60°C was determined to be optimal for annealing, permitting a two-step amplification protocol (denaturation at 95°C and annealing and extension at 60°C) and was used for the duration of this study.

Standard Curve and Melt Curve Analysis

To determine the linearity of this assay, a standard curve was generated using known quantities of *C. quinquecirrha* 16S rDNA copy number, in triplicate, from a range of 1.0×10^{10} to

1.0×10^1 in 10-fold increments in samples. This assay was confirmed to be linear over a 9-log scale (Figure 2), with excellent reproducibility and a single-peak melting curve $T_M = 74.5^\circ\text{C}$ (Figure 3). Both the standard curve and the melting curve were analyzed using the ABI StepOne Plus. Every subsequent qPCR amplification included both an identical standard curve and melt curve analysis on every plate run in this project. This permitted an absolute determination of 16S rDNA quantitation on all samples processed.

eDNA Samples

The amount of both temporal and spatial variability of 16S eDNA seen on both the 500- μm and 100- μm mesh filters was large (Figures 4 and 5). eDNA putatively corresponding to ephyrae and planula larvae was detected throughout the bay; however, the largest quantities of 16S copy number were detected to the north of Barnegat Inlet. The western side of the bay also tended to have higher concentrations of 16S rDNA than did the corresponding sites on the eastern side of the bay.

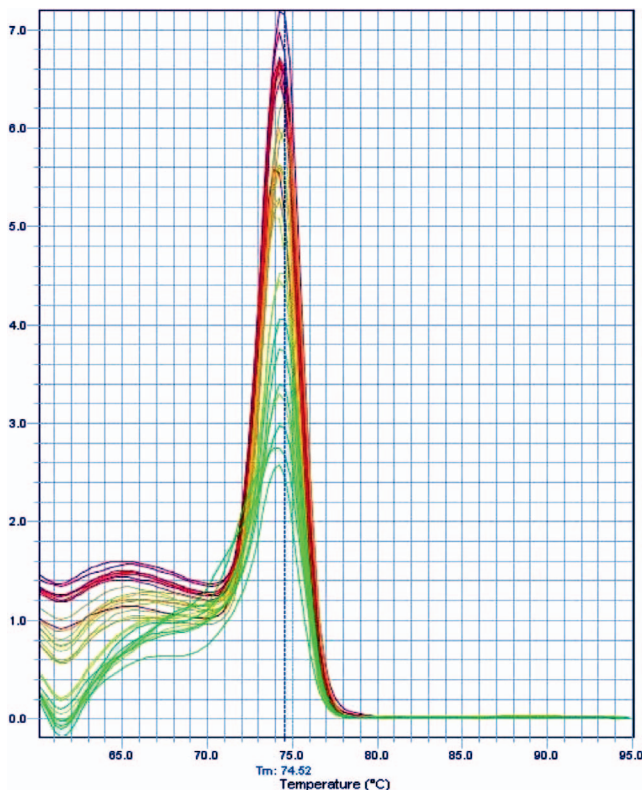


Figure 3. Melting curve generated from nine standards used in the *C. quinquecirrha*-specific qPCR reaction. The single peak, $T_m = 74.52^\circ\text{C}$, identifies that only a single molecular species has been amplified in the qPCR assay.

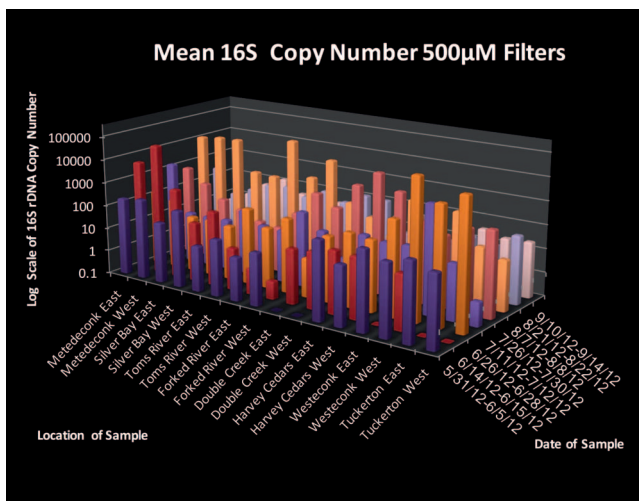


Figure 4. Total 16S rDNA on 500- μm mesh filters. Note: Double Creek East and West from 31 May 2012 to 5 June 2012 are not zero values but had no data collected; Tuckerton East and West from 14 June 2012 to 15 June 2012 are not zero values, but the qPCR reaction failed for these samples.

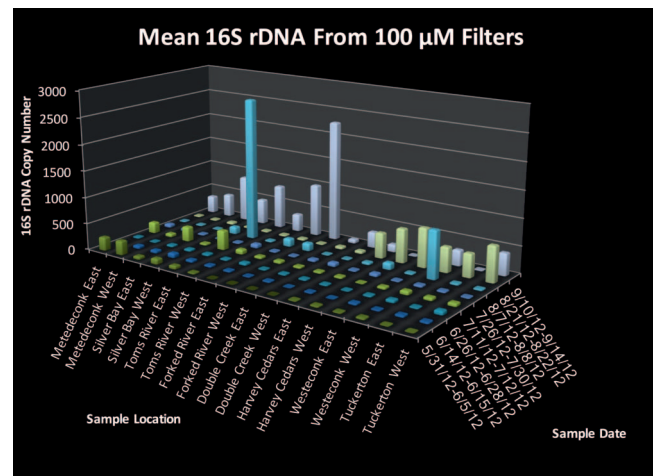


Figure 5. Total 16S rDNA on 100 μm mesh filters. Note: Double Creek East and West from 31 May 2012 to 5 June 2012 are not zero values, but had no data collected; Forked River East and West from 31 May 2012 to 5 June 2012 are not zero values, but the qPCR reaction failed for these samples.

Ephyrae Detection: 500- μm Filters

The highest densities of ephyrae, based on the 16S copy number on the 500- μm filters, were seen in late July and early August 2012 (Figure 4). The highest seasonal abundances occurred in the northern part of Barnegat Bay, in particular Metedeconk East and Metedeconk West. These two sites appear to have more than one peak in ephyrae, suggesting multiple synchronized strobilation events. The highest single level of 16S amplification on a 500- μm filter from an individual sample occurred in the southern portion of the Bay, Tuckerton West, with a quantity of 2.8×10^4 copies present in a 2- μL sample from mid-September 2012, but the site average on that date was considerably smaller (Figure 4). The overall levels of free 16S rDNA in the water column reflected a pulsing pattern, where an increase in ephyrae one week is usually followed by lower densities for about a month, at which point levels increase once again (Figure 4). As the season progressed, the overall levels of 16S eDNA detected on the 500- μm filters increased until late August, where they appear to resemble levels similar to the beginning of the season.

Planula Detection: 100- μm Filters

16S rDNA levels on the 100- μm filters varied spatially and temporally; however, the pattern was different from that of the 500- μm filters (Figure 5). The majority of the sampling sites in the bay had relatively uniform levels of planula larva detection based on the 16S copy number seen on the 100- μm filters (the filters could also be detecting *C. quinquecirrha* eggs, fertilized or unfertilized), with average copy numbers in the 400–600 range. Additionally, high levels of 16S were not detected until the end of the summer beginning in August, with all sites demonstrating some larval presence (Figure 5).

In the northern portion of the bay, the trend in peak 16S rDNA levels on the 100- μm filters suggests that the eastern sites may have a greater concentration of planula larvae than do the western sites (Figure 5). In the central and southern

Bay, the trend was the opposite, with western sites having a greater quantity of 16S rDNA on the 100- μ m filters. The three southernmost locations in the bay, Harvey Cedars, Westcunk Creek, and Tuckerton, experienced the greatest density of 16S rDNA in late August, as opposed to mid-September (Figure 5). These sites did, however, exhibit an increase in total 16S rDNA copy during mid-September, but not as high as the peak in late August. The presence of eDNA in these southern regions of the bay suggests that reproductive output from adults in the northern portion of the bay may be transported into the southern regions of Barnegat Bay. What is unknown is whether these larvae are successfully settling and metamorphosing into polyps.

DISCUSSION

Monitoring the health of marine and estuarine systems is essential, because these habitats provide important ecosystem services, yet remain under constant stress from factors such as eutrophication, ocean acidification, coastline modification, and overfishing. *Chrysaora quinquecirrha* and other gelatinous zooplankton are highly tolerant of low dissolved oxygen (Breitburg *et al.*, 1997), and increasing populations are strongly associated with degraded water quality (Kolesar *et al.*, 2010). Consequently, frequent or persistent blooms of adults may be an indicator of coastal water health (Link, 2005). However, the early life history stages of various scyphozoans are rarely quantified because traditional sampling methods are often unsuccessful (Lodge *et al.*, 2012). This study successfully demonstrates that this molecular methodology can detect early life history stages of *C. quinquecirrha*, making it possible to assess source polyp populations and recruitment locations throughout Barnegat Bay.

The use of modified species-specific primers targeting the 16S rDNA locus of *C. quinquecirrha* mtDNA was shown to amplify eDNA successfully with high efficiency and reproducibly. With both primers terminating on a nucleotide unique to *C. quinquecirrha*, the likelihood of nonspecific binding is greatly reduced (Dieffenbach, Lowe, and Dveksler, 1993). Both standard curves and melt curve data of amplified 16S rDNA gathered with each qPCR run confirm that the amplification occurring in the qPCR reaction is only that of *C. quinquecirrha*. These data were also supported by gel electrophoresis showing that the primer set CQF/CQR1 did not amplify other cnidarians common in Barnegat Bay.

The larger quantities of 16S eDNA seen in the northern part of Barnegat Bay were expected, because this area is known to have broadly dispersed, large populations of *C. quinquecirrha* (Bologna *et al.*, 2017). However, the oscillatory pattern associated with strobilation of ephyrae demonstrates that multiple events occurred during the summer. The results demonstrate three distinct strobilation events that occurred in late May, late July, and late August (Figure 4). Although many triggers for mass strobilation of scyphozoans are known (Condon *et al.* 2001; Prieto *et al.* 2010; Purcell, Hoover, and Schwarck, 2009; Purcell *et al.*, 1999), the data cannot identify any single water quality characteristic that would explain the pattern observed. However, and perhaps more importantly, the presence of ephyrae throughout the season (Figure 4) indicate continuous production of individuals and may indicate a

complex polyp strategy integrating food availability and water quality. On the other hand, the minimal detection of planula larvae for the majority of the summer reflects the distribution of sexually mature adults in the bay. For the summer of 2012, adult *C. quinquecirrha* populations were highest in early August (Bologna *et al.*, 2017), which coincides with the previous peak of ephyrae in mid-late July (Figure 4). This then corresponds to the detection of planulae in late August to mid-September (Figure 5) and confirms the life cycle complexity of this species.

The detection of 16S rDNA from *C. quinquecirrha* in the southern portion of Barnegat Bay was an important, albeit somewhat surprising, finding (Figures 4 and 5). Adult sea nettles are rare south of Barnegat Inlet but are an annual summer resident in the northern part of the bay, where poor tidal flushing restricts the turnover of water. The occurrence of early life history stage *C. quinquecirrha* south of Barnegat Inlet may be reflective one or a combination of several factors: (1) polyp colonies, although previously undetected (Bologna, 2011), may exist in the southern Bay, but tidal flushing or other influences may help to remove these individuals from the system; (2) recently observed adult populations within lagoonal communities in the southern bay may serve as a source of early life history stage *C. quinquecirrha*, which are now migrating into the bay; (3) *C. quinquecirrha* is establishing populations in the southern portion of the bay, but population density may not be at a level in which large adults are seen with high frequency. The effect that these early life history stage sea nettles will have on the southern Bay is unknown. Their presence may have previously been undetected because they are difficult to observe or sample. On the other hand, these founding populations may be a sentinel to a shift in the populations of gelatinous zooplankton present in the bay.

A lack of long-term data regarding jellyfish blooms makes understanding the large, and seemingly increasing, populations of jellyfish difficult to assess (Condon *et al.*, 2012). Underwater cameras, acoustic monitoring, and blue-water diving are all methods that can be used to detect the presence and abundance of jellyfish (Condon *et al.*, 2012). This study presents a sensitive, real-time qPCR method that can successfully and accurately determine the presence and relative abundance of *C. quinquecirrha* eDNA in water samples from Barnegat Bay. qPCR assays conducted in marine or brackish environments are rare and generally thought to be less reliable because of a larger biomass-to-water ratio and hydrodynamic influences, but work by Thomsen *et al.* (2012a) has demonstrated otherwise, and the results of this study concur. In addition, a few other studies have been successful at extracting marine mammal DNA from salt water (Foote *et al.*, 2012) and assessing marine ichthyofauna (Kelly *et al.*, 2014; Thomsen *et al.*, 2012b). The success of the methodology used in this study holds great potential to affect the way marine systems are surveyed and studied, because samples can be collected with relative ease, and the diversity of knowledge gained through these simple water samples is extensive. This technique may also help to determine whether, in fact, gelatinous zooplankton populations are rising, by acting as a survey technique to systems vulnerable to invasion and colonization by *C. quinquecirrha* or other species. This assay can also be used to detect

polyp settlement on hard substrates (J.J. Gaynor and G. Shchegolev, unpublished data) and determine where this species is overwintering, which will likely be important in managing *C. quinquecirrha* populations. It should be noted that these determinations give a total, raw 16S copy number in filtered samples. To translate the 16S copy number into the actual number of individual ephyrae or planulae must await future studies that quantify the number of 16S copies relative to cell number in each developmental stage, before these data can be translated into predictive models of settlement or blooms of adults.

CONCLUSIONS

The qPCR assay developed and optimized in the study is species specific for the 16S rDNA region of the mtDNA of *C. quinquecirrha*. It is linear over a 9-log range and generates efficient and reproducible results. This study also demonstrated that eDNA can be successfully extracted, preserved, and amplified when taken from brackish water to generate usable community data indicators. The conclusion that previously undetected early life history stage *C. quinquecirrha* exist in the southern part of Barnegat Bay is important to understanding how gelatinous zooplankton move and are transported within a system, as well as how they may invade new systems. It is important to note this study is apparently the first comprehensive field-based survey mapping effort, both spatially and temporally, that details the early life history stages of a scyphozoan in a brackish estuary. Quantitative molecular data on the distribution of these free-swimming early stage *C. quinquecirrha* may prove useful in both understanding and managing blooms of sea nettle medusae in Barnegat Bay. By applying molecular techniques to assess ecosystem health, hard-to-sample populations likely will be better understood, resulting in improved efforts to preserve important ecosystems.

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