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Diet assessment of the Atlantic Sea Nettle *Chrysaora quinquecirrha* in Barnegat Bay, New Jersey, using next-generation sequencing

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Abstract

Next-generation sequencing (NGS) methodologies have proven useful in deciphering the food items of generalist predators, but have yet to be applied to gelatinous animal gut and tentacle content. NGS can potentially supplement traditional methods of visual identification. *Chrysaora quinquecirrha* (Atlantic sea nettle) has progressively become more abundant in Mid-Atlantic United States' estuaries including Barnegat Bay (New Jersey), potentially having detrimental effects on both marine organisms and human enterprises. Full characterization of this predator's diet is essential for a comprehensive understanding of its impact on the food web and its management. Here, we tested the efficacy of NGS for prey item determination in the Atlantic sea nettle. We implemented a NGS 'shotgun' approach to randomly sequence DNA fragments isolated from gut lavages and gastric pouch/tentacle picks of eight and 84 sea nettles, respectively. These results were verified by visual identification and co-occurring plankton tows. Over 550 000 contigs were assembled from ~110 million paired-end reads. Of these, 100 contigs were confidently assigned to 23 different taxa, including soft-bodied organisms previously undocumented as prey species, including copepods, fish, ctenophores, anemones, amphipods, barnacles, shrimp, polychaete worms, flukes, flatworms, echinoderms, gastropods, bivalves and hemichordates. Our results not only indicate that a 'shotgun' NGS approach can supplement visual identification methods, but targeted enrichment of a specific amplicon/gene is not a prerequisite for identifying Atlantic sea nettle prey items.

Keywords: *Chrysaora quinquecirrha*, gut content, jellyfish diet, next-generation sequencing

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Introduction

Jellyfish blooms in recent years have become conspicuous components of worldwide marine ecosystems, particularly during productive summer months, often to the detriment of both marine organisms and human enterprises (Mills 2001; Brodeur *et al.* 2002, 2008; Purcell 2012). Jellyfish are opportunistic, voracious predators of zooplankton and ichthyoplankton (fish larvae and eggs) (Purcell 1997; Brodeur *et al.* 2008) and have the potential to alter planktonic food webs. As a consequence, recent decades have seen a dramatic increase in research dedicated to determining their effect on marine ecosystems as related to commercially important species (e.g. fish). The apparent proliferation of massive jellyfish blooms along with range extensions has been associated with anthropogenic stresses such as overfishing, eutrophication, climate change, translocation and habitat modification (Richardson *et al.* 2009; Purcell 2012; Condon *et al.* 2013). However, the current data are inconclusive in regard to whether jellyfish are actually globally increasing (Gibbons & Richardson 2013). For example, both Brotz *et al.* (2012) and Duarte *et al.* (2013) suggest near-global increasing trends, while Condon *et al.* (2012) found no evidence. Most recently, Condon *et al.* (2013) suggested jellyfish populations appear to follow decadal oscillations (~20 years) with no

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significant increase over the last 140 years. However, Condon *et al.* (2013) also suggest a significant, but weak increase since the 1970s. Clearly, there is no consensus and much of the confusion centres around the lack of long-term data sets (Condon *et al.* 2012; Gibbons & Richardson 2013).

Trophic relationships of most gelatinous animals (animals belonging to Cnidaria or Ctenophora) are poorly known (Purcell *et al.* 2007; Purcell 2012). As a consequence, it is unclear as to what role most jellyfish play in benthic and pelagic food webs (Condon *et al.* 2012). Additionally, most gelatinous species are assumed to be pelagic generalist/opportunists. Full characterizations of gelatinous animal diets are essential for a comprehensive understanding of their impacts on the food web and their management (Purcell 1997, 2009; Pauly *et al.* 2009).

The Atlantic Sea nettle (*Chrysaora quinquecirrha*) is naturally distributed along the coast of the western Atlantic Ocean (Morandini & Marques 2010). In recent years, sea nettles have become progressively more abundant in Mid-Atlantic State estuaries, suggesting these coastal ecosystems are possibly experiencing fundamental shifts in planktonic trophic web structure (Purcell *et al.* 2007). For example, the warming waters of the Chesapeake Bay (USA) have resulted in a *C. quinquecirrha* population 'explosion' that has had devastating ecological and economic effects (Cargo & King 1990; Delano 2006; Purcell *et al.* 2007). Further to the north, the shallow estuary Barnegat Bay (Ocean County, New Jersey, USA) is likewise experiencing a population 'explosion' of *C. quinquecirrha*. Barnegat Bay is a highly eutrophic brackish water system with nutrients arriving in the bay via run-off and atmospheric inputs (Kennish *et al.* 2007). Prior to the mid-1990s, *C. quinquecirrha* was virtually unknown from the bay, but this sea nettle now appears to be a permanent resident (Crum *et al.* 2014).

Gelatinous prey identification has historically involved either direct observation or the collection of adults, the excision and preservation of the gastric pouches and tentacles and then visual inspection of contents under a microscope in the laboratory. Molecular techniques have recently been devised and utilize Sanger sequencing or next-generation sequencing (NGS). DNA can be extracted from the gut contents of the predator and then sequenced. The DNA sequences are then compared to reference DNA databases for prey item identification. NGS methodologies are powerful tools for deciphering the food items of generalist predators at a scale and accuracy previously unimaginable (reviewed in Pompanon *et al.* 2012) NGS methodologies are capable of producing sequence data several orders of magnitude greater than ever obtainable using more traditional Sanger sequencing. As a consequence, a more efficient and thorough investigation into potential prey items is possible.

Molecular techniques are potentially far more appealing for prey item identification than standard visual identification methods (Pompanon *et al.* 2012). For example, larval forms of many marine species remain undescribed or are not easily identifiable to the species level. Prey DNA sequence data can be readily identified to the species level when searched against reference databases (e.g. National Center for Biotechnology Information) if present in the database, while visual identification often requires an expert in morphology and years of training. Additionally, visual identifications are often of low resolution (e.g. family level or above), time-consuming and, more significantly, require prey items to be intact and/or in the very early stages of digestion prior to the destruction of physical characteristics.

Visual identification can prove to be problematic when predators have potentially high digestive clearance rates, as is the case with *C. quinquecirrha* (3.5 ± 1.1 h for copepods; Purcell 1992) or prey does not possess structural components (e.g. ctenophores). NGS methods should be able to detect prey items well after visual identification becomes impossible and thousands of sequences can be identified within a day. Here, we test the efficacy of utilizing NGS technologies in the identification of *C. quinquecirrha* prey items in Barnegat Bay to start to unravel predator–prey interactions. We successfully demonstrate that a 'shotgun' approach to sequencing gelatinous prey items found in the gut and/or on tentacles can supplement visual identification methods and is potentially equally as effective as other NGS methods that use targeted enrichment of a specific gene (e.g. *COI*).

Methods

Gastric lavage

We sampled eight adult jellyfish from three Barnegat Bay localities (two from Forked River West, three from Toms River West, and three from Silver Bay East) using nets, ladles and/or buckets on 30 July 2013 (Appendix S1, Supporting information). Depths ranged from the surface to 1.5 m on average. In an attempt to remove as much bycatch as possible, specimens were rinsed three times with sterile artificial seawater at a salinity of 19 parts per thousand (Crystal Sea Marinemix, Marine Enterprises International, LLC) filtered through 0.45-μm filters. Specimens were then placed upside down on clean dissecting trays and bell diameter measured. Approximately 3 mL of sterile seawater was pipetted into the oral cavity to wash out the gastric

pouches. Contents were immediately sucked back up and placed into sterile 15-mL tubes with 100% ethanol to yield a 70% (v/v) ethanol. This procedure was repeated up to three times per jellyfish with all samples from the same jellyfish pooled. Sample tubes in the field were placed on ice and subsequently stored in a −80 °C laboratory freezer until DNA isolation. Each of the pooled gastric lavage samples from individual jellyfish was extracted separately.

Macroscopic gastric pouch/tentacle pick

Eighty-four juvenile and adult *Chrysaora quinquecirrha* individuals were collected from southern region (Double Creek; 19 individuals), mid-region (Forked River; 19 individuals) and northern region (near Toms River to Silver Bay; 46 individuals) of Barnegat Bay (Appendix S1, Supporting information). Individuals were collected in June (7 individuals), July (55 individuals), and August (22 individuals) 2013 to provide a broad temporal assessment of diet. Individuals were immediately preserved in 95% ethanol to stop digestion and returned to the laboratory.

Preserved *C. quinquecirrha* were initially rinsed across a 250-μm mesh sieve and transferred to watch bowls where they were dissected under a binocular dissecting microscope. All visible prey items were removed from the gastrovascular cavity as well as the tentacles. One half of the total gastric pouch/tentacle picks were set aside for DNA isolation. Individual prey items in the remaining half sample were enumerated and identified to lowest possible taxonomic level using a dissecting microscope. Gosner (1971), Bousfield (1973), Robins & Ray (1986), Johnson & Allen (2012) were used for prey item identification.

DNA isolation

All samples allocated for DNA isolation were stored at −80 °C in 70% (v/v) ethanol. Gut lavage and gastric pouch/tentacle pick samples were centrifuged at 16 000 ***g*** for 30 min. Ethanol was decanted, and pellets were briefly dried in a Speed-Vac to remove traces of ethanol. DNA pellets were then extracted using a CTAB/NaCl protocol (Winnepenninckx *et al.* 1993) with the following modifications. All extractions were carried out in 500 μL volumes in 1.7-mL microcentrifuge tubes and homogenized by grinding for 30 s with a micropestle (Eppendorf). Homogenized samples were incubated at 60 °C for 60 min, and RNA was digested by incubation with RNase A (Sigma-Aldrich; 10 μg for 30 min at 37 °C) prior to precipitation. DNA was precipitated with 2/3 volume of isopropanol, pelleted in a microfuge (16 100 g for 10 min at 4 °C), washed twice with ice-cold 70% (v/v) ethanol, briefly dried in a Speed-Vac, and resuspended in 20 μL of TE (10 mM Tris, 0.1 mM EDTA, pH 8). DNA concentrations and $OD_{260/280}$ ratios were determined in a NanoDrop ND-1000.

NGS library preparation and sequencing

Two pooled libraries were prepared for NGS sequencing: gastric lavage and gastric pouch/tentacle pick. The gastric lavage pooled library consisted of eight samples and the gastric pouch/tentacle pick library consisted of one half of the total gastric pouch/tentacle pick sample (84 individuals) used in the macroscopic analysis. The DNA samples were sent to GeneWiz, Inc. (South Plainfield, NJ; http://www.genewiz.com/), for library preparation and sequencing. GeneWiz prepared each library using the Illumina NEBNext® Ultra™ DNA Library Prep Kit (San Diego, CA, USA). DNA shearing to the targeted 250 bp was accomplished using the Covaris S220 (Woburn, MA, USA). End repair and A-tailing, adapter ligation and PCR-mediated indexing, and enrichment then followed, following Illumina's protocols. The two gut content DNA libraries were multiplexed with a RNA library from another project and paired-end sequenced (2 × 100) on the Illumina HiSeq2500 platform. This resulted in 64 134 235 (gastric lavage) and 50 670 651 (mean gastric pouch/tentacle pick) paired-end reads (NCBI SAMN05929792, PRJNA349266).

Filtering and assembly

Raw reads were quality-filtered using the NGSQC-TOOLKIT_v2.3.2 (Patel & Jain 2012). We kept only full-length reads with PHRED quality scores >30. Consequently, 61 075 232 (gastric lavage) and 48 136 837 (gastric pouch/tentacle pick) paired-end reads were retained for further analyses.

Three separate assemblies were performed as follows: gastric lavage, gastric pouch/tentacle pick and combined. Gastric lavage and gastric pouch/tentacle pick consisted of only reads associated with the given library. The combined analysis consisted of all quality-filtered paired-end reads (109 201 158 reads) from each library (gastric lavage and gastric pouch/tentacle pick). Paired-end reads were assembled using the CLC Genomics Workbench (http://www.clcbio.com/products/clc-genomics-workbench/) de novo assembler. Word size and bubble size were automatically calculated with a minimum contig length of 200 base pairs (bp). Once the initial contigs were assembled, each of the reads were then mapped back to the contigs (mismatch cost = 2; insertion cost = 3, deletion cost = 3; length fraction = 0.5; similarity fraction = 0.8), which were subsequently updated.

Annotation

The combined build contigs were BLAST (Altschul *et al.* 1990) searched against the NCBI nucleotide sequence database using standalone BLASTN 2.2.29+ and the preformatted nt database (downloaded 4/13/14). BLAST searches used default settings except for outfmt = 5 (xml) and max_target_seqs = 5. More stringent and less stringent searches were performed by adjusting the general and scoring parameters (e.g. expect threshold, gap costs), but did not alter prey item identification (not shown). The contigs from gastric lavage and gastric pouch/tentacle pick builds were BLAST searched (same settings as above) against the combined contigs for annotation and comparative purposes.

Taxon identification of gut content

BLASTN XML results from the combined build were imported into MEGAN 5.5.4 (Huson *et al.* 2007) for visual inspection. Given our expectation of many more contigs than useful identifiable sequences, we used very stringent LCA (lowest common ancestor) and analysis parameters (Min Score = 500 [bit score], Max Expected = 0.01 [e-value], Top Percent = 5.0, Min Support Percent = 0.0, Min Support = 1, LCA per cent = 100.00, Min Complexity = 0, Use Minimal Coverage Heuristic) with hopes of more quickly identifying useful sequence data. For the purposes of species level identification, we focused on genes that are both well represented in the NCBI database across the breadth of potential prey species and have previously been used to delineate species boundaries (e.g. barcoding genes: *12S*, *16S*, *18S*, *28S*, *COI*). Nonspecies level assignments were based on sequence similarity, number of available sequences and length of BLAST hit.

We are not confident in species level prey item identification for nonbarcoding genes at this time given the paucity of fully sequenced prey item genomes. The amount of gene conservation between homologous genes in different prey species is unknown, and there is no consistency in what other genes have been sequenced. As a consequence, even a 100% match to a nonbarcoding gene may result in misidentification (see Appendix S2, Supporting information for Engraulidae [anchovies] example). Also, we cannot rule out the possibility of paralogy. As more genomes are sequenced over the coming years, the use of nonbarcoding genes may become a viable alternative and this data set can be revisited at that time.

A prey species was considered to be present in the tentacle pick and/or gut lavage if we assigned a species name to a contig with >98% sequence similarity to the aforementioned barcoding genes (in addition to MEGAN settings), and/or the same species was visually identified in the gut content/tentacle picked samples. We also only considered species level identification for taxa that are known to be present in the Northeast Atlantic Ocean and surrounding estuaries/bay (known Barnegat Bay invasive species included). If the gene regions identified were only present in a limited number of potential prey species and we were unable to verify by visual inspection, the sequences were scored as having higher taxonomic rankings.

Identification of gelatinous animal prey was slightly different because they preserve poorly and are digested quickly by *Chrysaora*. Furthermore, it is impossible to verify whether scyphozoan (jellyfish) contigs were from predator or prey so all of these sequences were treated as predator DNA contamination. Verification of *Mnemiopsis* as a prey species came from PCR. For all other gelatinous prey species, species identification was based on multiple contig hits to the same organism for multiple barcoding genes and known presence in the Northeast Atlantic Ocean and surrounding estuaries/bay.

Molecular verification of Mnemiopsis as a prey species

Mnemiopsis was abundant in our plankton tows and lift nets and has previously been identified as a *Chrysaora* prey species (e.g. Purcell & Cowan 1995). A subsample of *Chrysaora* individuals collected from several regions of Barnegat Bay (Silver Bay, Toms River, Cattus Island; $N = 17$) were sampled for the presence of *Mnemiopsis* DNA in their guts. Specifically, a species-specific region of the *COI* for *Mnemiopsis leidyi* was sequenced from these samples.

DNA was extracted by boiling samples for 10 min in 100 μL of 5% (w/v) Chelex 100 in 50 mM Tris base (pH 11). GenBank sequence (NC_016117; Pett *et al.* 2011) was used to design the PCR primers (F 5′-TGTC GCCCAAATTACTGTTTC-3′; R 5′-TGACGGGGTAAAC CTCATAAA-3′) to amplify a 682-base pair fragment.

All 20 μL PCRs (ABI Veriti) were carried out with ChoiceTaq Mastermix (Denville Scientific, Metuchen, NJ) using the following protocol: initial denaturation at 95 °C for 2 min; 30 cycles of 20 s at 95 °C (denaturation), 20 s at 55 °C (annealing) and 30 s at 72 °C (extension); and a final extension at 72 °C for 7 min. PCR products were visualized on a 1% agarose gel and verified using automated Sanger sequencing (ABI3130).

Molecular quantification of BLAST hits

Precise quantification of prey species counts is difficult because the exact prey species abundance is unknown. Furthermore, in the case of experimentally controlled

diet analyses, the number of reads does not appear to correlate well with known diet proportions (e.g. Deagle *et al.* 2013). Despite these potential drawbacks, we looked at the quantitative signature across the BLAST-identified markers to approximately quantify the relative abundance of prey species from the proportion of reads associated with each of the assembled BLAST-identified contigs. To assess possible 'predator contamination,' BLAST assignments of contigs to scyphozoans (jellyfish) were analysed. All BLAST-identified scyphozoan sequences were treated as predator contamination because only a few scyphozoan genomes have been sequenced.

Reads and contigs assigned to organisms with origins obviously not associated with the diet of *Chrysaora* (e.g. tetrapod, bacteria, plant, virus) were likewise quantified. These sequences likely represent the microbiome of predator or prey, the prey's prey and/or eDNA (environmental DNA) found in the bay water.

Plankton tows

Zooplankton tows were conducted during the collection events ($N = 32$) across the sites and dates of collection. Triplicate 363-μm zooplankton nets were towed at minimally engaged engine speed for 1 min. Length of tow was standardized using a mechanical flow meter to assess the distance travelled. As such, the known cross-section of the net with a known speed for the tow duration allowed volume quantification for each sample. After collection in the field, ctenophores were counted while the sample was field sieved because they do not preserve well and this was the only way to get an accurate assessment of their distribution in the plankton tows. Zooplankton were preserved in ethanol and stained with Rose Bengal for ease of identification and quantification in the laboratory. Each sample was returned to the laboratory for identification and enumeration. All samples were standardized to numbers per cubic metre to assess the potential prey composition of the water column associated with collection events. As the diet analysis was conducted on the conglomerate sample, all zooplankton tow data were pooled for comparative purposes.

Results

Assembly

The gastric lavage build yielded 385 477 contigs [N50 = 1046 (scaffold regions included), average contig length = 713 bp; Table S1, Supporting information]. The gastric pouch/tentacle pick build yielded 292 224 contigs [N50 = 1799 (scaffold regions included), average contig length = 986 bp; Table S2, Supporting information]. The combined analysis yielded 552 296 contigs [N50 = 880 (scaffold regions included), average contig length = 646 bp; Table S3, Supporting information]. Assembled contigs and build summaries for each of the builds can be found in Dryad: http://dx.doi.org/10.5061/dryad.84jr7.

BLAST annotation and visual verification

The BLAST search of the combined data set identified 534 427 contigs with BLASTN hits to the nr database (Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7). Of these, 6961 sequences were assigned in MEGAN with 3907 potential prey specie contigs (Table 1; Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7). Manual inspection of these sequences identified 100 contig sequences (Table S4, Supporting information; Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7) with BLAST hits to the barcoding genes with >98% sequence homology, bit scores >500 and e-values <0.01 (Table 2; Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7). Of these, ten (10) were visually confirmed to various taxonomic levels (Table 2). Care was taken to ensure that the assignment of each of the 100 contig sequences was accurate to the lowest taxonomic level possible (see Appendix S2, Supporting information for an example). More identified taxa were recovered from the gut lavage (18) than the gastric pouch/tentacle pick samples (15) with 10 taxa being recovered from both libraries (Table 2; Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7). Varying the cut-off e-values for the removal of poor quality reads had no effect on the identification of prey items (results not shown).

Molecular quantification of BLAST hits

The per cent of contribution to the MEGAN assignments differed depending on whether reads or contigs were quantified. Seventy-three per cent (reads) and 44% (contigs) of all MEGAN assignments belonged to nonprey species including scyphozoans (jellyfish) (Fig. 1; Appendix S4, Supporting information). Reads assigned to any particular group tended to make up a lower percentage of total assignments as compared to contig assignments (Figs 1 and 2; Appendix S4, Supporting information). However, scyphozoans composed nearly 50% of all MEGAN-assigned reads but only 0.80% of all assigned contigs (Fig. 2; Appendix S4, Supporting information). No single nonprey assignment using either reads or contigs was >18% of the total MEGAN assignments (Figs 1 and 2; Appendix S4, Supporting information). Ray-finned fishes, flatworms, round worms composed nearly 40% of all read and contig MEGAN prey

Table 1 Summary of the number of contigs assigned by MEGAN using a 'shotgun' approach

Group	MEGAN contig assignments
Teleostomi (fish)	2806
Tetrapoda (tetrapods)	2752
Bacteria	2005
Hexapoda (insects and closest relatives)	923
Viridiplantae (green algae, land plants)	775
Cnidaria (jellyfish, hydra, sea anemones, corals)	615
Fungi	485
Alveolata (protists)	236
Echinodermata (sea urchins, sand dollars, sea cucumbers, sea lilies)	221
Crustacea (crustaceans)	186
Mollusca (bivalves, gastropods, cephalopods)	183
Nematoda (roundworms)	158
Amoebozoa (amoebas)	155
Platyhelminthes (flat worms)	147
Hemichordata (acorn worms)	103
Ctenophora (comb jellies)	73
Cephalochordata (lancelets)	63
Chelicerata (mites, scorpions, and relatives)	63
Tunicata (tunicates)	58
Annelida (ringed worms)	56
Euglenozoa flagellate protozoa)	54
Viruses	50
Placozoa (nonparasitic multicellular animal)	43
Porifera (sponges)	41
Cryptophyta (single-celled algae)	39
Choanoflagellida (flagellate eukaryotes)	31
Archaea	29
Chondrichthyes (cartilaginous fishes)	24
Stramenopiles (mainly algae)	22
Heterolobosea (amoebae)	21
Parabasalia (flagellated protists)	12
Myxiniformes (hagfish)	9
Bilateria indet.	8
Haptophyceae (division of algae)	8
Myriapoda (millipedes, centipedes and relatives)	8
Opisthokonta indet. (animal and fungus kingdoms)	8
Rhodophyta (red algae)	7
Onychophora (velvet worms)	4
Petromyzontiformes (lampreys)	3
Brachiopoda (brachiopods)	2
Rotifera (wheel animals)	2
Fornicata (unicellular heterotrophic flagellates)	1
Jakobida (heterotrophic flagellates)	1
Xenacoelomorpha (marine worms)	1
Xenoturbellida (marine worm-like species)	1
Rhizaria (unicellular eukaryotes)	1

assignments (Figs 1 and 2; Appendix S4, Supporting information; Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7).

Visual identification

Visual inspection of the dissected individuals yielded 10 common and relatively distinct groups of organisms including fish eggs and larvae, Harpacticoida, Calanoida, Polychaeta, Brachyura, Mysidacea, Cladocera, Bivalvia and gastropod egg sacks/larvae. In general, most taxa showed substantial signs of digestion, but in some cases, distinct characteristics were present which allowed more detailed identification (e.g. *Alitta* (=*Nereis*) jaws). Based on the taxonomic characteristics present, several organisms were identifiable only to Phyla, while others had sufficient morphological characteristics identifiable to Genus (Table 2). When comparing the proportional abundance of taxa in the diet with those collected from zooplankton tows, several interesting patterns emerge (Fig. 3; Appendix S4, Supporting information). The dominant taxon present in the tows was Calinoida (86%) with similar high abundances from dissected individuals (64%). However, *Chrysaora quinquecirrha* diet showed disproportionately high abundance of fish eggs, fish larvae, Harpactacoida and Polychaeta relative to available planktonic prey and may reflect selective capture and retention. Specifically, the high per cent of harpacticoid copepods (7.5%) and adult polychaetes (3.2%) present in dissected individuals demonstrates active benthic feeding and corresponds to field observations of *C. quinquecirrha* actively swimming to the benthos and dragging its tentacles along the sediment surface (P. A. X. Bologna, personal observation).

Molecular verification of Mnemiopsis as a prey species

Two of the seventeen *Chrysaora* samples collected were positive for *M. leidyi* DNA (11% [Appendix S3, Supporting information]).

Discussion

Understanding the dynamics of generalist predator–prey relationships in marine systems can be difficult, but is essential for a complete understanding of food web interactions and carbon cycling. In the case of gelatinous animals, visual identification often fails to identify all prey items, especially other gelatinous zooplankton, that have been preyed upon (e.g. *Mnemiopsis leidyi*) due to their high digestion rates (Purcell 1992). As a consequence, our understanding of the flow of energy through the ecosystem could be biased.

Table 2 *Chrysaora quinquecirrha* prey items identified in both the gut lavage and gastric pouch/tentacle pick libraries. Numbers indicate total number of assigned contigs used DNA assignments. Visual identification represents taxonomic level discernible. UI indicates unidentified material collected from the gastrovascular cavity from dissected individuals

Final Classification	Common Name	Gene(s)	Gut lavage	Gastric pouch/ tentacle pick	Number of Assigned Contigs	Visually Identified
Pisces						
Anchoa mitchilli	Bay anchovy	*COI* and other miscellaneous mitochondrial genes		X	15	Engraulidae
Cnidarians						
Ctenophora						
Mnemiopsis leidyi	Sea walnut	*18S*; *28S*; *COI* and other miscellaneous mitochondrial genes	X	X	10	UI
Actiniaria						
Diadumene leucolena	White anemone	*28S*	X		1	
Nynantheae	Anemone possibly white anemone	*COI* and other miscellaneous mitochondrial genes	X		17	
Possibly *Nematostella vectensis*	Starlet sea anemone	Miscellaneous nuclear genes	X	X	9	UI
Crustaceans						
Amphipoda						
Ampithoe valida	Amphipod #1	*COI*; *18S*	X		5	
Crangonyctidae	Amphipod #2	*28S*	X		1	
Cirripedia	Barnacle	*28S*	X		1	
Copepoda						
Calanoida						
Acartia tonsa	Calanoid copepod #1	*ITS1*, *5.8S rRNA*, *ITS2*; *COI*	X	X	4	*Acartia*
Pseudodiaptomus coronatus	Calanoid copepod #2	*18S*; *28S*	X	X	2	Calanoida
Cyclopoida	Cyclopoid copepod	*18S*	X		1	
Mysida						
Americamysis bahia	Opossum shrimp	*18S*	X	X	2	Mysidae
Annelida						
Polychaeta						
Alitta (=*Nereis*) *succinea*	Polychaete clam worm	*28S*; *COI*	X	X	5	*Alitta*
Goniadidae	Polychaete worm #2	*28S*	X		1	
Trematoda						
Lepocreadiidae	Trematode	*18S*	X	X	2	UI
Platyhelminthes						
Stylochidae	Flatworm	*28S*	X	X	3	Platyhelminthes
Echinodermata						
Asterozoa	Sea stars or brittle star	*28S*		X	1	UI
Echinozoa	Echinoderm	*28S*		X	1	UI
Mollusca						
Gastropoda						
Nudibranchia	Nudibranch	*18S*	X	X	3	Gastropoda
Possibly Euthyneura	Sea snails and slugs	Heat shock protein	X		1	
Bivalvia						
Mercenaria mercenaria	Hard clam	*COI*; *18S*		X	4	*Mercenaria*
Veneridae other than *Mercenaria*	Clam #2	*COI*		X	1	Bivalvia
Hemichordata	Possible acorn worm	BAC sequences	X	X	4	Hemichordata

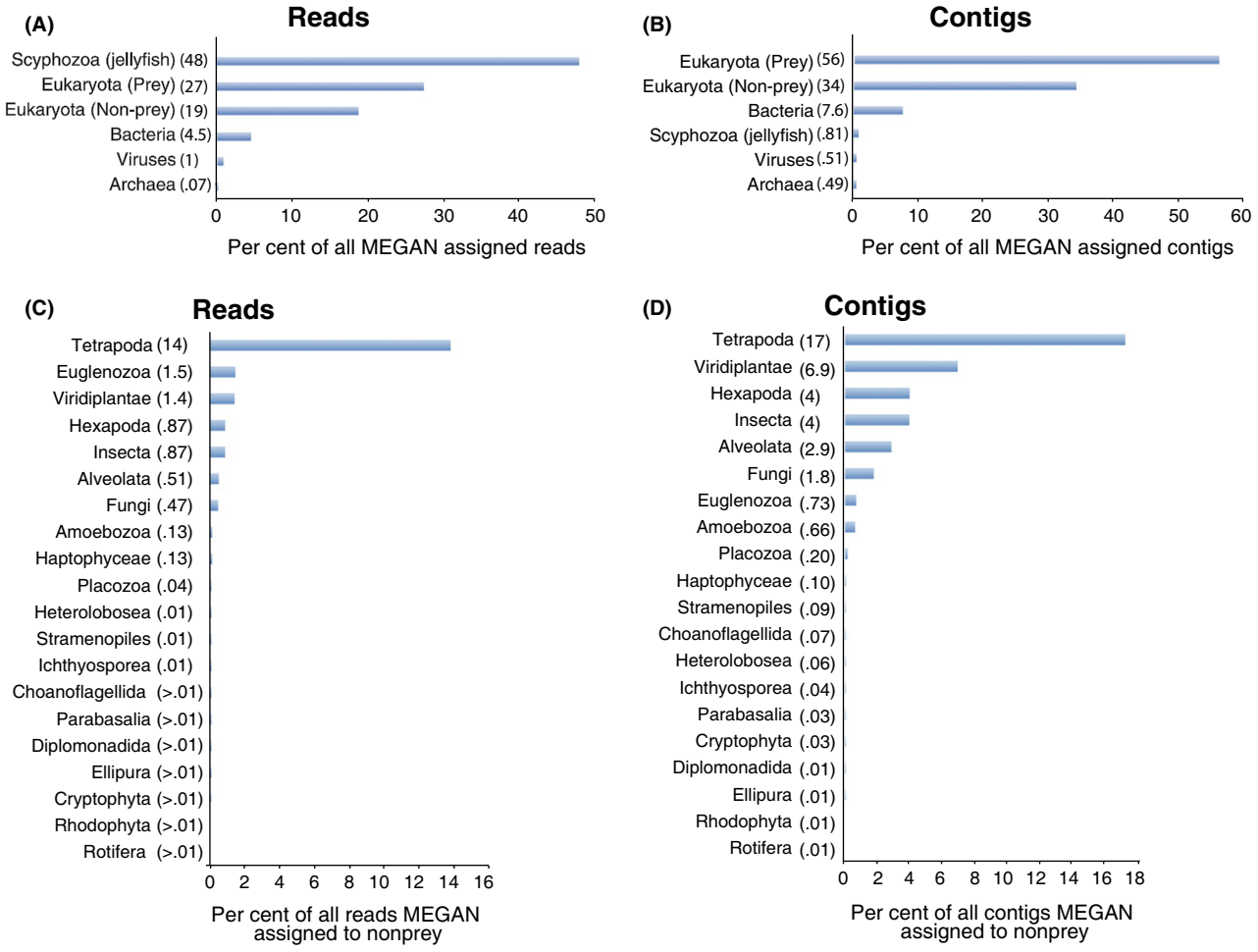

Fig. 1 MEGAN assignments. Percentages indicated in brackets. (A) Per cent of all MEGAN-assigned reads. (B) Per cent of all MEGAN-assigned contigs. (C) Per cent of all MEGAN-assigned reads to nonprey. (D) Per cent of all contigs MEGAN assigned to nonprey. Rotifera (wheel animals), Rhodophyta (red 'algae'), Cryptophyta (algae), Ellipura (springtails and coneheads), Diplomonadida (flagellates), Parabasalia (flagellated protists), Choanoflagellida (free-living unicellular/colonial flagellate eukaryotes), Ichthyosporea, Stramenopiles (algae), Heterolobosea (protozoans), Placozoa (placozoan), Haptophyceae (algae), Amoebozoa (amoebas), Alveolata (Protists), Insecta (insects), Hexapoda (insects, springtails, coneheads, two-pronged bristletails), Viridiplantae (green plants), Euglenozoa (flagellate protozoa), Tetrapoda (tetrapods). [Colour figure can be viewed at wileyonlinelibrary.com].

Most studies that have looked at the diets of predators have utilized PCR prior to NGS for targeted amplicon enrichment (reviewed in Pompanon *et al.* 2012). Blocking primers of some sort are often used to prevent amplification of the predator's own DNA (see Piñol *et al.* 2014 for advantages of not using blocking primers). Here, we chose a 'shotgun' approach whereby we bypassed the targeted enrichment step and directly deep sequenced gastric lavage and gastric pouch/tentacle pick DNA isolations. Our 'shotgun' method offers potential advantages over NGS-targeted enrichment approaches in that all prey items have the potential of being sequenced. Targeted enrichment approaches are primer-dependent and can fail to amplify taxa where there is a poor match between primer and prey even with universal primers (Piñol *et al.* 2014). Our method also has the advantage in that DNA degradation of diet samples is not as consequential as in PCR amplification, which requires an intact template. Furthermore, if one factors in labour and time costs, our method is more economical (personal observations). We bypass primer design and PCR amplification, which makes our method ultimately quicker and cheaper. Additionally, total NGS costs will likely decrease in the future. To our knowledge, this is the first NGS dietary study of a jellyfish.

Diet studies in general will always benefit from multiple lines of evidence, as no single method will be able to delineate the complete diet (Hargrove *et al.* 2012; Pompanon *et al.* 2012). When possible, multiple

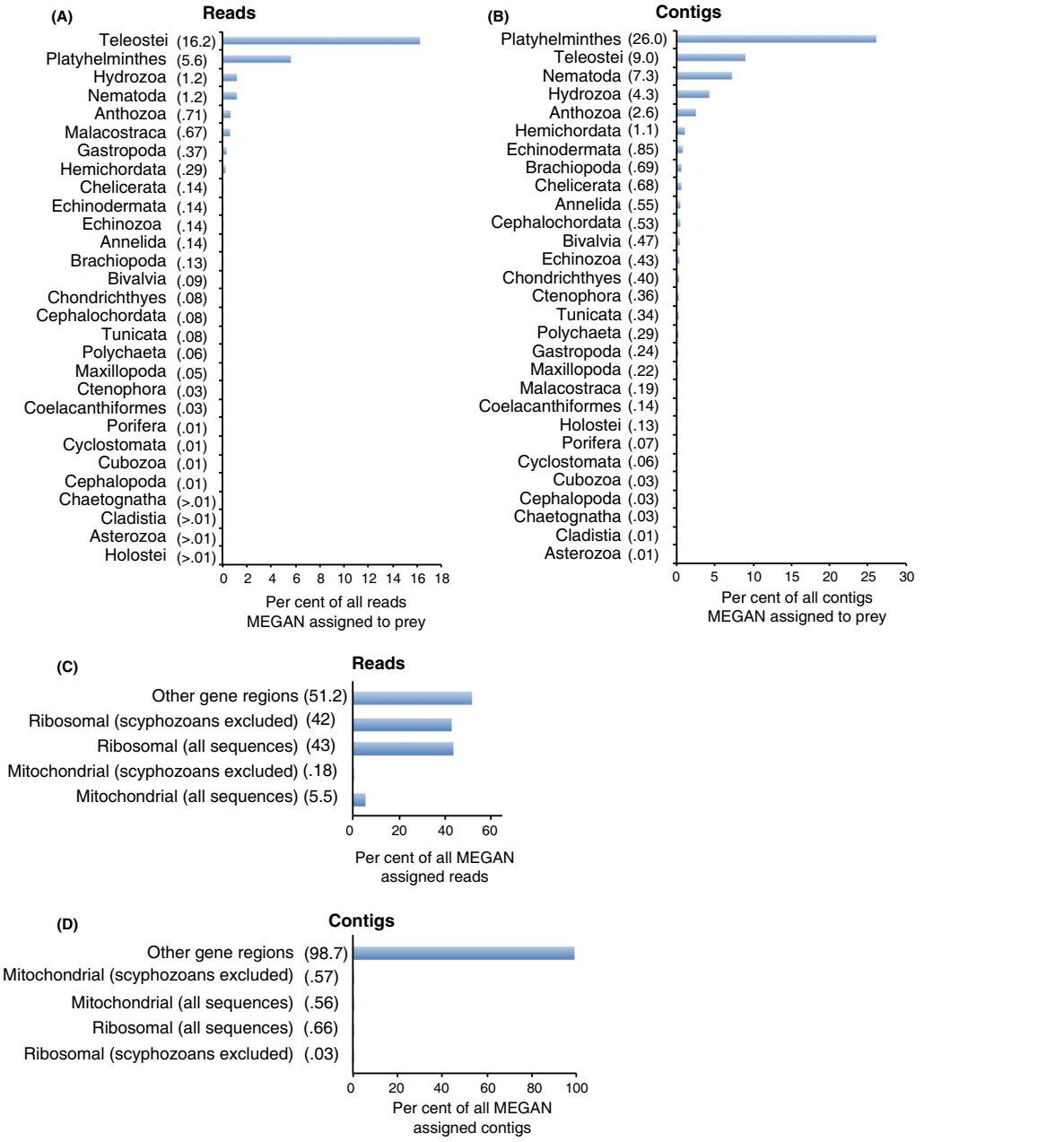

Fig. 2 MEGAN assignments. Percentages indicated in brackets. (A) Per cent of all reads MEGAN assigned to prey. (B) Per cent of all contigs MEGAN assigned to prey. (C) Per cent of all assigned MEGAN reads. (D) Per cent of all assigned MEGAN contigs. Holostei (bowfins, gars), Asterozoa (seastars, brittle stars), Cladistia (bichirs, reedfish), Chaetognatha (arrow worms), Cephalopoda (squids, octopuses, cuttlefish), Cubozoa (box jellyfish), Cyclostomata (jawless fishes), Porifera (sponges), Coelacanthiformes (coelacanth), Ctenophora (comb jellies), Maxillopoda (barnacles, copepods, mystacocarids, tantulocarids, branchiurans, ostracods, related groups), Polychaeta (annelid worms), Tunicata (tunicates), Cephalochordata (lancelets), Chondrichthyes (cartilaginous fishes), Bivalvia (clams, oysters, mussels, scallops), Brachiopoda (lamp shells), Annelida (ringed worms), Echinozoa (sea urchins, sea cucumbers), Echinodermata (sea stars, sea urchins, sea cucumbers, related groups), Chelicerata (horseshoe crabs, sea spiders, arachnids), Hemichordata (acorn worms, pterobranchians), Gastropoda (snails, slugs), Malacostraca (crabs, shrimp, beach hoppers, krill, pill bugs, mantis shrimp), Anthozoa (corals), Nematoda (roundworms), Hydrozoa (hydrozoans), Platyhelminthes (flatworms), Teleostei (ray-finned fishes). [Colour figure can be viewed at wileyonlinelibrary.com].

methods should be used. However, DNA-based identifications have potential advantages over visual identification methods. DNA-based methods have a larger temporal time frame for identification as they rely on DNA and not the physical characteristics, which erode quickly during the digestion process (Pompanon *et al.*

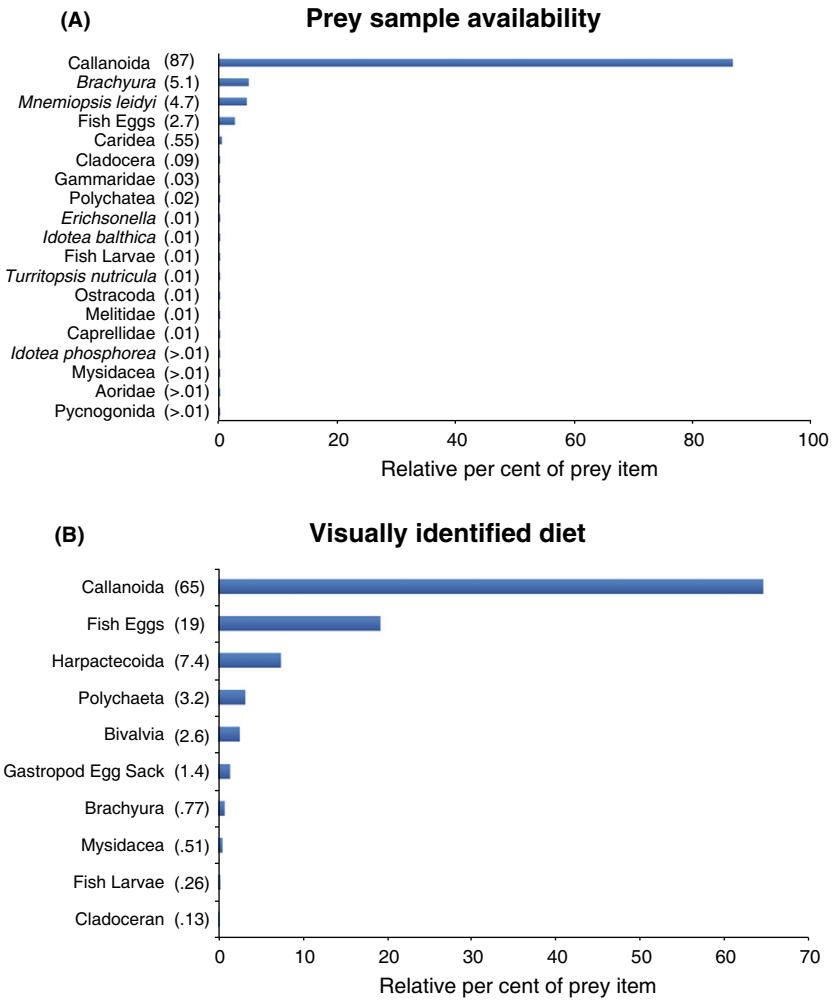

Fig. 3 Relative per cent of prey items available and visually identified. Percentages indicated in brackets. (A) Relative per cent of prey items available. (B) Relative per cent of prey items visually identified in diet. Pycnogonida (sea spiders), Aoridae (amphipods), Mysidacea (shrimp-like crustaceans), *Idotea phosphorea* (seaweed isopods), Caprellidae (amphipods), Melitidae (amphipods), Ostracoda (seed shrimp), *Turritopsis nutricula* (jellyfish), *Idotea balthica* (isopod), *Erichsonella* (isopod), Polychatea (annelid worms), Gammaridae (amphipod), Cladocera (water fleas), Caridea (caridean shrimp), *Mnemiopsis leidyi* (sea walnut), *Brachyura* (crab), Callanoida (copepods). [Colour figure can be viewed at wileyonlinelibrary.com].

2012). Additionally, greater taxonomic resolution is possible even by a novice taxonomist. As DNA databases grow, earlier DNA-based studies can be revisited allowing for the identification of new taxa and/or further resolution. Lastly, DNA-based approaches do not require as large of a sample size as visual identification methods given their higher sensitivity if samples are strategically collected over temporal scales (Carreon-Martinez *et al.* 2011). This is of significant importance when there are only limited resources or for rare or endangered taxa.

Diet of Chrysaora quinquecirrha

The diet of at least six of the thirteen recognized *Chrysaora* species (sensu Morandini & Marques 2010) has been characterized including *C. quinquecirrha* in the Chesapeake Bay (Table 3). *C. quinquecirrha* is a keystone predator (Piraino *et al.* 2002; Purcell & Decker 2005) and cruising forager (Ford *et al.* 1997). *C. quinquecirrha* prey on a multitude of organisms and preferred prey items can change from ephyra to medusa (Purcell 1992). In the Chesapeake Bay, prey items other than zooplankton make up an important part of *C. quinquecirrha*'s diet (individuals >45 mm) as zooplankton cannot meet nitrogen demands (Purcell 1992). A consensus is starting to develop indicating that *Chrysaora* species may not be generalist predators (Riascos *et al.* 2014; P. A. X. Bologna, personal observation). Our molecular diet assessment is in general agreement with prior studies in regard to the type of prey species (Table 3).

Our diet analyses of *C. quinquecirrha* from Barnegat Bay suggest that this species consumes both benthic and pelagic organisms and may play an important role in benthic–pelagic coupling (nutrient cycling). We identified at least two polychaete worms (*Alitta succinea*, Goniadidae) and visually observed large polychaetes in gastric pouches. The mean depth of Barnegat Bay is 1.5 m with a maximum depth of 6 m (Kennish 2001). There are a couple of possibilities that explain the presence of benthic organisms in the gut of *C. quinquecirrha*. The elongated tentacles and oral arms of

Table 3 Literature review of gut contents identified in different *Chrysaora* sp.

Species	Gut contents	Source
C. melanaster	Fish	Hamner 1983; Brodeur *et al.* 2002; Zavolokin *et al.* (2008); Gorbatenko *et al.* (2009); Purcell (1991)
	Theragra chalcogramma eggs, larvae, juveniles	
	unidentified eggs, larvae, juveniles	
	Cnidarians	
	unidentified	
	Sarsia tubutesa	
	S. nipponica	
	Hydromedusae	
	Ctenophores	
	unidentified	
	Crustaceans	
	Copepods	
	eggs, naupilia	
	calanoids	
	Calanus glacialis	
	Neocalanus plumchrus	
	N. cristatus	
	Eucalanus bungii	
	Metridia okhotensis	
	Bradyidius pacificus	
	Pseudocalanus minutus	
	Acartia longiremis	
	Decapods	
	Crab larvae	
	Chionoecetes spp. megalopae	
	Euphausiids	
	Thysanoessa inermis	
	T. raschii	
	hyperiid amphipods	
	Themisto libellula	
	T. pacifica	
	Vibilia	
	mysids	
	Tunicates	
	larvaceans	
	Chaetognaths	
	Sagitta elegans	
	Mollusks	
	pteropods	
	Limacina helicina	
	Limacina spp.	
C. quinquecirrha	Fish	Purcell (1992); Purcell *et al.* (1994); Gorbatenko *et al.* (2009); Purcell (1991)
	Fundulus spp. larvae	
	Anchoa mitchelli eggs	
	Ctenophores	
	Mnemiopsis leidyi	
	Beroe cucumis	
	Crustaceans	
	cirriped larvae	
	cladocerans	
	copepods	
	copepodites, nauplii, adults	
	calanoids	
	Acartia tonsa	
	Polychaetes	
	benthic larvae, adults	

Table 3 *Continued*

Species	Gut contents	Source
	Mollusks	
	bivalve larvae, veligers*	
	gastropods veligers	
	Rotifers	
	Protozoa	
C. cf. caliparea	Fish	Kanagaraj *et al.* (2011)
	Eggs, larvae, juveniles	
	Cnidarians	
	Hydromedusae	
	Ctenophores	
	Crustaceans	
	amphipods	
	cirripeds	
	copepods	
	undetermined	
	calanoids	
	Acrocalanus	
	Acartia spp.	
	Paracalanus	
	Centropages	
	cyclopoids	
	Oithona spp.	
	Corycaeus	
	harpacticoids	
	Microsetella	
	Macrosetella	
	decapods	
	euphausiids	
	mysids	
	stomatopods	
	Polychaetes	
	Mollusks	
	bivalves	
	gastropods	
C. hysoscella Reassigned to *C. fulgida* (Morandini & Marques 2010)	Fish	Flynn & Gibbons (2007); Purcell (1991)
	larvae	
	Cnidarians	
	Hydromedusae	
	Amphinema digitale	
	Clytia gregaria	
	Cosmetira pilosella	
	Obelia spp.	
	Chrysaora	
	Scaphomedusae	
	Aurelia aurita	
	Ctenophores	
	Crustaceans	
	eggs, larvae	
	crab megalopae†, zoea	
	amphipods	
	gammarids	
	chaetognaths	
	cirrepid nauplii	
	cladocerans	
	copepods	
	cumaceans	
	decapods	

Table 3 *Continued*

Species	Gut contents	Source
	Palaemon sp.	
	isopods	
	mysids	
	Polychaetes	
	larvae	
	benthic forms	
	Echinoderms	
	ophiuroid larvae	
	Dinoflagellates	
	Noctiluca	
C. fuscescens	Cnidarians	Suchman *et al*. 2008
	hydromedusae	
	siphonophores	
	Ctenophores	
	Crustaceans	
	Cladocerans	
	Copepods	
	Calanoids	
	Acartia	
	Aetideus	
	Calanus	
	Candacia	
	Centropages	
	Clausocalanus	
	Eucalanus	
	Lucicutia	
	Metridia	
	Paracalanus	
	Pseudocalanus	
	Rhincalanus	
	Scolecithricella	
	Tortanus	
	Cyclopoids	
	Oithona	
	Corycaeus	
	Euphausiids	
	eggs, larvae	
	Euphausia pacifica	
	Thysanoessa	
	spinifera	
	Tunicates	
	doliolids	
	larvaceans	
	salps	
	Polychaetes	
	larvae of benthic	
	taxa	
	Mollusks	
	bivalve larvae	
	gastropod larvae	
	pteropods	
C. plocamia	Fish	Riascos *et al*. (2014)
	eggs, larvae	
	Engraulis ringens eggs	
	Cnidarian	
	larvae	
	hydromedusae	

Table 3 *Continued*

Species	Gut contents	Source
	Crustaceans	
	amphipods	
	Caprella sp.	
	cirripeds	
	larvae	
	cladocerans	
	copepods	
	unidentified	
	calanoids	
	cyclopoids	
	harpacticoids	
	decapods	
	larvae	
	Emerita analoga	
	euphausiids	
	unidentified	
	Nematoscelis sp.	
	Isopods	
	unidentified	
	Excirolana braziliensis	
	ostracods	
	Tunicates	
	thaliacean salps	
	Polychaetes	
	larvae, adults	
	Mollusks	
	bivalves	
	larvae, juveniles	
	gastropods	
	larvae, juveniles	
	Bryozoan	
	larvae	
	Porifera	
	larvae	

*Bivalve veligers can survive ingestion (Purcell *et al.* 1991).
†*Cancer* spp. megalopae are known to parasitize medusae (Suchman *et al.* 2008).

C. quinquecirrha could be passively coming in contact with benthic prey as they scrape along the bay floor or there are diel vertical migrations of either prey or predator. We have observed *C. quinquecirrha* in the field actively swimming to the benthos and dragging their tentacles through seagrass beds and along unvegetated benthic habitats with active retraction of oral arms (P. A. X. Bologna, personal observation). In the shallow tributaries of the Chesapeake Bay, medusa prey includes benthic polychaetes (Purcell 1992) and our results concur that these may be important trophic links for *C. quinquecirrha*. This hypothesis will have to be further tested via quantitative predator–prey data. Flynn & Gibbons (2007) suggest benthic production in Walvis Bay Lagoon, Namibia, increased in response to the prior collapse of pelagic fisheries. As a consequence, the diet of *C. hysoscella* (reassigned to *C. fulgida* by Morandini & Marques 2010) now consists of a large number of benthic prey (e.g. polychaetes). For *C. fulgida*, it remains to be determined whether this is due to diel vertical migrations of the prey or predator.

Crustaceans can make up a large proportion of the gelatinous diet and can be dependent on ichthyoplankton densities (Purcell *et al.* 1994b; Purcell 1997). Our analysis identified at least seven different crustaceans including amphipods, copepods, barnacles and mysid shrimp (Table 1). In the Chesapeake Bay, copepods make up most of the diet of both *C. quinquecirrha* and *M. leidyi* (Purcell 1992; Purcell *et al.* 1994a,b). Barnegat Bay *C. quinquecirrha* populations also prey upon the calanoid copepod *Acartia tonsa* in addition to at least two other copepod species (Table 2).

We positively identified at least four molluscan genera in both Gastropoda (2) and Bivalvia (2; Table 2). The identification of the economically important hard clam *Mercenaria* indicates a trophic link, but the potential impacts on the already hard hit shellfish industry (Bricelj *et al.* 2012) are unknown. However, in the Chesapeake Bay, *C. quinquecirrha* medusae are not significant predators of the bivalve veliger larvae *Crassostrea virginica* (oyster), *Mytilus edulis* (mussel) and *Mulinia lateralis* (clam) (Purcell *et al.* 1991). The sea nettle medusae capture veliger larvae but fail to digest them. All of our assembled bivalve contigs are associated with the gastric pouch/tentacle picked samples. It remains to be determined what stage of development these bivalves were at and whether they were being ingested or egested at the time of collection.

Cnidarians and ctenophores are also important components of *Chrysaora*'s diet (Table 3). We positively identified at least three anemones, and to our knowledge, this is the first time anemones have been recognized as part of *Chrysaora*'s diet. This is likely due to their small size and gelatinous composition, making visual identification difficult. Similar to the Chesapeake Bay sea nettle populations (Purcell & Cowan 1995), the ctenophore *M. leidyi* makes up part of *C. quinquecirrha*'s diet in Barnegat Bay. *C. quinquecirrha* can completely eliminate *M. leidyi* from Chesapeake Bay tributaries (Purcell & Cowan 1995) and cause trophic cascades (Purcell & Decker 2005). *M. leidyi* heavily predates bivalve veliger larvae (Purcell *et al.* 1991), and in the northwestern Black Sea, *M. leidyi* has the highest clearance rate when feeding on bivalve veligers (400 L ind.$^{-1}$ day^{-1}) (Finenko *et al.* 2014). *M. leidyi* is also a much more proficient copepod predator. The predation of *M. leidyi* by *C. quinquecirrha* may increase bivalve veliger larvae and copepod survival rates, indirectly benefiting zooplanktivorous fish (Purcell *et al.* 1991; Purcell & Decker 2005).

Jellyfish can be fish predators (Purcell 1985, 1997; Arai 1988; Bailey & Houde 1989; Purcell & Arai 2001), fish competitors (Arai 1988; Purcell 1997; Purcell & Arai 2001) and/or intermediate hosts of fish parasites (Arai 1988; Purcell *et al.* 2000). Commercially important fish larvae can account for a significant portion of the gelatinous animal diet (Purcell 1997; Purcell & Arai 2001). Gelatinous predators directly compete for the same zooplankton food source as ichthyoplankton (Purcell & Arai 2001). As compared to other zooplankton, fish eggs and larvae are generally larger and are often unable to escape encounters with gelatinous predators (Purcell *et al.* 1994b). As a consequence, *C. quinquecirrha* preferentially selects fish eggs and larvae to all other prey types (Fancett 1988; Purcell *et al.* 1994b; Purcell & Arai 2001) and in high concentrations *C. quinquecirrha* can have substantial effects on ichthyoplankton (Purcell 1992). BLAST annotation attributed thousands of contigs to fish (Table 1, Dryad: http://dx.doi.org/10.5061/dryad.84jr7). Although the vast majority of fish contigs are currently unidentifiable beyond Teleostei, we did find sufficient support for the identification of the Bay anchovy (*Anchoa mitchilli*), the most common fish in estuaries of the U.S. Atlantic coast (Houde & Zastrow 1991). In the Chesapeake Bay, *C. quinquecirrha* is an important predator of *Anchoa mitchilli* (Purcell *et al.* 1994b). More specific amplification techniques targeting a specific region such as *COI* could be necessary to determine exactly how many species are actually present in our samples.

Our diet analysis also identified echinoderms, flukes, flatworms and hemichordates (Table 2). Echinoderms have previously been identified in the gut of *C. hysoscella* but not *C. quinquecirrha*. Flukes, flatworms and hemichordates have yet to be identified as *Chrysaora* prey items due in part possibly to their soft bodies and probable high digestion rates, all limiting factors of visual identification methods.

Limitations

High-throughput sequencing methodologies are capable of producing millions of sequences, but like all technological advances, there are limitations. Some limitations are biological and include the genetic and cellular make-up of the organism (e.g. taxon-specific variation in GC content, copy number, relative DNA concentrations within a tissue type), unavoidable technical biases associated with DNA extraction, DNA pooling, library preparation (e.g. differential PCR amplification bias), differential DNA survival in the gut, the type of NGS platform (e.g. Illumina, Ion Torrent) and the bioinformatics pipeline used in the processing of reads (Martin-Laurent *et al.* 2001; Deagle & Tollit 2007; Amend *et al.* 2010; Porazinska *et al.* 2010; Quail *et al.* 2012; Deagle *et al.* 2013).

Potential criticisms of our 'shotgun' methodology include the likely amplification of an overwhelming number of sea nettle and nonprey items (e.g. bacteria, viruses). However, this is not of concern in this study in part due to the depth of sequencing (nearly 110 million paired-end reads). Piñol *et al.* (2014) also found that NGS produces informative sequences, but in their case, they used universal primers for targeted enrichment without blocking primers. Viral and bacterial contigs accounted for less than a third of our total sequences assigned by MEGAN (Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7). Of the nearly 12 500 sequences assigned by MEGAN, only 33 were attributed to Cnidaria (Tables 1 and 2; Dryad doi: http://dx.doi.org/10.5061/

dryad.84jr7). Of the cnidarian sequences, nine were manually validated as belonging to *Nematostella*, one to *Diadumene* and five to the predator *Chrysaora* including a nearly complete mitochondrial genome. Sequences identified as *Aurelia* (1 sequence) were not validated beyond Cnidaria given the BLAST hit sequence similarity, BLAST hit length and level of the gene's discriminatory power. The seventeen MEGAN-assigned *Metridium* sequences are not *Chrysaora* and were all assigned to Nynantheae as *Diadumene* mitochondrial genes have not been sequenced. It should also be noted that some of the *Chrysaora* sequences could indeed be prey items, but this cannot be definitively determined with a DNA-based approach. DNA-based methods are also not immune to secondary predation whereby one predator eats another one who still has prey of its own in its gut (Sheppard *et al*. 2005). Lastly, there may also be accidental by-catch, which we have addressed with extensive rinsing of sea nettles prior to gut lavages. These potential biases are well recognized.

Other biases in DNA-based diet analyses depend on the amount of degradation, which is linked with both temperature and time spent in the gut (Carreon-Martinez *et al*. 2011) as well as prey and predator size, prey type and number of prey present in the gut (Purcell 1989, 1992; reviewed in Purcell 1997). In *C. quinquecirrha*, higher rates of predation and clearance rates are strongly associated with prey density and temperature, respectively (Purcell 1992). This should not pose as much of a problem for a 'shotgun' approach as even the most heavily degraded prey item has a chance of being sequenced given that an intact template sequence is not needed.

Despite the aforementioned potential pitfalls, we were able to identify, at minimum, 23 operational taxonomic units that are associated with gut content and/or oral arms of *C. quinquecirrha*. Even though only nine prey items were identified to the species level, we are confident they are correctly identified. Furthermore, the lack of taxonomic resolution will only decrease through time as more and more genomes are sequenced. Virtually all of our contigs had a good match to a NCBI sequence at a taxonomic level on par with visual identification methods (family or above).

Most importantly we recognize that this study is not a complete diet assessment of *C. quinquecirrha*. The prey items identified in the gastric pouch, and tentacle picked samples represent a snapshot of prey items eaten in the late morning to early afternoon (based on time of collection and known digestion rates) over the course of 26 days during July and August. The gut lavages come from a single day of collecting and were biased towards larger individuals (81.4 mm + 27.6, range = 37–120 mm). The only possible exceptions include large polychaete worms and fish that were possibly taken the night before and given their large size take longer to digest.

Our restricted sampling times have likely biased our results. For example, in the Chesapeake Bay, *C. quinquecirrha* prey concentrations are higher during the night, probably due to higher prey densities, and include benthic organisms (Purcell 1992). Furthermore, in the Chesapeake Bay, prey items of ephyrae and small medusa (<6 mm live diameter) consisted mainly of rotifers and protozoans (Purcell 1992). For the ephyrae and small medusa, there was negative selection for copepod nauplii, polychaete larvae and gastropod veligers but positive selection for rotifers (Purcell 1992). In Namibia, the total prey consumed by *C. fulgida* was greater during the day, but the average diversity was greater during the night (Flynn & Gibbons 2007). Complete characterization of the *C. quinquecirrha* diet will require strategically collected gut/tentacle content samples taken over a 24-h period throughout the year from individuals of all size classes.

To our knowledge, this is the first study to apply NGS technology to analyse the prey items of a gelatinous predator. The use of NGS for the identification of gelatinous animal gut/tentacle content identification offers a powerful alternative to traditional methods of visual identification. Here, we have used a 'shotgun' approach to randomly sequence DNA fragments isolated from gut contents and items picked from the tentacles. We have shown that the shotgun approach is useful to identify sea nettle prey down to a level not usually possible using visual identification methods. Furthermore, at minimum, nine new *C. quinquecirrha* prey species were identified. These include sea anemones, barnacles, trematodes, flatworms, nudibranchs, sea snails/slugs and possibly a hemichordate. Visual identification methods will continue to play a key role in identifying gelatinous prey items but, in conjunction with DNA-based methods, will ultimately result in a more robust trophic level reconstruction.

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J.J.G., P.A.X.B. and R.W.M. conceived and designed the study; J.J.G. and P.A.X.B. collected gut lavages and gastric pouch/tentacle picks; P.A.X.B. identified dissected prey and zooplankton samples; J.J.G. and R.W.M. performed DNA isolations; R.W.M. analysed data and wrote the manuscript with input from J.J.G. and P.A.X.B.

Data accessibility

NGS raw read data: NCBI SRA: SAMN05929792, PRJNA349266.

Gastric lavage assembled contigs and build summary: Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7

Gastric pouch/tentacle picks assembled contigs and build summary: Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7

Combined gut lavage and gastric pouch/tentacle picks assembled contigs and build summary: Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7

BLAST Search results for combined gut lavage and gastric pouch/tentacle picks: Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7

MEGAN results for combined gut lavage and gastric pouch/tentacle pick contigs: Dryad doi: http://dx.doi.org/10.5061/dryad.84jr7

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 GPS coordinates of collected samples

Appendix S2 Issues of using nonbarcoding genes

Appendix S3 Contig and Read Summaries

Appendix S4 Molecular verification of *Mnemiopsis*

Table S1 Total yield and quality of DNA isolation

Table S2 Gastric Pouch/Tentacle Pick Build Summary

Table S3 Combined Gastric Lavage Build and Gastric Pouch/Tentacle Pick Summary

Table S4 MEGAN assignments