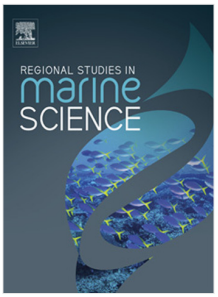
Journal Pre-proof



DNA metabarcoding unveils niche overlapping and competition among Caribbean sea urchins

Ruber Rodríguez-Barreras, Filipa Godoy-Vitorino, Kim Præbel, Owen S. Wangensteen

PII: S2352-4855(20)30665-4
DOI: https://doi.org/10.1016/j.rsma.2020.101537
Reference: RSMA 101537

To appear in: *Regional Studies in Marine Science*

Received date : 29 February 2020
Revised date : 26 September 2020
Accepted date : 7 November 2020

Please cite this article as: R. Rodríguez-Barreras, F. Godoy-Vitorino, K. Præbel et al., DNA metabarcoding unveils niche overlapping and competition among Caribbean sea urchins. *Regional Studies in Marine Science* (2020), doi: https://doi.org/10.1016/j.rsma.2020.101537.

DNA metabarcoding unveils niche overlapping and competition among Caribbean sea urchins

Ruber Rodríguez-Barreras[1], Filipa Godoy-Vitorino[2], Kim Præbel[3], and Owen S. Wangensteen[3]

1- Department of Biology, University of Puerto Rico at Bayamón; Zona Industrial Minillas, 170, PR-174, Bayamón, Puerto Rico, 00959-1919.

2- Department of Microbiology and Medical Zoology, University of Puerto Rico School of Medicine; Paseo Dr. José Celso Barbosa, San Juan, 00921

3- Norwegian College of Fishery Science, UiT the Arctic University of Norway, Tromsø, Norway.

*Corresponding author: ruber.rodriguez@upr.edu

Abstract: Detailed information of trophic interactions among consumer–resources in food webs is usually limited due to the lack of accurate identification of eaten food resources. The use of DNA-metabarcoding has been proven useful for molecular identification of the numerous taxa present in stomach contents. Here, we characterize the diet and trophic behavior of four sea urchin species inhabiting shallow waters of Puerto Rico using this molecular technique. We extracted, sequenced, and analyzed DNA from the gut content of a total of 60 individuals collected at three sites at the northeastern coast of Puerto Rico. Our results demonstrated that seaweeds were the dominant food source for the four sea urchin species at all three sites, but also small protists, fungi and metazoans were important components of sea urchin's diet. Interspecific differences in diet were also found among sites. PERMANOVA analysis detected significant differences among species (Pseudo-F= 1.755, $p < 0.001$), and among sites (Pseudo-F= 2.52, $p < 0.001$). A SIMPER analysis showed that in all cases the main taxa causing differences between species and sites were macroalgae

(Rhodophyta, Chlorophyta and Ochrophyta) with some contribution of small eukaryotes (Apicomplexa and Bacillariophyta). This diet characterization in sea urchins revealed a generalist omnivore behavior, but with a clear dominance of algae as a main dietary component. Thus, we found a potential inter-specific competition due to niche overlapping, which seems to be more common than initially thought.

Keywords: Sea urchins; food webs; metabarcoding; trophic analysis; diet analysis; Caribbean

INTRODUCTION

Understanding trophic interactions is essential in ecology, and this is particularly true in marine ecosystems where a high number of species share the same habitat and occupy apparently similar trophic positions (McCutchan et al. 2003). These similar trophic positions occur under different patterns of spatial variability due to local biotic and abiotic interactions (Van Dover et al. 1992). The ecological understanding of trophic interactions among consumer–resources in food webs is limited, mostly due to the lack of accurate identification of ingested food resources (De Barba et al. 2014). Studies that estimate the relative contribution of different food resources in the diet have important implications for a wide range of ecological dynamics from individuals to ecosystems, but also for conservation purposes since maintaining species interactions is essential for ecosystem functioning (Leray et al. 2015). The feeding behavior of keystone species has a strong impact on ecosystem structure and functioning and revealing the food resources utilized by these keystone species provide essential information on the food web dynamics (Lasley-Rasher et al. 2015). Sea urchins are primarily omnivorous with preference for herbivorism,

being important grazers in reef ecosystems (Lessios 2016). This close trophic relationship with algae and marine vascular plants make echinoids a keystone species for the maintenance and stability of shallow marine benthic ecosystems (Pretch and Pretch 2015; Pérez-Portela et al. 2020).

A simple visual analysis of gut contents under a microscope usually results in a narrow view of the food spectrum. Some food items remain indigestible; however, others become unidentifiable (Symondson 2002). Gut contents (soft bodied and digested specimens) of animals often contain a high quantity of unidentifiable material that is otherwise difficult to identify with traditional microscopic identification (McClenaghan et al. 2015). Stable isotope analysis (SIA) has also become an important tool in ecological analysis of diet (Wangensteen et al. 2011, Rodríguez-Barreras et al. 2015, Cabanillas-Terán et al. 2016), but with some taxonomical limitations. To overcome these difficulties, a promising new avenue for unveiling consumers' diet is offered by DNA-metabarcoding (Taberlet et al. 2012). Recent applications of high- throughput sequencing (HTS) such as metabarcoding for molecular biodiversity assessment, help identify numerous taxa based on DNA samples from the environment or community (Wangensteen et al. 2018a, Siegenthaler et al. 2019). Thus, DNA metabarcoding is emerging as a powerful tool to estimate prey diversity and composition from gut contents in consumer samples, yielding millions of sequences from PCR amplicons (Leray et al. 2013, Barnes and Turner 2016). The development of this highly effective molecular technology facilitates the dietary analysis from gut/fecal genomic DNA, while minimizing the interaction with animals (Valentini et al. 2009, De Barba et al. 2014, Kemp et al. 2019), and increasing the speed of the analyses. DNA metabarcoding stands as the best technique to accuratetly identify food

resources, reducing field efforts and costs of traditional monitoring (Berry et al. 2015, Kartzinel and Pringle 2015, Leray and Knowlton 2015).

Sea urchins are considered among the most common benthic grazers in tropical marine ecosystems (Moberg and Folke 1999), where they can exert a strong influence in the community structure (Wangensteen 2013, Agnetta et al. 2015, Steneck 2020). The sea urchins *Diadema antillarum* Philippi, 1845, *Echinometra lucunter* (Agassiz, 1863), *Lytechinus variegatus* (Lamarck, 1816), and *Tripneustes ventricosus* (Lamarck, 1816) are the most common species in Caribbean shallow water systems (Precht and Precht 2015, Tuohy et al. 2020). These species inhabit seagrass meadows and coral reefs, highly productive ecosystems common in littoral areas (Alvarado 2011). Sea urchins from shallow waters are usually classified as generalist herbivores or facultative omnivores feeding on algae or seagrass (Hendler et al. 1995). Former studies using traditional techniques of stomach contents of sea urchins, have found bryozoans, ascidians and hydroids among the contents of *T. ventricosus* (Barrios and Reyes 2009), and sponges, hydroids, bryozoans, nematodes, rotifers, gastropods, bivalves and copepods in *D. antillarum* (Lewis 1964, Herrera-López et al. 2003). These co-occurring species can coexist due to differences in feeding behavior and differences in internal assimilation mechanisms (Wolf et al. 2009).

The aim of this study was to characterize the diet and trophic behavior of four common sea urchin species inhabiting shallow waters of Puerto Rico. To do this, we applied DNA-metabarcoding of gut samples, using the universal mitochondrial marker cytochrome c oxidase I. We tested for differences in the diet composition among species and at different localities, to assess for possible trophic niche overlapping among these keystone species.

METHODS

Study sites

The Northern coast of Puerto Rico is characterized by a very narrow shelf and high-energy sandy beaches, due to the action of northeast trade winds and North Atlantic winter storms. Substrate composition of sites are mostly carbonate rocks. Due to the high annual precipitation levels, high sediment discharge from rivers is common in the north (U.S.G.S. 1996). Samples were collected during February and October of 2019 from three shallow-water sites (1-2 m depth) of the Northeastern coast of Puerto Rico: Cerro Gordo located in Vega Baja municipality (**CG**, 18°16'51.40"N - 65°17'12.21"W), Isla de Cabra in Toa Baja (**IC**, 18°47'27.72"N -66°13'76.15"W), and Mar Azul in Luquillo (**MA**, 18°23'18.46"N - 65°43'5.52"W).

Sample collection and processing

We collected six adult specimens of the sea urchins *Diadema antillarum*, *Echinometra viridis*, and *Tripneustes ventricosus* in each of the three sites, for a total of 18 individuals per species. We only collected three individuals of *Lytechinus variegatus* in CG and IC (six in total); since this species was not found at MA. These sample sizes are comparable to other molecular diet studies (e.g. Casey et al. 2019; Whitaker et al. 2019). Even though these sizes are somehow smaller than those usually reported in classical gut content analysis, the higher sensitivity of molecular techniques is expected to yield more representative samples from less individuals, since the numbers of detected taxa from each specimen will be orders of magnitude higher. The specimens of *E. lucunter* and *D. antillarum* were collected associated with hard-bottom biotopes (fringing reef), while *L.*

variegatus and *T. ventricosus* were gathered in a back-reef lagoon biotope covered by seagrass (*Thalassia testudinum*) meadows, upon which they are known to graze. Sampling was approved by the Department of Natural and Environmental Resources of Puerto Rico permit number DRNA: 2019-IC-003 issued to Ruber Rodríguez-Barreras. Sea urchins were placed in separate bags with seawater, placed in a foam cooler at the collection site and transported to the laboratory at the UPR Medical Sciences Campus, for processing. In total, 60 individuals were dissected as approved by IACUC permit A5301118 issued to Ruber Rodríguez-Barreras and Filipa Godoy-Vitorino. Gut pellets were extracted and stored in the freezer at -80°C until DNA extraction.

DNA extraction

Gut samples were homogenized at 3000 revolutions per minute (r.p.m.) for 2 min at room temperature in a bead beater (Biospec Products Bartlesville, OK, USA). DNA extractions were done using the Powersoil DNA isolation kit (Qiagen,Carlsbad, CA, USA) according to manufacturer's instructions. The Qubit® dsDNA HS (High Sensitivity) (Thermo fisher Scientific Waltham, Massachusetts, USA) was used to assess DNA concentrations of purified extracts. DNA extraction and pre-PCR preparations were performed in a separate laboratory from post-PCR procedures to avoid contamination.

DNA amplification and high-throughput sequencing

For the evaluation of the sea urchin gut contents (60 samples), DNA samples were amplified using a single set of primers, targeting the mitochondrial cytochrome c oxidase subunit I (COI) region (Leray et al. 2013, Wangensteen et al. 2018a). The Leray-XT primer set amplifies a 313-bp fragment of the COI region in a broad range of taxa including

metazoan and other eukaryotic groups (Wangensteen et al. 2018a). We used the forward primer mlCOIintF-XT (5'-GGWACWRGWTGRACWITITAYCCYCC-3') (Wangensteen et al. 2018), and the reverse primer jgHCO2198 (5'-TAIACYTCIGGRTGICCRAARAAYCA-3' (Geller et al. 2013). These primers have already been successfully applied for the characterization of both marine communities (Wangensteen et al. 2018a, 2018b, Atienza et al. 2020) and gut contents (Macías-Hernández et al. 2018, Siegenthaler et al. 2019a, Kemp et al. 2019). Eight-base oligo-tags (Coissac 2012) attached to the metabarcoding primers were added to the amplicons during a single PCR step, to label different samples in a multiplexed library. A variable number (2, 3 or 4) of fully degenerate positions (Ns) was added at the beginning of each primer, to increase variability of the amplicon sequences (Guardiola et al. 2015).

The PCR mix recipe for the Leray- XT primer set included 10 µl of AmpliTaq gold 360 Master mix (Applied Biosystems), 3.2 µg of Bovine Serum Albumin (Thermo Scientific), 1 µl of each of the 5 µM forward and reverse tagged primers (including 2 - 4 leading Ns and 8- bp sample tags), 5.84 µl H_2O and 2 µl extracted DNA template (standardized to 5 ng/µl) for each sample. The PCR profile included an initial denaturing step of 95°C for 10 min, 35 cycles of 94°C for 1 min, 45°C for 1 min and 72°C for 1 min, and a final extension step of 72°C for 5 min. PCR products (60 samples), including two negative controls and technical replicates of two selected samples, with sample tags attached, were pooled at equimolar concentration into one sample pool and purified using MinElute columns (Qiagen).

The library preparation was conducted using the Next-Flex PCR- free library preparation kit (BIOO Scientific). Library quantification was done using the NEBNext

qPCR quantification kit. The pool was sequenced (final molarity of 20 pM) along with 1% PhiX sequencing control on an Illumina MiSeq using v3 chemistry (2 × 250 bp paired-ends).

Bioinformatic data analyses

Bioinformatic analyses were conducted using the OBITools metabarcoding software suite (Boyer et al. 2016). Read quality assessment was done with FastQC and paired-end read alignment was done using illuminapairedend, reads with alignment quality score > 40 were retained. Demultiplexing and primer removal was done using ngsfilter. Aligned reads with a length of 303–323 bp and free of ambiguous bases were selected using obigrep and dereplicated with obiuniq. The uchime-denovo algorithm (Edgar et al. 2011), implemented in VSEARCH (Rognes et al. 2016) was then used to remove chimeric reads. Remaining COI sequences were clustered into molecular operational taxonomic units (MOTUs) using the SWARM 2.0 algorithm (Mahé et al. 2015) with a d value of 13, successfully applied for the same COI fragment in previous works (Siegenthaler et al. 2019a, Kemp et al. 2019, Garcés-Pastor et al. 2019). This algorithm offers a conservative solution to the high variability of the COI gene (Wangensteen and Turon 2017) for the removal of singletons. Taxonomic assignment of the representative sequences for each MOTU used the ecotag algorithm (Boyer et al. 2016), using a local reference database (Wangensteen et al. 2018a) containing filtered COI sequences retrieved from the BOLD database (Ratnasingham and Hebert 2007) and the EMBL repository (Kulikova et al. 2004). Supernumerary MOTUs arising from putative pseudogenes were collapsed using the LULU algorithm (Frøslev et al. 2017). To compensate for PCR sequencing errors, contaminants, and false positives (Alberdi et al. 2018), we used a total abundance filter, and MOTUs with a total abundance of < 5 reads were removed. All sequences assigned to bacteria and sea urchins were

removed. The final dataset of paired sequences, once quality-filtered, demultiplexed into samples, dereplicated into unique sequences, chimera and pseudogene scanned, and reduced to MOTUs with more than 5 reads and samples with >50 non-predator reads per sample, is available in the Mendeley data repository https://data.mendeley.com/datasets/52py3ps9cr/1

Non-metric multidimensional scaling analysis (MDS) ordinations using Bray-Curtis dissimilarities were performed to visualize potential similarities and differences among sites and localities. Multivariate analyses were conducted based on 4^{th} root-transformed relative abundance of reads (collapsed at the order level) using a two-way PERMANOVA with Bray-Curtis dissimilarities and 1,000 permutations (function: adonis) and pairwise PERMANOVAs (function: pairwise.adonis) were used for post-hoc analyses. Simper (function: simper) was also performed to determine which MOTUs were responsible for significant differences. All analyses used R version 3.1.3 (https://www.R-project.org/) with the package vegan, version 2.3- 5 (Oksanen et al. 2016). Heatmaps were built using custom scripts.

RESULTS

High-throughput DNA sequencing

The number of reads obtained from the MiSeq run after the filtering process (demultiplexing, quality-filtering, sequence- length filtering, and removal of bacterial reads) were 19,250,611. Of these, 18,105,032 reads (94% of the total) were assigned to the four sea urchin species (consumers). The remaining reads (including prey items) were obtained from 50 of the 60 sequenced samples and two PCR-negative controls. No MOTUs were removed during the blank correction, since no MOTU showed abundance in the PCR-negative controls

higher than 10% of the total reads of that MOTU. After removing a total of 6,499 MOTUs with less than 5 reads and samples that yielded less than 50 non-prey reads, we obtained a dataset including 153,393 reads distributed in 691 eukaryotic MOTUs and 50 samples, with an average of 3,068 prey reads/individual. The rarefaction curves for individual samples show that the numbers of detected prey MOTUs is not saturated for most of the samples, indicating that our dataset is not exhaustive and could benefit from further sequencing depth. This is a typical behavior of most COI trophic metabarcoding datasets (**Fig. S1**).

The COI primer pair amplified 24 phyla of algae, flowering plants, invertebrates, fungi and fishes. Of the 691 MOTUs detected, 60 were assigned to the Order level, 38 were assigned to the Class level, 70 were assigned to the family level, 80 to the genus level, and 69 to the species levels.

Description of the sea urchin diet

Seaweed food resources dominated in the four sea urchin species at all three sites, followed by small protists, fungi and metazoans (**Fig. 1**). The diet of the black sea urchin *D. antillarum* was dominated by plants with a relative abundance of 49.2 ± 0.4 %, followed by small protists 36.6 ± 8.7 % (**Table S1**). Fungi and metazoans were the least represented groups with 7.28 ± 5.8 % and 6.91 ± 2.3 % respectively. The diet of the sea urchin *E. lucunter* was dominated by plants with high relative read abundances 62.3 ± 5.1 %, followed by small protists 23.7 ± 5.4 %), metazoans 11.36 ± 5.1 %, and fungi 2.69 ± 1.7 %. The white sea urchin *T. ventricosus* showed high relative read abundances of plants with 65.2 ± 11.9 %, small protists 17.8 ± 13.1 %, fungi 10.1 ± 1.8 %, and metazoans 6.81 ± 1.6%. The diet of the green sea urchin *L. variegatus* was also dominated by plants with 43.2 ± 15.9 %, small protists 36.1 ± 0.42 %, fungi 17.2 ± 15.9 %, while metazoans were the

less represented with 3.54 ± 0.5 %. Intraspecific variation in food resources was also found, but in most individuals the seaweeds and other small eukaryotes represented the highest relative abundances (**Fig. S2)**.

Variation in sea urchin diet/food resources among sites

The heatmap results showed a mixed pattern of food resources with no specific trend, neither among sites nor among species, but with slight differences among relative abundance of dietary taxa (**Fig. 2**). There was no difference in the relative abundances of metazoans in the diet of the four sea urchins among sites. We found that sea urchins from Cerro Gordo contained the lowest relative abundance of metazoans 3.3 ± 1.3 %, while in Mar Azul sea urchins contained the highest relative abundance of metazoans 11.5 ± 1.6 %. Seaweed in the diet remained stable among sites with small changes in relative abundance, with a minimum found in Isla de Cabra 43.2 ± 12.0 %, while Cerro Gordo and Mar Azul were similar between them. In Isla de Cabra we found that fungi and small eukaryotes were particularly abundant, expressing their maximum relative abundances in that site (**Table S1**).

The PERMANOVA analysis detected significant differences among species (Pseudo-F= 1.755, $p < 0.001$), and among sites (Pseudo-F= 2.52, $p < 0.001$); nevertheless, the interaction between sites and species was not significant (Pseudo-F= 1.19, $p < 0.107$). The sea urchin species *L. variegatus* and *T. ventricosus* were not included in this analysis because the number of DNA samples was too low among all sites. Pairwise PERMANOVA results found that sea urchin gut contents were similar among species in Isla de Cabra and Cerro Gordo, but not in Mar Azul (**Table 1**). However, the diet/resources/stomach content differed among *E. lucunter*, *D. antillarum* and *T. ventricosus* at Mar Azul. Furthermore, we

also found spatial differences among sea urchin species but always between different species of different sites (**Table S2**).

The SIMPER analysis found that in all cases the main MOTUs causing differences between species and sites were macroalgae (Phyla Rhodophyta, Chlorophyta and Ochrophyta) and other small eukaryotes (Phyla Apicomplexa and Bacillariophyta). For *E. lucunter*, the major contributor to spatial differences within the species, was the Phylum Rhodophyta. Differences among species in Mar Azul were caused by differences in relative abundances of the Phylum Rhodophyta, as well as higher abundance of metazoans (arthropods and annelids), and the seagrass *Thalassia testudinum* in gut content of *D. antillarum*. The sea urchins *T. ventricosus* and *E. lucunter* differed because of differences in 8 MOTUs of the Rhodophyta and higher abundance of *T. testudinum* in *T. ventricosus*. Differences between *D. antillarum* and *T. ventricosus* in Mar Azul were also caused by MOTUs of the Phylum Rhodophyta as well as higher abundance of metazoans (mollusk, arthropods and annelids) in *D. antillarum* diet. MOTUs belonging to the Phylum Chlorophyta were not among the first 30 MOTUs causing differences among sea urchins in Mar Azul (**Tables S3a-S3g)**. The metric multidimensional scaling analysis (MDS) also found trophic similarities between the four sea urchin species in two of the three sites. Only in Mar Azul trophic segregation among *E. lucunter*, *D. antillarum* and *T. ventricosus* was observed, whereas in Cerro Gordo and Isla de Cabra the clusters of all sea urchin species remained overlapped (**Fig. 3**).

DISCUSSION

Overlapping vs niche-diversification

This study is the first attempt to provide a molecular diet assessment of four Caribbean Sea urchins, and demonstrates the suitability of using DNA metabarcoding to analyze diet composition and relative abundances of ingested items in these species. Niche overlapping found in this study suggests similarities in trophic position among these sea urchins, except in Mar Azul where sea urchins seem to co-exist in distinguishable polygons associated with differential use of food resources (**Fig. 3**). The lack of overlapping between the two species found in Mar Azul could be related to the availability of different food resources and a higher trophic position for *D. antillarum* related to the ingestion of more metazoans. The lack of overlapping diets has been observed in sea urchin populations before, supporting a more omnivorous feeding strategy, as proposed by Rodríguez-Barreras et al. (2015). We could also interpret our results in terms of habitat preservation, mainly due to differences in food resources consumption (see Cabanillas-Terán et al. 2016).

Trophic studies using stable isotopes found similar $\delta^{13}C$ and $\delta^{15}N$ values between *T. ventricosus* and *D. antillarum* (Cocheret de la Morinière et al. 2003, Rodríguez-Barreras et al. 2015, Rodríguez-Barreras et al. 2016), suggesting a similar trophic position for both species (Phillips et al. 2014), despite *T. ventricosus* being found in the seagrass beds behind the backreef zone where *D. antillarum* inhabits. Even though *T. ventricosus* usually grazes on *Thalassia testudinum* leaves, gut content analysis of *T. ventricosus* have found bryozoans, ascidians, and hydrozoans (Barrios and Reyes 2009). After all, the occurrence of small invertebrates in the diet of *T. ventricosus* may be the result of passive ingestion of epibionts (secondary predation), but still it represents an important source of nitrogen that gives this sea urchin a higher trophic position (arthropods and gastropods). *T. ventricosus*

also migrates and/or inhabits in the fore and back reef zones (Bechtel et al. 2004), which could increase the similarities between this species and *D. antillarum* and *E. lucunter*.

Coexistence of species may be possible due to differences in feeding behavior and internal assimilation mechanisms (Wolf et al. 2009), such as in Mar Azul, where ordination differences were found among *E. lucunter*, *T. ventricosus* and *E. lucunter* (**Table S2**). However, our results also showed niche overlapping among three of the four sea urchins at two of the three sites (**Fig. 3**), demonstrating food competition more than differences in feeding behavior. The homogenization of the local food resources pool could be leading to niche overlapping and competition, at least in Cerro Gordo and Isla de Cabra. Interspecific competition is common among sea urchins when food resources are limiting (Privitera et al. 2008, Wangensteen et al. 2011). The lack of samples for *L. variegatus* precludes the analysis between *T. ventricosus* and *L. variegatus*. However, at least for one site, both species exhibited no remarkable differences between them, even when *L. variegatus* is considered a more generalist species than *T. ventricosus* (Keller 1983). Further studies including more specimens of *L. variegatus* should be performed to clarify these relations.

Although *Diadema* and *Echinometra* inhabit in different zones that *Tripneustes* and *Lytechinus*, the trophic overlapping among most sea urchins may indicate a food resource similarity among the four species, instead of a traditional partitioning niche strategy to avoid niche competition among co-occurring species. Similarities between *T. ventricosus*, *D. antillarum* and *E. lucunter* in two of the three sites could be associated with low variability of local food resources despite these species usually inhabit different biotopes.

Omnivory

Sea urchins have been classified as primary foragers, but recent studies considered them as generalist consumers with a plastic feeding behavior (Vanderklift et al. 2006, Wangensteen et al. 2011, Rodríguez-Barreras et al. 2015 and 2016). Based on our results, the four sea urchins studied here can best be described as generalist omnivores. The COI results reveal a clear dominance of seaweeds in the sea urchin diet. For this reason, it has been generalized that sea urchins are among the most important herbivores in the Western Atlantic (Lessios 2016, Gizzi et al. 2020), capable of controlling seaweeds communities. However, seaweeds communities can also affect ingestion and assimilation food patterns in sea urchins (Cabanillas-Terán et al. 2019). The species *D. antillarum* is considered a keystone herbivore (Bodmet et al. 2015). Nevertheless, previous studies have found small-sized metazoans such as sponges, hydroids, bryozoans, nematodes, rotifers, gastropods, bivalves, and copepods in diet analyses, representing up to 32 % of the gut species-richness index (Tuya et al. 2001, Hernández et al. 2007, Cabanillas-Terán et al. 2009), suggesting that secondary predation of metazoans can contribute significantly to the diet of this species, and may not be an accidental ingestion after all. Stable isotope studies have suggested a more omnivorous trophic level for *D. antillarum* (Rodríguez-Barreras et al. 2015), reinforcing the importance of benthic invertebrates as potential nitrogen sources.

The potential omnivory of *D. antillarum* in the Caribbean (Rodríguez-Barreras et al. 2015; Rodríguez-Barreras et al. 2016), and a similar pattern found in the Mediterranean sea urchin *Arbacia lixula* (Wangensteen et al. 2011), challenge the widely accepted classification of sea urchins as herbivores. The high relative reads abundance from metazoans found in this study (**Fig. 1**) must be considered further evidence supporting a more generalist omnivorous diet for Caribbean Sea urchins. Relative abundances of

metazoans were similar among *D. antillarum*, *T. ventricosus* and *L. variegatus*. The sea urchin *E. lucunter* reached even higher relative abundances of metazoans than *D. antillarum*, suggesting a widespread omnivore strategy among sea urchins in the Caribbean.

The occurrence of small eukaryotes and fungi must be interpreted with caution, it is possible that these taxa were passively consumed during the ingestion, and more related with the epibiotic communities associated with algae and plant communities, symbiotic relationships or internal pathogens, and not food resources *per se*. Also, the detection of fish taxa in sea urchin diet should not be interpreted as whole individuals. Fish fragments, like scales or dead tissue, could be ingested while foraging, not by active or selective capture. Nevertheless, gastropods, arthropods, and worms could be captured and ingested alive as part of the food bulk.

Abiotic factors, such as salinity, grain size, pH, temperature, and tidal cycles, are important drivers for the spatial variability, but may also contribute to changes in the benthic communities (García et al. 2018, Limatola et al. 2020). Measurements of salinity, water temperature, and pH were taken as part of this study exhibiting similar values among the three sites. The lack of local variability on these physicochemical factors could be related to geographical proximity. Nevertheless, the fact that Cerro Gordo contained the lowest relative abundance of metazoans and more similarities among the other two sites could be linked with the existence of a mangrove lagoon canal 270 meters from the collecting site in CG, while IC and MA are less influenced by inland discharges from interior lagoons or streams. Mangrove systems provide nutrient inputs to nearshore waters (Rezende et al. 1990), promoting a robust seaweed community and more non-animal resources for sea urchins.

Advantages and disadvantages

This study has contributed to unraveling the trophic preferences of Caribbean Sea urchins, increasing the diversity of known digested items commonly referred as soft bodied metazoans, food resources usually classified as "unidentifiable items" using the traditional methods. Although DNA metabarcoding for diet identification is more expensive than traditional methods, this method has the potential to expand our knowledge of ecological interactions like predation, ecophysiology and dynamics to assess consumer/prey- related biases (Schnell et al. 2015, Siegenthaler et al. 2019a). However, DNA metabarcoding can be used on diet studies to improve diet content characterization, and relative abundances of digested material at reasonable costs. Diet assessment applying molecular tools like DNA metabarcoding represents a novel approach, with advantages over standard methods (Siegenthaler et al. 2019a, 2019b).

The lack of identification to the species level for much of the sequenced DNA fragments highlights the importance of continuous improvements of the reference databases to increase the taxonomic accuracy of the marker. A correct separation of gut contents (pellets) from the digestive tissues of individuals is also important. The hundreds of thousands of useful DNA reads found in this study, instead of millions, may be a result of a high proportion of gut tissue due to suboptimal separation procedures during sample preparation. However, our results show that DNA metabarcoding of trophic samples was more effective in determining the food resources used for sea urchins than traditional morphological methods (Herrera-López et al. 2003, Barrios and Reyes 2009), in line with other studies on the use of metabarcoding to identity food resources (Pompanon et al. 2012, De Barba et al. 2014).

To conclude, the present study represents baseline information for future evaluation of sea urchin diets. The molecular diet characterization of four Caribbean Sea urchin species revealed that these species have a generalist omnivorous behavior, with seaweeds as the dominant food items across species and at all three sites. But it was also revealed that small protists, fungi, and metazoans represent important components in sea urchin's diet. The existence of a potential interspecific competition due to niche overlapping seems to be more common than initially thought. Further studies would be necessary to evaluate the importance of small-sized metazoans in diet assimilation and contribution.

Acknowledgements

The authors are thankful to E. Tosado-Rodríguez and J. Hernández-González for their support during sampling in Puerto Rico, and their assistance in the laboratory processing samples and extracting DNA.

References

Alberdi, A., Aizpurua, O., Gilbert, M. T. P., & Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution*, 9(1), 134-147.

Alvarado, J. J. (2011). Echinoderm diversity in the Caribbean Sea. *Marine Biodiversity*, *41*(2), 261-285.

De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., & Taberlet, P. (2014). DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: application to omnivorous diet. *Molecular Ecology Resources*, 14(2), 306-323.

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, *17*(1), 1-17.

Barrios, J., & Reyes, J. (2009). Hábitos alimenticios de *Tripneustes ventricosus* (Lamarck, 1816) (Echinodermata, Echinoidea) en Isla Tortuga, Venezuela. *Foro Iberoam Rec Mar Acuicult II*, 583-589.

Bechtel, J. D., Gayle, P., & Kaufman, L. (2004). The return of *Diadema antillarum* to Discovery Bay: patterns of distribution and abundance, 367-375.

Berry, O., Bulman, C., Bunce, M., Coghlan, M., Murray, D. C., & Ward, R. D. (2015). Comparison of morphological and DNA metabarcoding analyses of diets in exploited marine fishes. *Marine Ecology Progress Series*, *540*, 167-181.

Bodmer, M. D., Rogers, A. D., Speight, M. R., Lubbock, N., & Exton, D. A. (2015). Using an isolated population boom to explore barriers to recovery in the keystone Caribbean coral reef herbivore *Diadema antillarum*. *Coral Reefs*, *34*(4), 1011-1021.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). OBITOOLS: A unix- inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*, 176–182.

Cabanillas Terán, N. (2009). Ecología y estatus trófico del erizo de mar *Diadema antillarum* (Philippi, 1845) en los fondos rocosos de las Islas Canarias (Gran Canaria, España).

Cabanillas-Terán, N., Loor-Andrade, P., Rodríguez-Barreras, R., & Cortés, J. (2016). Trophic ecology of sea urchins in coral-rocky reef systems, Ecuador. *PeerJ*, *4*, e1578.

Cabanillas-Terán, N., Hernández-Arana, H. A., Ruiz-Zárate, M. Á., Vega-Zepeda, A., & Sanchez-Gonzalez, A. (2019). Sargassum blooms in the Caribbean alter the trophic structure of the sea urchin *Diadema antillarum*. *PeerJ*, *7*, e7589.

Casey, J. M., Meyer, C. P., Morat, F., Brandl, S. J., Planes, S., & Parravicini, V. (2019). Reconstructing hyperdiverse food webs: Gut content metabarcoding as a tool to disentangle trophic interactions on coral reefs. *Methods Ecol. Evol. 10*, 1157–1170.

Coissac E. (2012) OligoTag: a program for designing sets of tags for next-generation sequencing of multiplexed samples. *Methods Mol Biol*. *888*, 13–31.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, *27*(16), 2194–2200. https://doi.org/10.1093/bioinformatic s/btr381

Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C. and Hansen, A. J. (2017) Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. *Nature Communications*, 8, 1188.

Garcés-Pastor, S., Wangensteen, O. S., Pérez-Haase, A., Pèlachs, A., Pérez-Obiol, R., Cañellas-Boltà, N., Mariani, S., Vegas-Vilarrúbia, T. (2019) DNA metabarcoding reveals modern and past eukaryotic communities in a high mountain peat bog system. *J Paleolimnol*, in press. doi: 10.1007/s10933-019-00097-x

García, E., Clemente, S., & Hernández, J. C. (2018). Effects of natural current pH variability on the sea urchin Paracentrotus lividus larvae development and settlement. *Marine environmental research*, *139*, 11-18.

Geller, J., Meyer, C., Parker, M., & Hawk, H. (2013). Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all- taxa biotic surveys. *Molecular Ecology Resources*, *13*, 851–861.

Gizzi, F., Jiménez, J., Schäfer, S., Castro, N., Costa, S., Lourenço, S., ... & Monteiro, J. (2020). Before and after a disease outbreak: Tracking a keystone species recovery from a mass mortality event. *Marine Environmental Research*, *156*, 104905.

Guardiola, M., Uriz, M. J., Taberlet, P., Coissac, E., Wangensteen, O. S., & Turon, X. (2015). Deep-sea, deep-sequencing: metabarcoding extracellular DNA from sediments of marine canyons. *PLoS One*, *10*(10), e0139633.

Hendler, G. (1995). *Sea stars, sea urchins, and allies: echinoderms of Florida and the Caribbean* (No. Sirsi) i9781560984504).

Hernández, J. C., Gil-Rodriguez, M. C., Herrera-López, G., & Brito, A. (2007). Diet of the "key herbivore" *Diadema antillarum* in two contrasting habitats in the Canary Islands (Eastern-Atlantic). *Vieraea*, *35*, 109-120.

Herrera-López, G. Reyes, A. C., Pérez, J. C. H., Lorenzo, J. G. G., & Hernández, N. G. (2003). Alimentación y diversidad algal en la dieta del erizo Diadema antillarum en Tenerife, Islas Canarias. *Revista de la Academia Canaria de Ciencias: Folia Canariensis Academiae Scientiarum*, *15*(3), 129-141.

Herrera-López, G., Reyes, A. C., Pérez, J. C. H., Lorenzo, J. G. G., & Hernández, N. G. (2003). Alimentación y diversidad algal en la dieta del erizo *Diadema antillarum* en Tenerife, Islas Canarias. *Revista de la Academia Canaria de Ciencias: Folia Canariensis Academiae Scientiarum*, *15*(3), 129-141.

Hughes, T. P., Reed, D. C., & Boyle, M. J. (1987). Herbivory on coral reefs: community structure following mass mortalities of sea urchins. *Journal of Experimental Marine Biology and Ecology*, *113*(1), 39-59.

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., ... & Pringle, R. M. (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences*, *112*(26), 8019-8024.

Keller, B. D. (1983). Coexistence of sea urchins in seagreas meadows: an experimental analysis of competition and predation. *Ecology*, *64*(6), 1581-1598.

Kemp, J., López-Baucells, A., Rocha, R., Wangensteen, O. S., Andriatafika, Z., Nair, A., Cabeza, M. (2019) Bats as potential suppressors of multiple agricultural pests: a case study from Madagascar. *Agric, Ecosys Environ* 269, 88-96. https://doi.org/10.1016/j.agee.2018.09.027

Kulikova, T., Aldebert, P., Althorpe, N., Baker, W., Bates, K., Browne, P.,… Apweiler, R. (2004). The EMBL nucleotide sequence database. *Nucleic Acids Research*, 32, D27–D30. https://doi.org/10.1093/nar/gkh120

Lasley-Rasher, R. S., Brady, D. C., Smith, B. E., & Jumars, P. A. (2015). It takes guts to locate elusive crustacean prey. *Marine Ecology Progress Series*, *538*, 1-12

Leray, M., & Knowlton, N. (2015). DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 2076–2081. https://doi.org/10.1073/pnas.1424997112

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V.,… Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, *10*, 1–14. https://doi.org/10.1186/1742-9994-10-34

Lessios, H. A. (2016). The great *Diadema antillarum* die-off: 30 years later. *Annual review of marine science*, *8*, 267-283.

Lewis, J. B. (1964). Feeding and digestion in the tropical sea urchin *Diadema antillarum* Philippi. *Canadian Journal of Zoology*, 42(4), 549-557.

Limatola, N., Chun, J. T., & Santella, L. (2020). Effects of Salinity and pH of Seawater on the Reproduction of the Sea Urchin Paracentrotus lividus. *The Biological Bulletin*, *239*(1), 000-000.

Macías-Hernández, N., Athey, K., Tonzo, V., Wangensteen, O. S., Arnedo, M. A., Harwood, J. D. (2018) Molecular gut content analysis of different spider body parts. PloS ONE 13, e0196589. doi:10.1371/journal.pone.0196589Mahé, F., Rognes, T., Quince, C., de Vargas, C., & Dunthorn, M. (2015). Swarm v2: Highly- scalable and high- resolution amplicon clustering. *PeerJ*, *3*, e1420. https://doi.org/10.7717/peerj.1420

McClenaghan, B., Gibson, J. F., Shokralla, S., & Hajibabaei, M. (2015). Discrimination of grasshopper (Orthoptera: Acrididae) diet and niche overlap using next- generation

sequencing of gut contents. *Ecology and Evolution*, *5*, 3046–3055. https://doi.org/10.1002/ece3.1585

McCutchan Jr, J. H., Lewis Jr, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, 102(2), 378-390.

Moberg, F., & Folke, C. (1999). Ecological goods and services of coral reef ecosystems. *Ecological economics*, 29(2), 215-233.

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2016). *Vegan: Community ecology package* (pp. 631–637).

Pérez- Portela, R., Riesgo, A., Wangensteen, O. S., Palacín, C. & Turon, X. (2020). Enjoying the warming Mediterranean: Transcriptomic responses to temperature changes of a thermophilous keystone species in benthic communities. *Molecular Ecology* 29, 3299–3315.

Phillips, D. L., Gregg, J. W. (2003). Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136, 261-269.

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., ... & Ward, E. J. (2014). Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology*, 92(10), 823-835.

Pompanon, F., Deagle, B. E., Symondson, W. O., Brown, D. S., Jarman, S. N., & Taberlet, P. (2012). Who is eating what: diet assessment using next generation sequencing. *Molecular ecology*, 21(8), 1931-1950.

Precht, L. L., & Precht, W. F. (2015). The sea urchin *Diadema antillarum*–keystone herbivore or redundant species?. *PeerJ PrePrints*, *3*, e1565v1.

Privitera, D., Chiantore, M., Mangialajo, L., Glavic, N., Kozul, W., & Cattaneo-Vietti, R. (2008). Inter-and intra-specific competition between *Paracentrotus lividus* and *Arbacia lixula* in resource-limited barren areas. *Journal of Sea Research*, 60(3), 184-192.

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, 7, 355–364.

Rezende, C. E., Lacerda, L. D., Ovall, A. R. C., Silva, C. A. R., & Martinelli, L. A. (1990). Nature of POC transport in a mangrove ecosystem: a carbon stable isotopic study. *Estuarine, Coastal and Shelf Science*, *30*(6), 641-645.

Rodríguez-Barreras, R., Pérez, M. E., Mercado-Molina, A. E., Williams, S. M., & Sabat, A. M. (2014). Higher population densities of the sea urchin *Diadema antillarum* linked to wave sheltered areas in north Puerto Rico Archipelago. *Journal of the Marine Biological Association of the United Kingdom*, 94(8), 1661-1669.

Rodríguez-Barreras, R., Cuevas, E., Cabanillas-Terán, N., & Branoff, B. (2016). Understanding trophic relationships among Caribbean sea urchins. *Revista de biologia tropical*, *64*(2), 837-848.

Rodríguez-Barreras, R., Cuevas, E., Cabanillas-Terán, N., & Sabat, A. M. (2015). Potential omnivory in the sea urchin Diadema antillarum?. *Regional Studies in Marine Science*, *2*, 11-18.

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahe, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Siegenthaler, A., Wangensteen, O. S., Benvenuto, C., Campos, J., & Mariani, S. (2019a). DNA metabarcoding unveils multiscale trophic variation in a widespread coastal opportunist. *Molecular ecology*, 28(2), 232-249.

Siegenthaler, A., Wangensteen, O. S., Soto, A. Z., Benvenuto, C., Corrigan, L., & Mariani, S. (2019b). Metabarcoding of shrimp stomach content: Harnessing a natural sampler for fish biodiversity monitoring. *Molecular ecology resources*, 19(1), 206-220.

Schnell, I. B., Sollmann, R., Calvignac-Spencer, S., Siddall, M. E., Douglas, W. Y., Wilting, A., & Gilbert, M. T. P. (2015). iDNA from terrestrial haematophagous leeches as a wildlife surveying and monitoring tool–prospects, pitfalls and avenues to be developed. *Frontiers in zoology*, *12*(1), 24.

Symondson, W. O. C. (2002). Molecular identification of prey in predator diets. *Molecular ecology*, 11(4), 627-641.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next- generation biodiversity assessment using DNA metabarcoding. *Molecular ecology*, 21(8), 2045-2050.

Tuohy, E., Wade, C., & Weil, E. (2020). Lack of recovery of the long-spined sea urchin Diadema antillarum Philippi in Puerto Rico 33 years after the Caribbean-wide mass mortality. *PeerJ*, *8*, e8428.

U.S.G.S. (1996). Atlas of ground-water resources in Puerto Rico and the U.S. Virgin Islands. Thalia D. Veve and Bruce E. Taggart (Edts). Water resources investigation report, 94-4198, 151 pp.

Valentini, A., Pompanon, F., & Taberlet, P. (2009). DNA barcoding for ecologists. *Trends in ecology & evolution*, 24(2), 110-117.

Van Dover, C. L., Grassle, J. F., Fry, B., Garritt, R. H., & Starczak, V. R. (1992). Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. *Nature*, *360*(6400), 153.

Vanderklift, M. A., & Kendrick, G. A. (2005). Contrasting influence of sea urchins on attached and drift macroalgae. *Marine Ecology Progress Series*, 299, 101-110

Wangensteen, O. S. (2013). Biology and phylogeography of the black sea urchin *Arbacia lixula* (Echinoidea: Arbacioida). PhD thesis. Universitat de Barcelona. 2013.

Wangensteen, O. S., Palacín, C., Guardiola, M., & Turon, X. (2018a). DNA metabarcoding of littoral hard- bottom communities: High diversity and database gaps revealed by two molecular markers. *PeerJ*, *6*, e4705. https://doi.org/10.7717/peerj.4705

Wangensteen, O.S., Cebrian, E., Palacín, C., Turon, X. (2018b) Under the canopy: community-wide effects of invasive seaweeds in marine protected areas revealed by metabarcoding. *Mar Poll Bull*, 127: 54–66. doi.org/10.1016/j.marpolbul.2017.11.033

Wangensteen, O. S., Turon, X., García-Cisneros, A., Recasens, M., Romero, J., & Palacín, C. (2011). A wolf in sheep's clothing: carnivory in dominant sea urchins in the Mediterranean. *Marine Ecology Progress Series*, 441, 117-128.

Wangensteen, O., & Turon, X. (2017). Metabarcoding techniques for assessing biodiversity of marine animal forests. In S. Rossi, L. Bramanti, A. Gori, & C. Orejas (Eds.), *Marine animal forests. The ecology of benthic biodiversity hotspots* (pp. 445–473). Cham, Switzerland: Springer International Publishing.

Whitaker, M. R., Baker, C. C., Salzman, S. M., Martins, D. J., & Pierce, N. E. (2019). Combining stable isotope analysis with DNA metabarcoding improves inferences of trophic ecology. *PLoS ONE 14*, e0219070.

Wolf, N., Scott, A., & Martínez del Rio C. (2009). Nutritional ecology: Ten years of experimental animal isotopic ecology. *Functional Ecology*, 23, 17-26.

Figures 1, 2 & 3

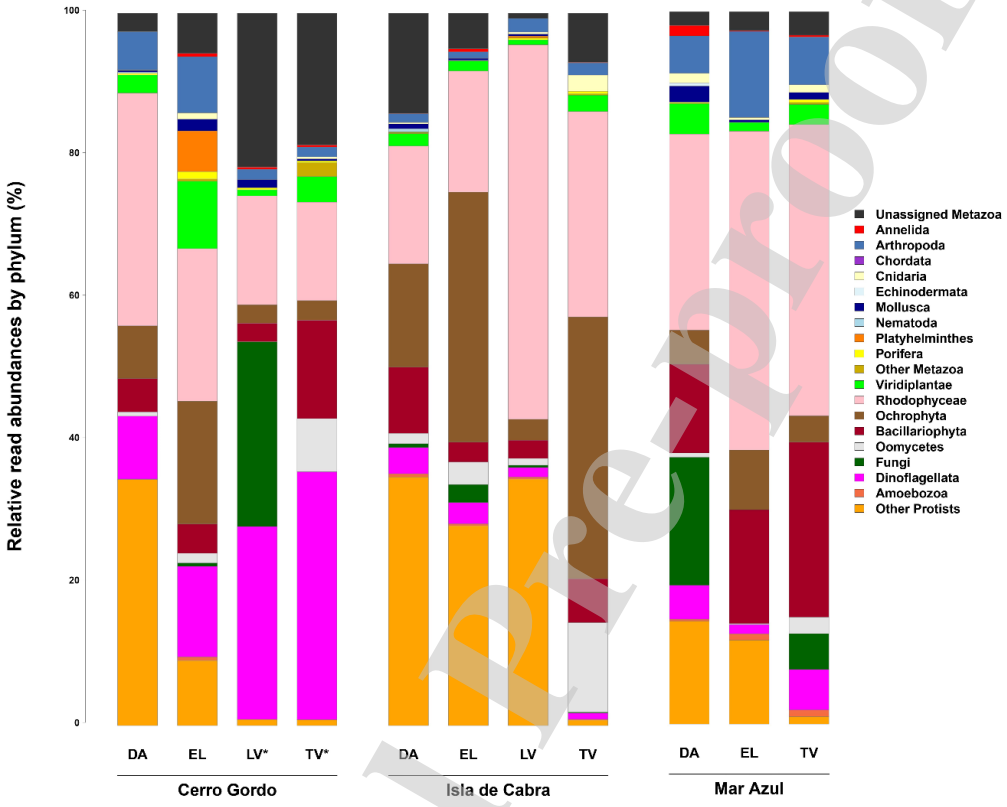


Figure 1. Relative abundances of MOTUs detected in stomach samples by COI metabarcoding of four Caribbean sea urchins *Diadema antillarum* (DA), *Echinometra lucunter* (EL), *Lytechinus variegatus* (LV), and *Tripneustes ventricosus* (TV) in Puerto Rico. Each bar represents one species, and sites are shown on top of the graph. (*) *L. variegatus* and *T. ventricosus* are represented for only one individual in Cerro Gordo.

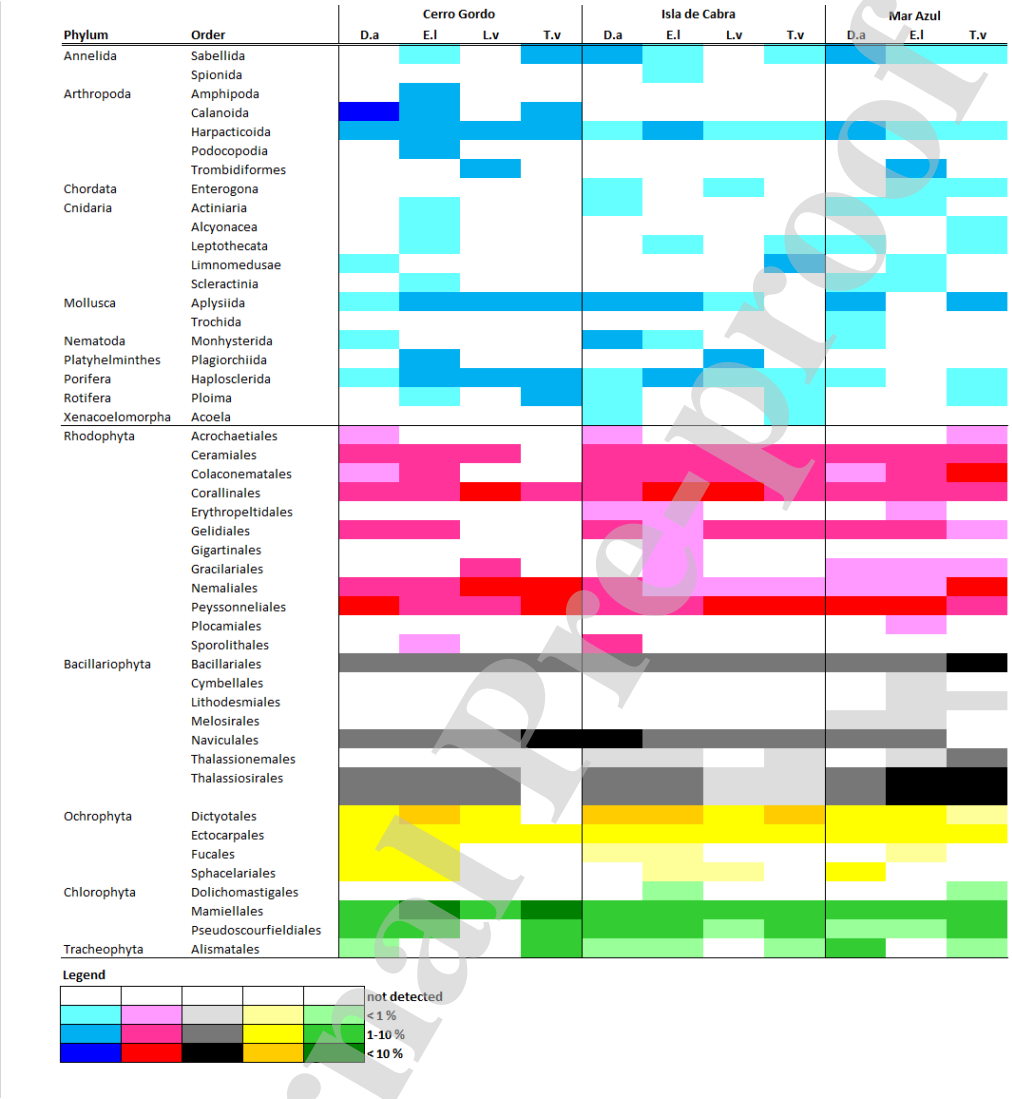


Figure 2. Heatmap of phyla and orders detected in samples of three sites in Puerto Rico. Colors represent differences in relative read abundances after DNA amplification using COI marker. Kingdoms are represented with a different set of color; blues for metazoans, reds and pinks for Rhodophyta, black and grays for Bacillariophyta, yellows for Ochrophyta, and greens for Chlorophyta and Tracheophyta.

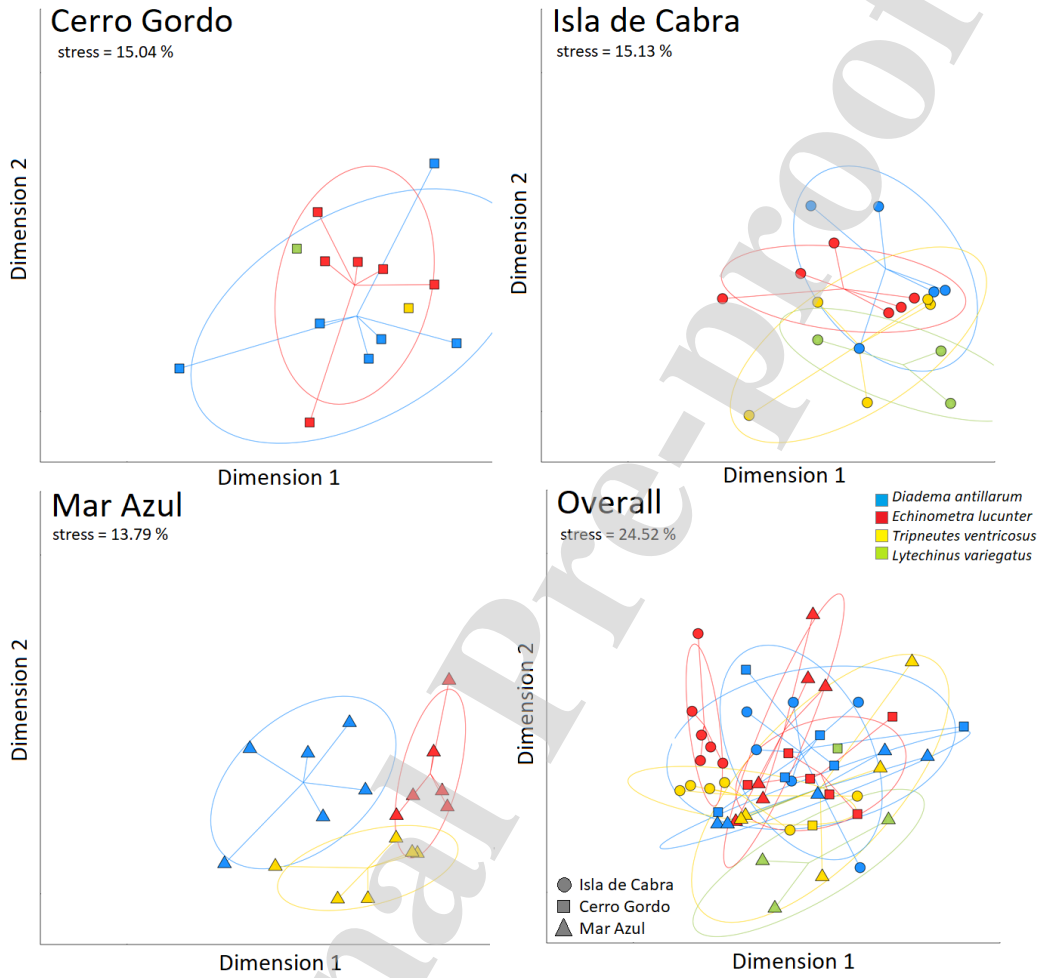


Figure 3. nMDS ordinations (Bray-Curtis) for cytochrome-c oxidase subunit I (COI) gene sequences of four sea urchins *Diadema antillarum*, *Echinometra lucunter*, *Lytechinus variegatus* and *Tripneustes ventricosus*, collected from three sites of Puerto Rico. Circles, triangles and squares identify individuals of each species. In each case, individuals of the same species grouped together.

Table 1. Two-way PERMANOVA results of differences between species within the same site. COI samples were not tested for sea urchin *Lytechinus variegatus* at any site, neither for *Tripneustes ventricosus* in Cerro Gordo, since DNA sample were one or cero. F is a pseudo-F and p the significant level.

Cerro Gordo	Samples	*D. antillarum*	*T. ventricosus*	*E. lucunter*
D. antillarum	6			
E. lucunter	6	F= 1.24, p= 0.17	-	
Isla de Cabra	Samples	*D. antillarum*	*T. ventricosus*	*E. lucunter*
D. antillarum	5			
T. ventricosus	5	F= 0.82, p= 0.60		
E. lucunter	6	F= 1.18, p= 0.24	F= 1.08, p= 0.34	
Mar Azul	Samples	*D. antillarum*	*T. ventricosus*	*E. lucunter*
D. antillarum	6			
T. ventricosus	6	F= 1.81, p= 0.04 *		
E. lucunter	6	F= 2.23, p= 0.01 *	F= 2.15, p= 0.02 *	

HIGHLIGHTS

- We assess the trophic ecology of four Caribbean sea urchin species using DNA metabarcoding.
- Our results reveal a generalist omnivore behavior, but with a clear dominance of algae in sea urchin diet.
- Interspecific differences in diet were also found among sites.
- Our results show a significant difference among species, and among sites.

AUTHOR STATEMENT

Manuscript title:

DNA metabarcoding unveils niche overlapping and competition among Caribbean sea urchins

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Thus, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication except in the Journal of Regional Studies in Marine Science. The name of each author must appear at least once in each of the following categories:

Conception and design of the study: Ruber Rodríguez Barreras and Owen Wangensteen

Acquisition of data: Ruber Rodríguez Barreras and Filipa Godoy-Vitorino

Pre-sequencing sample processing: Ruber Rodríguez Barreras and Filipa Godoy-Vitorino

Sample sequencing and data analysis: Owen Wangensteen and Ruber Rodríguez Barreras

Interpretation of data: Owen Wangensteen, Ruber Rodríguez Barreras, Filipa Godoy-Vitorino and Kim Præbel

Drafting the manuscript: Owen Wangensteen Ruber Rodríguez Barreras, Filipa Godoy-Vitorino and Kim Præbel

Revising the manuscript critically for important intellectual content: Owen Wangensteen Ruber Rodríguez Barreras, Filipa Godoy-Vitorino and Kim Præbel

Ruber Rodríguez-Barreras
University of Puerto Rico at Bayamón
Industrial Minillas 174 PR-170, Bayamón, PR, 00959
ruber.rodriguez@upr.edu

September 26th, 2020

To:
Jong Seong Khim, Ph.D.
Editor-in-Chief
Regional Studies in Marine Science

Dear Dr., Khim,

I want to confirm that the author enrolled in this research has no conflict of interest. There are not any financial and personal relationships with other people or organizations that could inappropriately influence this study. Thus, all authors have contributed in different ways to this manuscript. See the following table for details.

Authors	Conceptualization	Field work	DNA extraction	Samples processing	Data analysis	Writing process	Funding acquisition
Ruber Rodríguez Barreras	X	X	X	X	X	X	
Filipa Godoy Vitorino	X	X	X			X	X
Kim Præbel				X	X	X	X
Owen S. Wangensteen	X			X	X	X	X

Sincerely,

Ruber Rodríguez-Barreras