

# Sifting environmental DNA metabarcoding data sets for rapid reconstruction of marine food webs

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## Abstract

Marine ecosystems are changing rapidly due to ocean warming, overfishing and a raft of other anthropogenic impacts. Such changes are expected to disrupt productivity dynamics and alter marine food webs, with likely negative consequences for ecosystem services. It is, therefore, essential to devise and implement methods that can rapidly and inexpensively monitor changes in the marine food web structure. Unfortunately, conventional methods for surveying marine food webs are typically laborious, expensive and often destructive, resulting in only a small fraction of marine ecosystems being well studied, and an even smaller subset of them being studied through time. Here, we pilot a low-cost approach to reconstructing trophic networks of marine tropical, temperate and polar regions, using taxonomical inventories arising from published environmental DNA (eDNA) metabarcoding studies, and building trophic links based on primary literature information. Although the trophic webs obtained are a simplified approximation of those constructed with traditional methods, they generate realistic networks that fit with expectations, and allow ecological inference over time scales and costs that are orders of magnitude smaller than that traditionally achieved. We show the potential of a new application of environmental DNA analysis that promises to offer a rapid and scalable approach to gather vital information on ecosystem structure, hence boosting marine monitoring at a time of increasingly rapid environmental changes.

## KEYWORDS

anthropogenic impacts, biodiversity, ecosystem structure, eDNA, marine monitoring, trophic webs

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## Etymology of Ghoti

George Bernard Shaw (1856-1950), polymath, playwright, Nobel prize winner, and the most prolific letter writer in history, was an advocate of English spelling reform. He was reportedly fond of pointing out its absurdities by proving that 'fish' could be spelt 'ghoti'. That is: 'gh' as in 'rough', 'o' as in 'women' and 'ti' as in palatal.

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## 1 | FOOD WEBS IN THE CONTEXT OF MARINE MANAGEMENT

Food webs are biological portrayals of natural communities, focusing on networks of trophic interactions, and typically defined by their structure and dynamics (Link et al., 2005). Since Darwin's famous consideration of the "tangled bank" (Darwin, 1859), this idea of an intricate web of links among species has served as a paradigm in ecology and progressively gained consideration in conservation management, as a means to monitor the health of ecosystems and their responses to environmental changes (Bascompte, 2009; Rogers et al., 2010; Sala & Sugihara, 2005). Indeed, changes in species composition and relative abundance, in response to climatic and anthropogenic impacts may affect trophic relationships in several sections of a food web, altering energy flows at multiple trophic levels (Rogers et al., 2010). Events such as ocean warming and acidification (Koenigstein et al., 2016), overfishing (Mullon et al., 2005), habitat destruction (Layman et al., 2007), invasions of alien species (Papacostas & Freestone, 2019) can all reduce or modify biodiversity, potentially disrupting trophic interactions and food web structure (Kimbro et al., 2009; Myers et al., 2007), and leading to detrimental consequences for ecosystem functions and services (Chen et al., 2016; Daskalov, 2002).

Though some of the earliest trophic web studies had considered marine habitats (Hardy, 1924; Petersen, 1918), food web analysis has had greater popularity in terrestrial and freshwater contexts, due to the intrinsic difficulties that marine systems present (Link et al., 2005). Indeed, the study of marine food webs has always been challenging for several reasons: expensive sampling cost and inaccessibility (Bicknell et al., 2016), species dispersed over broad areas due to physical (Carr et al., 2003) and biological processes (Block et al., 2011), higher functional diversity and trophic web complexity compared to terrestrial habitats (Cohen, 1994; Link et al., 2005).

To better understand the complexity of oceans, marine conservation and fisheries management have, over decades, gradually shifted their focus from single-species to ecosystem-based approaches (Garcia, 2003; Jennings, 2005), and in this context, trophic interconnections have become increasingly important descriptors in the quest to evaluate and reduce the ecological impacts of human activities (Rogers et al., 2010; Sala & Sugihara, 2005). However, a major barrier to the implementation of food web descriptors in marine management strategies is the requirement of comprehensive data sets on consumer–resource relationships for the species within the food web (Moloney et al., 2011), especially for the ones at lower trophic levels. Therefore, further information on species composition and their interactions is critical to inform policy decisions and assess the efficacy of conservation/management interventions (Douvere & Ehler, 2011). Considering the velocity of anthropogenic impacts (Halpern et al., 2019), the necessity for rapid assessment and monitoring of marine trophic webs is at odds with the time and resources required to generate the necessary data, making management action less effective. Thus, it remains a priority to improve and

refine the tools to reconstruct marine food webs for monitoring marine ecosystems.

## 2 | A PATH FOR MARINE FOOD WEB RECONSTRUCTION

### 2.1 | From stomach contents to stable isotopes

The initial attempts to unravel the complexity of marine ecological networks were based on in situ observational studies (Bailey et al., 2007) and stomach content analysis (Amundsen & Sánchez-Hernández, 2019; Hyslop, 1980). Although these continue to have an important role in marine ecology, they often remain time-consuming, subject to bias (Amundsen & Sánchez-Hernández, 2019; Baker et al., 2014) and generally require large sample sizes and lethal sampling (Vinson & Budy, 2011). In 1978, in a momentous shift in the field of ecology, DeNiro and Epstein stated that "organisms are what they eat, isotopically" (DeNiro & Epstein, 1978). This paved the way for the expansion of a novel way of studying trophic links, based on the recording of Carbon ( $\delta^{13}\text{C}$ ) and Nitrogen ( $\delta^{15}\text{N}$ ) stable isotope ratios of consumers, which were found to mirror those of their diets (DeNiro & Epstein, 1978, 1981; Fry, 2006). Isotope analysis has had a remarkable impact on food web studies, by improving diet reconstruction (e.g. Matley et al., 2018), elucidating niche space (e.g. Layman et al., 2007) and building food webs (e.g. Fry, 2006). Their key advantage is the fact that isotopic signatures reflect resource consumption over an extended period (up to several months), hence being more suited for developing robust models of trophic structure.

Despite this, stable isotopes have some limitations in the study of complex environments such as entire marine ecosystems, as they are constrained by metabolic assumptions, prey identification resolution, and the invasive sampling required. Indeed, isotopic enrichment is subject to many variables (Fry, 2006; Lecomte et al., 2011; Post et al., 2007), and a common problem is that the range of possible signatures is too narrow for capturing the astounding diversity of food sources in the ocean. Importantly, only a small number (for trophic studies, typically  $^{13}\text{C}$  and  $^{15}\text{N}$ ) of isotopes are generally used, which do not allow accurate estimation of proportions if the number of prey types is larger than four or five, or if sources have similar isotopic signatures (Moore & Semmens, 2008; Phillips & Gregg, 2003). Furthermore, the accuracy of trophic networks depends on the resolution of node selection: that is, not just "who eats whom" but "who is who" (Martinez, 1993). More specifically, species identification involves highly specialized, long-term, collaborative taxonomic work, which does not always lead to clear taxon resolution (Roslin & Majaneva, 2016), assigning the various web nodes to categories that are often taxonomically and functionally too broad to be fully informative (Perez-Matus et al., 2017). Finally, the stable isotope approach requires repeated invasive temporal sampling, in order to capture the whole range of potential prey items in the system under study, making it time-consuming, costly and highly reliant on complementary techniques (Nielsen et al., 2018; Vinson & Budy, 2011).

## 2.2 | Metabarcoding in dietary studies

Over the last decade, there has been a rapid expansion of massively parallel, high-throughput sequencing technologies (Bik et al., 2012; Shokralla et al., 2012), which have drastically reduced the cost and time required for generating large and comprehensive species inventories. The approach of simultaneously identifying several taxa from bulk samples through the parallel sequencing of DNA barcodes (the DNA metabarcoding approach) (Bohmann et al., 2014; Taberlet et al., 2012; Valentini et al., 2016) facilitates a faster and more standardized sampling, enhancing spatial and temporal resolution, and reducing reliance on traditional taxonomic approach (Cordier et al., 2017; Pawlowski et al., 2018; Seymour et al., 2020).

Furthermore, DNA metabarcoding has been rapidly embraced for the study of animal diets (Bohmann et al., 2014), hence vastly expanding the range of diversity available for scrutiny in gut contents of consumers, using a tool that is universal across the tree of life (Meyer et al., 2020; Siegenthaler et al., 2019). DNA-based stomach content analysis approaches have boosted interest in dietary studies, particularly owing to the higher taxonomic accuracy afforded, which can improve our understanding of trophic links that would otherwise remain obscure using traditional methods (De Barba et al., 2014; Meyer et al., 2020; Nielsen et al., 2018). This has been especially important for species whose diet is particularly problematic to assess, for instance, due to highly diverse consumer–resource systems composed of cryptic species (Leray et al., 2015), or as a result of prey deterioration in dietary remains such as stomach contents, regurgitates and scats (e.g. Kaunisto et al., 2017; McInnes et al., 2017; Nielsen et al., 2018), or for species that forage widely in remote environments and are therefore difficult to study (Walters et al., 2019).

The accuracy and affordability of DNA barcoding open new research avenues for the study of trophic interactions by allowing comparative studies of trophic networks in space and time (Smith et al., 2011). First attempts to reconstruct a whole trophic web via DNA metabarcoding have been made in well-studied ecosystems (Casey et al., 2019; Smith et al., 2011) and low-diversity terrestrial environments such as the Arctic (Wirta et al., 2015). DNA metabarcoding also offers unprecedented resolution in unravelling feeding associations among hosts and parasitoids (Smith et al., 2011), generalist consumers and their resources (De Barba et al., 2014; Meyer et al., 2020; Wirta et al., 2015), and omnivorous predators, within large, hyper-diverse food web (Casey et al., 2019). Limitations of this approach are the need to capture, subject to stress and, in most cases, sacrifice the studied organisms at the nodes of the prospective interaction network, as well as the requirement to use correction factors to account for multiple sources of bias (Thomas et al., 2016).

## 2.3 | Tapping into a new source: eDNA metabarcoding studies

One further step into the expanding world of DNA monitoring may offer opportunities to untie diet studies from the constraints

of collecting animals from wild populations (Clare, 2014). In recent years, the emergence of environmental DNA (eDNA) is having an explosive impact on biodiversity research, ushering in an era of non-invasive, efficient, whole-ecosystem surveying (Bakker et al., 2019; Berry et al., 2019; Seymour et al., 2020). Environmental DNA is defined as a mixture of genetic material, including entire cells and extracellular DNA, retrieved from a variety of environmental samples such as sediment, water and air (Barnes & Turner, 2016; Pawlowski et al., 2020; Taberlet et al., 2012).

Novel metabarcoding studies based on eDNA extracted from marine water and sediments have shown their effectiveness in detecting differences in metazoan community composition through space (e.g. Jeunen et al., 2019; Sigsgaard et al., 2020), time (Berry et al., 2019; Djurhuus et al., 2020) and across anthropogenic impact gradients (Bakker et al., 2017; DiBattista et al., 2020). Collecting water or sediment eDNA samples may soon enhance the reach of existing surveys, as well as reduce the costs of data collection through the involvement of recreational (UNIG) and commercial fishing vessels (Russo et al., 2020). Of all the biodiversity methods, eDNA surveys are among the least destructive, as there is no requirement of handling organisms (Boussarie et al., 2018), which in the oceans typically range from tiny to enormous and are often elusive and difficult to locate and capture. DNA metabarcoding also offers the level of methodological universality able to screen biodiversity across virtually all trophic levels in a community (Bourlat et al., 2013).

Despite its obvious advantages, like every other method, eDNA metabarcoding has its limitations, as cautioned by several authors (Cristescu & Hebert, 2018; Hansen et al., 2018). Among these, false positives (i.e. species detected but not present in the sampling area) may arise from contamination along the analytical workflow (sampling, DNA extraction, amplification, sequencing, etc.), or through transport and resuspension (Barnes & Turner, 2016). False negatives (i.e. species present in the sampling area but not detected) may also result in important species being “missed” due to the inefficiency of the primers used. Most importantly, perhaps, gaps in the publicly available DNA sequence reference data bases may still hinder the taxonomic identification of key organisms (Collins et al., 2019), while differences among bioinformatic processes may still account for changes in biodiversity reconstruction (Flynn et al., 2015). Nevertheless, countermeasures are incessantly devised, leading to increasingly robust eDNA procedures across habitats (Goldberg et al., 2016).

The rapidly expanding popularity of eDNA studies means that an unprecedented number of large data sets are being generated non-invasively and made available to the scientific community upon publication. These data can be used to explore the trophic structure of marine communities inhabiting certain areas and may lead to useful, integrated information for management interventions.

Here, we propose a novel approach for the reconstruction of trophic networks of marine communities, by using marine eDNA metabarcoding data sets from recently published studies and identifying all possible consumer–resource interactions through a literature review-based approach. First, we harvested the published eDNA

metabarcoding data and reconstructed trophic webs from three tropical ecosystems subjected to different levels of anthropogenic disturbance: Bahamas, a shark sanctuary; Turks & Caicos, where fishing pressure is increasing in recent years, and Jamaica, known for longer-term depletion of fish populations (Bakker et al., 2017, 2019). We then selected data sets from a temperate rocky coast in southern New Zealand (i.e. Aramoana; Jeunen et al., 2019) and a polar habitat in the Canadian Arctic (i.e. Iqaluit; Lacoursière-Roussel et al., ), to explore the generality of the proposed approach across latitudes and different climatic zones. Subsequently, we collated species inventories for each community, focussing on macro-eukaryotic taxa, which generally have a better-understood taxonomy and greater significance for management and conservation policy. We then constructed a pairwise consumer-resource interaction matrix for all the taxa within the ecosystem (i.e. nodes of the reconstructed trophic network; see Table S1), determining all possible trophic links of the community. To obtain this information, we sifted through thousands of records from different online data bases (Web of Science, Google Scholar and FishBase) and identified 136 scientific publication (Table S2) for demonstrated trophic interactions at the species level (i.e. binary links in the food web). For simplification, in the query, we did not include cannibalistic, parasitic or other symbiotic interactions. When information on a specific taxon was not available, information from the immediately higher taxonomic level was used (example provided in Appendix S1). Basal resources that represent a variety of trophic pathways in marine environments were chosen to set the base of the food web (Briand et al., 2016): sedimentary organic matter (SOM) as detrital particles sedimented into the ocean floor (Cresson et al., 2012), particulate organic matter (POM), including phytoplankton, bacteria and organic particles suspended in the water column (Volkman & Tanoue, 2002), and multicellular autotrophs.

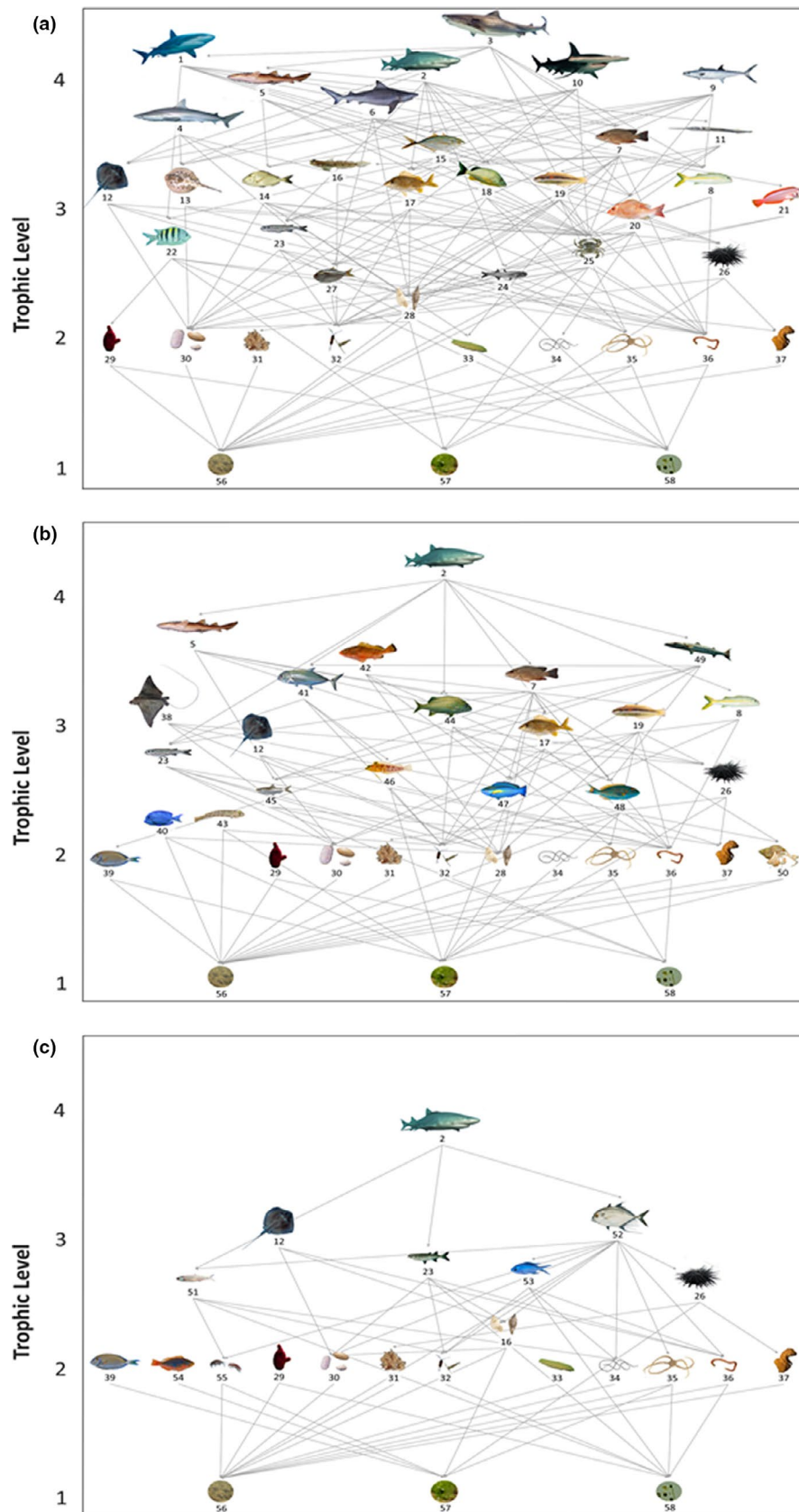
Matrices were then imported into R, and the *cheddar* package (Hudson et al., 2013) was used to visualize trophic webs and measure their network properties, which included: link density (i.e. the number of links for each node), measures of trophic height, such as

average chain (i.e. the average chain length of all paths from each node to a basal resource) and longest chain (i.e. the average of the longest chain length from each node to a basal resource, weighted for the trophic level of the node) (Hudson et al., 2013). Using Cytoscape v 3.8.0 (Shannon et al., 2003), we evaluated topological parameters of the food webs, as neighbourhood connectivity (i.e. the average number of neighbours for each node) a measure that shows the degree of connectivity of nodes in a network. (Maslov & Sneppen, 2002). All statistical analyses were performed in R v 3.3.0 (<https://www.R-project.org/>).

Representation of the three tropical trophic networks based on sites experiencing low (Bahamas), medium (Turks & Caicos), and high (Jamaica) levels of anthropogenic pressure are shown in Figure 1 (a, b, and c, respectively). The number of nodes reflected the different levels of anthropogenic impact, with the Jamaican trophic web having almost half ( $N = 23$ ) the number of nodes compared to the network of Bahamas ( $N = 40$ ) and the trophic web of Turks and Caicos having an intermediate number ( $N = 34$ ). The link density also followed a similar pattern, with the more impacted network having just over half ( $L/N = 2.26$ ) the number of interactions compared to the less impacted web ( $L/N = 4.00$ ) and the Turks & Caicos network showing an intermediate value ( $L/N = 3.03$ ).

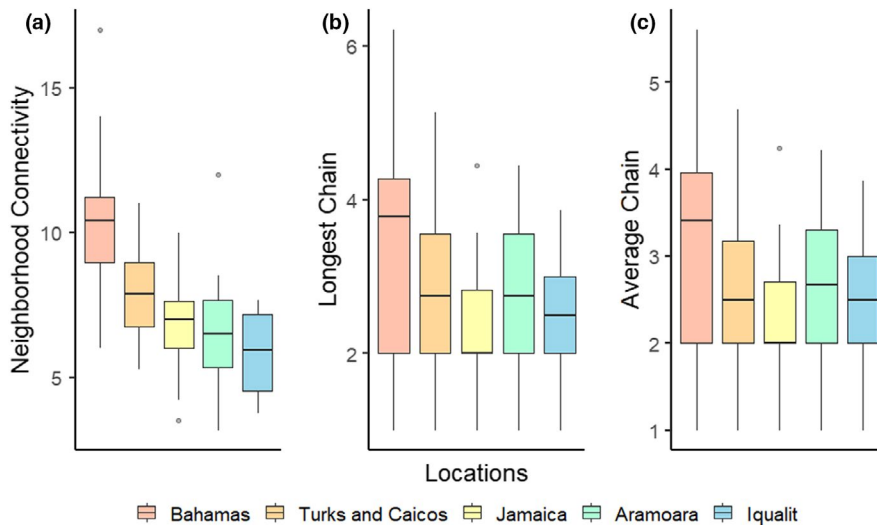
Neighbourhood connectivity and length-chain parameters all significantly varied among the three Caribbean networks (Figure 2a). In general, the nodes from the Bahamas network were more connected (mean  $\pm$  SE) (neighbourhood connectivity =  $10.29 \pm 0.32$ ), exhibiting statistically (Kruskal-Wallis test:  $H = 70.99$ ,  $df = 4$ ,  $p < .0001$ ) higher values of neighbourhood connectivity compared to both Turks & Caicos and Jamaica (Figure 2a). The Bahamas food web also displayed significantly longer longest chain (Figure 2b) and average chain path (Figure 2c), with greater values compared to those in the other two more impacted locations (Kruskal-Wallis test:  $H = 14.55$ ,  $df = 4$ ,  $p = .005$  and Kruskal-Wallis test:  $H = 13.61$ ,  $df = 4$ ,  $p = .008$ ; respectively).

**FIGURE 1** Visual representation of the trophic relationships in (a) Bahamas, (b) Turks and Caicos and (c) Jamaica reef habitats. 1—Caribbean reef shark (*Carcharhinus perezi*, Carcharhinidae), 2—Lemon shark (*Negaprion brevirostris*, Carcharhinidae), 3—Tiger shark (*Galeocerdo cuvier*, Carcharhinidae), 4—Blacknose shark (*Carcharhinus acronotus*, Carcharhinidae), 5—Nurse shark (*Ginglymostoma cirratum*, Ginglymostomatidae), 6—Bull shark (*Carcharhinus leucas*, Carcharhinidae), 7—Grey snapper (*Lutjanus griseus*, Lutjanidae), 8—Yellowtail snapper (*Ocyurus chrysurus*, Lutjanidae), 9—*Scomberomorus* sp. (Scombridae), 10—Great hammerhead (*Sphyrna mokarran*, Sphyrnidae), 11—Redfin needlefish (*Strongylura notata*, Belonidae), 12—Southern stingray (*Hypanus americanus*, Dasyatidae), 13—Yellow stingray (*Urobatis jamaicensis*, Urotrygonidae), 14—*Calamus* sp. (Sparidae), 15—Bar jack (*Caranx ruber*, Carangidae), 16—Dash goby (*Ctenogobius saepepallens*, Gobiidae), 17—White grunt (*Haemulon plumieri*, Haemulidae), 18—Bluestriped grunt (*Haemulon sciurus*, Haemulidae), 19—*Halichoeres* sp. (Labridae), 20—Lane snapper (*Lutjanus synagris*, Lutjanidae), 21—Rosy razorfish (*Xyrichtys martinicensis*, Labridae), 22—Seargent-major (*Abudefduf saxatilis*, Pomacentridae), 23—Hardhead silverside (*Atherinomorus stipes*, Atherinidae), 24—Reef silverside (*Hypoatherina harringtonensis*, Atherinidae), 25—Brachyura, 26—Echinoidea, 27—Atlantic menhaden (*Brevoortia tyrannus*, Clupeidae), 28—Gastropoda, 29—Ascidiacea, 30—Bivalvia, 31—Bryozoa, 32—Copepoda, 33—Holothuroidea, 34—Nematoda, 35—Ophiuroidea, 36—Polychaeta, 37—Porifera, 38—Whitespotted eagle ray (*Aetobatus narinari*, Myliobatidae), 39—Barber surgeonfish (*Acanthurus bahianus*, Acanthuridae), 40—Blue tang surgeonfish (*Acanthurus coeruleus*, Acanthuridae), 41—Horse-eye jack (*Caranx latus*, Carangidae), 42—Red hind (*Epinephelus guttatus*, Serranidae), 43—*Gnatholepis* sp. (Gobiidae), 44—French grunt (*Haemulon flavolineatum*, Haemulidae), 45—Redear herring (*Harengula humeralis*, Clupeidae), 46—*Malacotenus* sp. (Labrisomidae), 47—Princess parrotfish (*Scarus taeniopterus*, Scaridae), 48—*Sparisoma* sp. (Scaridae), 49—Great barracuda (*Sphyrna barracuda*, Sphyrnidae), 50—Paguridae, 51—Anchoa, 52—Carangidae, 53—Blue chromis (*Chromis cyanea*, Pomacentridae), 54—Redband parrotfish (*Sparisoma aurofrenatum*, Scaridae), 55—Amphipoda, 56—S.O.M., 57—Algae, 58—P.O.M. (figure appears in colour in the online version only). Photo credits provided in Appendix S2



Food webs were also reconstructed using data from the cold temperate (Aramoana; Figure 3a) and the polar (Iqaluit; Figure 3b) ecosystems, in order to illustrate the generality and global scope of

the proposed approach. According to general expectations of poleward reduction of marine biodiversity (Hillebrand, 2004), we also observed fewer nodes ( $N = 23$ ;  $N = 18$ ; respectively; Aramoana and



**FIGURE 2** Food web properties for Bahamas (red), Turk & Caicos (orange), Jamaica (yellow), Aramoana (green), Iqaluit (blue) and results of Kruskal-Wallis test among locations: (a) Neighbourhood Connectivity ( $H = 70.99$ ,  $df = 4$ ,  $p < .0001$ ); (b) Longest Chain ( $H = 14.55$ ,  $df = 4$ ,  $p = .005$ ) and (c) Average Chain ( $H = 13.61$ ,  $df = 4$ ,  $p = .008$ ) (figure appears in colour in the online version only)

Iqaluit) and about half the link density ( $L/N = 2.57$ ;  $L/N = 2.28$ ) in the food webs from colder climatic regions, compared to the less disturbed tropical example ( $L/N = 4.00$ ). Connectivity and length chain parameters were also lower in the cold-water habitats (Figure 2).

### 3 | BENEFITS, CAVEATS AND FUTURE STEPS

In the last decade, the rapid expansion of eDNA metabarcoding in a marine biomonitoring context has generated countless examples of its effectiveness in assessing marine biodiversity (e.g. Aglieri et al., 2020; Boussarie et al., 2018; Sigsgaard et al., 2020). Despite a growing body of literature in freshwater habitats (e.g. Compson et al., 2019; Seymour et al., 2020), just a few research studies have explored how eDNA metabarcoding could be beneficial for a community network analysis perspective in marine environments (DiBattista et al., 2020; Djurhuus et al., 2020; Minicante et al., 2019). The present exercise shows that it is possible to further extend the boundaries of eDNA analysis into the field of trophic ecology, obtaining simplified yet realistic representations of different marine trophic webs, at a speed and scales that would be inconceivable through any other means, and, crucially, at virtually no cost, owing to the availability of published eDNA-based taxon inventories.

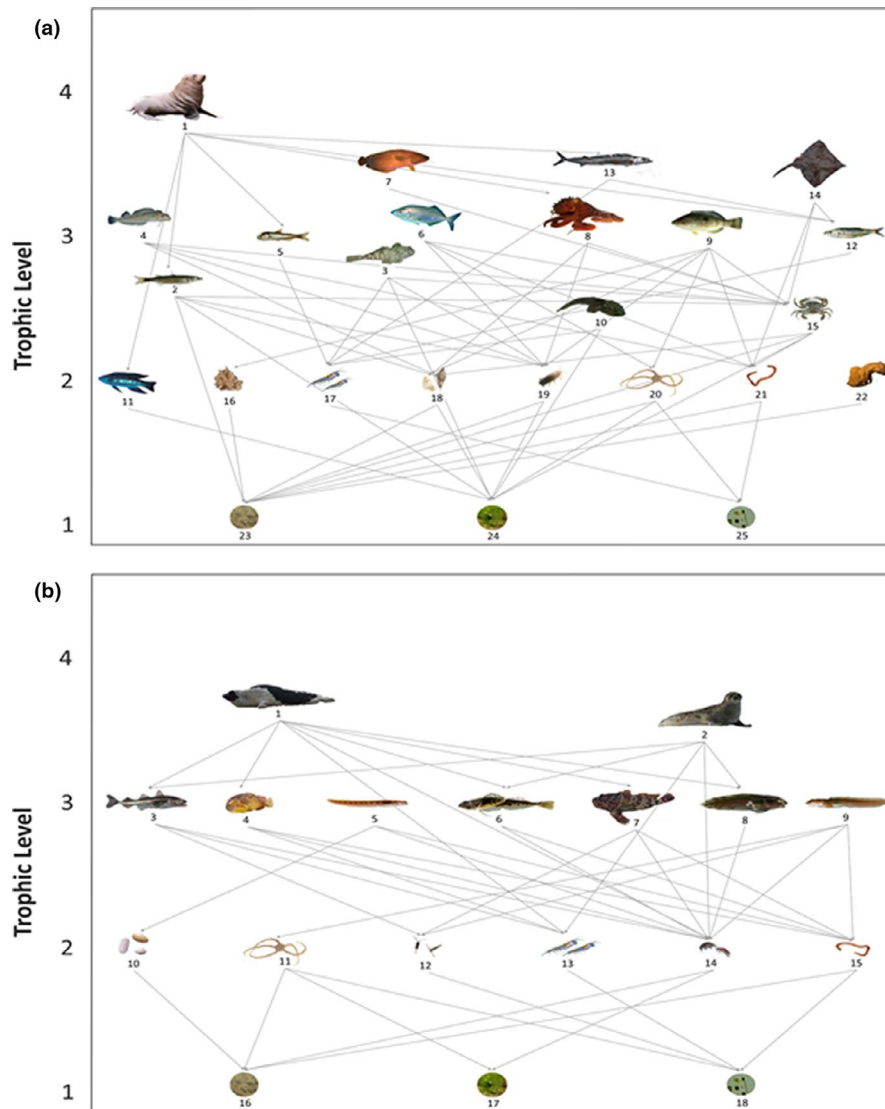
We provided a reconstruction of tropical, temperate and polar marine food webs to explore the feasibility of this approach across the latitudes of the planet, where different abiotic and biotic conditions occur, and results fit with what is known about the selected climatic zones. The reconstruction of the polar food web in Iqaluit was unable to differentiate among the trophic positions of polar fishes (all shown at the same level in the trophic web, Figure 3b). This is probably mainly due to the nature of this environment, where traditional sampling effort is too limited for allowing accurate estimation of biodiversity, consequently, resulting in scant knowledge of species-specific diets (Archambault et al., 2010; Darnis et al., 2012). This dearth of information, especially for taxa at lower trophic levels, reduces the resolution of the food web, which may affect robust

conclusions and consequent applications. This caveat is well recognized in trophic ecology studies (Casey et al., 2019; Smith et al., 2011) and several studies in recent years have increased efforts to build more comprehensive DNA libraries for the identification of species, with the aim to fill the knowledge-gap pertaining to species interactions in less-studied systems (e.g. Walters et al., 2019).

At more regional scales, the food webs reconstructed in the three Caribbean island nations reflect the known levels of ocean stewardship existing in the region (Bakker et al., 2017, 2019), with the diversity in apex sharks and functional redundancy in mesopredator sharks and large piscivores, leading to an increase in complexity of trophic interaction and food web structure in healthy tropical reef ecosystems (Barley et al., 2020; Gilarranz et al., 2016; Roff et al., 2016). It is clear that such a 'coarse' approach to food web reconstruction would require some level of investment in validation using diet analyses for at least some key nodes of the network; yet, eDNA-based reconstructions would allow rapid, affordable, low-effort replicates of food web snapshots that could cover a much wider area over a much shorter period.

Interestingly, even with the proposed simplified approach, it is possible to identify noteworthy features of food webs, in a comparative framework. For instance, the topology (neighbourhood connectivity), trophic chain length (longest chain and average chain) and the trophic position of key apex predators [e.g. Lemon shark (*Negaprion brevirostris*, Carcharhinidae)] all differ between ecosystems under distinct anthropogenic impacts (Figure 1). The simplification of food webs is a common indicator for ecological degradation in marine environments (Coll et al., 2008; Gilarranz et al., 2016), so, even with the caveat of required validation and data set expansion, this approach appears promising.

Further examination of top- and mesopredators reveals that different reef sharks in the Bahamas have trophic positions similar to previous studies reviewed by Roff et al., 2016 (see Table S3). There is a variation in the trophic position of some species [e.g. Blacknose shark (*Carcharhinus acronotus*, Carcharhinidae), Bull shark (*Carcharhinus leucas*, Carcharhinidae)] that could be due to the different prey composition and abundance among studies or, more



**FIGURE 3** (a) Visual representation of the trophic web and its trophic relationships in the rocky shore ecosystem of Aramoana. 1—New Zealand fur seal (*Arctocephalus forsteri*, Otariidae), 2—Yellow-eye mullet (*Aldrichetta forsteri*, Mugilidae), 3—Thornfish (*Bovichtus variegatus*, Bovichtidae), 4—Common triplefin (*Forsterygion lapillum*, Tripterygiidae), 5—Blackhead lanternfish (*Lampichthys procerus*, Myctophidae), 6—Bastard trumpeter (*Latridopsis forsteri*, Latridae), 7—Rock cod (*Lotella rhacina*, Moridae), 8—New Zealand octopus (*Macroctopus maorum*, Octopodidae), 9—Spotty (*Notolabrus celidotus*, Labridae), 10—Maori chief (*Notothenia angustata*, Nototheniidae), 11—Butterfish (*Odax pullus*, Odacidae), 12—New Zealand blueback sprat (*Sprattus antipodum*, Clupeidae), 13—Snoek (*Thyrstites atun*, Gempylidae), 14—New Zealand rough skate (*Zearaja nasuta*, Rajidae), 15—Brachyura, 16—Bryozoa, 17—Euphausiacea, 18—Gastropoda, 19—Isopoda, 20—Ophiuroidea, 21—Polychaeta, 22—Porifera, 23—S.O.M., 24—Algae, 25—P.O.M. (b) Visual representation of the trophic web and its trophic relationships in Iqaluit. 1—Harp seal (*Pagophilus groenlandicus*, Phocidae), 2—Ringed seal (*Pusa hispida*, Phocidae), 3—Polar cod (*Boreogadus saida*, Gadidae), 4—Atlantic spiny lump sucker (*Eumicrotremus spinosus*, Cyclopteridae), 5—Fish doctor (*Gymnelus viridis*, Zoarcidae), 6—Arctic staghorn sculpin (*Gymnocanthus tricuspis*, Cottidae), 7—Twohorn sculpin (*Icelus bicornis*, Cottidae), 8—Inquiline snailfish (*Liparis inquilinus*, Liparidae), 9—*Lycodes* sp. (Zoarcidae), 10—Bivalvia, 11—Ophiuroidea, 12—Copepoda, 13—Euphausiacea, 14—Amphipoda, 15—Polychaeta, 16—S.O.M., 17—Algae, 18—P.O.M. (figure appears in colour in the online version only). Photo credits provided in Appendix S2

likely, for the difference in the approach used to estimate trophic levels. In fact, diet composition studies use quantitative proportions of prey categories and their respective trophic level, gleaned from stomach contents and stable isotopes analysis (Hussey et al., 2014). Conversely, our suggested approach, which uses binary eDNA data, considers all trophic interactions with the same weight, which could distort the complexity of food web structure (Bersier et al., 2002),

but still provide a realistic portrayal of tropical reef marine communities through vastly reduced practical and financial investment.

The recent, explosive popularity of eDNA studies means that: (i) a large number of studies are being carried out on marine communities across the world; ii) these studies are producing large DNA-based taxonomic inventories, stored in public repositories, and available to the scientific community at no

extra cost. Although, as said above, an in-depth understanding of a trophic web requires direct, painstaking validation of predator-prey links through gut content, metabarcoding and isotopic analyses, these remain costly, lengthy, infrequent and therefore insufficient for large scale application. The use of opportunistic network analyses built on eDNA metabarcoding studies could represent a valuable asset to generate an extensive baseline of simplified food web reconstructions, which could help environmental scientists and practitioners monitor a larger portion of our seas and flag possible ongoing anthropogenic disturbance (DiBattista et al., 2020; Gilarranz et al., 2016). These rapidly generated networks have the potential to unveil processes such as the loss of keystone species (Wu et al., 2020), the impact of expanding/invasive species (Saebi et al., 2020), the detrimental impacts of environmental changes on top predators (Sagarese et al., 2017), the depletion of forage taxa that sustain commercially important resources (Lassalle et al., 2011), the ecosystem-level effect of marine protection (Casselberry et al., 2020), and a variety of other ecological processes that are often latent and difficult to unpick over timescales that are relevant to management.

It remains clear that more research should be devoted to assessing the biases of the proposed approach, such as the distortion caused by the use of binary data and the reliance on literature data. Further work should also focus on assessing the temporal robustness of the reconstructions based on eDNA taxon inventories, and standardized methods should be devised for the selection of the nodes and the comparisons with traditional methods. Yet, in a world where biodiversity loss and ecosystem disruptions remain major challenges, it would be unwise not to tap on multiple, free-to-use, large data sets that continue to be generated, relentlessly, from all ocean regions and habitats.

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## CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

## DATA AVAILABILITY STATEMENT

Primary sequence and diet data are already available in the literature and cited either in the paper or reported in Table S2. Diet matrices for analysis replication are provided in Table S1.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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