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HOW IS PELAGIC *SARGASSUM*—ASSOCIATED BIODIVERSITY ASSESSED? INSIGHTS FROM THE LITERATURE§

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Abstract: Over the past decade unprecedented blooming of pelagic *Sargassum* has occurred across the Equatorial Atlantic from West Africa to the Caribbean. Although pelagic *Sargassum* mats are considered beneficial in the open ocean, providing valuable habitat for a diverse array of endemic and associated species, they also inundate coastal areas and cause a plethora of management challenges for fisheries, tourism, nearshore coastal ecosystems, public health and the socioeconomic welfare of coastal communities. In—water harvesting has been suggested as a desirable management solution to prevent shoreline inundation, but destruction of the associated biodiversity is a concern with this approach and has not been adequately examined. Furthermore, in—water harvesting methods within the Tropical Atlantic and Caribbean have been ad hoc and highly variable with no established sampling protocol. Here we review 30 published studies detailing methods to collect information on the biodiversity associated with pelagic *Sargassum*. Nets, hook and line, video recordings, bare—hands and plastic bags have all been used to collect epiphytic, clinging and free—swimming fauna associated with *Sargassum*. Net sampling was the predominant method; however, in the absence of a standardized approach a wide range of net types and sizes were used. Similarly, separation, identification and preservation methods were all unstandardized. This review highlights the need for standardization and provides the first set of guidelines for the collection and assessment of *Sargassum*—associated biodiversity. Nevertheless, these approaches are labor intensive and require extensive replication in time and space to produce a reasonable assessment of the biodiversity associated with the *Sargassum* community.

Key words: epiphytic fauna, clinging fauna, free—swimming fauna, literature review

Introduction

Floating mats of *Sargassum* (hereon referred to simply as 'pelagic *Sargassum*') typically comprise several morphotypes of 2 holopelagic species (*Sargassum natans* and *S. fluitans*) and provide essential habitat, refuge, nursery ground and foraging habitat for a wide variety of associated and endemic species (Butler et al. 1983, Witherington et al. 2012, Moser and Lee 2012, Martin 2016, Martin et al. 2021). Much of this knowledge comes from assessments in the Sargasso Sea where pelagic *Sargassum* has existed for centuries (Parr 1939, Fine 1970, Butler et al. 1983, Lapointe et al. 2014), and significant steps have been taken to protect it (Laffoley et al. 2011).

Unlike in the Sargasso Sea, the mass blooming of pelagic *Sargassum* across the North Equatorial Recirculation Region (NERR) of the Atlantic presents both a potential asset and a hazard for Caribbean and West African countries (UNEP 2021). Over the past decade unprecedented blooms of pelagic *Sargassum* in the NERR have resulted in devastating shoreline inundations, with mass accumulation and decomposition of the seaweed along the coasts of Caribbean and West African countries (Milledge and Harvey 2016). Clean—up efforts across the region has cost millions of dollars to national economies, strained local resources, and significantly affected coastal livelihoods (Milledge and Harvey 2016, Chávez et al. 2020). While pelagic *Sargassum* mats function as healthy biodiverse ecosystems at sea, the mass shoreline inundations present a plethora of management challenges for fisheries, tourism, public health and nearshore coastal ecosystems (Milledge and Harvey 2016, Ramlogan et al. 2017, van Tussenbroek et al. 2017, Resiere et al. 2018, Oxenford et al. 2019). Furthermore, mass inundations from this newly established source region are now considered the new 'normal' to which countries must adapt (Desrochers et al. 2020).

In—water removal has been suggested as a desirable management solution to prevent the multiple negative impacts associated with shoreline inundation and the problems associated with clearing the seaweed from onshore, especially from sandy beaches which can be significantly damaged by repeated removal efforts and use of heavy equipment (Hinds et al. 2016, Chereau 2019, Dutch Caribbean Nature Alliance 2019, Webber and Maddix 2021). Furthermore, considerable attention is now being given to valorization of pelagic *Sargassum* (Desrochers et al. 2020, Oxenford et al. 2021, UNEP 2021) and in—water harvesting is likely to be an attractive option for obtaining fresh 'clean' *Sargassum* for applications that require high quality fresh *Sargassum*, given the issues associated with separating fresh from partially decayed *Sargassum* (Oxenford et al. 2021).

However, destruction of the associated biodiversity is a concern with in—water removal, as elaborated by the Dutch Caribbean Nature Alliance (2019) who state that there is a need for

§*This article is based on presentations given in November 2021 at the virtual 74th meeting of the Gulf and Caribbean Fisheries Institute and in November 2022 at the 75th meeting of the Gulf and Caribbean Fisheries Institute, Ft. Walton Beach, FL.*

a clear set of guidelines when harvesting pelagic *Sargassum* at sea to minimize any disturbance to or loss of marine life. Despite these concerns, the potential impacts of in–water collection of pelagic *Sargassum* on the associated biodiversity have not been adequately examined to provide appropriate guidance for this management intervention. *Sargassum* is well known to support diverse taxa (Coston–Clements et al. 1991, Casazza and Ross 2008, Laffoley et al. 2011, Moser and Lee 2012, Witherington et al. 2012), thus the impact of in–water harvesting could be significant. To date, the lack of consistency in biodiversity assessment studies in the tropical Atlantic and Caribbean limits the understanding to the patterns of diversity and thus the ability to understand the potential impact of large–scale removal. However, Monroy–Velázquez et al. (2019) noted that pelagic *Sargassum* found close to the coastline had diminished diversity, perhaps due to intensive foraging by coastal fishes and seabirds; within this context, removal of pelagic *Sargassum* may not pose a significant threat.

The faunal community associated with pelagic *Sargassum* is known to consist of sessile and motile organisms which can be found attached to, within and beneath floating mats (Weis 1968, Dooley 1972, Monroy–Velázquez et al. 2019, Martin et al. 2021). This associated community plays an important role in pelagic food webs (Laffoley et al. 2011, Martin et al. 2021). Even the most inconspicuous sessile epifauna have an important role to play, as they provide feeding opportunities for higher trophic level organisms (Martin et al. 2021) which may have commercial importance. Assessing the sessile epiphytic fauna can also prove useful for age determination of the pelagic *Sargassum* (Stoner and Greening 1984, Shadle et al. 2019) and subsequently help determine which valorization applications may or may not be appropriate. For this reason, biodiversity assessments should pay attention to both sessile and motile associated fauna, noting that there will always be inherent and significant variability in pelagic *Sargassum* according to the morphotype, age, and prevailing and past patterns of recruitment to the *Sargassum* (Stoner and Greening 1984, Martin et al. 2021).

This study reviews the variety of methods used to collect, identify and analyze the biodiversity associated with pelagic *Sargassum* that have been documented in the published literature. The purpose of this review is to provide guidance to Caribbean and West African countries on the steps they can take to assess pelagic *Sargassum*–associated biodiversity in easy, practical and reproducible ways so that data collected over time and across the region are comparable.

Materials and Methods

Literature search process

To identify the most relevant literature, 22 scientific and environmental databases (Supplementary Table S1) were searched to identify primary studies focused on biodiversity of pelagic *Sargassum* communities. Key search phrases used to identify relevant articles included: '*Sargassum* AND Biodiversity', '*Sargassum* AND Faunal Communities', 'Sargasso Sea AND Faunal Communities', '*Sargassum* AND Fauna' and 'Sargasso Sea AND Biodiversity'. Under each search phrase, only journal articles and M.S. or Ph.D. theses were selected for further consideration. In addition to the listed databases (Supplementary Table S1), the *Sargassum* Reference Repository hosted by the Centre for Resource Management and Environmental Studies (CERMES), at the University of the West Indies in Barbados, was also used to identify relevant articles. Selected documents from the database search and the repository were imported into Zotero Reference Manager and SRA–Dedupe–Ui was used to remove duplicates.

The reference lists of all relevant papers selected from the database and repository search were then loaded into an excel document to search for additional articles regardless of publication type. After relevant articles were identified using this 'snowballing' technique, all duplicate articles were removed (Supplementary Figure S1).

Selection and exclusion criteria

Article titles with key words relating to the topic under investigation were selected and all non–relevant records were excluded. After initial selection, the abstracts of articles were read to determine relevance. Non–*Sargassum* algae and detached benthic *Sargassum* species are structurally different from pelagic species; thus, the methods used to collect non–pelagic species may not be appropriate for adequately assessing pelagic *Sargassum* influx events. Articles selected for the assessment process focused only on the assessment of biodiversity associated with pelagic *Sargassum* (Supplementary Table S2).

Articles which looked exclusively at the genetic diversity of pelagic *Sargassum* species (i.e., *S. natans* or *S. fluitans*) were excluded from this study. For articles which examined the biodiversity of species associated with pelagic *Sargassum* mats compared to open water, only methods used to assess the biodiversity of pelagic *Sargassum* mats were considered. No limitations were placed on the year of publication and, as a result, articles included in this review dated from 1968 to the time of writing.

Data analysis

In this paper, 30 articles were selected and each article was reviewed carefully to answer 5 research queries covering aspects relating to the collection, identification and assessment of pelagic *Sargassum*–associated species, sample method and post–collection handling:

1. What type of *Sargassum*–associated biodiversity is identified?
2. What method(s) are used to collect pelagic *Sargassum* associated biodiversity?
3. How are the samples sorted and preserved?
4. How are the species identified?
5. How are the data analyzed?

Biodiversity associated with pelagic *Sargassum* mats is classified into 3 main groups: epiphytic fauna, clinging fauna, and free–swimming fauna which expands from the 'sessile' and 'motile' groups described by Weis (1968). Epiphytic fauna refers to any sessile animal which grows on pelagic *Sargassum*, such as hydroids, tube worms and encrusting bryozoans, etc. Clinging fauna refer to animals that exhibit very limited range of movement and spend their time clinging to or climbing around the *Sargassum*, such as crustaceans, polychaetes, molluscs, flatworms, the *Sargassum* frogfish, seahorses and turtle hatchlings. Free—swimming fauna refer to species that swim inside and beneath the *Sargassum* thalli such as juvenile turtles, adult turtles, vertical migrating fishes and pelagic fishes.

TABLE 1. *Net types and specifications used to collect pelagic Sargassum-associated biodiversity. Reference numbers refer to numbered studies listed in Supplementary Table S3.*

Net type	Frame opening (m²)/ Net length (m)	Mesh size (mm)	Number of studies	Reference
Hand operated	0.07-0.25 m^2	0.3-13	21	1, 2, 3, 4, 5, 7, 9, 11, 13, 16, 17, 20, 21, 22, 23, 24, 25, 27, 28
Towed	0.28-2.64 m^2	0.3-25	10	2, 4, 10, 18, 19, 22, 23, 24, 26
Encircling	30.5 m by 5.2 m	1-12.5	4	7, 19, 29, 30

Results and discussion

The selected articles were diverse in their aims; some studies examined the relationship between the pelagic *Sargassum* mat morphology and associated biodiversity (Moser et al. 1998) or the species associated with pelagic *Sargassum* compared to open water (Casazza and Ross 2008), and others investigated pelagic *Sargassum* for free—swimming, clinging and epiphytic fauna (Fine 1970, Dooley 1972, Butler et al. 1983, Wells and Rooker 2003, Monroy—Velázquez et al. 2019). Across the 30 reviewed articles, 37% (11 articles) collected information on epiphytic fauna, 43% (13 articles) on clinging fauna and 77% (23 articles) reported on free—swimming fauna (Supplementary Table S3).

Collection methods

Five different methods have been reported in the literature to collect biodiversity associated with pelagic *Sargassum*: a variety of nets, hook and line, video recording, bare—handed collections, and collection of fauna with plastic bags (Supplemental Table S4). Overall, nets were used across the majority of studies (93%; 28 articles), with hook and line (11%; 3 articles), video recordings (7%; 2 articles), hand collections (7%; 2 articles) and plastic bags (4%; 1 article) being used to a lesser extent. Using the identified methods, biodiversity samples were collected from floating clumps of pelagic *Sargassum* (82%), along transects (11%) set at varying distances from shore, and from beach—cast pelagic *Sargassum* (7%).

Net types

Three distinct groups of nets were employed: hand operated nets such as dipnets, landing nets and hand nets, towed nets such as bongo nets, neuston nets, trawls and plankton nets, and encircling nets such as purse seines. Over the different studies, nets varied in size both across and within net types (Table 1).

Of the 3 net types, hand operated nets were the most used with 70% of the studies (Table 1) using various sizes to capture organisms across the 3 biodiversity groups. Researchers using other net types also demonstrated selective use of hand operated nets when the density of pelagic *Sargassum* was too thick for other net types (Casazza and Ross 2008) or when the seas were rough and caused mats to scatter (Dooley 1972). Towed and encircling nets were the second and third most frequently reported collection method (respectively), and were used predominantly for the capture of free—swimming fauna. Hook and line (including one multi—hook longline), and video recordings were only used to assess free—swimming fauna, and hand collections were used solely to collect pelagic *Sargassum* for the assessment of epiphytic fauna (Figure 1).

This preliminary assessment provides an overview of the potential sampling options researchers could use to investigate the biodiversity associated with pelagic *Sargassum*. However, no clear guidance emerges on which net type and/or size should be used. There was no indication why some researchers chose hand operated nets whilst others chose towed nets to sample organisms across all 3 biodiversity groups simultaneously.

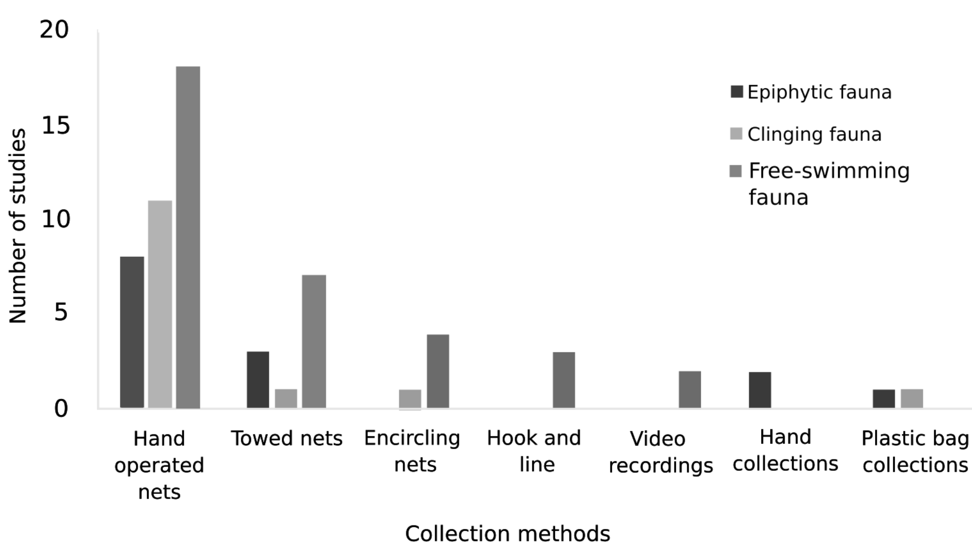

FIGURE 1. *Methods used across studies (n=30) to assess various types of epiphytic fauna, clinging fauna and free-swimming fauna associated with pelagic Sargassum.*

Other gear

Organisms associated with pelagic *Sargassum* are found throughout the mass of floating thalli, as well as several meters below it (Casazza and Ross 2008, Martin 2016). However, sampling depth was not specified in the majority (80%) of studies. In the few studies where depth was specified, net sampling took place within the upper surface waters (0 – 5 m) regardless of net type, whilst the predominantly used hand operated nets were likely to have been limited to the upper layer of floating *Sargassum*. For example, Martin (2016) stated that hand operated nets are not very effective at targeting the larger more mobile species, especially those found at greater depths below the seaweed. Similarly, Moser et al. (1998) reported a high abundance of juvenile and cryptic fish in their dipnet collections, but minimal capture of larger species.

To assess larger free—swimming fauna, Moser et al. (1998) and Casazza and Ross (2008) used a combination of dip net sampling and video recordings. Free floating cam recorders and remotely operated vehicles (ROVs) were deployed approximately 1 m below pelagic *Sargassum* mats. Both these studies indicated a higher presence of juvenile fish within pelagic *Sargassum* mats with larger fish being found in 'layers' below the mat. These studies provide insights into the distribution of species associated with *Sargassum* mats and can help researchers decide which sampling technique they require based on the target biodiversity.

Bearing in mind that studies which solely use video recordings are only able to assess free—swimming organisms below and/or adjacent to the mat, and studies which use nets are limited to the smaller more cryptic organisms, combining net sampling with video observations will play an important role in providing a better understanding of the biodiversity associated with *Sargassum* influx events. Given the inefficiencies of net sampling when targeting free—swimming fauna, biodiversity assessments of pelagic *Sargassum* should include the use of underwater video footage to give a more representative quantitative view of the overall biodiversity associated with pelagic *Sargassum*. While broad assessment methodologies will provide a better understanding of the pelagic *Sargassum* community, motile species may never be fully assessed and are likely to escape in the event of large—scale harvesting in any case.

Treatment of samples

Separation and preservation

Subsequent to collection, samples are generally sorted on board research vessels or taken back to laboratories for sorting and identification. To separate collected motile organisms some studies immediately placed the collected pelagic *Sargassum* in a bag containing seawater (Schell et al. 2016) or freshwater (Stoner and Greening 1984) where it was vigorously shaken for several minutes to remove organisms. Alternatively, collected samples were soaked in buckets of freshwater (Dooley 1972, Monroy—Velázquez et al. 2019) to induce osmotic shock and encourage the release of organisms attached to the pelagic *Sargassum*.

After rinsing, the residual water is typically filtered through a mesh sieve ranging between 0.28—1 mm in size. Dislodged organisms collected in the mesh are then fixed with 5 or 10% formalin (Weis 1968, Bortone et al. 1977, Niermann 1986, Calder 1995, Huffard et al. 2014) or immediately preserved in 40% isopropanol (Bortone et al. 1977, Casazza and Ross 2008), 70% ethanol (Bortone et al. 1977, Martin 2016, Schell et al. 2016, Monroy—Velázquez et al. 2019, Martin et al. 2021), or 95—96% ethanol (Settle 1993, Comyns et al. 2000, Taylor 2015, Shadle et al. 2019, Mendoza—Becerril et al. 2020) for later analyses. In rare cases, fish species were preserved with dry ice (Wells and Rooker 2003, Wells and Rooker 2004).

Species identification

Organisms associated with *Sargassum* were identified to genus/species level in all of the studies; however, the reference manuals/guides used were only noted by a minority (17%) of studies, with the most frequently used being Morris and Mogelberg (1973, Table 2). In addition to the above manuals/guides, several studies alluded to the use of dissecting (Calder 1995, Schell et al. 2016, Martin et al. 2021) and compound (Schell et al. 2016) microscopes to aid in the morphological identification of species.

Quantitative assessment of biodiversity

To date, studies on pelagic *Sargassum*—associated fauna have been diverse in their aims, and have included objectives to investigate species diversity and evenness, test the relationship between *Sargassum* biomass and species richness and density, test the effect of environmental parameters on observed patterns, assess video footage for relative abundance, and quantify epiphytic fauna. This section provides a brief overview of the key assessment procedures used by the reviewed studies.

Species richness/density

Several studies assessed the relationship between pelagic *Sargassum* biomass and associated species using a beam balance (Dooley 1972) or spring scales (Settle 1993, Schell et al. 2016; Taylor et al. 2017, Martin et al. 2021) to record weights, or a volume displacement method (Fine 1970, Stoner and Greening 1984). Studies examining clinging and free—swimming fauna have defined species richness as the number of species per wet weight of *Sargassum*, and species density as the number of individuals per wet weight of *Sargassum*. Calder (1995) examined species richness of epiphytic fauna (hydroid species) using displaced volume in lieu of weight.

Video recordings

Only 2 studies (Moser et al. 1998, Casazza and Ross 2008) have used video recordings as a collection method. While Casazza and Ross (2008) provided a qualitative overview of the species present and recorded their behaviors, Moser et al. (1998) estimated relative abundance using 2 species time methods: Rapid Visual Technique (RVT) and Visual Fast Count (VFC). Since RVT and VFC both rely on a weighted scoring system to estimate the relative abundance of species, 10—min video footage was broken into 2—min segments and the RVT scores (weighted by order of encounter) and VFC scores (weighted by expected frequency) were calculated. Relative abundance was calculated by dividing the score of each species by the sum of

TABLE 2. *Identification manuals/guides used by various studies to aid identification of fauna associated with pelagic* Sargassum.

Manual Author	**Manual Title**	**Studies**
LeCroy (2002)	An Illustrated Identification Guide to the Nearshore Marine and Estuarine Gammaridean Amphipoda of Florida (vol. 2)	Monroy-Veláquez et al. 2019
LeCroy (2004)	An Illustrated Identification Guide to the Nearshore Marine and Estuarine Gammaridean Amphipoda of Florida (vol. 3)	Monroy-Veláquez et al. 2019
Kensley and Schotte (1989)	Guide to the Marine Isopod Crustaceans of the Caribbean	Monroy-Veláquez et al. 2019
Chace (1972)	The Shrimps of the Smithsonian-Bredin Caribbean Expeditions with a Summary of the West Indian Shallow-water Species (Crustacea: Decapoda:Natantia)	Monroy-Veláquez et al. 2019
Williams (1984)	Shrimps, Lobsters, and Crabs of the Atlantic Coast of the Eastern United States, Maine to Florida	Monroy-Veláquez et al. 2019
Castillo-Rodríguez (2014)	Biodiversidad de Moluscos Marinos en Mexico	Monroy-Veláquez et al. 2019
de León-González et al. (2009)	Poliquetos (Annelida: Polychaeta) de México y América Tropical	Monroy-Veláquez et al. 2019
Froese and Pauly (2011)	Fishbase	Monroy-Veláquez et al. 2019
Morris and Mogelberg	Identification Manual to the Pelagic *Sargassum* Fauna	Martin 2016, Taylor et al. 2017, Martin et al. 2021
Calder (1988)	Shallow-water Hydroids of Bermuda: The Athecatae	Mendoza-Becerril et al. 2020
Schuchert (2012)	North-west European Athecate Hydroid andTheir Medusae	Mendoza-Becerril et al. 2020
Coston-Clements et al. (1991)	Utilization of the *Sargassum* Habitat by Marine Invertebrates and Vertebrates- a Review	Shadle et al. 2019

scores for all species (Moser et al. 1998).

Quantifying epiphytic fauna

Many of the earlier studies that investigated the epiphytic fauna associated with pelagic *Sargassum* gave a qualitative account of their findings. These studies recorded the presence or absence of species (Weis 1968), visually determined rare, common, or dominant organisms (Weis 1968, Calder 1995) or categorized the 'age' of *Sargassum* based on the number of organisms associated with the pelagic *Sargassum* (Butler et al. 1983, Calder 1995).

Later studies (Huffard et al. 2014, Shadle et al. 2019, Mendoza—Becerril et al. 2020) quantitatively analyzed epiphytic fauna using percentage cover. Although each of these studies expressed the associated epiphytic fauna as percentage cover, slightly different approaches were used. Huffard et al. (2014) estimated percentage cover by placing each *Sargassum* strand in a tray and taking a photograph directly above the *Sargassum*. This photograph was then cropped into growth zones (previously described by Ryland (1974)) and ImageJ was used to place 250 random dots on each image to ensure at least 25 dots fell onto the bladder, stem and leaf of the *Sargassum*. Estimates of percent cover were calculated by recording the number of randomly placed dots that fell on epiphytic taxa versus those that fell on *Sargassum* with no epiphytes. Similarly, Shadle et al. (2019) took digital images (front and back) of the *Sargassum*; however, this was achieved with a Zeiss dissecting microscope that was fitted with a Canon digital camera. iSolution Lite software was then used to find the total area of the *Sargassum* sample as well as the basal area covered by each type of encrusting epiphyte present; once both measures were obtained, the total epiphytic percentage cover was calculated. Mendoza—Becerril et al. (2020) on the other hand, investigated the hydroid cover on *Sargassum* by placing the thallus between 2 clear rectangular acrylic plates which were subdivided into 1 x 1 cm squares. Percentage cover of hydroids was estimated by counting the number of squares occupied by *Sargassum* and those occupied by hydroids on both sides of the plate.

To determine the total biomass of epiphytic fauna, pelagic *Sargassum* samples were oven dried and weighed with epiphytic fauna still intact. Once the initial weight was obtained, epiphytic fauna was scraped off with the use of forceps and the sample was reweighed. The difference in the 2 weights was used as the total dry weight for epiphytic fauna (Shadle et al. 2019).

Recommendations

There is a real need to conduct site—specific biodiversity studies that are comparable across sites and over time. The type of question to be answered will dictate the best method and level of replication required and each method will have their own set of challenges. When conducting biodiversity assessments to better understand and mitigate the impacts of in—water harvesting, it is important to:

1. Acknowledge the inherent and significant variation associated with pelagic *Sargassum* communities. Components within the *Sargassum* community vary in time

and space within the Atlantic and the Gulf of Mexico (Butler et al. 1983, Stoner and Greening 1984, Martin et al. 2021). To determine seasonality of the fauna associated with pelagic *Sargassum* in the Tropical Atlantic, intensive sampling efforts will be required.

2. Understand the limitations of biodiversity assessments. Given the variation in *Sargassum* communities, it is ill—advised to generalize the findings of one biodiversity assessment to identify a specific time or place where *Sargassum* should be harvested. With that said, Monroy—Velázquez et al. (2019) reported that once pelagic *Sargassum* is close to shore the quantity and diversity of associated fauna is diminished. The rationale is that pelagic *Sargassum* in shallower water is intensively foraged by fishes, thus its removal prior to imminent stranding can be accepted as having the least impact. However, spatial configuration, nearshore slope, the presence of reefs and hydrography of the different coastlines will likely influence the distance from shore where biodiversity decreases.
3. Recognize that not all fauna within the *Sargassum* community will be affected in the same way from in—water harvesting. Large vagile fish are likely to be the least impacted taxa, given their capacity to escape, if large—scale removal is developed. Similarly, sea birds that use *Sargassum* mats for foraging (Haney 1986, Moser and Lee 2012) will also disperse when approached by harvesting vessels. Clinging fauna are more likely to be captured during harvesting events, and special efforts will be required to reduce the impacts on endangered sea turtle species. Epiphytic communities such as hydroids and tubeworms may be of lesser concern than clinging species with inherent rarity (i.e., Sargassum Frogfish, pipefish and juvenile sea turtles). However, the presence of attached flyingfish egg masses (Oxenford et al. 2019) does elevate concern for impact on the epiphytic communities, and preliminary scans to assess the presence of egg masses may be required prior to in—water harvesting.

Based on the reviewed literature, preliminary guidelines for developing a simple, easy to follow protocol for the collection and assessment of pelagic *Sargassum*—associated biodiversity that is suitable for wide use by a variety of researchers and other stakeholders is presented here.

Targeted biodiversity group

Prior to data collection, the targeted biodiversity should be classified as epiphytic, clinging or free—swimming fauna. These broad groupings will help to determine which collection methods are likely to be most suitable.

Given the nature of epiphytic and clinging fauna, both biodiversity groups can be collected using similar net types, as the results are likely to be much less sensitive to differences in net types and/or sizes than when assessing free—swimming fauna. Free—swimming fauna, on the other hand, may require nets with larger frame openings, larger mesh sizes and the additional use of video footage. Tools and techniques used to investigate the free—swimming fauna associated with pelagic *Sargassum* mats will depend on whether assessments are striving to 1) simply record the presence or absence of a species or 2) record the sizes of individuals associated with pelagic *Sargassum*. In the first instance net sampling may not be required as this can be assessed with the use of video. However, in the second case, researchers may want to employ a mixed approached (net and video sampling) bearing in mind that net type and size have the potential to bias results.

Net requirements

Whilst hand operated, towed and encircling nets can all be used to collect organisms across the 3 biodiversity groups, biodiversity assessments should strive to use the net type most suitable for each group. For example, if researchers only want to assess epiphytic and/or clinging fauna, hand operated nets may be the most suitable since these organisms are found attached and clinging to the pelagic *Sargassum*. On the other hand, a study assessing free—swimming fauna should consider using either towed or encircling nets, since large vagile species are likely to escape hand operated nets.

In addition to net type and size, it is also necessary to consider mesh size when conducting biodiversity assessments. According to Tanaka and Leite (1998), a mesh size of 0.5 mm is sufficient for capturing amphipods and gastropods, however the presence/abundance of larger fish may be underestimated (Moser et al. 1998). This is because water resistance is greater for smaller mesh sizes, resulting in slower pulling/towing speeds and thus allowing fast free—swimming fauna to escape. For this reason, it is important that collection protocols for pelagic *Sargassum*—associated biodiversity carefully consider the targeted biodiversity group(s) and their net requirements. Using the net specifications identified in this review, Table 3 provides a list of recommended net types, net sizes and mesh sizes which can be used to collect organisms across the 3 biodiversity groups.

Sampling depth

The sampling depths (0—5 m) used in this review is a reasonable range for future studies, and these depths can extend to deeper waters if the target group for the assessment is free—swimming fauna. These depths are not required when targeting epiphytic and clinging fauna; however, biodiversity assessments of clinging and epiphytic fauna should account for the entire depth/thickness of the mat being sampled.

Separating organisms from pelagic Sargassum

Once collected, the entire *Sargassum* sample should be placed in a container of seawater and transported to the laboratory. To separate the collected fauna from the *Sargassum*, the entire contents of the container should be placed in buckets of freshwater to induce osmotic shock. Alternatively, smaller samples can be placed in bags of freshwater and shaken vigorously to remove clinging and small free—swimming fauna. The rinse water in either case should be filtered through a mesh sieve to collect all detached organisms. Within the reviewed

TABLE 3. *List of recommended net specifications to be used to collect epiphytic, clinging and free-swimming fauna from pelagic Sargassum. Recommendations are based on the findings of 30 reviewed articles and serve as a starting point for future biodiversity assessments.*

Biodiversity group	Recommended net types(s)	Recommended frame opening (m^2)/ net length (m)	Recommended mesh size
Epiphytic fauna	Hand operated nets	0.07–0.25 m^2 No optimal size identified	0.5 mm
Clinging fauna	Hand operated nets	0.28–2.64 m^2 No optimal size identified	0.5 mm
Free-swimming fauna	Towed nets	0.28–2.64 m^2 No optimal size identified	3–25 mm No optimal size identified
Free-swimming fauna	Encircling nets Based on a single study	30.5 m by 5.2 m No optimal size identified	12.5 mm No optimal size identified

literature, sieves used to collect organisms after rinsing ranged between 0.28–1 mm mesh size. Keeping the mesh size <1 mm will reduce the likelihood of losing any of the small and often cryptic clinging fauna (i.e., amphipods, shrimps, molluscs) associated with the collected pelagic *Sargassum*. Ideally, the sieve used to collect organisms from the rinse water should be equal to or finer than the mesh size used to collect organisms in–water. Unlike clinging and free–swimming fauna, epiphytic fauna will remain attached to the pelagic *Sargassum*, and can be assessed without removal.

Identifying species

The manuals listed in Table 2 can serve as a starting point for the identification of fauna associated with pelagic *Sargassum*. Morphological identifications can be further validated with the use of genetic approaches using DNA barcoding libraries such as the Barcode of Life DataSystems (BOLD) and GenBank (Ratnasingham and Hebert 2007, Benson et al. 2017). In cases where it is equally important to identify the morphotypes of pelagic *Sargassum* in addition to their associated biodiversity, Parr (1939), Schell et al. (2015) and Martin et al. (2021) provide useful accounts of the various morphotypes.

Preserving specimens

If the species cannot be identified immediately, samples should be preserved for later analysis. Although several of the reviewed articles indicated the use of formalin and alcohol solutions to preserve organisms, there was no indication as to why a particular substance or concentration was chosen. According to Collins (2014), formalin is preferred for taxonomic purposes because it preserves tissue morphology over long periods. However, organisms can be preserved directly in 70% ethanol as it is an effective biocide. Ethanol concentrations >70% may result in the dehydration of samples, and those <70% will not be an effective biocide. This review suggests the use of 70% ethanol as opposed to formaldehyde solutions for preserving epiphytic and clinging fauna until they can be identified, as ethanol is more cost effective, less toxic and readily accessible across Caribbean and West African countries. Additionally, specimens preserved in ethanol can be used for molecular taxonomic identification, whereas those preserved in formalin cannot. On the other hand, large free–swimming pelagic fish captured during biodiversity assessments should be photographed and released back into the ocean. Sea turtles should only be captured with the proper permits and under specific regulations.

Other considerations

Acknowledging the benefits of combined net and video sampling, assessments of pelagic *Sargassum*–associated biodiversity will likely include underwater footage when assessing free–swimming fauna. Within the reviewed studies, cameras and ROVs were both used to visualize the free–swimming fauna associated with pelagic *Sargassum*; however, within the Caribbean and West Africa context it may be more feasible to conduct underwater assessments using cameras since access to ROVs is likely to be limited across countries and more costly.

Conclusion

With increasing numbers of *Sargassum* researchers and actors across the Caribbean and West Africa, there is a pressing need for the development of a clear set of guidelines for the collection and assessment of pelagic *Sargassum*–associated biodiversity. This would allow data to be collected across different countries in easy, practical and reproducible ways. The adoption of such guidelines would help to develop a clearer sense of the homogeneity or heterogeneity of pelagic *Sargassum* communities and achieve comparable results for future studies. It should be noted, however, that while the recommendations provided in this study are straightforward and require very little cost, extensive replication in time and space is required to achieve a reasonable assessment of the biodiversity associated with the pelagic *Sargassum* community.

Biodiversity associated with pelagic *Sargassum* varies over time and space, and therefore impacts associated with in–water harvesting and the trade–offs against coastal impacts will also vary. Studies comparing the biodiversity associated with pelagic *Sargassum* in nearshore and offshore environments are needed to better understand when, where and if in–water harvesting should occur. While establishing a clear set of guidelines for biodiversity assessments cannot directly inform managers of the impact(s) of bulk removal of massive quantities of pelagic *Sargassum* at sea, guidelines can improve conservation efforts by clarifying the value of *Sargassum* to the broader pelagic ecosystems of the Tropical Atlantic and the Caribbean Sea.

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