

A COMPARISON OF MORPHOLOGICAL FEATURES BETWEEN FRESHLY COLLECTED AND SILICA BEADS-DEHYDRATED MACROALGAL SAMPLES IN THE CENTRAL GREAT BARRIER REEF, AUSTRALIA

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ABSTRACT

Accurate and reliable identification of macroalgae has a crucial role in a range of research relating to the interaction and function of macroalgae in reef ecosystems. The dependency on identifying macroalgae on fresh samples places severe constraints on conducting this research leading to potential biases due to the selection of sites either close to shore or in the vicinity of fully equipped research stations. Therefore, this study aimed to determine if could reliably be used silica bead-dehydrated samples to identify genera characteristics that are only visible in sectioned material. Fresh macroalgal samples were collected at Nelly Bay, compared to Magnetic Island. Materials and sections were to previously collect macroalgal samples and sections dehydrated in silica beads while in the field and reconstituted in filtered seawater for identification. Each sample was identified using histological sections supported with visual tools (photograph, herbaria). In this study, macroalgal identified samples from all species to genus with characteristic features recognizable within both fresh and reconstituted samples. No artifacts were present in reconstituted macroalgal samples compared to freshly collected samples. Therefore, it is suggested that using silica beads as a preservation field method for collecting and identifying macroalgal samples is a suitable and accurate alternative method to the use of fresh samples, which eliminates the time and distance constraints associated with fresh samples.

Keywords: Great Barrier Reef, Histological sectioning, Macroalgae identification, Silica-beads preservation, Reconstituted

INTRODUCTION

Gambierdiscus toxicus is a toxic benthic dinoflagellate endemic to tropical and subtropical regions. It is responsible for ciguatera fish poisoning (CFP) - the most commonly reported seafood illness worldwide (Chinain, Gatti *et al.*, 2020). *Gambierdiscus* spp is commonly found epiphytically on macroalgae but can also be found on the surfaces of dead coral or in sediments (Kohli, Farrell *et al.*, 2015, Kibler, Davenport *et al.*, 2017). The prevalence of ciguatera is likely to increase under global climate change (Chinain, Gatti *et al.*, 2019) as predicted rising sea surface temperatures (SST) and ocean acidification is likely to increase available substrate for macroalgae.

Researchers have reported some macroalgae that *Gambierdiscus* spp appear to have a preference for *Gracilaria*, *Caulerpa*, *Sargassum*, *Halimeda*, *Ulva*, *Bryopsis*, *Codium*,

Turbinaria (Kohli, Farrell *et al.*, 2015, Rains and Parsons 2015, Kibler, Davenport *et al.*, 2017, Parsons, Brandt *et al.*, 2017, Boisnoir, Pascal *et al.*, 2019). Understanding species composition of macroalgae can be tricky, mainly due to their nature of intertwining/entangling with other marine macroalgae in their habitats. It may bring time-restrain and cause confusion if needed to separate the macroalgae during the site visit. In addition, the golden method for macroalgae sampling is to always use fresh samples. However, in carrying out sampling in large numbers and covering a very large geographic area, this method becomes very expensive and logistically difficult to implement; therefore preservation method is preferable.

The identification process is complex within the field for several reasons, including the necessity for microscopic equipment, sectioning tools, and flow-through aquaria to

maintain macroalgal samples for identification. The need for such specialized equipment can bias and limit sampling locations (representation of samples) to reef systems accessible to research stations that support the necessary equipment. Collection and identification of macroalgae in the field are restricted by high time-consuming methods that incorporated a combination of techniques, including photographic images, herbarium specimens, and maintenance of fresh macroalgal samples (Baker, Walters *et al.*, 2019, Davies, Gamache *et al.*, 2022, Rowan 2022). Therefore, it is essential to develop an efficient and reliable preservation method that can be utilized in the field and enables accurate identification of macroalgal samples on return to laboratory facilities independent of time and distance constraints.

This study aimed to identify genus marine macroalgal samples collected at Nelly Bay, Magnetic Island, and determine if re-constituted dried macroalgal samples induced morphological artifacts in comparison to freshly collected ones macroalgal samples. By doing this we are hoping to examine preservation method using silica beads has the same advantage to accurately identify the macroalgal samples.

MATERIALS AND METHODS

Study site and data collection

A total of 12 fresh macroalgal samples were collected from the inshore reef at Nelly Bay, Magnetic Island (18°10'S, 146°50'E) on 30 August 2012 (**Figure 1**). Fresh macroalgal samples were collected on the snorkel and were placed into separate zip-lock bags, ensuring to leave the base of the macroalgal attached to the substrate to allow re-growth. While in the field (Nelly Bay), fresh samples were: (1) washed in filtered seawater (FSW) to dislodge any epiphytic fauna and flora; (2) photographed; (3) a herbarium prepared; (4) and fragments of each macroalgal sample were fixed in formaldehyde acetic acid (FAA) solution. On return to James Cook University (JCU), fixed macroalgal samples were processed for histological sectioning, and herbaria were placed into a 60°C oven for seven days.

Morphological features for identifying fresh macroalgal samples were compared with macroalgal samples of the same genera dehydrated with silica beads (these were previously collected as part of the Ph.D. research on ciguatera causing toxic benthic dinoflagellate by Leanne Sparrow in 2009 field

trip). During the 2009 field trip, macroalgal samples were collected at two mid reef sites: Lodestone and Keeper Reefs (18°35'S, 146°20'E) and one inshore reef at Pioneer Bay, Orpheus Island (18°33'S, 146°29'E) (**Figure 1**). Macroalgal samples were collected in the field (as described above for sampling in Nelly Bay) and then processed in the laboratory facilities at the Orpheus Island Research Station (OIRS). Each macroalgal sample was photographed, their wet weight recorded, and portions were stored at -80°C (genetic analysis), dried in silica beads, and herbarium prepared.

All collection sites were located within the central Great Barrier Reef (**Figure 1**). All macroalgal samples collected at Nelly Bay and dehydrated samples in silica beads from the 2009 field trip were prepared at JCU for histological sectioning and staining following the protocol established for preserved samples from the 2009 field trip (Nadarajan 2011, Niedermaier 2011).

Identification

Each macroalgal sample was identified to genus level (species where possible) using two identification keys (Heimann, 2012) in association with prepared slides, herbaria, and photographs. After identification, morphological features from fresh macroalgal samples were compared to preserved samples.

Histological procedures

The macroalgal samples dehydrated with silica beads during the 2009 field trip were first reconstituted in filtered seawater for 24hrs, before fixing them in formaldehyde acetic acid (FAA). Calcified algae were decalcified in 10% HCl solution before fixing. After at least 24 hours in FAA, samples were processed following standard protocols (Ellis, 1999).

All samples were dehydrated in a graded ethanol series (70%, 80%, 90%, 95%, and 100%) followed by two changes of 100% ethanol for optimal dehydration prior to each sample being placed into a molded cassette. A number of 5 µm sections were made using a manual rotary microtome, and six slides were prepared for each sample from Nelly Bay (two slides from each sample from the 2009 field trip); which were then stained with either Mayer's Haematoxylin or Young's Eosin-Erythrosin (H&E) or Alcian Blue Safranin O (AB-S). All slides were dried in a 60°C oven for 24 hours before images were obtained using Olympus DP70 camera and BX51 microscopes.

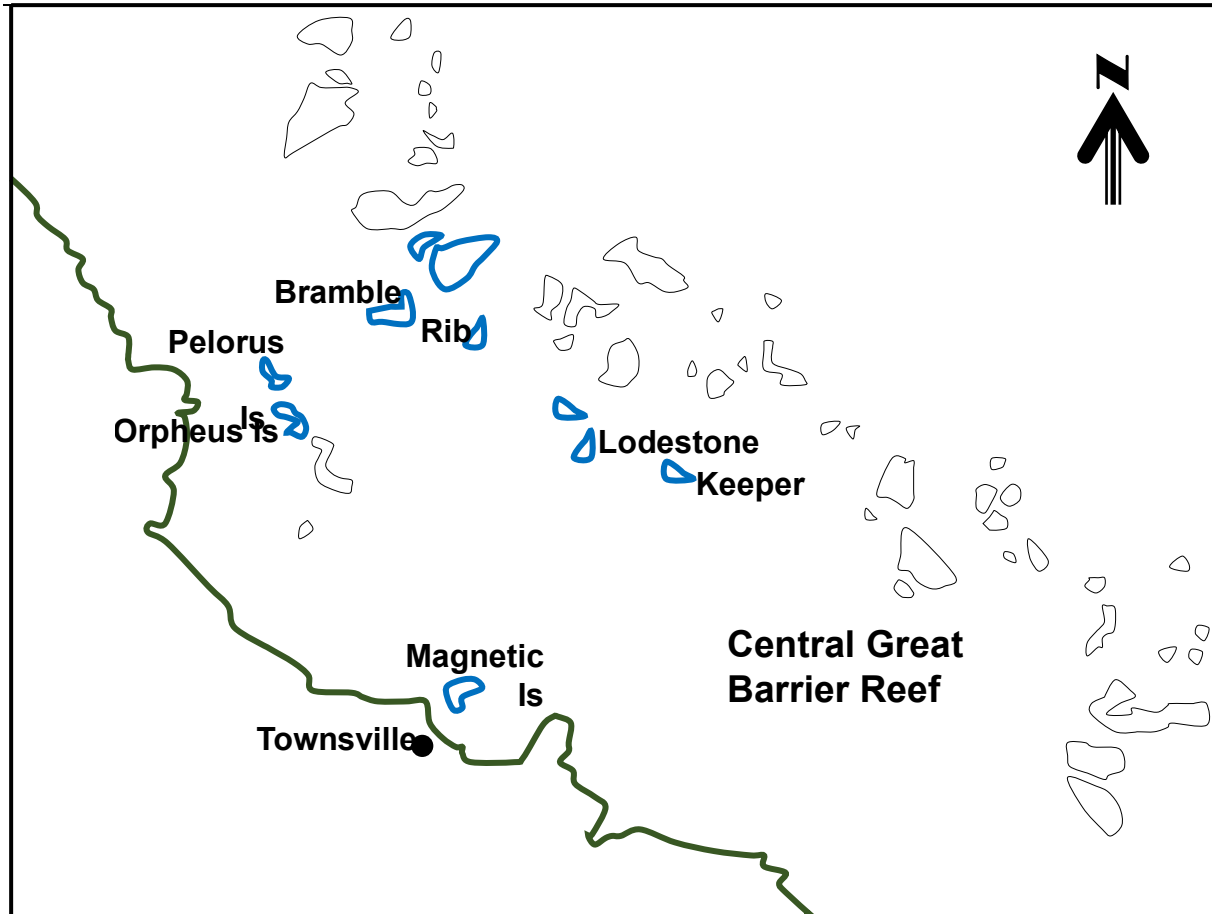


Figure 1. Map of collection sites for fresh and reconstituted macroalgal samples in the Central Great Barrier Reef, Australia (Source: Leanne Sparrow).

RESULTS AND DISCUSSION

A total of 12 fresh macroalgal samples were collected within nine genera, of which three were calcified, *Halimeda* sp., *Galaxaura* sp., *Amphiora* sp. All three seaweed phyla were

represented (**Table 1**). Identification to species level was only achievable for some genera in the Rhodophyta (Supplement: Appendix 6 and 8). These were compared to preserved samples that were reconstituted from the 2009 field trip (**Table 1**; Supplement: Appendix 1-9).

Table 1. Fresh macroalgal samples collected from Nelly Bay, Magnetic Island in the central GBR and collection sites for preserved samples of the same genera from the 2009 field trip

Chlorophyta		Phaeophyta		Rhodophyta	
Fresh	Preserved	Fresh	Preserved	Fresh	Preserved
<i>Halimeda</i> sp.	Keeper Reef	<i>Padina</i> sp.	Pioneer Bay	<i>Halymenia</i> sp.	Keeper Reef
		<i>Sargassum</i> sp.	Pioneer Bay	<i>Halymenia</i> cf. <i>florensia</i>	Keeper Reef
		<i>Turbinaria</i> sp.	Pioneer Bay	<i>Galaxaura</i> sp.	Lodestone Reef
		<i>Dictyota</i> sp.	Pioneer Bay	<i>Laurencia</i> sp.	Lodestone Reef
			Pioneer Bay	<i>Laurencia</i> cf. <i>filiformis</i>	Lodestone Reef
			Pioneer Bay	<i>Laurencia</i> cf. <i>intrica</i>	Lodestone Reef
			Pioneer Bay	<i>Amphiora</i> sp.	Lodestone Reef
					Keeper Reef

No artifacts were identified when reconstituted samples were compared with the same genera from fresh macroalgal samples (**Table 2**).

Characteristic morphological features for identification were comparable between reconstituted and fresh macroalgal samples

(Table 2, Supplement: Appendix 1-9). In particular, the cortex and utricle within the discoid segments of *Halimeda* sp. were distinct in both samples (Supplement: Appendix 1). A longitudinal section of *Sargassum* sp. clearly showed the conceptacle and the paraphyses and antheridia (Supplement: Appendix 3); similarly, for the Phaeophyta, *Dictyota* sp. the longitudinal sections were comparable between samples (Supplement: Appendix 5). In contrast,

the cortex in the reconstituted sample for of the rhodophyte *Galaxaura* sp. was partially damaged, but the pseudoparenchymatous cellular structure was still identifiable within both fresh and reconstituted samples (Supplement: Appendix 7); and the characteristic apical cell within the sunken pit of *Laurencia* spp was also comparable between dehydrated and fresh samples (Supplement: Appendix 8).

Table 2. A comparison of identifying morphological features between fresh and re-constituted macroalgal samples collected from the central GBR

Phylum	Genus	Fresh	Reconstituted	Artefacts
Chlorophyta	<i>Halimeda</i> sp.	Plates calcified; segment discoid	Plates calcified; segment discoid	None
Phaeophyta	<i>Padina</i> sp.	Thallus less erect; Branches large, firm, flattened, fan-shaped; Outer margin inrolled	Thalli erect; Branches large, firm, flattened, fan-shaped; Outer margin inrolled	None
Phaeophyta	<i>Sargassum</i> sp.	Thallus less erect, massive, not reticulate; Branches solid, not membranous, compressed, leaf-like structure, lateral; Hair absent; Vesicle stalked	Thalli erect, massive, not reticulate; Branches solid, not membranous, compressed, leaf-like structure, lateral; Hair absent; Vesicle stalked	None
Phaeophyta	<i>Turbinaria</i> sp.	Thallus less erect; Branches massive, solid, flattened, terete, hair absent, float like the blade, turbinate lateral	Thallus less erect; Branches massive, solid, flattened, terete, hair absent, float like the blade, turbinate lateral	None
Phaeophyta	<i>Dictyota</i> sp.	Thallus less erect; Branches massive, solid, flattened, membranous, strap-shaped, dichotomously, without midrib	Thallus less erect; Branches massive, solid, flattened, membranous, strap-shaped, dichotomously, without midrib	None
Rhodophyta	<i>Halymenia</i> sp.	Thallus uncalcified, crustose, multiple row distal, continuous, flattened, multiaxial, uniseriate, not hollow, axes visible,	Thallus uncalcified, crustose, multiple row distal, continuous, flattened, multiaxial, uniseriate, not	None

Turupadang, A Comparison Of Morphological Features

Phylum	Genus	Fresh	Reconstituted	Artefacts
			hollow, axes visible,	
Rhodophyta	<i>Galaxaura</i> sp.	Thallus calcified, jointed, dichotomous, cylindrical, rigid, surface smooth	Thallus calcified, jointed, dichotomous, cylindrical, rigid, surface smooth	None
Rhodophyta	<i>Laurencia</i> sp.	Thallus uncalcified, single distal row, continuous, uniseriate absent, apical cell surrounded by tricoblast	Thallus uncalcified, single distal row, continuous, uniseriate absent, apical cell surrounded by tricoblast	None
Rhodophyta	<i>Amphiroa</i> sp.	Thallus calcified, free erect, compact, dichotomous, cylindrical, diameter > 200 µm	Thallus calcified, free erect, compact, dichotomous, cylindrical, diameter > 200 µm	None

The identifying features were more distinct under Alcian Blue Safranin (ABS) staining compared to the Mayer's Haematoxylin and Young's Eosin-Erythrosin (H&E) (Supplement: Appendix 1-9). Although residues formed with the ABS stain for the fresh *Padina* sample (Supplement: Appendix 2a), this did not identify the characteristic morphological features. Sectioning of the Rhodophyta, *Halymenia* spp was difficult due to its gelatinous thallus, and staining of sections for both re-constituted and fresh was poor (Supplement: Appendix 6); however, staining was staining of a whole-mount for the re-constituted sample appeared to be successful (Supplement: Appendix 6c).

Accurate macroalgal identification is vital in the profiling and monitoring ciguatera prevalence, including the assessment of likely macroalgal substrate preferences. It is challenging to identify macroalgal substrates to genera while in the field as identifying characteristics are frequently cellular-based – this requires sectioning of macroalgae for microscopic observation. Field samples collected from the central Great Barrier Reef (GBR) were dehydrated in silica beads before being reconstituted in filtered seawater and histological sections prepared for identification to genera (Nadarajan, 2011, Niedermaier, 2011). These samples were compared with

histological sections of fresh macroalgal samples collected in my study to assess the reliability of this field method. This study found that identifying characteristics were comparable between fresh and reconstituted macroalgal samples. These include the characteristic apical cell in a sunken pit and the non-distinct axial filament in *Laurencia* spp, and the medullary cells in the intergenicula, which are characteristically dissimilar in length *Amphiroa* (Heimann 2012, Lim 2020). Currently, fresh samples are collected from the field in macroalgal research and need to be directly returned to the laboratory for identification (Nakahara, Sakami *et al.*, 1996, Parsons, Settlemer *et al.*, 2011, Turner, Poon *et al.*, 2017) It is suggested that this method is necessary as intact and undisturbed samples will increase the opportunity of successful identification to low taxonomic level (e.g., species-level) (Hallegraeff, Anderson *et al.*, 1995, Fetscher, Busse *et al.*, 2009). The use of silica beads to dehydrate field samples may be a reliable alternative method that supports accurate and efficient identification.

Degradation of fresh macroalgal samples limits the number of samples collected and the distance of field collection sites from a laboratory with supportive equipment for identification. This study did not identify any

artifacts in comparing morphological characteristics between reconstituted and fresh macroalgal samples. Identification of macroalgae in this study included samples from all species and calcified and non-calcified specimens, which involved a range of morphological and cellular identifying structures. This suggests that using silica beads for on-site dehydration of macroalgal samples may be a suitable and alternative method to eliminate the current limitation of distance to the field site and the total number of samples collected. Recent field trips for the collection of macroalgal samples in association with ciguatera research used silica beads to dehydrate macroalgal samples for transport and storage – this enabled sampling at sites remotely located from suitable laboratory facilities, including the outer reef on the GBR and Harvey Bay, Queensland (pers. comm. L. Sparrow). Although currently, researchers prefer to use fresh macroalgal samples due to the required high degree of accuracy in identification (Parsons, Settlemyer *et al.*, 2011, Parsons, Brandt *et al.*, 2017, Jauzein, Açaf *et al.*, 2018), dehydration of field-collected macroalgal samples using reusable silica beads may be an alternative method that minimizes the current dependency on fresh samples and eliminate current distance limitations to field collection sites.

Identification of macroalgal species is an integral part of research studying the interaction of marine organisms, including macroalgal palatability by herbivorous organisms, herbivorous fish feeding preferences, the role of macroalgal species in the degradation and recovery of coral reefs, and the impact of global warming on macroalgal range expansion and community and associated organisms. The application of the field-collection dehydration method described in this study will positively contribute to this range of marine research. This study has demonstrated that it will be a valuable tool that will reduce the current dependency on the distance to suitable laboratory facilities and enable improved sampling design relating to minimum macroalgal sampling numbers for collection and identification.

CONCLUSIONS AND RECOMENDATION

The study identified the marine macroalgal samples to genus level, and artefacts were not present in reconstituted macroalgal samples compared to freshly collected samples. Using silica beads as a field preservation method for collecting and identifying macroalgae samples is a suitable and accurate alternative method.

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