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PRACTICAL TOOLS

Calcification accretion units (CAUs): A standardized approach for quantifying recruitment and calcium carbonate accretion in marine habitats

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Abstract

1. Standardized metrics that quantify a component of ecosystem functioning are essential for evaluating the current status of coastal marine habitats and for monitoring how ecologically important ecosystems are changing in response to global and local environmental change. Calcification accretion units (CAUs) are a standardized tool for quantifying net calcium carbonate accretion, early successional community structure, recruitment of algae and sessile invertebrates and other response metrics that can be determined from image analyses in coastal marine habitats.
2. CAUs are comprised of paired-settlement tiles that are separated by a spacer. This design mimics the presence of different representative habitats that are common in most marine systems such as exposed benthic surfaces, cryptic spaces inaccessible to grazers and shaded overhangings. The protected space between the tiles facilitates recruitment and inclusion of cryptic taxa in community assemblage estimates. After a period of deployment, CAUs are photographed for image analysis and then decalcified to quantify calcium carbonate accretion rates.
3. The CAU methodology provides a cost-effective, standardized protocol for evaluating structure and function in marine benthic habitats. We illustrate how CAU data can be used to compare accretion rates and the relative proportion of carbonate polymorphs in ecosystems across the globe.
4. Here we provide a comprehensive standard operating procedure for building, deploying and processing CAUs, to ensure that a consistent protocol is used for accurate data collection and cross-system comparative studies.

KEYWORDS
accretion, artificial substrate units, calcification, calcium carbonate, ecosystem monitoring, recruitment, settlement tiles

1 | INTRODUCTION

Standardized approaches for evaluating ecosystem functioning are essential for characterizing the current status of coastal marine habitats. Such measurements allow for cross-system comparisons and provide a rigorous approach for understanding how ecologically valuable ecosystems are responding to rapid environmental change (Van Rein et al., 2009). Coastal degradation of marine habitats continues to accelerate due to local and global human impacts, and as a result the development and implementation of consistent protocols and strategies to monitor disparate habitats has become increasingly important (Jackson et al., 2001; McCauley et al., 2015). Evaluation of benthic community composition, such as tracking changes in cover or abundance of community members over time, is a commonly used metric in ecosystem monitoring. These data provide valuable insights into the structure of community assemblages (i.e. diversity, abundance), and although they can provide proxies for ecosystem health and function (Bellwood et al., 2019), they do not inherently scale up to ecosystem processes (Brandl et al., 2019). Direct measurements of emergent ecosystem properties, such as biological production, biogeochemical cycling or calcium carbonate ($CaCO_3$) accretion, are therefore needed to determine spatial and temporal changes in marine ecosystem functions (Hatcher, 1997).

Emergent ecosystem properties represent the sum of all biotic, abiotic and interactive processes that shape communities (Hatcher, 1997) and provide a more holistic assessment of how an ecosystem is functioning (Brandl et al., 2019). Calcium carbonate ($CaCO_3$) accretion is an example of an emergent ecosystem property in marine habitats that directly represents key ecosystem functions such as habitat vertical growth, stabilization, expansion and erosion (Hatcher, 1997). Although net accretion of carbonate is particularly important in calcifier-dominated habitats (e.g. coral and oyster reefs), it can be used as an indicator of ecosystem function in a suite of marine habitats across the globe.

Marine taxa ranging from bivalves and arthropods to corals and algae secrete external calcium carbonate shells and skeletons that persist even after the organism dies (Lowenstam & Weiner, 1989). The net accumulation of these carbonate structures over time, as well as other mechanisms of carbonate precipitation, is the product of abiotic and biotic constructional (i.e. $CaCO_3$ production) and erosional processes (Perry et al., 2008). The resulting carbonate accretion facilitates the growth and persistence of habitat frameworks that in turn support a diversity of associated species. Evaluating current and future rates of carbonate accretion is becoming increasingly important because ocean acidification impairs biogenic calcification and has the potential to decrease ecosystem accretion rates, which threatens the proper functioning of calcifier-dominated habitats. By monitoring net accretion, we can first establish baseline ecosystem rates and then develop a quantifiable and predictive understanding of the ecosystem-wide consequences of ocean acidification (Perry & Alvarez-Filip, 2019).

Calcification accretion units (CAUs) employ the commonly used 'settlement tile' methodology to collect a representative population of a marine community (Field et al., 2007) and build on it by including cryptic habitat space and incorporating the quantification of new carbonate accretion (Price et al., 2012; Vargas-Angel et al., 2015) (Figure 1). In a typical study with settlement tiles, panels are deployed in a focal habitat where they are colonized by resident taxa for varying periods of time. Tiles are then analysed for community composition, diversity or recruitment success (Edmunds et al., 2015; Thomason et al., 2002). However, a single standardized method for settlement tile deployments does not currently exist. Tiles are made of diverse materials, ranging from terracotta to plastic, and are deployed for different periods of time, which can influence the taxa that settle on the plates (Field et al., 2007). The substrate type,

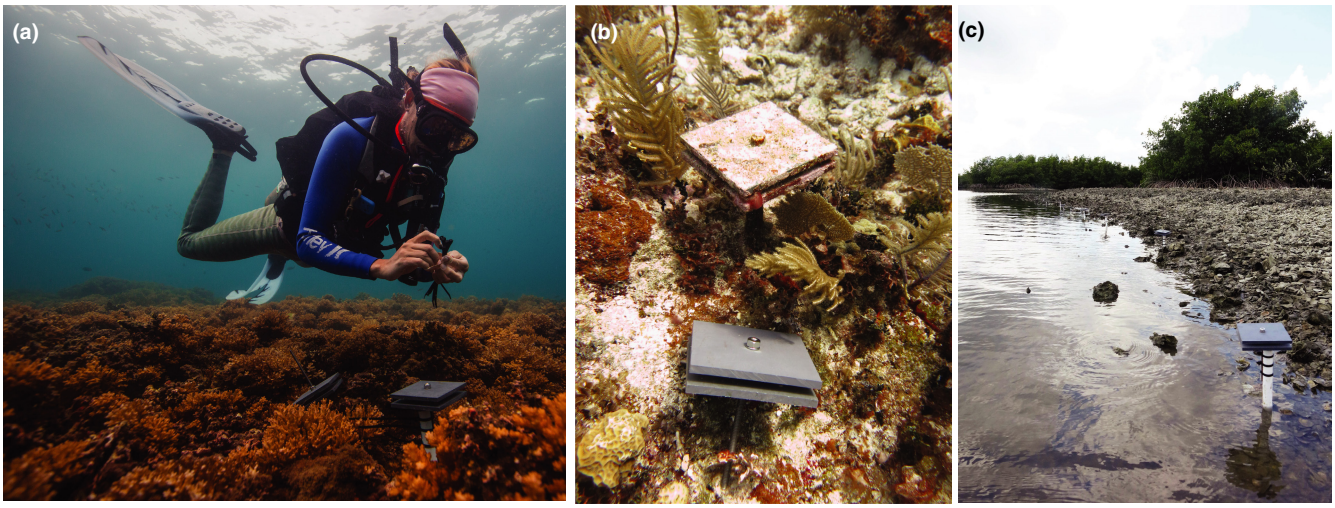

FIGURE 1 CAU field deployments. Calcification accretion units (CAUs) are deployed in coastal marine habitats where they are used to evaluate early successional community structure and net calcium carbonate accretion as an indicator of ecosystem function. (a) CAUs are deployed by a SCUBA diver on a coral reef in the Eastern Tropical Pacific. (b) A new CAU ready to be swapped with a unit that was deployed for 1 year on a coral reef in Belize. (c) CAUs were deployed on PVC poles in soft bottom substrate on an oyster reef in the Indian River Lagoon in Florida, shown at low tide. Photo credit: Sean Mattson (a) and Maggie Johnson (b, c)

duration of deployment, size of tile and sampling frequency often depends on the research question of interest, and because the approach varies by habitat and research group, the data are often not directly comparable (Field et al., 2007). Although a wide range of settlement tile data currently exist in the literature, much of it cannot be used to make direct spatial and temporal comparisons because of fundamental differences in methodology.

The CAU methodology offers a standardized protocol for constructing, deploying and processing settlement tiles, and builds on the classic single 'settlement tile' design by including a space that facilitates recruitment by cryptic taxa. Cryptic taxa contribute to biodiversity in marine habitats, but are often overlooked and underestimated by standard settlement tile protocols (Plaisance et al., 2011; Ransome et al., 2017). CAUs further elevate the typical settlement tile approach by incorporating a standardized technique to quantify the amount of $CaCO_3$ that accumulates on the tiles during the deployment through decalcification of tile communities and then calculation of the non-calcified (also referred to as fleshy) and calcified biomass of sessile taxa. Additionally, photographs of CAU tiles are used to evaluate community assemblages and can be used for any other image-based questions such as quantifying the recruitment of key taxa (i.e. corals) or invasive species.

The methodology of CAUs was introduced by Price (2010), as a modification of a design implemented in Raimondi and Morse (2000). The first use of CAUs as paired-settlement tiles for quantifying $CaCO_3$ accretion was in Price et al. (2012), where CAUs were deployed on remote coral reefs of the central tropical Pacific. The data revealed how environmental characteristics, such as pH, influence reef accretion rates and community structure (Price et al., 2012). Since then, CAUs have been used by the National Oceanic and Atmospheric Administrations (NOAA) coral reef monitoring programs in the Pacific and Caribbean, and by local programs in a handful of other locations (Alvarado-Rodríguez et al., 2019; Randi et al., 2021; Reis et al., 2016). CAUs share commonalities with another standardized approach for quantifying recruitment of benthic taxa over different spatial and temporal scales, Autonomous Reef Monitoring Structures (ARMS) (Knowlton et al., 2010). ARMS have been implemented globally by programs including NOAA's coral reef monitoring program, the Smithsonian Natural History Museum and the ARMS Marine Biodiversity Observation Network (ARMS-MBON) (Obst et al., 2020). However, the methodologies employed by ARMS and CAUs differ in important way. ARMS possess multiple levels of enclosed and semi-enclosed PVC plates (23 × 23 cm) and are processed with the goal of assessing biodiversity using molecular approaches (e.g. DNA barcoding) (Leray & Knowlton, 2015; Plaisance et al., 2011). CAUs are smaller in size and simplified in complexity, with a streamlined and less time-intensive workflow that is specifically designed to quantify carbonate accretion, as well as coarse community composition via morphological identifications. CAUs and ARMS provide complementary but unique insights into different aspects of benthic community structure and dynamics.

The CAU technique is being adopted readily by different research groups and programs, in part because the method is elegant, cost-effective and less time-intensive than other approaches. Yet, a complete protocol is not widely available. To facilitate the broad usage of CAUs and to ensure that the protocol being implemented by different groups is indeed standardized, we describe a comprehensive standard operating procedure (SOP). Here we provide the instructions for constructing CAUs and the standard procedure for CAU deployment, retrieval and data collection.

2 | LIMITATIONS OF CAUs

CAUs facilitate the collection of standardized data across habitats and provide insight to ecosystem functioning and early successional community structure. However, some caveats to the CAU methodology should be considered when implementing them in research and monitoring programs. CAUs are colonized by contemporary communities and represent early successional stages of sessile benthic communities; thus, accretion estimates from the CAUs represent 'new' accretion in the system. Furthermore, because the CAUs are not protected from biotic and abiotic pressures (e.g. herbivory, predation), any carbonate accumulation represents net accretion (i.e. the net sum of carbonate deposition and erosion). If a researcher is interested in exploring gross carbonate accretion metrics, the CAU methodology could be augmented with simple exclusion cages (Lewis, 2016; Smith et al., 2010). Another consideration is that CAUs represent a relatively small surface area relative to available benthic habitat. To ensure that CAUs are representative of the adjacent benthos, the number of replicates should be optimized per site.

The CAU methodology includes a protected space intended to facilitate settlement and inclusion of cryptic taxa. As a flat, homogenous surface (i.e. space between two sanded PVC tiles), this design is inherently limited in structural complexity. Microtopography and three-dimensional surface structure can influence settlement and recruitment processes (Edmunds et al., 2014; Nozawa et al., 2011), and thus the flattened, protected habitat of the CAU surfaces likely yield an under-representation of true cryptic diversity and may influence subsequent community dynamics and function (Brandl & Bellwood, 2016). Targeted studies that incorporate structural complexity into the CAU design, such as the different substrate types used in ARMs panels (e.g. plastic pond mesh), would shed light on the role of surface complexity in shaping early successional community dynamics. An additional consideration when using CAUs for estimations of biodiversity is that taxa are identified to coarse functional groups through image annotations, and image analyses underestimate biodiversity, particularly of cryptic taxa (Casey et al., 2021). The utility of the CAU design is that it could easily be augmented to quantify biodiversity using molecular techniques, such as the DNA metabarcoding approach used with ARMS (Leray & Knowlton, 2015).

As with any methodology, appropriate implementation is contingent on the research question of interest. CAUs provide a standardized method for evaluating present-day net carbonate accretion as an indicator of ecosystem function and can be applied in virtually any benthic marine habitat. By using a closely standardized protocol,

valuable data can be compared across different habitats, regions and ecosystems over time.

3 | CAU DESIGN

3.1 | CAU tile construction

CAUs are generally cost-effective because they are constructed from materials commonly available via local hardware stores or commercial suppliers (Figure 2a; Table 1), and the materials can be recycled for subsequent deployments after appropriate cleaning. CAUs use polyvinyl chloride (PVC) as the standardized substrate for the tiles, a material that facilitates settlement of biotic assemblages similar to the surrounding substrata and is both inert and acid-tolerant (Hixon & Brostoff, 1985). Although pre-cut tiles may be ordered, large sheets of PVC plastic can be purchased and cut into the requisite 10×10 cm size using standard carpentry tools. The steps for constructing and assembling CAUs are outlined in Figure 2, and a complete materials' list with example part numbers from a representative commercial supplier (e.g. McMaster Carr) is presented in Table 1. Each tile side, or PVC plastic sheet (prior to

FIGURE 2 CAU assembly and deployment. (a) Materials for constructing CAUs can be purchased at local hardware stores or from commercial hardware suppliers. (b) One CAU unit consists of two 10×10 cm PVC tiles stacked on top of each other and separated by a plastic spacer. (c) Stainless steel nuts, lock washers, tiles and spacers are threaded on to a stainless steel threaded rod. (d) One CAU unit recently deployed on a stainless steel stake on a coral reef in Belize. Upper case letters in images correspond to part numbers listed in Table 1 and the asterisk indicates an optional tag

TABLE 1 Components for CAU assembly, including part numbers from a representative commercial supplier

Figure 2 label	Component	McMaster Carr Part #
A	Super-Corrosion-Resistant 316 Stainless Steel Hex Nut, 1/4″-20 Thread Size	94804A029
B	316 Stainless Steel Split Lock Washer for 1/4″ Screw Size, 0.26″ ID, 0.487″ OD	92147A029
C	Type I PVC (grey sheet, ¼″ thick) Full sheet is 48″ × 96″	874K215
D	Nylon Unthreaded Spacers, 1/2″ OD, 3/8″ Length, for 1/4″ Screw Size	94639A570
E[a]	Plastic Seamless Pigeon Band	National Band & Tag Company: Style 2408
F	Type 316 Stainless Steel Fully Threaded Stud, 1/4″-20 Thread, 6″ Long	90575A570
Type 316 Stainless Steel Threaded Rod, 3/8″-16 Thread, 2-1/2 Feet Long		93250A266
316 Stainless Steel Cast Wire Rope Clamp for 3/8″ Rope Diameter—Not for Lifting		3017T46
1 1/4″ diameter schedule 40 PVC Pipe Standard-Wall Unthreaded Rigid PVC Pipe for Water, 1-1/4 Pipe Size, 5 Feet Long		48925K94

[a]Indicates an optional component that could be used to tag and track specific CAU units.

cutting), is sanded to roughen the surface and facilitate recruitment (Hixon & Brostoff, 1985). Sanding is recommended with a rotary sander fitted with a medium grit sanding disc (e.g. size 80), and each tile should be sanded until surfaces are homogenous. Sheets of PVC can then be cut to 10 × 10 cm tiles and a centre hole drilled with a drill press and ¼″ drill bit.

3.2 | CAU assembly

CAU units are made of two 10 × 10 cm PVC tiles separated by a 1-cm acrylic spacer, with each pair of two tiles constituting one CAU unit (Figure 2b). Tiles, spacers and 316 stainless steel lock washers and nuts are threaded onto a 6″-stainless steel rod (Figure 2c). The outer nuts and lock washers are hand tightened with a wrench to hold the tiles in place. The lock washers prevent slippage during deployment and hold the tiles directly in line with each other. The acrylic spacer between the two tiles of one unit creates a protected habitat that facilitates recruitment of cryptic taxa (Figure 1b). Acrylic spacers and stainless steel hardware can be cleaned with a mild acid solution and used in subsequent deployments, which further contributes to the cost-effectiveness of the methodology. If tracking specific units through time is necessary, bird bands with identification numbers (e.g. seamless pigeon leg bands) can be threaded onto the top or bottom of the CAU assembly (Figure 2c). All CAUs should be assembled in the laboratory prior to field deployments. If cryptic biodiversity is a primary question of interest, additional structures or materials, such as the plastic pond filter mesh used in ARMS (Knowlton et al., 2010), could be included to increase microtopographic complexity.

4 | FIELD METHODOLOGY

4.1 | Deployment

At each study site, six CAU replicates are deployed (actual number of replicates should be based on within-site variability in the metrics of interest when possible), with replicate units separated by 2–3 m. CAUs are attached to either stainless steel stakes or PVC poles with zip ties or rope clamps and positioned ~0.25 m above the substrate (Figure 1). The attachment method for CAUs should be determined by substrate type and can vary as long as the CAUs are secure and approximately level. In hard bottom habitats, such as a contiguous carbonate reef, stainless steel stakes can be hammered into the reef, and the stake secured further with marine epoxy (Aquamend). Stakes can be sharpened (e.g. with a lathe) to facilitate insertion into very hard substrates. In highly porous reef habitat, such as the dead *Pocillopora* framework common in the Eastern Tropical Pacific, CAUs can be attached to longer PVC poles (~1.5 m in length) that are hammered into dead substrate. In soft bottom habitats, such as sandy patches or seagrass beds, PVC poles ~1 m in length typically afford sufficient stability (Figure 1c). In mild-to-moderate disturbance environments, CAUs can be attached to stakes or PVC poles with at least three zip ties, cinched as tightly as possible. In higher energy habitats, CAUs should be secured to stakes with wire rope clamps that ensure CAUs maintain a level orientation during the deployment. Subtidal CAUs are deployed at each site by SCUBA divers, and sites are carefully recorded with GPS coordinates to facilitate relocation.

4.2 | Retrieval

The standard deployment duration for CAUs is 1 year, although this can be shortened to suit specific research questions or extended to longer periods for remote sites. For retrieval, a SCUBA diver or snorkeler removes the attachments for each CAU (i.e. rope clamps removed using wrenches or zip ties cut with snips) and places each CAU into a separate gallon Ziplock bag with seawater. Keeping organisms on the CAUs alive until processing is important to preserve organismal integrity (e.g. pigmentation) and aid species identification; direct sunlight and exposure to extreme temperature variability should be avoided. Units should be kept in well-flushed and oxygenated seawater until processing (5-gal

bucket or cooler), with the top of Ziplock bags open where necessary to facilitate gas exchange.

5 | LABORATORY PROCESSING

5.1 | Immediate processing

CAUs are immediately disassembled upon return to laboratory facilities. Motile invertebrates are hand picked off each tile (unless they are of specific interest) and tiles are gently agitated in seawater to remove excess sediment. Each tile is submerged in seawater and each side of the tile is photographed with a scale bar and label. (Tiles can also be briefly submerged in freshwater to facilitate removal of motile invertebrates). High-quality photographs are essential for accurate image analyses. CAUs should be fully submerged to allow arborescent taxa to stay upright during photos. Adequate lighting should be supplied by overhead lights or external strobes, and tiles should be placed on a white background (Figure 3a). Tiles are then rinsed in fresh water, wrapped in aluminium foil (with the top open to let moisture escape) and dried at 60°C for 2–5 days, or until a constant weight is achieved. Tiles may be frozen and maintained at −20°C until a drying oven is available. Makeshift ovens can be built using heat lamps, aluminium foil and wooden or plastic boxes, but ensure fire danger is mitigated before use. Tiles should be stored in airtight bags or containers until subsequent processing (Figure 3b).

5.2 | Recruit enumeration and identification

While many taxa can be identified and enumerated using image-based analyses, we strongly recommend using the actual dried tiles and a stereomicroscope to quantify the recruits of scleractinian corals and other small, cryptic taxa. For these detailed identifications, tiles are viewed under varying levels of magnification to search for one or two polyp settlers and a needle tool is used to 'poke' the substrate to help identify hard corals from other benthic invertebrates. The total number of recruits can be quantified, along with an estimate of their size (mm) and taxonomic affiliation (when possible). This approach has been designed specifically for evaluating the recruitment of scleractinian corals, but can be applied similarly to other focal taxa of choice. Individuals that require molecular analyses to verify identification can be subsampled and preserved at this step. Any tools used in collecting samples for molecular identification (e.g. DNA metabarcoding) should be sterilized following standard protocols, such as soaking in 10% bleach and rinsing in sterile water (Casey et al., 2021).

FIGURE 3 Post-deployment CAU processing. (a) Immediately after retrieval, CAUs are disassembled in the laboratory and photographed while submerged in seawater. Each side of each tile is photographed, and then individual tiles are wrapped in foil and dried to a constant weight at 60°C. (b) Dried CAU tiles are stored in airtight bags or containers until decalcification. (c) Tiles are submerged in 5% hydrochloric acid until all calcium carbonate is dissolved. (d) Remaining organic biomass is scraped from tiles and the biomass and neutralized acid solution are vacuum filtered through pre-weighed cellulose filters

5.3 | Decalcification

After the tiles are dried, they can be stored indefinitely in an airtight container away from light. If stored, tiles should be dried again briefly (1–2 hr) before subsequent processing. To begin processing, tiles should be removed from the drying oven, allowed to cool briefly (~1 hr) and then weighed to the nearest 0.01 g (total dry weight). Each tile is then submerged in 5% hydrochloric acid (HCl) to dissolve $CaCO_3$ (Figure 3c) in an acid-tolerant container (e.g. plastic). Commercially available HCl is sufficient for decalcification (laboratory grade is not necessary). The duration of decalcification varies depending on the amount of biomass on tiles and can range from 1 to 5 days. Acid should be refreshed when it appears neutralized and no longer actively dissolves $CaCO_3$ (no visible bubbling). Tiles are decalcified when all $CaCO_3$ is dissolved and hard structures, like shells or carbonate casings, are soft and additional acid refreshments no longer result in further bubbling. Care must be taken when working with HCl, and all institutional PPE requirements and guidelines for working with corrosives should be closely adhered to. Decalcification can produce noxious fumes and should be done in a space with adequate air exchange (e.g. protected space outside, fume hood, etc.).

5.4 | Post-decalcification processing

Once decalcified, tiles are scraped with a straight edge razor blade to remove residual biomass from tiles, and tiles are rinsed with DI water. The slurry (mix of neutralized HCl and fleshy biomass) is then vacuum filtered through pre-weighed 11-μm cellulose filters (Whatman) using a ceramic funnel. In preparation, all filters should be numbered with a permanent marker, dried at 60°C for ~1 hr, cooled briefly and then weighed. This is the initial (bare) filter weight, which will be subtracted from final filter weights to calculate the actual non-calcified (hereafter referred to as fleshy) biomass.

Decalcification containers should be lightly rinsed with DI water to remove residual biomass, and the sides of the funnel should be lightly rinsed during filtration to allow all biomass to accumulate directly on the filter. The slurry is vacuum filtered until all liquid is removed (Figure 3d). The vacuum is then turned off and the vacuum seal is broken by gently lifting the corner of the filter with forceps. The filter with biomass is gently removed with the forceps, folded in half and then wrapped in a foil envelope (as above for the tiles, leaving the top open to allow moisture to escape). If the acid slurry is not fully neutralized, the residual HCl in the filter can react with the foil, in which case wax paper should be placed between the filter and foil to protect the integrity of the filter and sample. Filters with fleshy biomass, as well as the scraped bare tiles, are then dried as described above. Bare tiles should be rinsed with DI water prior to drying and labelled appropriately.

After the drying period, bare tiles and filters with dried biomass are allowed to cool briefly and weighed as described above. The bare tile weights and filter with dried biomass weights are both used in the calculation of accretion rates. The fleshy biomass of each tile is calculated by subtracting the bare filter weight from the weight of the dried filter with biomass (filter with dried biomass − bare filter = fleshy biomass). This represents the non-calcified, organic portion of the community on each tile. The calcified biomass is then calculated by subtracting the bare tile weight from the total dried tile weight and then subtracting the total fleshy biomass (total dry weight − bare tile − fleshy biomass = calcified biomass).

Net accretion (i.e. calcification) rate is determined by summing the calcified biomass of both tiles in a CAU unit and then normalizing to the surface area of the tiles in a CAU unit (400 cm^2) and duration of deployment in years. Net calcification per CAU unit is expressed as g $CaCO_3$ cm^{-2} $year^{-1}$. Rates can then be averaged across all CAUs within a site to calculate site-level net accretion rates.

The core data resulting from the decalcification process are as follows: calcified biomass, fleshy biomass (also referred to as non-calcified biomass) and total dried biomass. An example dataset of carbonate accretion rates and non-calcified biomass from coral and senescent reefs in the Atlantic and Pacific are presented in Table 2, illustrating the utility of CAUs for global comparisons. Statistical analyses with these data should properly evaluate model assumptions, as some data will be prone to non-normal distributions. For example, recruit count data (or other focal taxa) may be zero-inflated. In instances where transformation does not result in a normal distribution, statistical models that incorporate the appropriate distribution could be used (e.g. linear model with gamma distribution).

6 | IMAGE ANALYSIS

When possible, image analyses should be conducted prior to decalcification to allow the opportunity for cross-referencing image annotations with the actual tiles. At this step, recruits identified during the enumeration stage can be used to validate and inform image annotations. Image analysis can be conducted in any software that allows for random distribution of points, and identification of taxa underneath points, such as CPCe (Coral Point Count with Excel extensions) (Kohler & Gill, 2006) or the online platform CoralNet (https://coralnet.ucsd.edu) (Beijbom et al., 2015). Images should be cropped and corrected where necessary to facilitate identifications. Image analysis is performed with 25–100 randomly stratified points overlaid on each tile image (actual number of points should be determined using a power analysis and will depend on the questions of interest and the variability that exists in the system). The taxon or substrate underneath each point is identified to the finest taxonomic resolution possible. Species can be later grouped by functional group and carbonate polymorph for subsequent community analyses (Price et al., 2012; Vargas-Angel et al., 2015). Specific identifications in the image analyses will vary by location. However, each taxon should be tagged with a minimum of the following designations: accretion group (calcified, non-calcified, abiotic) > functional

TABLE 2 Exemplar data from CAUs deployed on coral and senescent reefs in the Pacific and Atlantic. The core data yielded from the CAU protocol include calcified biomass, which is representative of calcium carbonate accretion rates, non-calcified (or fleshy) biomass and the per cent of the calcifier community comprised of different $CaCO_3$ polymorphs

Location	Ocean region	Ecosystem	Calcified biomass		Fleshy biomass		% Carbonate polymorph				Reference
			g cm^{-2} $year^{-1}$	g m^{-2} $year^{-1}$	g m^{-2} $year^{-1}$	g cm^{-2} $year^{-1}$	Aragonite	Calcite	High-Mg Calcite	Other	
Pedre de Leste	South-western Atlantic	Inner Shelf Reef	0.044	437	0.014	137	1.7	2.1	96.2	0	Reis et al. (2016)
Abrolhos Archipelago		Outer Shelf Reef	0.075	745	0.012	120	1.9	0.6	97.4	0	
Parcel de Abrolhos			0.046	455	0.001	91	9.0	0.9	90.1	0	
Queimada Grande Reef	South-western Atlantic	Senescent Reef	0.013	127	0.006	56	43.4	13.2	37.7	6.2	Randi et al. (2021)
Palmyra Atoll	Central Pacific	Coral Reef	0.070	701	–	–	13.2	0.01	86.7	0	Price et al. (2012)
Kingman Reef			0.089	894	–	–	–	–	–	–	
Jarvis Island			0.194	1942	–	–	–	–	–	–	
Baker Island	Central Pacific	Coral Reef	0.072	718	–	–	–	–	–	–	Vargas-Angel et al. (2015)
Howland Island			0.082	820	–	–	–	–	–	–	
Palmyra Atoll			0.074	738	–	–	–	–	–	–	
Kingman Reef			0.085	849	–	–	–	–	–	–	
Jarvis Island			0.104	1040	–	–	–	–	–	–	

group > carbonate polymorph > genus > species (when possible). An example of functional group designations that have been used with CAUs deployed on coral reefs is presented in Table 3 (Price et al., 2012).

From the image annotations, the per cent cover of individual taxa as well as the per cent cover of major functional groups and the proportion of taxa comprised of different $CaCO_3$ polymorphs (aragonite, calcite, high-mg calcite) can be calculated (see example data in Table 2). Abundance data are usually presented by 'habitat type' with the surface (top tile), cryptic (in between tiles) and overhang (underneath bottom tile) habitats reported separately. Alternatively, data can be reported as total per cent cover for the whole CAU unit. The data can be mined later to answer additional research questions, such as recruitment of specific taxa (e.g. invasive species, coral recruits but see above). A schematic of the suggested workflow for the core metrics in the standardized CAU protocol is presented in Figure 4.

TABLE 3 Example of categories that can be used for image analyses of CAUs deployed on coral reefs. Communities will vary by location and habitat and should be amended as needed; however, taxon should be categorized by accretion group, functional group, $CaCO_3$ polymorph and genus and species where possible. Carbonate polymorphs can vary within genera and should be verified for each species

Accretion group	Functional group	Taxa	$CaCO_3$ polymorph
Calcifier	Calcified invert	Bryozoan	Mg Calcite
		Coral	Aragonite
		Tube worm	Aragonite
		Mollusc	Calcite
Calcifier	Calcified algae	Coralline algae	High-Mg Calcite
		Peyssonnelia	Aragonite
		Calcified macroalgae	Aragonite
Non-calcifier	Fleshy invert	Sponge	Variable *(some with calcite spicules)*
		Tunicate	—
		Fleshy tube worm	—
	Fleshy algae	Fleshy macroalgae	—
		Encrusting fleshy algae	—
		Turf	—
Calcifier	Substrate	Carbonate	Variable
Abiotic		Sediment	—
Non-calcifier		Biofilm	—

FIGURE 4 CAU protocol schematic. Suggested workflow for the standardized collection and processing of CAUs for community structure and carbonate accretion data

7 | CONCLUSION

Habitat degradation continues to escalate in coastal marine habitats, and methods that implement standardized protocols to evaluate spatial and temporal changes in ecosystem functioning are becoming increasingly important. The increasing adoption and utilization of CAUs, as well as other standardized methods such as ARMS, further speaks to the need for a clear and consistent protocol that can be used by different research groups and programs. Although settlement tiles have long been used for recruitment studies in marine research, the CAU method we detail here provides an opportunity to elevate the data collected from settlement tiles beyond community assemblage and to incorporate a metric of ecosystem function. By increasing the availability and transparency of the standardized CAU

protocol, we hope this tool can be adopted by more research groups and monitoring programs to enable large-scale cross-system studies and collaborative research to fill much needed gaps in our knowledge of coastal marine ecosystem performance now and in the future.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

N.N.P. and J.E.S. developed the original design and implementation of CAUs.; M.D.J. created figures and wrote the manuscript. All co-authors edited the manuscript and approved of the final submission.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

This manuscript contains no data.

ORCID

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REFERENCES

Alvarado-Rodríguez, J. F., Nava, H., & Carballo, J. L. (2019). Spatio-temporal variation in rate of carbonate deposition by encrusting organisms in different reef microhabitats from Eastern Pacific coral reefs. *Journal of the Marine Biological Association of the United Kingdom*, *99*, 1495–1505.

Beijbom, O., Edmunds, P. J., Roelfsema, C., Smith, J., Kline, D. I., Neal, B., Dunlap, M. J., Moriarty, V., Fan, T. Y., Tan, C. J., Chan, S., Treibitz, T., Gamst, A., Mitchell, B. G., & Kriegman, D. (2015). Towards automated annotation of benthic survey images: Variability of human experts and operational modes of automation. *PLoS ONE*, *10*, e0130312.

Bellwood, D. R., Streit, R. P., Brandl, S. J., & Tebbett, S. B. (2019). The meaning of the term 'function' in ecology: A coral reef perspective. *Functional Ecology*, *33*, 948–961. https://doi.org/10.1111/1365-2435.13265

Brandl, S. J., & Bellwood, D. R. (2016). Microtopographic refuges shape consumer-producer dynamics by mediating consumer functional diversity. *Oecologia*, *182*, 203–217.

Brandl, S. J., Rasher, D. B., Cote, I. M., Casey, J. M., Darling, E. S., Lefcheck, J. S., & Duffy, J. E. (2019). Coral reef ecosystem functioning: Eight core processes and the role of biodiversity. *Frontiers in Ecology and the Environment*, *17*, 445–453. https://doi.org/10.1002/fee.2088

Casey, J. M., Ransome, E., Collins, A. G., Mahardini, A., Kurniasih, E. M., Sembiring, A., Schiettekatte, N. M., Cahyani, N. K. D., Wahyu Anggoro, A., Moore, M., & Uehling, A. (2021). DNA metabarcoding marker choice skews perception of marine eukaryotic biodiversity. *Environmental DNA*, *3*, 1229–1246.

Edmunds, P. J., Nozawa, Y., & Villanueva, R. D. (2014). Refuges modulate coral recruitment in the Caribbean and the Pacific. *Journal of Experimental Marine Biology and Ecology*, *454*, 78–84.

Edmunds, P. J., Steneck, R., Albright, R., Carpenter, R. C., Chui, A. P. Y., Fan, T. Y., Harii, S., Kitano, H., Kurihara, H., Legendre, L., Mitarai, S., Muko, S., Nozawa, Y., Padilla-Gamino, J., Price, N. N., Sakai, K., Suzuki, G., van Oppen, M. J. H., Yarid, A., & Gates, R. D. (2015). Geographic variation in long-term trajectories of change in coral recruitment: A global-to-local perspective. *Marine and Freshwater Research*, *66*, 609–622. https://doi.org/10.1071/mf14139

Field, S. N., Glassom, D., & Bythell, J. (2007). Effects of artificial settlement plate materials and methods of deployment on the sessile epibenthic community development in a tropical environment. *Coral Reefs*, *26*, 279–289. https://doi.org/10.1007/s00338-006-0191-9

Hatcher, B. G. (1997). Coral reef ecosystems: How much greater is the whole than the sum of the parts? *Coral Reefs*, *16*, S77–S91. https://doi.org/10.1007/s003380050244

Hixon, M. A., & Brostoff, W. N. (1985). Substrate characteristics, fish grazing, and epibenthic reef assemblages of Hawaii. *Bulletin of Marine Science*, *37*, 200–213.

Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., Bradbury, R. H., Cooke, R., Erlandson, J., Estes, J. A., Hughes, T. P., Kidwell, S., Lange, C. B., Lenihan, H. S., Pandolfi, J. M., Peterson, C. H., Steneck, R. S., Tegner, M. J., & Warner, R. R. (2001). Historical overfishing and the recent collapse of coastal ecosystems. *Science*, *293*, 629–638. https://doi.org/10.1126/science.1059199

Knowlton, N., Brainard, R. E., Fisher, R., Moews, M., Plaisance, L., & Caley, M. J. (2010). Coral reef biodiversity. In A. McIntyre (Ed.), *Life in the world's oceans: Diversity distribution and abundance* (pp. 65–74). John Wiley & Sons.

Kohler, K. E., & Gill, S. M. (2006). Coral Point Count with Excel extensions (CPCe): A Visual Basic program for the determination of coral and substrate coverage using random point count methodology. *Computers and Geosciences*, *32*, 1259–1269. https://doi.org/10.1016/j.cageo.2005.11.009

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*, *112*, 2076–2081.

Lewis, L. (2016). *Consumers on coral reefs: Variation in the structure and function of herbivorous echinoid communities and their consequent effects on benthic dynamics* (Ph.D.). Biological Oceanography, University of California San Diego.

Lowenstam, H. A., & Weiner, S. (1989). *On biomineralization*. Oxford University Press.

McCauley, D. J., Pinsky, M. L., Palumbi, S. R., Estes, J. A., Joyce, F. H., & Warner, R. R. (2015). Marine defaunation: Animal loss in the global ocean. *Science*, *347*, 6219. https://doi.org/10.1126/science.1255641

Nozawa, Y., Tanaka, K., & Reimer, J. D. (2011). Reconsideration of the surface structure of settlement plates used in coral recruitment studies. *Zoological Studies*, *50*, 53–60.

Obst, M., Exter, K., Allcock, A. L., Arvanitidis, C., Axberg, A., Bustamante, M., Cancio, I., Carreira-Flores, D., Chatzinikolaou, E., Chatzigeorgiou, G., & Chrismas, N. (2020). A marine biodiversity observation network for genetic monitoring of hard-bottom communities (ARMS-MBON). *Frontiers in Marine Science*, *7*, 1031.

Perry, C. T., & Alvarez-Filip, L. (2019). Changing geo-ecological functions of coral reefs in the Anthropocene. *Functional Ecology*, *33*, 976–988. https://doi.org/10.1111/1365-2435.13247

Perry, C. T., Spencer, T., & Kench, P. S. (2008). Carbonate budgets and reef production states: A geomorphic perspective on the ecological phase-shift concept. *Coral Reefs*, *27*, 853–866. https://doi.org/10.1007/s00338-008-0418-z

Plaisance, L., Caley, M. J., Brainard, R. E., & Knowlton, N. (2011). The diversity of coral reefs: What are we missing? *PLoS ONE*, *6*, e25026.

Price, N. (2010). Habitat selection, facilitation, and biotic settlement cues affect distribution and performance of coral recruits in French Polynesia. *Oecologia*, *163*, 747–758. https://doi.org/10.1007/s00442-010-1578-4

Price, N. N., Martz, T. R., Brainard, R. E., & Smith, J. E. (2012). Diel variability in seawater pH relates to calcification and benthic community structure on coral reefs. *PLoS ONE*, *7*, e43843. https://doi.org/10.1371/journal.pone.0043843

Raimondi, P. T., & Morse, A. N. C. (2000). The consequences of complex larval behavior in a coral. *Ecology*, *81*, 3193–3211. https://doi.org/10.1890/0012-658(2000)081[3193:tcoclb]2.0.co;2

Randi, C. B., Becker, A. C., Willemes, M. J., Perry, C. T., Salgado, L. T., de Carvalho, R. T., Motta, F. S., de Moura, R. L., de Moraes, F. C., & Pereira-Filho, G. H. (2021). Calcium carbonate production in the southernmost subtropical Atlantic coral reef. *Marine Environmental Research*, *172*, 105490.

Ransome, E., Geller, J. B., Timmers, M., Leray, M., Mahardini, A., Sembiring, A., Collins, A. G., & Meyer, C. P. (2017). The importance of standardization for biodiversity comparisons: A case study using autonomous reef monitoring structures (ARMS) and metabarcoding to measure cryptic diversity on Mo'orea coral reefs, French Polynesia. *PLoS ONE*, *12*, e0175066. https://doi.org/10.1371/journal.pone.0175066

Reis, V. M. D., Karez, C. S., Mariath, R., de Moraes, F. C., de Carvalho, R. T., Brasileiro, P. S., Bahia, R. D. G., Lotufo, T. M. D. C., Ramalho, L. V., de Moura, R. L., & Francini-Filho, R. B. (2016). Carbonate production by benthic communities on shallow coralgal reefs of Abrolhos Bank, Brazil. *PLoS ONE*, *11*, e0154417.

Smith, J. E., Hunter, C. L., & Smith, C. M. (2010). The effects of top-down versus bottom-up control on benthic coral reef community structure. *Oecologia*, *163*, 497–507. https://doi.org/10.1007/s00442-009-1546-z

Thomason, J. C., Letissier, M. D. A., Thomason, P. O., & Field, S. N. (2002). Optimising settlement tiles: The effects of surface texture and energy, orientation and deployment duration upon the fouling community. *Biofouling*, *18*, 293–304. https://doi.org/10.1080/08927010210000034409

Van Rein, H. B., Brown, C. J., Quinn, R., & Breen, J. (2009). A review of sublittoral monitoring methods in temperate waters: A focus on scale. *International Journal of the Society for Underwater Technology*, *28*, 99–113.

Vargas-Angel, B., Richards, C. L., Vroom, P. S., Price, N. N., Schils, T., Young, C. W., Smith, J., Johnson, M. D., & Brainard, R. E. (2015). Baseline assessment of net calcium carbonate accretion Rates on US Pacific reefs. *PLoS ONE*, *10*, e0142196. https://doi.org/10.1371/journal.pone.0142196

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