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Quantification Approaches from Marine Imagery

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Summary

Monitoring of benthic habitats requires the ability to be able to survey ecosystems using methods which are not only cost effective, but also efficient, appropriate for the habitat type and the purpose of the survey. The scope of the present report was to summarise various commonly employed quantification techniques used to analyse still imagery, gaining knowledge and guidance from the literature, and presented in a format which can be used as guidance when planning survey work. A literature review was undertaken systemically using the PRISMA framework using a keyword search to identify literature. From the relevant literature identified during the review, information regarding quantification approaches, advantages/disadvantages, etc. were extracted and terms standardised to allow comparability. Five main quantification categories were summarised, ranging from qualitative to fully quantitative methods: Presence/Absence, SACFOR, Point count, Frequency of Occurrence and abundance (count, biomass and seabed cover).

For each of the quantification techniques, a clear definition, with both the advantages and disadvantages provides a useful comparison between techniques and when they may be appropriate to use. Deciding which technique is appropriate is linked to the purpose of the survey/monitoring, so pre-survey consideration is essential to ensure the best use of data. For example, while Presence/Absence (P/A) is the least time consuming of the techniques, it provides the lowest resolution of data and fewer statistical tests can be applied. Although, if using indicator species to monitor changes in ecosystem, P/A quantification can be the most effective technique to use. The literature review identified the lack of use of standardised terms when reporting quantification techniques applied to studies, making interpretation difficult at times, and often little or no justification of choice of methods were reported. Several case studies, as exemplars of the most-commonly used techniques are provided, that help inform design of monitoring studies to appropriately survey ecosystems using methods which are not only cost effective, but also efficient and appropriate for the habitat and taxa type.

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1 Introduction

In ecology, to better understand spatial patterns, monitor changes and assess potential impacts, which inform management strategies, quantification of benthic biota is essential (Molloy *et al.* 2013). With recent advances in technology, there has been a rapid expansion of the use of photography to quantify the megabenthos (Durden *et al.* 2016b; Benoist *et al.* 2019a; Simon-Lledo *et al.* 2020), with some survey platforms able to capture thousands of images over a few hours of deployment (Pizarro *et al.* 2013). The use of digital imagery-based techniques to sample benthic habitats poses significant advantages over traditional trawl assessments as it is now evident that trawl assessments underestimate both numerical and biomass density (e.g. 20–200-fold underestimation of biomass density (Benoist *et al.* 2019b) and 20–60-fold underestimation of numerical density (Morris *et al.* 2014)).

Photographic surveys can collect high quality information on benthic communities, including the presence/absence of taxa, direct counts of individuals or colonies, seabed areal cover estimates, and estimates of individual/colony size (Hill & Wilkinson 2004; Dumas *et al.* 2009; Trygonis & Sini 2012). The use of imagery to sample benthic communities and broad-scale habitat features has significantly increased in recent decades (Vevers 1951; Jaffe *et al.* 2001; Solan *et al.* 2003; Benoist *et al.* 2019a) as it can be deployed more effectively over larger study areas compared to physical sampling. Imagery is a sampling tool with many advantages: it is non-destructive, it may reduce costs compared to other methods, and it may produce more accurate and precise data than alternative options (Morris *et al.* 2014). As a result of this increase in efficiency, photographic surveys can enable collection of larger volumes of images for more accurate estimations of faunal abundance (Durden *et al.* 2015). Advancements in digital imagery techniques have also brought about the development of image annotation software (Gomes-Pereira *et al.* 2016) which allows large quantities of data to be analysed and has been used in greater than 500 publications. Despite the progress that has been made in standardising benthic environmental monitoring at national, regional, and international levels and the increased use of digital imagery, there is still a lack of guidance of the use of quantification methods applied to imagery.

The basis of field ecological studies is an attempt to capture the natural variability in organism abundances at various spatial and temporal scales (Baring *et al.* 2016). Adequate survey design and consideration of data quantification methods are important, as they affect the accuracy of results. (see Durden *et al.* 2016a for more details). The best survey designs are those that feature evenly dispersed sampling effort (images and transects) over environmental gradients in the survey area (Foster *et al.* 2014). Prior to undertaking an image-based survey or monitoring programme there are two key concepts which are important to consider in terms of survey design and subsequent interpretation of survey data, which may be of particular concern for photographic studies: pseudo replication (Hurlbert 1984) and autocorrelation (Legendre 1993) – see Durden *et al.* (2016a) for more details. For most ecological sampling programs, it is widely accepted that an increase in the number of replicate samples usually results in, up to a point, lower standard error and improved sampling precision (Andrew & Mapstone 1987; Bros & Cowell 1987).

Sub-sampling is a useful way to reduce the time required for processing of samples but the associated potential for lower sampling precision must be considered and evaluated explicitly. Several potential pitfalls associated with sampling for organism abundances using different sample sizes need to be considered (Andrew & Mapstone 1987). For example, the potential inability of small sampling units to obtain a reasonable indication of relative abundances at larger scales, and the chance of increased observer fatigue and decreased precision with very large sampling units (Andrew & Mapstone 1987). Both problems can lead to the over-estimation of species with large abundances and under-estimation of rarer ones (Andrew & Mapstone 1987). Many authors highlight the need for appropriate pre-survey

planning and pilot studies; without pre-planned exploration of levels of variability the risk of Type II errors increases (where no effect is detected due to lack of statistical power), leading to false conclusions (Fairweather 1991).

The concept of using digital imagery to generate samples has largely evolved from terrestrial and shallow-water applications, where this approach has been widely used and recommended for rapid ecological assessments (Goldberg & Foster 2002; Preskitt *et al.* 2004). After image collection, the quantification of benthic taxa in large volumes of imagery is most often carried out manually by analysts in a process termed annotation. Even automated systems (i.e. deep learning) must be trained and mediated by human input, potentially involving the manual annotation of thousands of images (Durden *et al.* 2016b). Analysts commonly access and annotate imagery using annotation software (see Gomes-Pereira *et al.* (2016) for examples).

The reliability of the method used to quantify benthic taxa is intrinsically linked to precision (how variable one sample is from the next) and bias (the difference between the expected value of a quantification method and the true value of the parameter being estimated). The design of the survey being undertaken and post-survey decisions for image analysis are important considerations. To be able to quantify variability of biota, several considerations are necessary such as:

- (a) the quantification method employed, and the sampling effort applied to scoring each image,
- (b) at a transect level, both the number of images considered, and the method of selection of such derived images,
- (c) the number of replicate transects in an area, and
- (d) the design and length (i.e. distance) of the transects (Houk & Van Woessik 2006).

The image selection approach (e.g. random vs. systematic sampling) within a transect will also result in a different spread of samples and may affect efficiency, bias, and variance (Perkins *et al.* 2016).

Quantification of taxa from still imagery can be performed using several techniques, each with their own potential benefits and disadvantages. The quantification methodology used for annotation is generally based on the scientific questions asked of the dataset, however various constraints will affect how viable each option might be. For example, the local environment or taxa being counted (e.g. colonial/covering organisms, or solitary individuals). On a densely populated rocky reef, colonial taxa can be annotated by exhaustively drawing around their extents and using software to calculate their seabed cover (cover is a dimensionless measure of local extent), or it may be more efficient to count them using a Frequency of Occurrence grid (number of positive occurrences/maximum possible number of occurrences). Further constraints may arise from image resolution and quality, camera angle (e.g. oblique, or downward facing), which may affect the analyst's ability to resolve taxon identity. Identification of taxa is generally performed by multiple analysts working on a single set of images, so standardisation and agreement in methodology is essential to produce reliable datasets (Durden *et al.* 2016b).

There remains a basic need to understand which methods of quantifying benthic taxa from still imagery are the most efficient in terms of cost (time and funds) and benefit (precision and accuracy) (Drummond & Connell 2005). Consideration of quantification techniques is important as different techniques can provide varying conclusions from the same assemblage structure (Knott *et al.* 2004; Trygonis & Sini 2012). Drummond and Connell (2005) undertook a literature review to compare the costs (time and funds) and benefits (precision and accuracy) of commonly used quantification methods to estimate percentage

cover of sessile marine organisms. Of the papers examined (n = 216; from 25 ecological journals between 1993 and 2001), only 5.5% drew on published methods or prior knowledge to support the selection of quantification method (e.g. visual vs. digital vs. point-intercept), the configuration of point intercepts (random vs. regular) and/or the intensity of sampling (number of point-intercepts). The study concluded that no specific method was used more commonly and that there was a need to understand how various methods compare in their estimation of the sample mean and variance.

Overall, the aim of the present guidance document is to consolidate existing knowledge of quantification method for benthic taxa from still images of still/frame-grab images) as well as to deliver additional perspectives to better advise and support the marine monitoring scientist who wishes to use marine imagery to monitor marine benthic habitats. While some quantification approaches require the use of specific annotations methods (which may not be covered in the document), others could be achieved using a variety of different annotation approaches - a review of different annotation approaches are not covered within this report. Through a critical literature review, an overview of quantification approaches that have been applied to digital imagery were examined to determine the most used approach, their advantages and disadvantages, and their most applicable environment (if stated). Information from a wide range of quantification techniques such as abundance, frequency of occurrence grids, and point count (intercept), from aquatic (predominantly marine) environments, will enable use of this knowledge to inform best practice for marine benthic imagery in the context of monitoring.

2 Gap analysis

2.1 PRISMA framework

To meet the overall project aim, a literature review was conducted via a critical review analysis to extract and synthesise relevant information on use of benthic faunal quantification techniques from digital imagery.

The web search engine Google Scholar (GS) was used to conduct the searches, as it is freely accessible and wider reaching than subscription-based or ‘paywall’ databases such as Web of Science or Scopus. Google Scholar also enables incorporation of wider relevant literature such as reports, guidelines and standards (i.e. non-peer reviewed literature where subscription-based databases may not contain such information; Martín-Martín *et al.* 2021).

2.1.1. Keywords

Several search terms were selected based on focussed relevant key terminology. The final search was carried out using the following terms:

“digital image AND enumerate* AND/OR annotate* AND abundance AND benthic* AND fauna AND marine AND/OR terrestrial* frame AND photo AND/OR still* -litter”* (n = 4000)

Boolean operators were used to incorporate multiple search terms:

A capitalised “AND” combines multiple search terms, “OR” allows for inclusion of alternative terms. The asterisk function “*” attaches to the stem of a word and searches for any word that includes that stem, such as the words ‘annotate’ or ‘annotation.’

A justification statement of “-litter” was used which was required in this literature review to exclude research that focussed on litter studies (n = 1000).

URL:

https://scholar.google.com/scholar?q=digital+image*+AND+enumerate*+AND%2FOR+annotate*+AND+abundance+AND+benthic*+AND+fauna++AND+marine+AND%2FOR+terrestrial*+frame+AND+photo+AND%2FOR+still*+-litter+&hl=en&as_sdt=0%2C5&as_ylo=2006&as_yhi=2009

The literature review conducted was based on the PRISMA framework for reporting of systematic reviews (Page *et al.* 2021). The PRISMA framework allows for transparent reporting of the inclusion (and justification of exclusion) of search literature, based on key search terms. A transparency of decision-making using a triage approach of relevance is provided (See Figure 1). Based on the returned literature from the keyword search, a screening stage enabled decisions for inclusion or exclusion based on non-relevant literature as well as justification statements on levels of relevance (see Table 1). Only literature ranked from scores 2–3 were included within the final review (Objective 1); limited low-ranking literature (score 1) were included as exemplars that provided information on background to the technique(s) employed. Selected texts based on relevant studies that may, or may not, have been identified via the Google Scholar search terms were added to the screening stage (a technique commonly applied in PRISMA-based literature reviews (Page *et al.* 2021).

Table 1. Qualitative ranking system based on relevance of evidence.

Justification	Score
Highly relevant; contextual and case-study specific with detailed information on statistical approaches and analysis	3
Mid-level relevance: contextual examples/case-study based/enumeration approaches/detailed methodology on deployment and use rather than statistical analysis	2
Low relevance (e.g. background description/workshop discussion-based reporting)	1
Not relevant to faunal quantification techniques for marine imagery	0

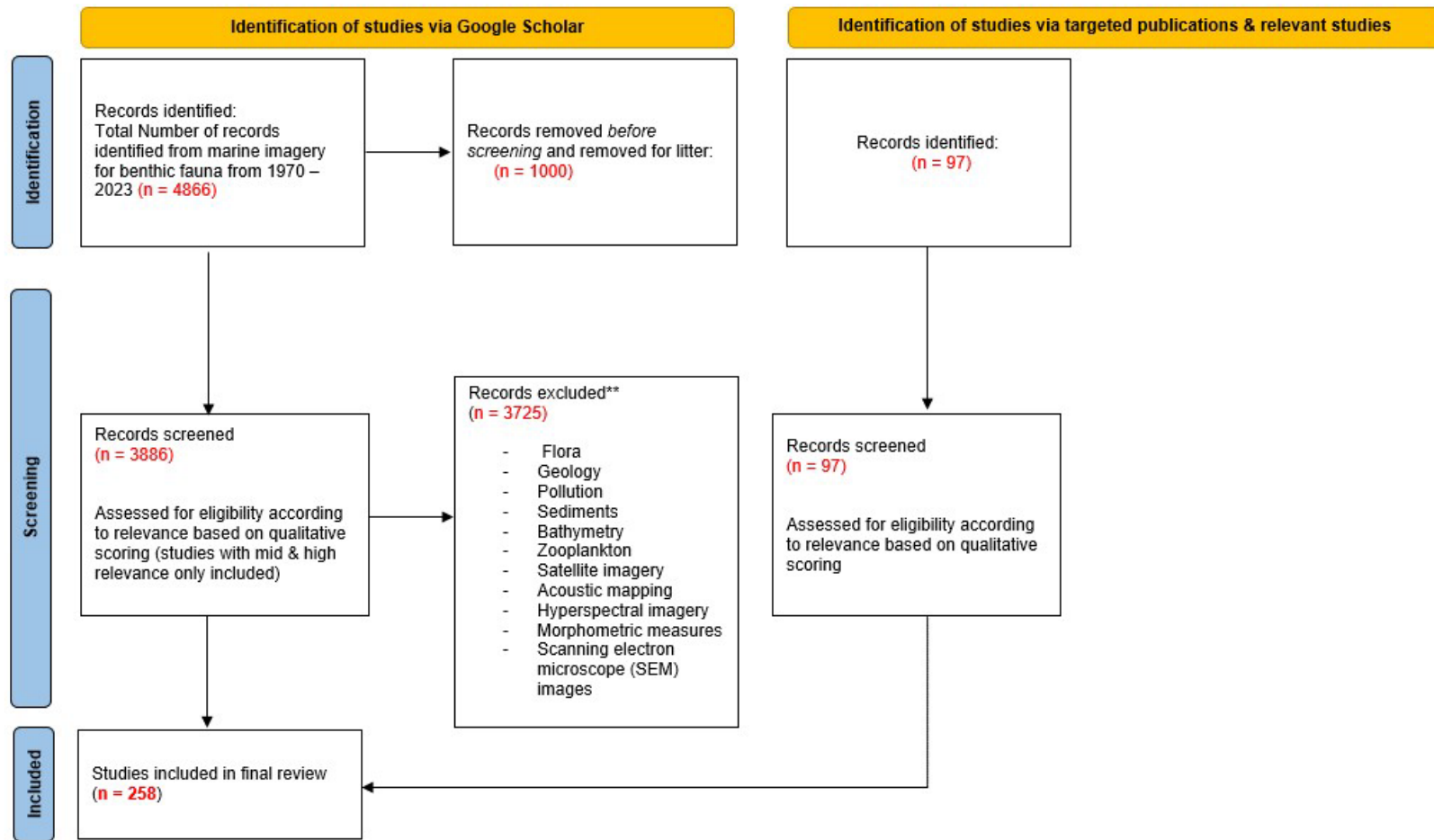


Figure 1. PRISMA flow diagram on systematic review on Faunal Enumeration Techniques for Marine Imagery (FETMI). Framework from Page *et al.* (2021).

A broad range of literature was captured from the PRISMA review, ranging from protocols, review papers (where no methods were undertaken but described and recommended), surveys (e.g. monitoring reports) to experimental peer-reviewed publications (where data were collected). The final literature items (n = 258) selected from the search were reviewed and entered into a proforma (Objective 1) to capture relevant information (e.g. quantification method, any advantages/disadvantages of methods employed, country (of authors' institute), for what data were used, etc). Sources lacked standardised reporting of quantification method(s), with the interchangeable use of synonyms a common trend. To overcome such a limitation, a hierarchical standardised terminology (extracted from the literature) was applied to each of the entries in the proforma (see Figure 2) to allow data to be easily extracted. For those sources which referred to more than one quantification method, a row was entered for each method in the proforma.

Extraction of information from the literature was challenging due to un-standardised, sparse, and incomplete reporting of methods in many instances, especially inconsistent use of terminology. Commonly employed quantification methods were classified according to overarching theme and nested subcategories within the theme (Figure 2) to allow standardisation to produce the gap analysis.

Table 2. Quantification classification and list of associated terms.

Quantification method	Subheading	Synonyms (from literature)
Abundance	Seabed cover	<ul style="list-style-type: none"> ● Coral cover ● Benthic cover ● Colony surface area ● Percentage coral cover ● Benthic percentage cover(age) ● Percentage cover of organisms ● Percent coverage of fauna ● Substrate cover ● Area cover ● Surface area ● Habitat space for benthic organisms
	Count	<ul style="list-style-type: none"> ● Density ● Specimen count ● Complete count ● Direct count ● Species count ● Faunal composition ● Individual count ● Individual colony count ● Count ● Stationary Point Count (SPC)
	Biomass	<ul style="list-style-type: none"> ● Biomass density
Point Count	Regular (Stratified)	<ul style="list-style-type: none"> ● Coral Point Count ● Hit Point Method
	Random	<ul style="list-style-type: none"> ● Coral Point Count
Presence/Absence		<ul style="list-style-type: none"> ● Presence/Absence (P/A)
Frequency of Occurrence		<ul style="list-style-type: none"> ● Points ● Grids
SACFOR		<ul style="list-style-type: none"> ● SACFOR

Quantification method classification

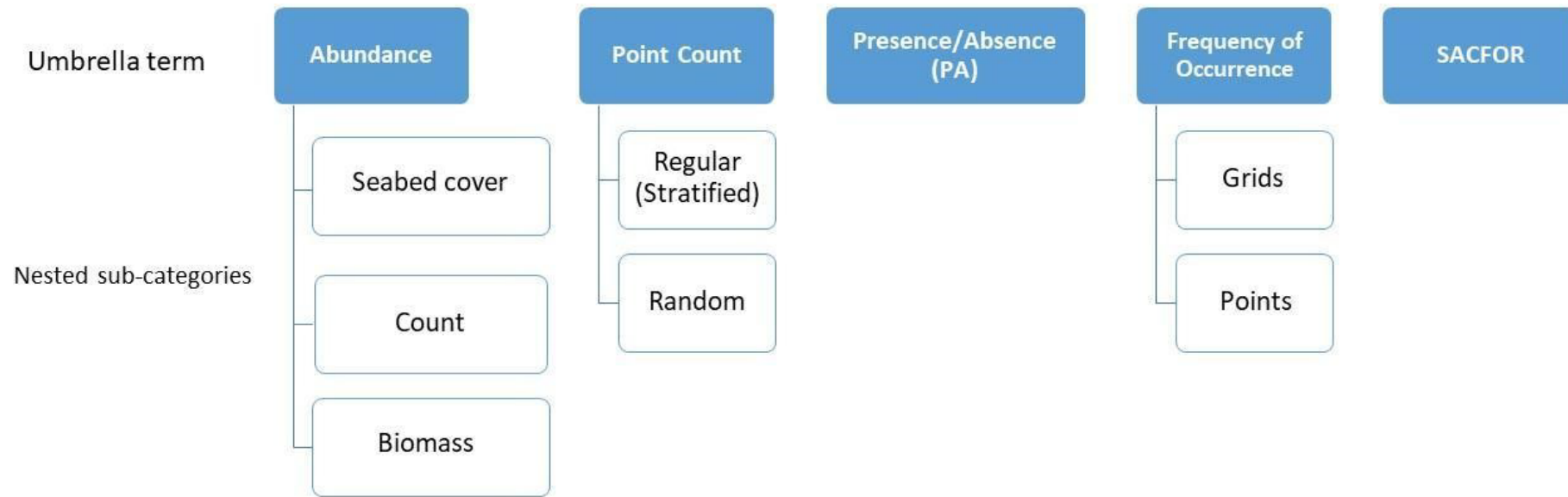


Figure 2. Quantification methods classified by key terms (derived from Objective 1 literature) standardised for synonyms: Abundance (covering count, seabed cover, biomass), Point Count, Presence/Absence, Frequency of Occurrence.

3 Quantification techniques

From the literature review, information was extracted for each of the main quantification methods to provide a definition, background description and application. Each of the quantification methods are described below, together with case studies that have employed them. Table 3 summarises the advantages, disadvantages, and considerations (extracted from the literature) for each method to aid ease of comparison between these methods.

3.1 Presence/Absence

Presence and absence (P/A) is a common quantification methods used for species distribution models (SDM) and records if a taxon is present in a sample but does not record the relative abundance of the taxa. Presence-only data is often used in 'species' distribution modelling and/ or it is critically important to distinguish between 'true absence' and 'not recorded'. Data can either be recorded as presence only records or include absence data. Absence data can either 'true' or 'inferred' absences records, where true absence are those where a species is explicitly recorded as being absent and inferred (pseudo-absence) being where no record of a species has been recorded. Collected true absence data can be challenging, hence many SDM use pseudo-absences (Wang *et al.* 2023).

The consequence of this quantification approach is that it limits the range of statistical analyses that can be applied to the data, e.g. greatly reducing the power to detect change in a community (Turner *et al.* 2016). This makes it less suitable for monitoring change at a community level, but it may be a critical effective/efficient approach for the presence of an indicator species (Perkins *et al.* 2016). Without the inclusion of proportional representation of taxa in P/A quantification, it is not possible to gauge the relative importance of species, however, the method is rapid, may be subject to relatively minimal errors in the dataset, and is readily comparable between time points and sites (Turner *et al.* 2016).

When considering the choice of a quantification method for change detection, its statistical power is an important consideration. That power is defined as the extent to which a test can correctly detect a real effect when it occurs. Conventionally, 80% power is the minimum to reliably avoid a failure to detect a real difference, with a statistical significance threshold of 5% (Saunders *et al.* 2011). Most presence-absence designs have a low statistical power to detect population declines of less than 20–50%. The low power of presence-absence designs is especially problematic if a small number of sites are surveyed, if encounter rates are low (i.e. if the population is sparse or survey effort is low), if the population is highly variable spatially, or if the population decline is widespread across many sites, rather than concentrated at a subset of sites (Strayer 1999).

Another aspect is P/A quantification is the use of indicator species to monitor change in ecosystems. An indicator species is defined as a species or group of species chosen as an indicator of, or proxy for, an overall ecosystem condition/response (Niemi & McDonald 2004; Van Rein *et al.* 2009) and are used in environmental management as a means of monitoring changes in ecosystems (Gillett *et al.* 2015). When choosing an indicator species, the selection of which species to use may reflect the presence of a conspicuous, easily identifiable, and quantified taxa, while for others it may be of ecological importance, such as key community structuring element (keystone species) (Saunders *et al.* 2011). These indicator or keystone species may be listed as priority or protected species, and the use of dominant space occupying target indicator species can be used to effectively monitor at lower data resolutions (Perkins *et al.* 2016). The use of indicator species (be it a single taxon, or a group of taxa) can reduce the need for specialist taxonomic skills, whilst reducing the overall time required for field sampling (Saunders *et al.* 2011).

Indicator taxa for monitoring are sensitive to environmental change and should meet several further criteria that demonstrate their value as an indicator of broader change, pollution, or other factor(s) of interest. These criteria include a well-known and stable taxonomy, natural history, ease of survey and data handling, broad geographic distribution of higher taxonomic levels, and patterns of biodiversity reflected in other taxa (Pearson 1994).

See case study 01 (page 20)

3.2 SACFOR

Semi-quantitative abundance scales typically contain 5–7 broad categories, allowing for coarse abundances to be recorded both quickly and accurately, but lack the precision of quantitative methods (Hawkins & Jones 1992). Work undertaken by Fischer-Piette (1936), an early pioneer of semi-quantitative scales, used a selection of similar scales to assess the biogeographic range of intertidal organisms, followed by Southward and Crisp (1954) who initially developed a log-based abundance scale, which evolved into the ACFOR scale (Abundant, Common, Frequent, Occasional, Rare) in 1958. Drawing on that specific scale, the UK Marine Nature Conservation Review (Hiscock 1990) subsequently developed the SACFOR (Superabundant, Abundant, Common, Frequent, Occasional, Rare) scale to allow rapid assessment of marine habitats, communities and species around the UK (Strong & Johnson 2020).

Count and cover data are recorded separately, using the same six classes, with cover using the growth form of the species (i.e. ‘massive/turf’ or ‘crust/meadow’) and the count scale modified for body shape (less than 1, 1–3, 5–15, greater than 15 cm). The cover classes are separated by a base-2 logarithmic scale, i.e. cover doubles between classes. The counts classes are on a base-10 logarithmic scale, i.e. density changes 10-fold between classes (Strong & Johnson 2020). This logarithmic scale can complicate the detection of subtle changes in species/communities using the SACFOR scale, and thus make it unsuitable for, at least some, monitoring programmes (Eleftheriou & McIntyre 2005).

A clear advantage of the SACFOR scale is that it allows for the rapid assessment of fauna, including skilled *in situ* assessments by divers and coastal observers, and is beneficial for broad comparisons between different sites (Turner *et al.* 2016). It is not suitable for detecting finer scale trends in benthic communities where full assessment of percentage cover and numerical density would be preferential (Turner *et al.* 2016). SACFOR does allow simultaneous assessment of species quantified as either seabed cover or numerical density using a common scale (Noble-James *et al.* 2020; Strong & Johnson, 2020). Data collected using the SACFOR scale can be converted to numerical / rank categories to enable further analyses (Howarth *et al.* 2011) which allows for the analysis of count and cover data with equal weighting, removing the influence of body size and growth form from the final analysis (Strong & Johnson 2020).

A potential observer bias can occur in the assigning of organisms to size categories (e.g. hermit crabs of the genus *Pagurus* may be assigned to the 1–3 or 3–15 cm categories), impacting the resultant SACFOR score. It is recommended that when assigning organisms to size classes, the typical adult maximum size of the species or group is used to improve consistency (Turner *et al.* 2016), this could be achieved by having a standard look-up table to assign size categories to specific species.

See case study 01 (page 20)

3.3 Frequency of Occurrence

Frequency of Occurrence Grids (also known as grid projection, grid cell count, frequency of observation, visual method, visual estimation), are where a sampling unit (quadrat, image) is divided into smaller, equally sized grid cells, and the number of cells assigned to a species is interpreted as a percentage of the total number of cells (Dethier *et al.* 1993; Benedetti-Cecchi *et al.* 1996; Frascchetti *et al.* 2001).

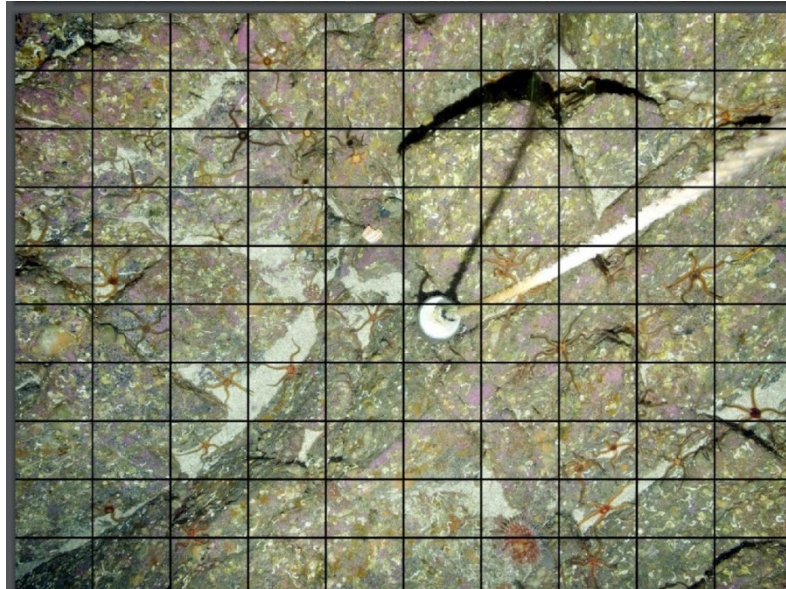


Figure 4. Image illustrating a frequency grid overlain on an image to allow quantification.

Frequency of Occurrence provides a measure of the spread of a taxon in a specified area. This is typically undertaken using a grid of equal cell sizes but is less frequently carried out using points. This means that large taxa present in small numbers will be represented to a greater extent in data collected using frequency or percentage cover estimates than abundance estimates (Beaumont *et al.* 2007). For example, if one large lamellate sponge is present in an image, only one occurrence would be reported using count quantification, but using the Frequency of Occurrence method, twenty squares may be recorded (equalling 20%) for the same sponge.

Frequency counts are more commonly used in terrestrial studies (e.g. Greig-Smith 1964) than marine epifaunal studies. Despite the relative lack of application of the Frequency of Occurrence technique in benthic monitoring studies, this technique offers potential benefits. Firstly, this method of quantifying community structure can be used for colonial and solitary species simultaneously, whereas seabed cover estimations and abundance counts are typically quantified separately (Beaumont *et al.* 2007). Less dominant taxa may be better represented using this method compared to abundance quantification, potentially making any subtle changes to community structure more readily detectable in a long-term benthic study (Van Rein *et al.* 2011a).

The concept of using Frequency of Occurrence grids is often referred to in the literature as the “visual method” or “visual estimation” (e.g. Dethier *et al.* 1993; Frascchetti *et al.* 2001). Dethier *et al.* (1993) examined user subjectivity in detail and concluded that the tendency to overestimate abundance of species with a low percentage of coverage was similar across different observers using visual estimates.

See case study 02 (page 21) and case study 03 (page 22)

3.4 Point Count

Point count, also known by many synonyms (point-intercept, random point count, Coral Point Count) is where percent cover is estimated by recording the taxa under a fixed number of points, or intercepts (e.g. Strong 1966; Kennelly & Underwood 1993). It is a commonly used method for sampling environments *in situ* and from imagery (Drummond & Connell 2005). Coral Point Count with Excel extensions (CPCe) was designed specifically to calculate statistical coral coverage quickly and efficiently over a specified area (Kevin *et al.* 2006) and has been used extensively for shallow-water studies, particularly tropical coral reefs.

The point-count quantification approach originated from terrestrial plant surveys and has been applied to marine benthic surveys in recent years (Perkins *et al.* 2022). For photographic surveys, points are overlain on an image (using projectors in older studies, or more recently using software) and the taxa under each point annotated, with the resultant data used to quantify percent cover (Perkins *et al.* 2022). There are instances where point count is used to record covering organisms only (e.g. shallow-water coral reef species cover) or may be used to record all taxa in the image (both sessile and colonial). Points can be placed in a uniform, random, or stratified manner (Foster 1991; Meese & Tomich 1992; Leonard & Clark 1993; Van Rein *et al.* 2011a, b; Trygonis & Sini 2012; Perkins *et al.* 2022). Cover is then used as a surrogate for the numerical or biomass density of target organisms across the survey area (Perkins *et al.* 2022). In applying this method, it is important to consider the number of points used, the relative abundance of the taxa of interest, the spatial arrangement of the points (e.g. random vs. regular), and the extent (size) of the individual organisms/colonies under study (Perkins *et al.* 2022).

The ability of the point-count method to detect individual taxa has been related to data resolution: the greater the number of point-intercepts sampled, the greater the sensitivity, thus the more taxa recorded – but the data-extraction effort also increases with resolution (Dethier *et al.* 1993; Van Rein 2011b). It should be noted that for rarer taxa (especially where strong spatial aggregation is shown), the point count method can lead to significant underestimation of cover (if not completely miss the taxa). Increasing sampling intensity (number of points and/or number of images) will improve the capacity to detect rare and cryptic species (Foster *et al.* 1991; Meese & Tomich, 1992; Dethier *et al.* 1993) but may require a prohibitively high sampling effort to gain sufficient precision in cover estimates to track changes (Dethier *et al.* 1993; Perkins *et al.* 2016). It is possible that, in some cases, this could lead to incorrect conclusions about trends because of high uncertainty around cover estimates.

Several studies have investigated sampling design issues around the point-count approach (e.g. Ryan 2004; Perkins *et al.* 2016; Montilla *et al.* 2020), making recommendations around the level of sampling required, with sample sizes often being prohibitively high when target species are rare or extremely patchy (Dumas *et al.* 2009). Consistent density of points maybe desired and could be achieved either by standardising the size of the image (e.g. Van Rein *et al.* 2011a) or by the density of points by area (e.g. using Coral Point Count (CPCe) overlaid points can be set so that a consistent number of points relative to the area are used (if zoomed in/out) (Thornycroft 2012)).

Another important consideration when applying the point count method is the spatial arrangement of the points, and whether the habitat has heterogeneously distributed taxa. Given that almost all species and biotopes exhibit spatially aggregated taxa, it is very likely that the spatial distribution of organisms will influence the assemblage described by some techniques (i.e. the random grid technique may over- or under-estimate the presence of heterogeneously distributed taxa, particularly when the number of points used is small (Beaumont *et al.* 2007)). Perkins *et al.* (2022) identified that increased sampling was required (both the number of images, and the number of points) with increased aggregation

(clustering) of taxa. It is claimed that randomly distributed points prevent any systematic bias associated with the regular arrangement of organisms (Andrew & Mapstone 1987) while regularly distributed points are claimed to be more representative of spatial variation (Greig-Smith 1983).

In general, but not always (objective specific), greater areal coverage is likely to be more useful than increasing resolution (number of points) within individual images, hence annotating many images over annotating a larger number of points, is generally recommended (Drummond & Connell 2005; Perkins *et al.* 2016).

Given that point count only samples a proportion of a sampling unit, it is likely to be more appropriate for habitats that have dominant space occupiers, such as hard corals in the tropics or kelp in temperate regions, which have high seabed cover (Drummond & Connell 2005; Dumas *et al.* 2009; Trygonis & Sini 2012; Perkins *et al.* 2022). This could also be applied to habitats with high cover taxa in deep-sea environments, such as cold-water coral reefs and dense sponge aggregations. This may be of relevance for those habitats that are listed under existing and 'under development' Essential Ocean Variables (Hard coral cover and composition, seagrass cover and composition, macroalgal canopy cover and composition, and mangrove cover and composition (see GOOS 2021)).

Overall, the point-count method has the advantage of being simple, quick, inexpensive, consistent (Aronson *et al.* 1994; Preskitt *et al.* 2004; Drummond & Connell, 2005; Jokiel *et al.* 2005; Van Rein *et al.* 2011a). Despite the clear advantages, it has poor sensitivity when it comes to detecting small taxa with low abundance and bias towards larger-area cover species (Edmunds & Flynn 2018). Another potential disadvantage of the point-count method is low data density per unit area if an inadequate point density is used (Drummond & Connell 2005) which could lead to an underestimation of species richness relative to the other techniques (Meese & Tomich 1992; Dethier *et al.* 1993; Beaumont *et al.* 2007).

3.4.1. Recommendations to determine sample effort

1. Consider the use of point count strategy testing to determine the optimal number and spatial distribution of points – note this step is likely to be highly case-sensitive, driven by both the environment and organisms under study and the specific objectives of the study.
2. Where biological diversity metrics are to be calculated from the resultant data, consider the use of rarefaction techniques (see, e.g. Gotelli & Chao 2013; Nicholas & Chao 2013) to determine what annotation effort level (number of points × number of images) would be most appropriate to a particular survey/study objective (see case study 04; Van Rein *et al.* 2011b)

While an increase in the number of points assessed per image increases the potential precision, it becomes more time consuming (i.e. costly). Therefore, it is recommended that some time is spent establishing the most appropriate point count strategy (points × images) for any given combination of environment, organisms, and ultimate objective. As noted above, formal assessments of these trade-offs can be made by reference to rarefaction techniques (see case study 04; Van Rein *et al.* 2011b) and via auto-similarity/self-similarity assessments (Schneck & Melo 2010); as employed in photographic assessments by Benoist *et al.* (2019a) and Taormina *et al.* (2020a).

See case study 04 (page 24)

3.5 Abundance

3.5.1 Abundance count

A scale describing the total number of organisms in a sample and expressed as numerical density (as number of individuals per unit area). Annotating for abundance is the most time consuming of the quantification methods but allows for the greatest variety of statistical analyses (Turner *et al.* 2016).

Abundance count is better for capturing small taxa present in large numbers than percent cover estimates. Conversely, large taxa present in small numbers will be represented to a greater extent in data collected using percentage cover or frequency estimates than abundance estimates (Beaumont *et al.* 2007). Perkins *et al.* (2022) showed that numerical density was likely to provide a higher statistical power to detect change over other enumeration methods, such as percent cover and presence/absence, particularly for morphospecies that are erect solitary organisms rather than encrusting.

Studies that employ abundance counts (numerical density) as measures of assemblage structure often exclude colonial organisms, such as hydroids, encrusting bryozoans, and colonial ascidians, from analysis of the data because of the difficulty in quantifying their abundances (Beaumont *et al.* 2007). There are two approaches to overcome this; combining seabed cover and numerical density on to a common scale to allow it to be statistically analysed combined (Stevens & Connolly 2004; Howell *et al.* 2010; Torres-Moye 2012) or using numerical biomass.

It should be noted, that while the ability to combine the numerical density and seabed cover onto a single scale allows statistical analysis of the combined dataset, which for community analysis is beneficial, this method will affect the resolution of data.

3.5.2 Numerical biomass

Biomass is a key ecological variable that informs the fields of conservation, environmental quality assessment, resource management, and the study of the stocks and flows of mass and energy through ecosystems (e.g. Tomlinson *et al.* 2014). In ecology, numerical biomass has typically been quantified from physical specimens from sledge and trawl nets (Gage & Bette, 2005), but trawling has long been known to provide semi-quantitative sampling of megabenthic biomass (See Durden *et al.* 2016c) and hence under-represents biomass estimates. Substantially higher standing stocks of deep-sea megabenthos, compared to trawl data, have been revealed from large photographic surveys (Morris *et al.* 2014; Durden *et al.* 2015), thus highlighting the advantage of using imagery to improve quantification of biomass (Durden *et al.* 2016c). There is general agreement of the importance of biomass as key variable (given its central role in assessing stocks and flows of mass and energy through marine ecosystems) in the essential biodiversity variable (EBVs) by global biodiversity observing system (GEO BON) and in the biology and ecosystem essential ocean variables (EOVs) by global ocean observing system (GOOS) and DOOS (Benoist *et al.* 2019b). Biomass directly, or indirectly feature in several EBVs, and assessment of these EBVs is considered relevant to CBD Aichi Biodiversity Targets (4–12, 14–15) (GEO BON 2011; Pereira *et al.* 2013).

The use of a taxon-specific length-weight relationship (LWR) approach is commonly employed in both the analyses of photographic surveys (e.g. Durden *et al.* 2015, Case study 06) and of trawl catches (e.g. Robinson *et al.* 2010) but requires adequate prior data for the taxon of interest (Benoist *et al.* 2019b). As LWRs are specific to taxa, data simply do not exist for the vast majority of megafaunal species, and this is especially true for deep-sea taxa. Two limitations of the general application of this approach are the limited baseline data

to draw from, as well as the error that may occur when applying the length-weight relationship due to seasonal variations (Benoist *et al.* 2019b). LWRs are potentially subject to systematic, temporal and spatial variation, and may be highly taxon-specific. Consequently, the use of LWRs out of temporal, spatial, or taxonomic, context may result in substantial systematic error (Benoist *et al.* 2019b).

The LWR method holds the advantage of being both simplistic and its ability to generate biomass estimates for individual specimens, such as are required in the study of individual-based body-size spectra (Edwards *et al.* 2017; Laguionie-Marchais *et al.* 2020) or any research involving the structuring role of body size in ecosystems (e.g. Sewall *et al.* 2013; Lewis *et al.* 2018; Durden *et al.* 2019).

Major works have been undertaken to compile datasets holding LWR data, for example, Wei *et al.* (2010) have provided a highly cited global benthic LWR biomass (from bacteria to megabenthos) database, which has been used as the basis of other major works, e.g. to predict future trends of seafloor biomass in response to climate change (Jones *et al.* 2014). As these biomass data encompass records based on bottom-trawl catches and on photographic surveys, this may potentially introduce mismatches in the spatial scale observed, and in the body sizes and the taxonomic groups assessed (Benoist *et al.* 2019b).

This is particularly problematic for environmental assessments conducted in poorly sampled areas where physical samples of the megafauna are rare, or even absent (e.g. deep-sea mining resource exploitation) (e.g. Gates *et al.* 2017; Durden *et al.* 2018; Stratmann *et al.* 2018), demonstrating a growing need for a more tractable method of taxon-independent biomass estimation (Benoist *et al.* 2019b). To attempt to overcome the disadvantage of the LWR being a taxon-dependent method, and hence of limited use in poorly sampled regions, a taxon-independent method of estimating specimen biovolume, as an estimator of biomass, from photographic observations can be used. The generalised volumetric method does not rely on previously collected measurements but can be obtained by taking two body size measurements from the specimen (image), equivalent cylindrical diameter (*ECD*) and equivalent cylindrical length (*ECL*) (see case study 06 for more details of this method) and biomass can then be calculated from imagery.

3.5.3 Seabed cover

Region-based area or percent coverage estimations, where the absolute or relative cover of a species is ascertained through *in situ* visual estimates or digitised images is a commonly applied method (Garrabou *et al.* 1998, 2002; Meese & Tomich 1992; Pech *et al.* 2004; Teixidó *et al.* 2002; Teixidó *et al.* 2011). Many synonyms are used to describe quantification seabed cover of taxa, such as areal cover, planar area, percent cover, which may be annotated using the same tool (i.e. polygon/freehand tool), but the way the data are expressed can vary (i.e. as numerical density or percentage cover of an image). The most reported measure of cover is percent cover and will be further outlined below.

An estimate of percent cover gives a measure of the area covered by a taxon in a specified sample or image area. Percent cover can be calculated by visual estimation, the overlay of grids, or most commonly by the overlay of points (Perkins *et al.* 2022). Measures of percentage cover, either using some form of random point or visual estimate, are amongst the most frequently used techniques in the determination of epibenthic assemblage structure (e.g. Foster *et al.* 1991; Dethier *et al.* 1993; Beaumont *et al.* 2007). With the development of annotation software (e.g. BIIGLE), polygon tools are becoming more widely used to estimate cover of taxa and can be expressed as either percent cover or numerical density.

Percent cover can act as a partial proxy measure for both numerical abundance and biomass abundance (Chiarucci *et al.* 1999; Parravicini *et al.* 2010) and if all taxa are

quantified using cover, this eliminates the need to combine data from different measurement scales. Large taxa present in small numbers will be represented to a greater extent in data collected using percentage cover or frequency estimates than individual abundance estimates, while small taxa present in large numbers will be represented to a greater extent in abundance counts than in percent cover estimates (Beaumont *et al.* 2007).

Dethier *et al.* (1993) showed visual estimates of percentage cover to be more repeatable and more sensitive than random point estimates (Beaumont *et al.* 2007). In some cases, visual estimation of percentage cover was more accurate than random point quadrat techniques (Dethier *et al.* 1993; Van Rein *et al.* 2011a). Within this report, note that point count and frequency of occurrence quantification methods also provide estimated percent cover.

See case study 05 (page 26)

Table 3. Tabular summary of quantification methods review.

Name: Count (expressed as density: no. individuals per unit area)
Method: Estimating the abundance count of organisms in an assemblage gives a measure of the number of individuals of a taxon present within a specified area (Beaumont <i>et al.</i> 2007).
Advantages: Great likelihood of recording rare taxa. Numerical density is likely to provide higher statistical power to detect change over seabed cover or Presence/Absence approaches, particularly for morphospecies that are erect solitary organisms rather than encrusting (Perkins <i>et al.</i> 2022). Allows the greatest range of statistical analyses to be conducted on the resultant data (Turner <i>et al.</i> 2016).
Disadvantages: More time consuming. Potential challenge of combining with seabed cover data (i.e. standardisation to a common scale and potential loss of resolution).
Considerations: How abundance count data will be treated post-analysis and combined with seabed cover data when that is necessary. Small taxa present in large numbers will be represented to a greater extent in abundance counts than in percent cover estimates (Beaumont <i>et al.</i> 2007).
Name: Numerical biomass (The amount of biomass per unit area product of the living material in an organism)
Method: Estimating the biomass of taxa using body dimensions and/or biovolume.
Advantages: Provide higher biomass estimates than trawl samples (Durden <i>et al.</i> 2016c). Allows assessment of stocks and flows of mass and energy through ecosystems (Tomlinson <i>et al.</i> 2014). Biomass (directly or indirectly) is considered important for CBD Aichi Biodiversity Targets (GEO BON, 2011; Pereira <i>et al.</i> 2013). LWR method is simplistic and can generate biomass for individual specimens (Edwards <i>et al.</i> 2017). GVM does not rely on previously collected measurements as is the case with LWR (Benoist <i>et al.</i> 2019b). GVM with appropriate training has low inter-analyst error (Durden <i>et al.</i> 2016c).
Disadvantages: Time consuming (Benoist <i>et al.</i> 2019b). LWR method is lacking for some taxonomic groups/morphotypes (Benoist <i>et al.</i> 2019b). For the LWR method, it requires a huge research effort to compile data for all morphotypes (Benoist <i>et al.</i> 2019b). LWRs are potentially subject to systematic, temporal and spatial, variation, and may be highly taxon-specific (Benoist <i>et al.</i> 2019b).
Considerations: The presence or absence of large, rare individuals has a very significant impact on the estimation of total biomass density (Sanders 1960).

Name: Seabed (percent) cover (dimensionless relative area measure)
Method: An estimate of seabed area covered by a taxon / taxon (Beaumont <i>et al.</i> 2007).
Advantages: Can be used for colonial and solitary species simultaneously (Beaumont <i>et al.</i> 2007), allowing for more statistical tests.
Disadvantages: More time consuming than sub-sampling methods such as point count.
Considerations: May under-represent small taxa (in large numbers) compared to abundance (Beaumont <i>et al.</i> 2007). Large taxa present in small numbers will be represented to a greater extent using percent cover than abundance (Beaumont <i>et al.</i> 2007).

Name: Frequency of Occurrence (number of positive occurrences / maximum possible number of occurrences)
Method: Frequency counts give a measure of the spread of a taxon in a specified area (Beaumont <i>et al.</i> 2007) and can be extrapolated to a measure of seabed cover.
Advantages: Can be used for colonial and solitary species simultaneously (Beaumont <i>et al.</i> 2007). Over-expression of less-dominant benthic categories could make any subtle changes to community structure more readily detectable in a long-term benthic study (Van Rein <i>et al.</i> 2011a).
Disadvantages: Maybe more time consuming than point count (number of cells vs points dependent) as all cells are recorded for the presence of taxa.
Considerations: Large taxa present in small numbers may be represented to a greater extent using frequency than abundance (Beaumont <i>et al.</i> 2007). Size of cell needs to be considered as this will influence data resolution (Moore <i>et al.</i> 2019).

Name: Point count: percent cover is estimated using ratio of points recorded for each taxa
Method: Points are superimposed on quadrats in a uniform, random or stratified manner, and the number of points assigned to a species sum up to a percentage of the total number of points (Foster 1991; Meese & Tomich 1992; Leonard & Clark 1993).
Advantages: Fast and easy application (Aronson <i>et al.</i> 1994; Preskitt <i>et al.</i> 2004; Jokiel <i>et al.</i> 2005). Can effectively be used to capture large conspicuous fauna, with a lower sample per unit effort (i.e. number of points, if enough images are analysed (Perkins <i>et al.</i> 2016)). Less time consuming than abundance quantification (Turner <i>et al.</i> 2016).
Disadvantages: Potential to fail to record rare taxa (Dethier <i>et al.</i> 1993). Poor sensitivity in detecting small taxa with low abundance (Beaumont <i>et al.</i> 2007). Potential over-representation of larger-area cover species compared to abundance quantification (Edmunds & Flynn 2018). Under-estimation of species richness and lack of individual-related data (Edmunds & Flynn 2018). Low data density per unit area (Drummond & Connell 2005; Van Rein <i>et al.</i> 2011a).

Name: Point count: percent cover is estimated using ratio of points recorded for each taxa

Considerations: Number of points and number of images used are important and have important trade-offs. Use Point count strategy testing to determine optimal number and point distribution (Taormina *et al.* 2020a). Rarefaction curves and studies of auto-similarity are useful aids in strategy design (Schneck & Melo 2010; Van Rein *et al.* 2011b). Ensure an adequate number of replicate samples (i.e. images) as it has been noted that can be more important than the number of points per image (Drummond & Connell 2005; Perkins *et al.* 2016).

Name: SACFOR (relative abundance scale)

Method: A standardised semi-quantitative scale of numerical density and seabed cover.

Advantages: SACFOR allows simultaneous assessment of species quantified as either cover or density using the same scale (Noble-James *et al.* 2020; Strong & Johnson 2020). A quick quantification method (Turner *et al.* 2016). Potential reduction of noise in the data caused by field-of-view variance (Noble-James *et al.* 2020). Scale can be used to make useful broad comparisons between different sites (Turner *et al.* 2016). Semi-quantitative scales can be applied to larger areas; they are better able to detect rare species than less extensive methods (Strong & Johnson 2020). Although the broad cover and count classes lack precision, their breadth ensures a high level of accuracy and repeatability between users (Strong & Johnson 2020), leading to its consistent application between users and across a variety of habitats (Strong & Johnson 2020).

Disadvantages: Not suitable for detecting finer scale trends in benthic communities (Turner *et al.* 2016). May be subject to observer bias with allocation of organisms to different size categories (Turner *et al.* 2016). Often applied in a subjective manner leading to intra- and inter-observer variability over space and time, this can be reduced with experience, training and well defined field methods (Strong & Johnson 2020). Does potentially limit the range of statistical tests which can be used (Strong & Johnson 2020).

Considerations: Potentially not suitable for monitoring ecosystem change due to the nature of the logarithmic scale (i.e. substantial change would need to occur to be recognised on the SACFOR scale (Eleftheriou & McIntyre 2005)). To reduce variation in allocating size classes to specific taxa, an agreed (and automated) system will help to reduce observer bias.

Name: Presence/Absence

Method: Only presence or absence of a given taxon is recorded.

Advantages: The quickest method to extract data from imagery (Turner *et al.* 2016). Presence/absence data can easily be compared over years and between sites (Turner *et al.* 2016). Errors in the dataset are also likely to be few when compared to other methods of analysis (Turner *et al.* 2016). Can be used to effectively monitor change using indicator species (Perkins *et al.* 2016). Low resolution method which can be used to monitor effectively for rare taxa (Perkins *et al.* 2016).

Disadvantages: Limits the possibilities for statistical analysis (Turner *et al.* 2016). The statistical power to detect change in community structure is greatly reduced (Turner *et al.* 2016).

4. Case studies

4.1. CS01: SACFOR and P/A

Beaumont, J., Rowden, A. & Clark, M. 2012. Deepwater biodiversity of the Kermadec Islands Coastal Marine Area. Science for conservation 319. New Zealand Department of Conservation (DOC). <https://www.doc.govt.nz/globalassets/documents/science-and-technical/sfc319entire.pdf>

Two datasets from two cruises were utilised for the report. In 2005, a series of Pisces V submersible and Remote Operated Vehicle (ROV) dives were conducted on seamounts in the study area from the RV Ka'imikai-o-Kanaloa (KOK), New Zealand. The Pisces V was equipped with a stills camera set to automatically take images every 15 seconds, with only one image from each location analysed to avoid repetitive sampling, resulting in 366 images suitable for analysis. The TAN0205 scientific cruise was undertaken on the RV Tangaroa. A total of 300 images were captured from 14 stations on eight seamounts in the study area (in addition to the above-listed seamounts, GI4 and GI9 were visited). A Teledyne Benthos camera system was mounted in a rigid frame and took seafloor photographs when lowered to within 2 metres of the bottom. However, many of these photos were very dark and, because they were mostly in black and white, faunal identification was difficult. As a result, only 115 of these images were suitable for analysis. The combined datasets yielded 482 analysed images.

Still images were analysed for fauna using Image J software. Due to the lack of scaling information, full quantification was not possible, hence the semi-quantitative SACFOR scale was employed. For statistical analysis, data were analysed as both presence/absence and SACFOR. Although coarse groupings were often used for multivariate analysis, many species were identified to the lower taxonomic levels.

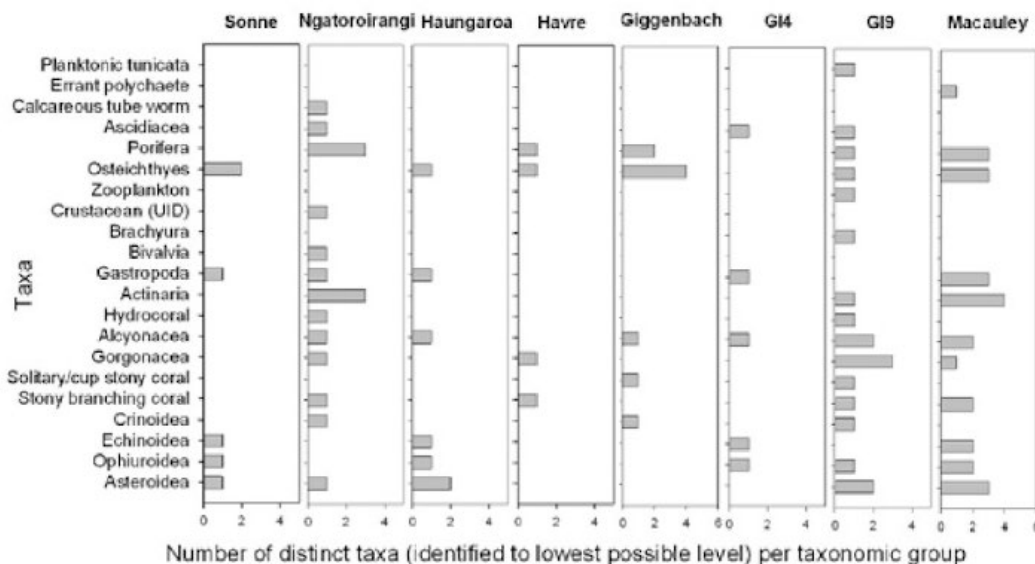


Figure 3. Graph showing the taxonomic diversity of fauna observed in the TAN0205 still images from each seamount. Image: Beaumont, *et al.* 2012.

The overall results of the study found little to no significant difference between the seamounts and the authors note that the sampling tools and strategies were not ideal for the purpose of providing a fully comprehensive description of the faunal assemblages in the study area, nor for appreciating the spatial variability in the composition of these

assemblages (including any small-scale differences in composition with changes in water depth). These findings were most likely a consequence of the coarse resolution of taxonomic identification, combined with a low number of usable images within each of the substrate subgroups for the still image datasets, and the quantification method - resulting in low power for the statistical tests. While the use of SACFOR can be a useful approach when data quality/quantity and time constraints do not allow for quantitative, the resolution of data are reduced (even if converted to numbers) and is not suitable for all monitoring, especially if time and image resolution allows for a more detailed quantification method (e.g. point count or abundance).

The study emphasises the importance of taking into consideration the sampling methods and being clear of the purpose of the work and choice of quantification as outline by Durden *et al.* (2016a).

4.2. CS02: Comparison of 4 quantification methods

Trygonis, V. & Sini, M. 2012. photoQuad: A dedicated seabed image processing software, and a comparative error analysis of four photoquadrat methods. *Journal of Experimental Marine Biology and Ecology* 424–425; 99–108. doi:10.1016/j.jembe.2012.04.018

The photoQuad software integrates a suite of 2D analyses used in marine biology and ecology for the study of sessile communities through photographic sampling. Trygonis and Sini (2012) used simulated data to compare different quantification techniques to quantify three distinct taxa. Data were collected during the summer of 2010, in a study to characterise coralligenous communities in the Aegean Sea (NE Mediterranean), located at an offshore reef off Lesbos Island, Greece (39°19.694' N 26°26.175' E). High resolution (12.1 megapixel) images were taken randomly within coralligenous assemblages at 20–30 m depth, using a PVC quadrat frame, covering an area of 25 × 25 cm.

The three species characteristic of Mediterranean coralligenous communities (Ballesteros 2006) chosen were: a sponge, *Agelas oroides*, a bryozoan, *Schizomavella* sp., and a scleractinian solitary coral, *Leptopsammia pruvoti*. For each of the three species, 30 images were drawn out of the available data pool that featured at least one individual occurrence; density (individuals per quadrat; Dsp) and absolute area (cm²; Asp) were measured using freehand drawn regions, and the respective empirical distributions of density and area per species were obtained. These data were then used to produce simulated data for method testing (see paper for more detail).

The experimental approach taken was designed to assess the accuracy and precision of four different measurement methods, and identify their sensitivity to species size, in their application to the estimation of absolute area and percentage coverage. Although a simplified simulation scenario, real data were used to produce realistic density and area patch values. The simulated datasets were analysed in photoQuad using the freehand tool (FH), Segmentation (SG), Grid cell counts (CL), referred to as Frequency of Occurrence grid within this report, and Stratified random point counts (RP) methods, to investigate and compare their bias in terms of accuracy and precision regarding the estimation of absolute area and percentage coverage. For this report, only FH, CL and RP are of interest as they compare the enumeration methods already discussed.

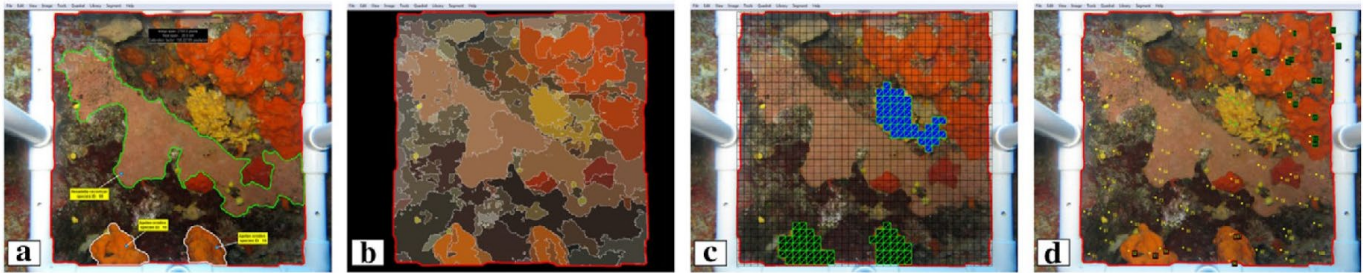


Figure 5. Composite figure of the PhotoQuad software illustrating the different tools: (a) Species markers (SC), freehand drawn regions (FH) and calibration marks. (b) Image map and region boundaries at intermediate level of segmentation (SG), scale 3 out of 4. (c) Grid cell counts (CL) with unit area equal to 0.5 cm². (d) Stratified random point counts (RP) with selected points assigned to different species. Image: PhotoQuad.

The analysis identified the bias in absolute area and percentage coverage estimates differed among the four measurement methods, and varying sensitivity to species size. The segmentation (SG) method showed the highest accuracy and lowest error variance, and although favourably biased by the current simulation design, highlight its performance in partitioning highly complex benthic images (Figure 5b). The method is robust to individual patch size except for extremely small regions. For overall performance, the Freehand tool (FH) method produced low scaled errors at species level that never exceeded 7.6% and 6.8% in area and coverage respectively. It was, however, less robust to individual size compared to the SG method, a result that agrees with the findings of Bernhardt and Griffing (2001).

Overall, the error analysis at species level indicates that, regarding the available simulated datasets, the segmentation method (SG) is superior, the freehand region (FH) method ranks second as it shows a lower variability across species compared to grid cell counts (CL), and the random point count (RP) method is accurate on large species, but inherently cannot output absolute area, while its scaled mean absolute errors are an order of magnitude greater than all other methods.

4.3. CS03: Comparison of point count and Frequency of occurrence method

Van Rein, H., Schoeman, D., Brown, C., Quinn, R. & Breen, J. 2011a. Development of low-cost image mosaics of hard-bottom sessile communities using SCUBA: comparisons of optical media and of proxy measures of community structure. *Journal of the Marine Biological Association of the United Kingdom*, **92**, 49-62. <https://doi.org/10.1017/S0025315411000233>

Van Rein *et al.* (2011a) compared visual estimate, point-count and frequency of occurrence grid quantification methods using standardised 25 × 25 cm photo-quadrats which were then mosaiced together (using 16 images) to provide 1 m² area for subsequent comparison of enumeration methods. Still and video imagery were collected sequentially during the same SCUBA dive from paired sets of 100 × 100 cm sampling quadrat frames, over a continuous two-week period in July 2008. A Nikon digital single-lens reflex (DSLR) camera in an Ikelite underwater housing with purpose-built 25 × 25 cm photo-quadrat frame extending 40 cm outwards from the lens, was used to collect the still image tiles which were then used to construct the photo-mosaics.

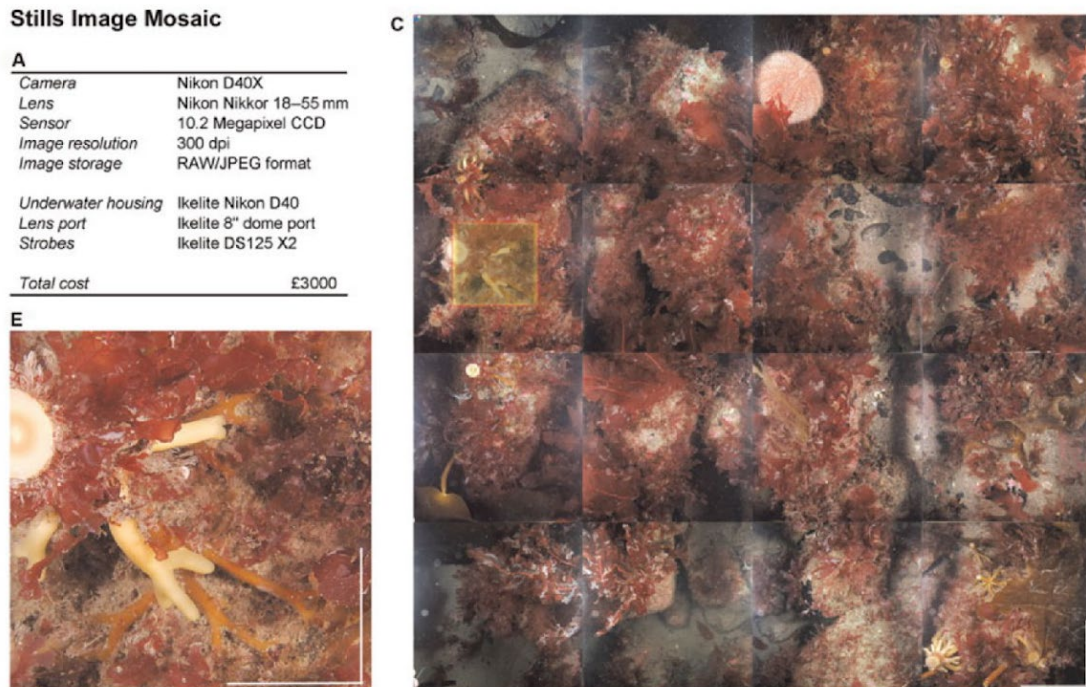


Figure 6. Image photo-mosaic using standardised image tiles combined to produce larger sample image for analysis. Image Van Rein 2011.

The visual estimate technique is a well-established method used worldwide, where the observer estimates percentage cover (Dethier *et al.* 1993; Leujak & Ormond 2007). Among the three techniques tested it was considered the most accurate, and therefore, the most reliable in terms of providing the best estimate of true community structure and composition (Dethier *et al.* 1993; Beaumont *et al.* 2007; Leujak & Ormond 2007). Therefore, data from this technique provided a baseline against which the other techniques were compared. In this study, biota within the entire image area were identified and recorded as present or absent to represent the community composition of each sample. Then a visual estimation of the percentage cover of each benthic category was conducted using the observer's judgement, to represent the community structure of each sample.

Two types of data were collected from each image, one to represent the community's morphological composition (community structure hereafter) and the other its unique assemblage. For community structure, species were assigned to broad benthic categories of coarse taxonomic resolution to represent their structural role within the community and recorded as percentage cover of the entire sampling area. These two data types were extracted from each of the still's mosaics, by a single observer, using three different data-extraction techniques in the following order: visual estimation of cover; visual estimation of cover by frequency of occurrence; and point-intercept cover estimation.

Despite the inherent differences in data extraction method, the impressions of community structure determined by the point count and visual estimate techniques were remarkably similar. The point count technique, however, clearly outperformed the others in terms of efficiency: community structure data were consistently extracted in half the time it took for the other techniques. However, the point count technique did have one distinct disadvantage relative to the others: low data density per unit area (Drummond & Connell 2005). As a result, species richness was underestimated relative to the other techniques.

In this study, gross differences in community structure determined by the visual estimate and Frequency of Occurrence count signified that the latter generated the least accurate data of

all techniques under investigation. In addition, these data showed the highest variability of all techniques tested. Therefore, despite having an efficiency, taxonomic benefit, and species richness comparable to that of the visual estimate technique, the authors concluded that the inherent overestimation effect and subsequent variability of cover estimations made by the Frequency of Occurrence technique made it unsuitable for monitoring use on the photo-mosaics. Data collected using the point count technique proved sensitive enough to detect change in the community with a lower number of replicates.

4.4. CS04: Comparing data density of point count quantification method

Van Rein, H., Schoeman, D., Brown, C., Quinn, R. & Breen, J. 2011b). Development of benthic monitoring methods using photoquadrats and scuba on heterogeneous hard-substrata: a boulder-slope community case study. *Marine and Freshwater Ecosystems*, 21, 676–689. DOI: 10.1002/aqc.1224

One of the main aims of this work was to test varying resolution (number of points) of the point-count quantification method. A total of 104 photo quadrats (cropped to a standardised size of 25 x 25 cm) were acquired from a depth of 30 m at the south-west shore of Rathlin Island, Northern Ireland.

Photoquadrats were analysed to record to dataset: community composition and structure were extracted. For this study, community composition was determined by quantification of taxa to the lowest taxonomic resolution possible. Community composition data were intended to accurately reflect taxonomic composition of sampled areas. Taxa were identified to functional groups (see paper for full details) intended to represent the structural components of the community that serve different ecological functions within the overall community. No motile or rare taxa were recorded during either quantification analysis of the images.

One hundred evenly spaced points were overlaid on images, even spacing chosen over random for ease of using non-automated system and community composition and structure recorded. (Software are now available for automated generation of points, allowing random and non-random point array to be analysed with ease.) To explore the effect that different data resolutions had on the data, 50 and 25 evenly spaced point-intercepts were sub-sampled from the 100-point data to construct two additional data sets: 50-point data (where each point counted for 2% coverage) and 25-point data (4% coverage per point).

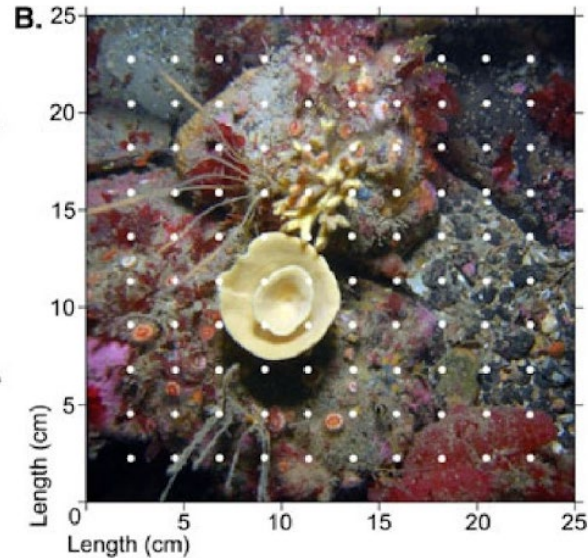


Figure 7. Example image illustrating application of 100 regular point data extraction used for study. Standardised image area was achieved by cropping all images to 25 x 25 cm. Image Van Rein *et al.* 2011

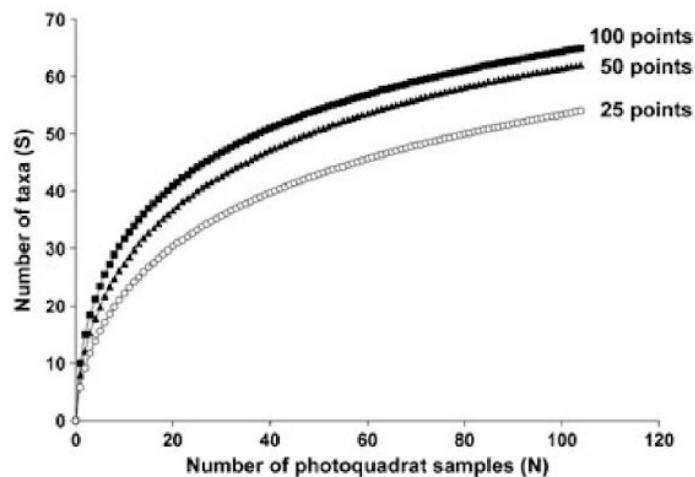


Figure 8. Average taxonomic accumulation curves' (random re-orderings of the photo quadrats) for photoquadrat sample images at different data resolution (i.e. number of points) to examine the effect of data resolution on the number of taxa recorded. It is clearly visible that 100 points records the highest number of taxa and reaches the asymptote faster. Image Van Rein *et al.* 2011.

In this study, increases in data resolution, from 25 to 100 points per 25 x 25 cm sample image, tended to increase the number of taxa and the number and distinctiveness of sub-communities identified at the site. Higher data resolutions also showed reduced levels of variability between replicate samples, which increased the relative sensitivity of the data to temporal changes in each sub-community. Overall, those data determined at higher data resolutions (i.e. 100 points) were considered more reliable for monitoring purposes (Andrew & Mapstone 1987). However, with regards to efficiency, it was not surprising that the high-resolution data were extracted with the lowest efficiency of all three data resolutions tested. This trade-off is typically encountered in monitoring method development, where the 'middle-ground' is often selected as a compromise between benefits (accuracy and precision) and costs (time and funds) (Brown *et al.* 2004; Drummond & Connell 2005). Alternatively, the 'middle ground' can be evaluated using more objective assessment measures, like

taxonomic benefit, which uses combined benefit and efficiency of survey methods to identify those that record taxa with the least effort per unit area (Van Rein *et al.* 2011b).

There is little consistency among studies regarding the optimum number of points per unit area of optical image (Brown *et al.* 2004; Preskitt *et al.* 2004; Page *et al.* 2006; Dumas *et al.* 2009). The optimum data resolution in this study, 50 points per 25 x 25 cm sample image (1 point every 12.5 cm²) is high relative to that used in other studies: 1 point per 13.2 cm² (Coles and Brown, 2007); 18 cm² (Vroom *et al.* 2005, 2010); 25 cm² (Page *et al.* 2006) 60 cm² (Preskitt *et al.* 2004); 69 cm² (Brown *et al.* 2004; Jokiel *et al.* 2005). It has been suggested that further increases in data resolution would have little additional positive effect but would more likely result in a reduction in data extraction efficiency (Bohnsack 1979; Dethier *et al.* 1993; Drummond & Connell 2005). Indeed, the lack of difference between the A1 and A2 dominant sub-communities determined using 100 and 50 points, respectively, provided further evidence that no additional benefits were gained by increasing the data resolution per sample. Note that these relative assessments are likely to be highly case sensitive (i.e. will depend on environment, fauna, objective).

4.5. CS05: Abundance (count and percent cover) combined datasets

Howell, K.L., Davies, J.S. & Narayanaswamy, B.E. 2010. Identifying deep-sea megafaunal 776 epibenthic assemblages for use in habitat mapping and marine protected area network design. *Journal of the Marine Biological Association UK*, 90(1), 33-68.

Howell *et al.* (2010) undertook community analysis of abundance data from still imagery from the UK deep-sea area (Figure 9) to define biological assemblages (Biotopes) for use in MPA design. In 2006 and 2007, 139 transects were undertaken using the same drop-frame camera system fitted with a video and stills camera (at oblique angles), resulting in 1987 used for analysis. All organisms greater than 1 cm were recorded as abundance counts except for colonial forms that were quantified as percent cover. The study represents what others quantify in terms of count and cover recorded separately, with the need to combine datasets. It should be noted that quantifying all taxa in this way is one of the most time consuming quantification methods (except for biomass).

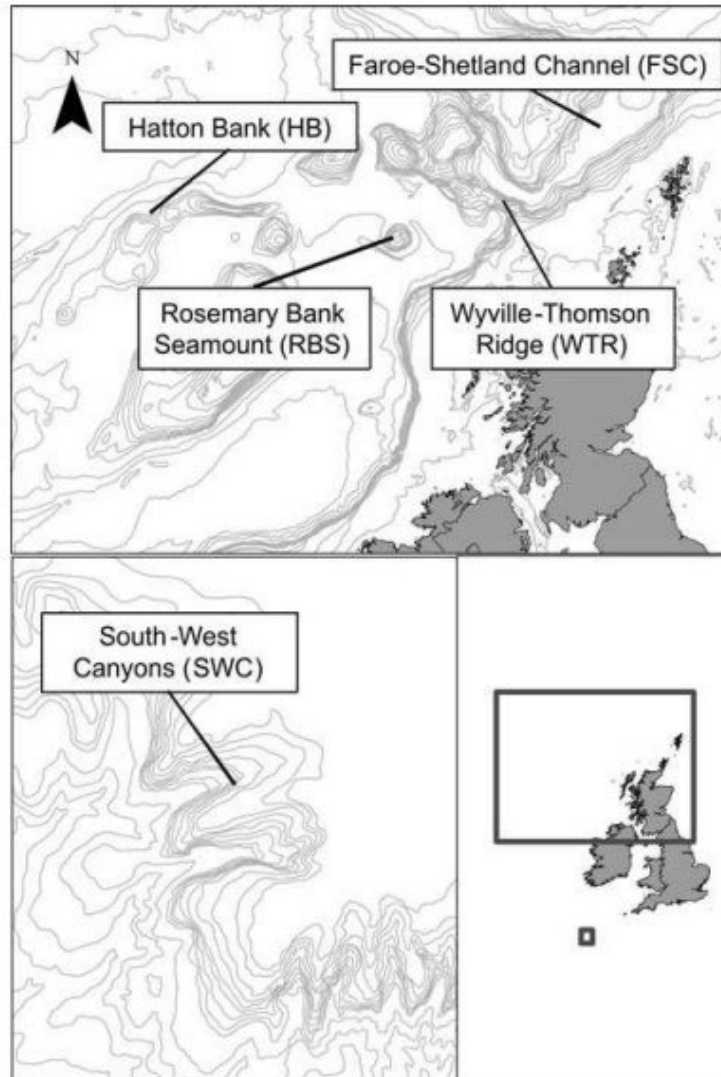


Figure 9. Areas sampled using a drop-frame camera system in the UK deep-water area in 2005-2007. Image: Howell *et al.* 2010.

To allow combination of the count and percent cover datasets, the variance in their point scale was inspected, with the abundance data having data ranged over a 0–1000 point scale, where percentage cover data ranged over a 0–100 point scale. Standardised abundance data were divided by 10 to bring the two datasets onto the same scale allowing them to be combined into a single dataset. Combined per cent cover and abundance data were analysed using PRIMER v.6 (Clarke & Warwick 2001). While the combination of abundance and cover dataset allows the data to be analysed together, there are disadvantages to be aware of:

- enumerating all taxa (count and percent cover) is time consuming and may not pose any advantage to using another quantification method which uses a single scale (records everything as percent cover), meaning that no transformation of the data are necessary.
- May not be a feasible method (given it is time consuming) for repeat monitoring if resources do not allow for full quantification.

Data were square root transformed and cluster analysis with group averaged linking was performed on Bray Curtis similarity matrix produced, to guide the identification of biological

assemblages at a scale relevant to mapping efforts (10s of m). Twenty-four biotopes were identified from the cluster analysis and full description given (see paper for more details).

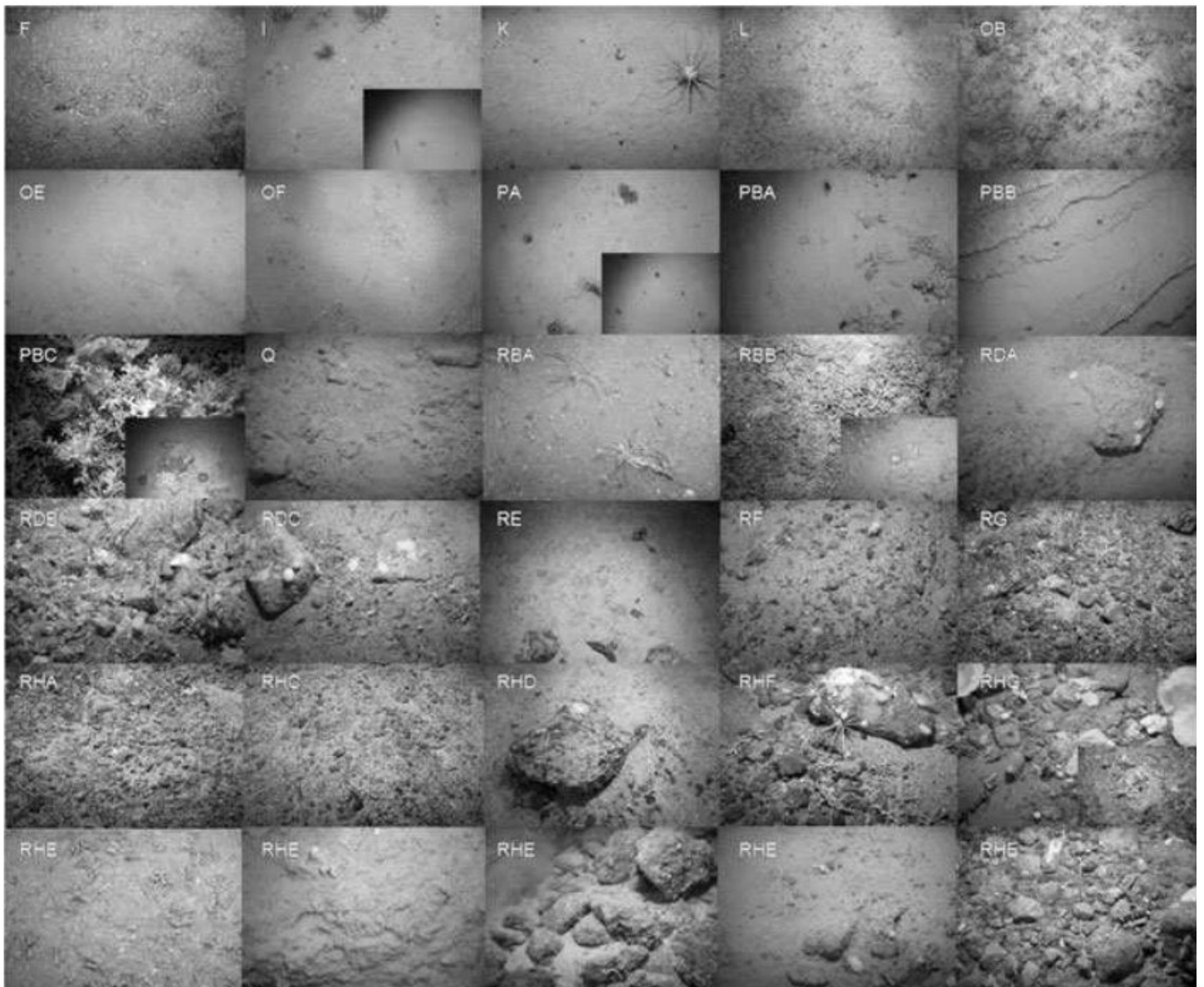


Figure 10. Biotopes defined from multivariate analysis of combined still imagery data. Howell *et al.* 2010.

4.6. CS06: Estimation biomass from imagery using a generalised volumetric method (GVM) biovolume method

Benoist, N. M. A., Morris, K. J., Bett, B. J., & Ruhl, H.A. 2019b. A generalised volumetric method to estimate the biomass of photographically surveyed benthic megafauna. *Progress in Oceanography*, 178. doi.org/10.1016/j.pocean.2019.102188

Benoist *et al.* (2019b) studied methods to quantify megafaunal biomass from photographs to compare the more traditionally used taxon-specific length-weight relationships (LWR), which relies of measurement from specimens to quantify megafaunal biomass against a generalised volumetric method (GVM) biovolume method, as an estimator of biomass.

For the photographic case study, seafloor imagery was acquired from three shelf-sea locations in the Celtic Sea (c. 100 m water depth), northeast Atlantic, using the AUV Autosub3. Images were taken at an altitude of 2.5 m above the seabed (fully field methods and subsequent image processing and assessment described by Morris *et al.* 2014; 2016). All benthic invertebrates were counted and identified to the lowest taxonomic unit. Three body dimensions were recorded per specimen (ECD, ECL, and SL) using the image analysis software Image-Pro Plus as illustrated in Figure 11 to allow calculation of biomass using the LWR and GVM method:

- (i) GVM equivalent cylindrical diameter (*ECD*)
- (ii) GVM equivalent cylindrical length (*ECL*)
- (iii) LWR standard linear body dimension (*SL*) as employed by Durden *et al.* 2016c

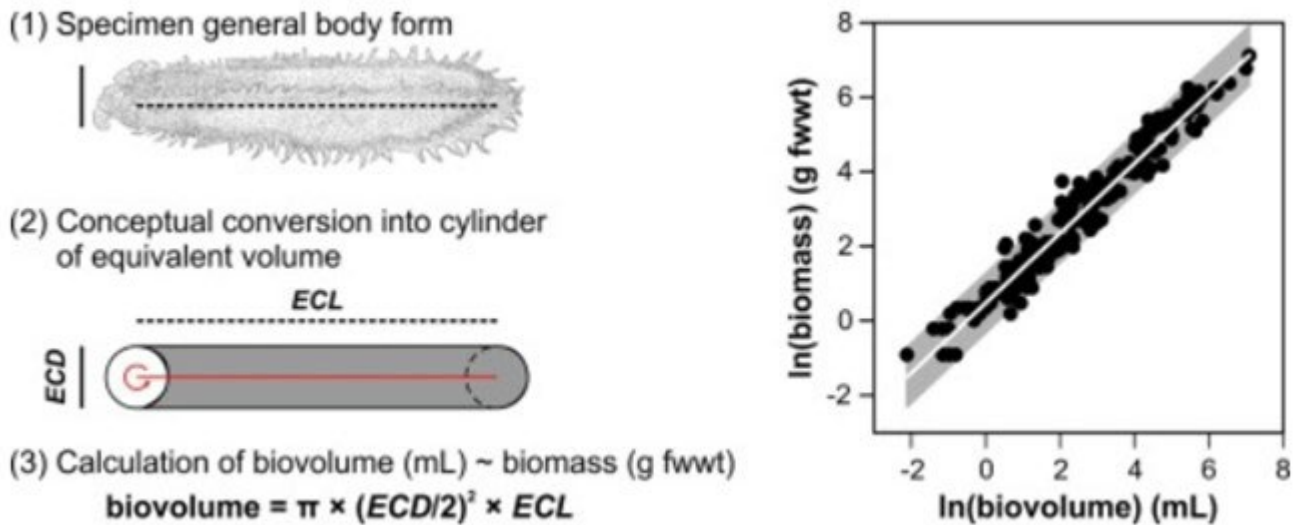


Figure 11. Illustrates the use of general body form to estimate volume for use in GVM based on a cylindrical body form. Image: Benoist *et al.* 2019.

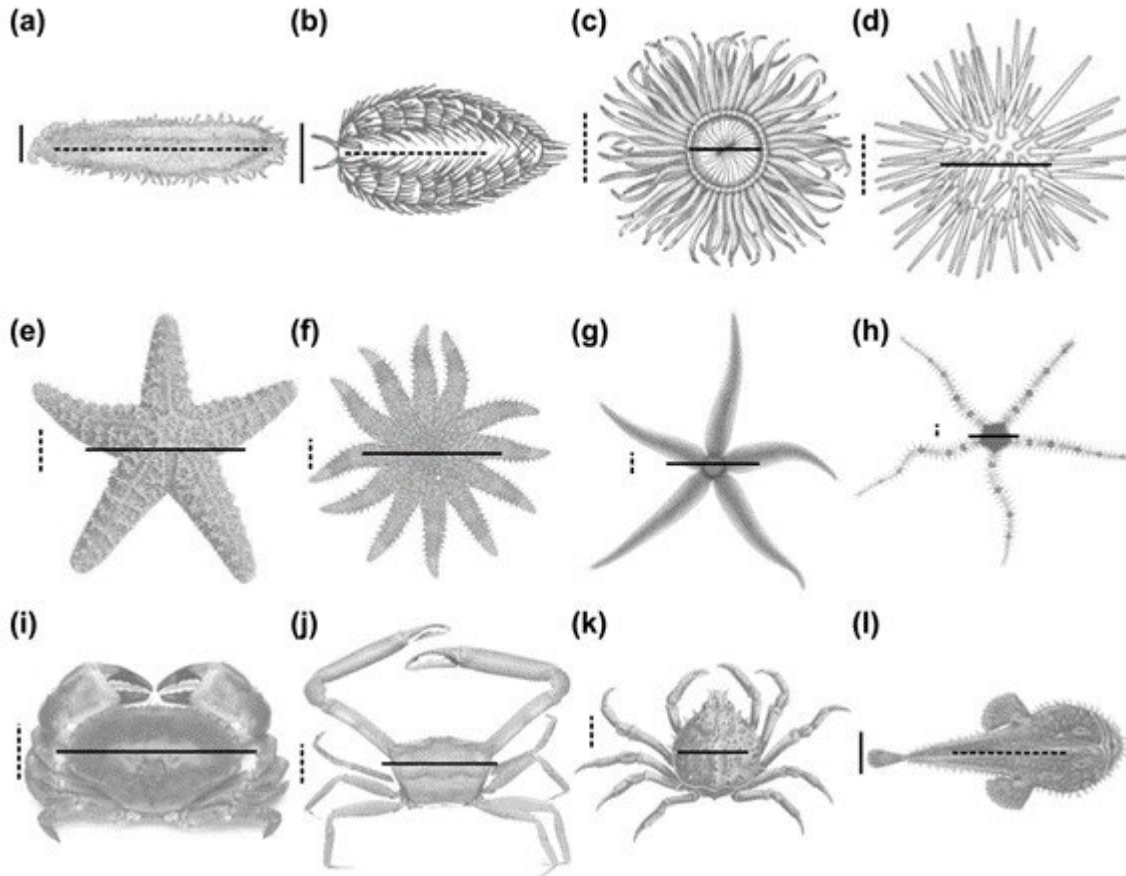


Figure 12. Two measurements required for the generalised volumetric method for example benthic megafauna body forms, equivalent cylindrical diameter (*ECD*; solid line) and equivalent cylindrical length (*ECL*; dashed line) shown. (a) Holothuroidea. (b) Polychaeta. (c) Anthozoa. (d) Echinoidea. (e–g) Asteroidea. (h) Ophiuroidea. (i–k) Brachyura. (l) Actinopterygii. Image: Benoist *et al.* 2019.

As noted by the authors, this method is simple for vermiform organisms (shaped like a worm) as is the case in Figures 12a and b, but can be readily translated for a wide range of morphologies. The GVM is readily adapted to various morphologies, including colonial, encrusting, or morphologically plastic forms (see paper for full guidance). Specimen body-size measurements (*ECD* and *ECL*) were converted using the GVM approach to estimate biovolume (V_E) and the *SL* measurement was converted to fresh wet weight biomass (M_E) using the LWR method:

$$M_E = a \times SL^b$$

where M_E is estimated body mass, *SL* is a defined standard linear body dimension, and *a* and *b* are taxon-specific constants obtained by log-log regression of measured body mass on *SL*, and consequently require adequate prior data for the taxon in question (e.g. Durden *et al.* 2016a).

Biovolume (V_E) and wet weight biomass (M_E) were compared. In total, 2896 specimens from eight phyla and 92 taxa were measured from photographs using both the GVM and LWR approach, for LWR, not all specimens could be compared due to lack of LWR data, hence for the data analysis, V_E and V_E -partial (only those identified that had comparable LWR data) were compared to estimated body mass derived using the LWR method.

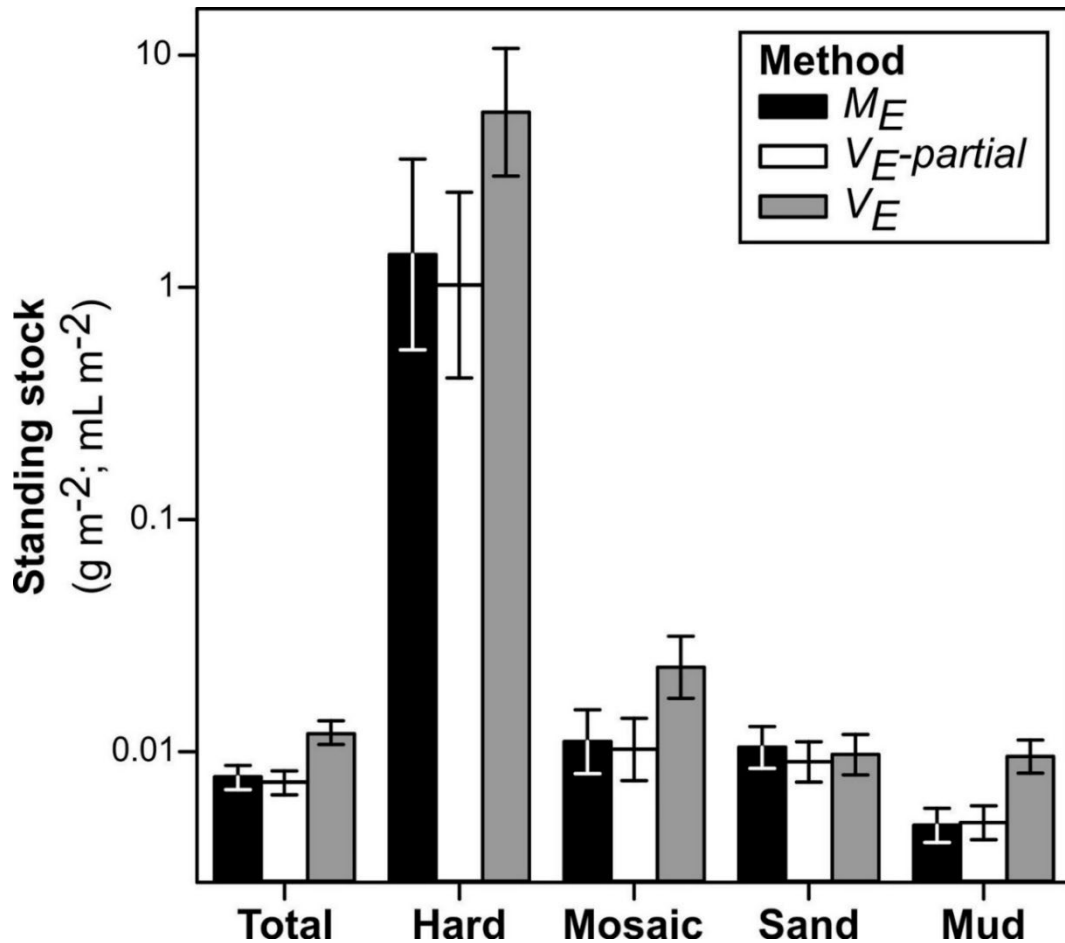


Figure 13. Celtic Sea megabenthos standing stock biomass by habitat type and estimation method. The mean value is presented with 95% confidence interval, as estimated using the length-weight-relationship (LWR) approach (ME , $g\ m^{-2}$) and the generalised volumetric method ($mL\ m^{-2}$), excluding (VE -partial) and including (VE) those taxa for which LWR estimation was not possible. Image: Benoist *et al.* 2019.

No statistically significant differences were detected between VE -partial and ME estimates for the total surveyed area, or within the individual habitat types encompassed by the survey (Figure 14). Similarly, both methods illustrated the same pattern and detected the same statistically significant differences between habitat types (VE -partial $F_{3,267} = 46.69$, $p < 0.001$; $ME\ F_{3,266} = 53.13$, $p < 0.001$). The study concluded that the biovolume GVM method posed advantages over the traditionally used LWR:

- Performance of the GVM is at least equal to the LWR data - estimated biovolume (VE -partial) was highly consistent with the biomass estimates (ME).
- GVM was able to assess c. 25% of taxa for which no LWR data were available (mainly bryozoans, sponges, and colonial cnidarians).
- A highly useful tool for assessing standing stock in marine regions lacking taxon-specific information (needed for the LWR method).
- In addition, the volumetric approach enables the assessment of those organisms that do not exhibit a distinctive body form or that are rarely sampled as complete entities (e.g. sponges, colonial and encrusting taxa).
- With appropriate training, little Inter-operator variability occurs as evidenced by Figure 14.

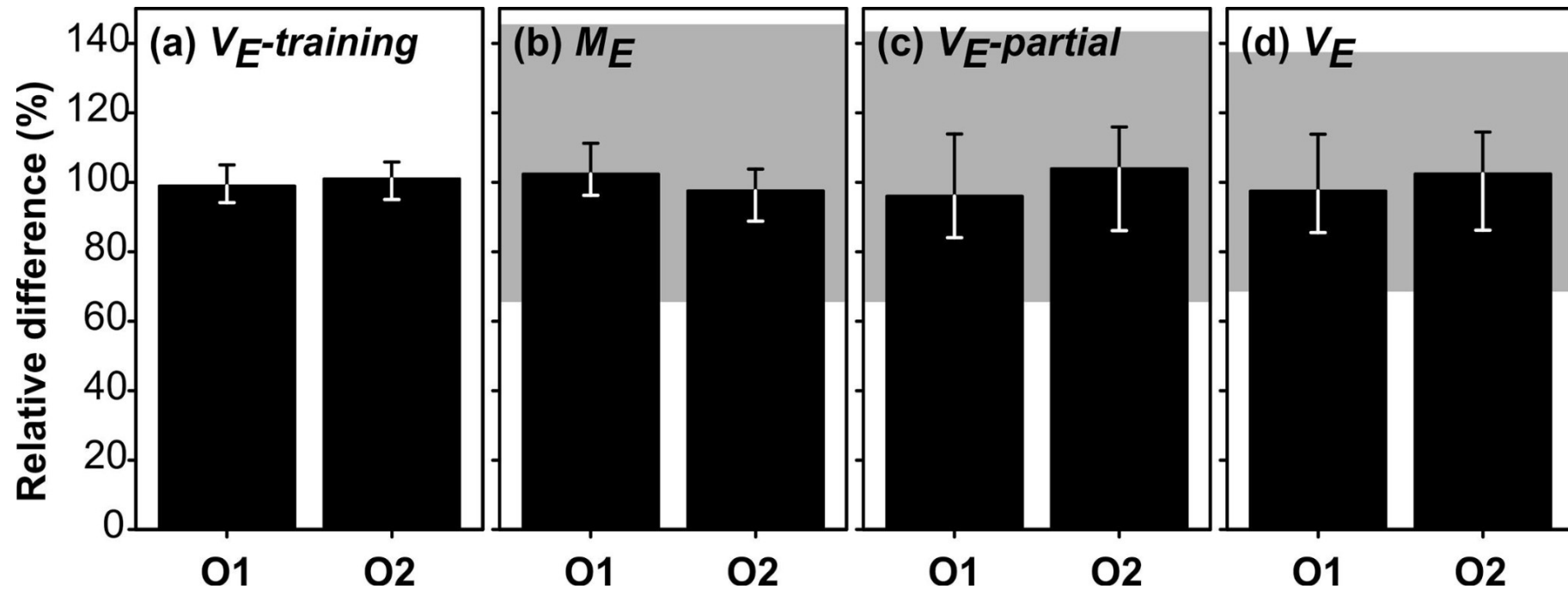


Figure 14. Inter-operator variability in the estimation of megabenthos standing stock biomass. Variability, as 95% confidence interval of individual operator mean value, is illustrated as relative difference (%) from the joint mean value (i.e. 100%) of the two operators (O1, O2). (a) Total biovolume estimated by the generalised volumetric method (GVM) for an initial training dataset (V_E -training). (b) Total survey biomass estimated using the length-weight-relationship (LWR) approach (M_E). (c–d) Total survey biovolume estimated using the GVM, excluding (c; V_E -partial) and including (d; V_E , mL) those taxa for which LWR estimation was not possible. The shaded bands (b–d) represent the variability, as 95% confidence intervals, of the full survey estimates of the corresponding standing stock parameter (i.e. M_E , V_E -partial, V_E). Image: Benoist *et al.* 2019.

4 Limitations and recommendations

A limitation of this report is that it does not include all studies that have been published on the quantification techniques. Similarly, a constraint of this work was the use of very specific keywords to identify literature on quantification approaches of digital (still) imagery. The lack of standardisation and consistency of reporting of data collection/annotation/quantified in the literature meant that some works were not found using the keyword searches. Some of this was overcome by adding citations from within the studies found through the keyword searches. During the first phase of running the keyword searches, it was apparent that there was a disparity in the number of articles reporting the various techniques. Due to the many synonyms found to describe the same technique, the second keyword search may not have found everything.

When reviewing the literature, there was an obvious lack of relevant information in the literature on the application of different quantification approaches in marine imagery, with limited discussion of the advantages and disadvantages and on which approach is best applied. To allow a more robust and standardised approach to imagery annotation, especially for the purpose of monitoring programmes this is essential and should be improved. In many instances the lack of consistent use of terminology and overall lack of detail when reporting of quantification methods, made interpretation of studies during the literature review challenging.

4.1 Considerations, prior to data collection

Prior to the collection of imagery data, several things should be considered to ensure the best quality 'fit for purpose' data are acquired:

1. *Purpose of study*: What taxa are being examined (this may determine which quantification approach to utilise). Need to consider community structure (i.e. if there are high proportions of rare species), need to carefully consider the annotation approach taken. For example, the Point count method can miss rare taxa, and would require a large sample effort.
2. *System settings*:
 - Lighting: ensure adequate and even illumination of the scene for greater ability identify taxa
 - Relative angle of camera to scene (may influence annotation method)
 - Low-oblique angle can improve ability to identify taxa (Saunders *et al.* 2011)
 - High-oblique/vertical downward facing, potentially better image scaling
 - Camera settings
 - Avoid use of autofocus (Saunders *et al.* 2011).
 - Camera platform speed, guard against motion blur (use flash, use global shutter sensor type)
 - Digital stills will inevitably be better than video frame-grabs due to their higher resolution and improved sharpness due to faster shutter speeds.
 - What seabed resolution of imagery is required (i.e. what taxonomic level/body size level is required)
 - If species identification is the highest priority, ensure good lighting, flash unit, calibration, etc.

- What is the minimum/maximum area needed per image/frame?
3. What statistical analyses are required; this will guide the quantification method chosen.
 4. Power analysis to determine detection of change to a community: Prior to carrying out a statistical power analysis, decisions should be made on the acceptable parameters under which undesirable change will be judged to have occurred, namely the power, significance level, and effect size (Saunders *et al.* 2011). Determine best sampling design (number of replicates and data density per sample) to determine optimal power and minimise both Type I (false positive) and II errors (false negative). By increasing the power of a test, it reduces Type II error risk but can increase the Type I error risk. This can be balanced by good sample design and altering the significance level. If the sample size (i.e. the number of images) is too small, the power is likely to be insufficient, while if the sample size is excessively large, the power will be adequate, but processing time will be increased (sampling effort considerations) (Bros & Cowell 1987).
 5. Sampling unit: It is important to consider the real extent surveyed and its implication for how well the largest/rarest individuals have been sampled, and how well the benthic body size classes are encountered, which is particularly important when undertaking biomass assessments (Ruhl *et al.* 2023). Consider unit size for sampling, e.g. will images cover a sufficient area to capture the taxa for the purpose of the survey, or is it more relevant to use mosaic of images to gain a greater unit area? (see Benoist *et al.* 2019a for example). Sampling unit could be defined by number of individuals rather than by area (Benoist *et al.* 2019a) using the auto-similarity curve approach (see Schneck & Melo 2010) or the assessment of multivariate dissimilarity-based standard error developed by Anderson and Santana-Garcon (2015).
 6. Pilot studies: For monitoring programme planning, an important consideration is the effect of size, abundance and patterns of distribution on the precision of quantification of biota; and the use of baseline surveys can be used to establish these key properties to ensure greater potential for collected appropriate data (Perkins *et al.* 2016).

4.2 Recommendations

4.2.1 Use of standardised terminology

With the current lack of consistent use and reporting of quantification techniques for annotating marine imagery, using standardised terms are recommended to allow consistent reporting and easy comparison of studies. It is proposed that the terminology used in this report forms the basis for a standardised terminology for quantification methods used in marine imagery.

4.2.2 Validation of methods

Recommendation from this guidance report would be to undertake validation of quantification methodology to determine the best approach for different environments, camera platforms, and objectives. Undertake a series of tests to trial different quantification approaches (including varying data resolution, i.e. number of cells for frequency of occurrence or number of points for point count), to fully understand the impact on various ecological metrics of such variations in method.

4.2.3 Testing site-specific protocols

For monitoring programs, detecting important trends when they occur is critical, and statistical power analysis provides a means of testing the ability of different designs and methodological approaches (Perkins *et al.* 2022). After validation of methods to determine minimum requirements for each of the quantification methods, applying these to different sites/habitats is important. This is particularly true for heterogeneous habitats which may be dominated by very different taxa which may require different quantification approaches to capture the community and ultimately change (Benoist *et al.* 2019a). For mosaic habitats (intermediate habitats), numerical density has been shown to be insensitive to unit size, while species richness and biomass density are linked to sample size (Benoist *et al.* 2019a). Validation of methods in a range of environments is a critically important step that must be conducted prior to initiating monitoring programs (Jokiel & Brown 2000). For example, determine the best approach for hard versus soft substratum habitats.

Testing statistical assumptions and the consequences of applying the different quantification approaches is another important consideration. For full transparency, it is important to identify the statistical limitations of applying the different quantification techniques presented in the report. It is important to fully understand how the quantification method affects the expression of community data, for example, point count quantification is known to under-represent or even miss rare taxa. And while using abundance count or cover quantification will capture rare taxa, when these data are combined (as described in section 3.6), the standardisation of the two scales can reduce the resolution of data obtained through the more time-consuming enumeration of all taxa. Test the combined use of other indices in addition to species abundance as this may enable measuring change in communities more sensitive - maybe for indicator taxa (Perkins *et al.* 2022).

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Appendix

PRISMA Framework

From this literature review, it was clear that there has been considerable growth in the use of digital imagery in marine science, not least in benthic studies (Appendix Figure A1). During the late 20th Century, these studies were exploratory and adopted the use of camera technology, with the evidence mostly consisting of PhD theses. Since the advent of technological advances such as compact digital cameras with underwater housing (e.g. GoPro in 2004), studies more than tripled in output with many studies dating from 2007 onwards and *ca.* greater than 20 per year (cross-reference Bicknell *et al.* 2016). It is apparent that annotation approaches and quantification methods were used in 2010 onwards; with manual counts employing Adobe/Excel (e.g. Coral Point Count) then digitally using software (e.g. BIIGLE 2009, Squidle+ 2015 – see Gomes-Pereira *et al.* 2016 and references therein). In terms of sampling the water column and quantification approaches, benthic approaches were observed in 2011–2014 with pelagic sampling growing in popularity as from 2019 onwards.

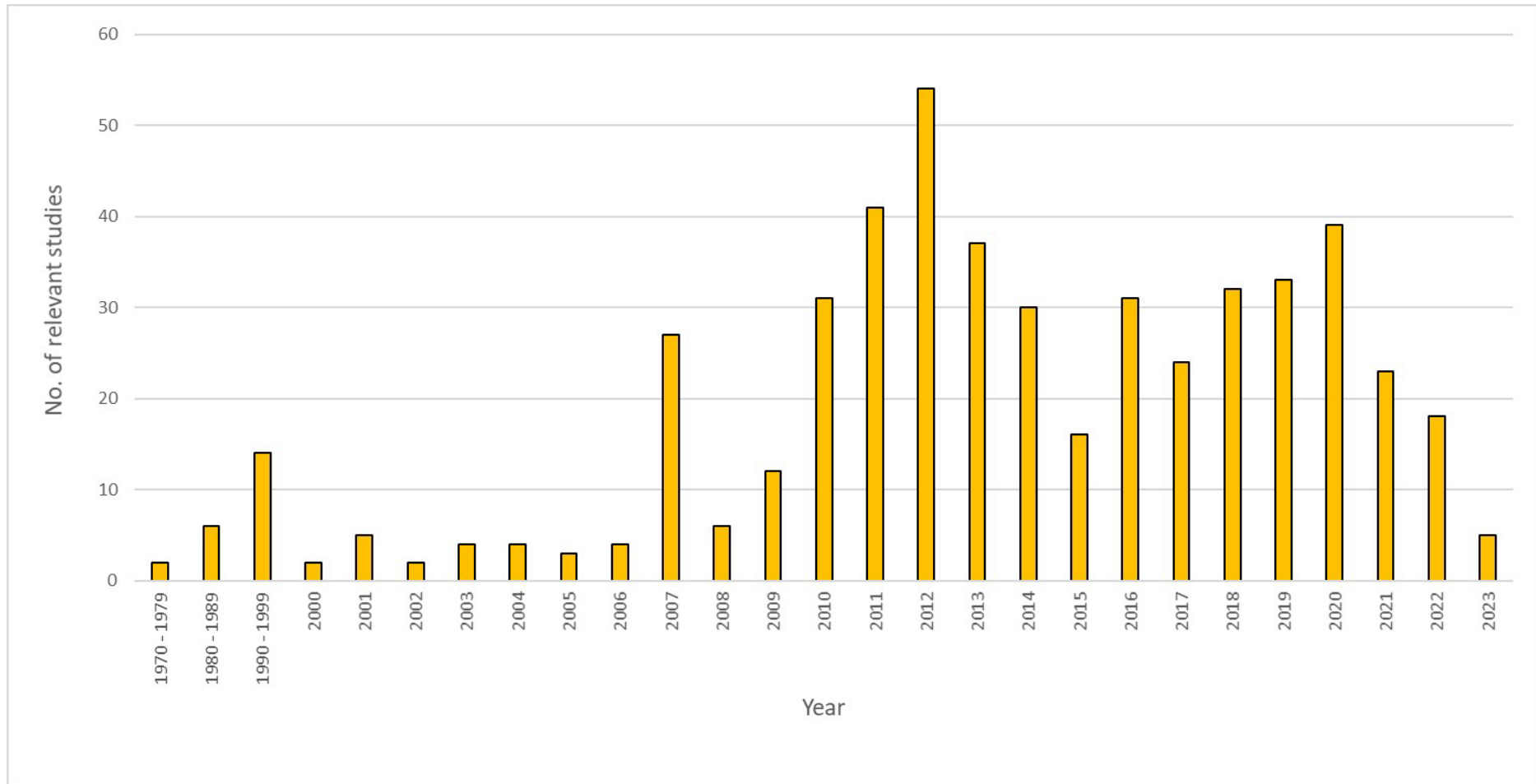


Figure A1. Relevant evidence of quantification techniques over time, highlighting the relative lack of publications reporting quantification method from imagery prior to 2007.

Within this literature review relevant contributing studies were observed from over 32 countries/overarching programmes (e.g. UNESCO/IUCN). The heat map of countries demonstrates the quantity of digital imagery research (relevant to this review) with the apparent world leaders, USA (n = 69), UK (n = 48), Australia (n = 43) Canada (n = 17) and New Zealand (n = 11) (Figures A2 & A3).

Table A1 displays the range of sampling platforms in use by various countries for digital imagery. Remote Operated Vehicles (ROVs) were the most utilised (n = 84), constituting ca. 24 % of all reported studies. The most reported sampling platforms after ROVs were SCUBA/Diver operated imagery (n = 67) and towed cameras (n = 40).

Table A2 shows the range of quantification methods by countries through use of digital imagery. Through this literature review, Abundance was the most employed method (n = 184), with more than double the amount of evidence compared to that of Point Count (n = 77) and SACFOR being the least employed (n = 7). The top ten countries employing such techniques account for 85% of all reported studies within this literature review, with USA, Australia and UK contributing over 60% to the evidence base (see Figure A3).

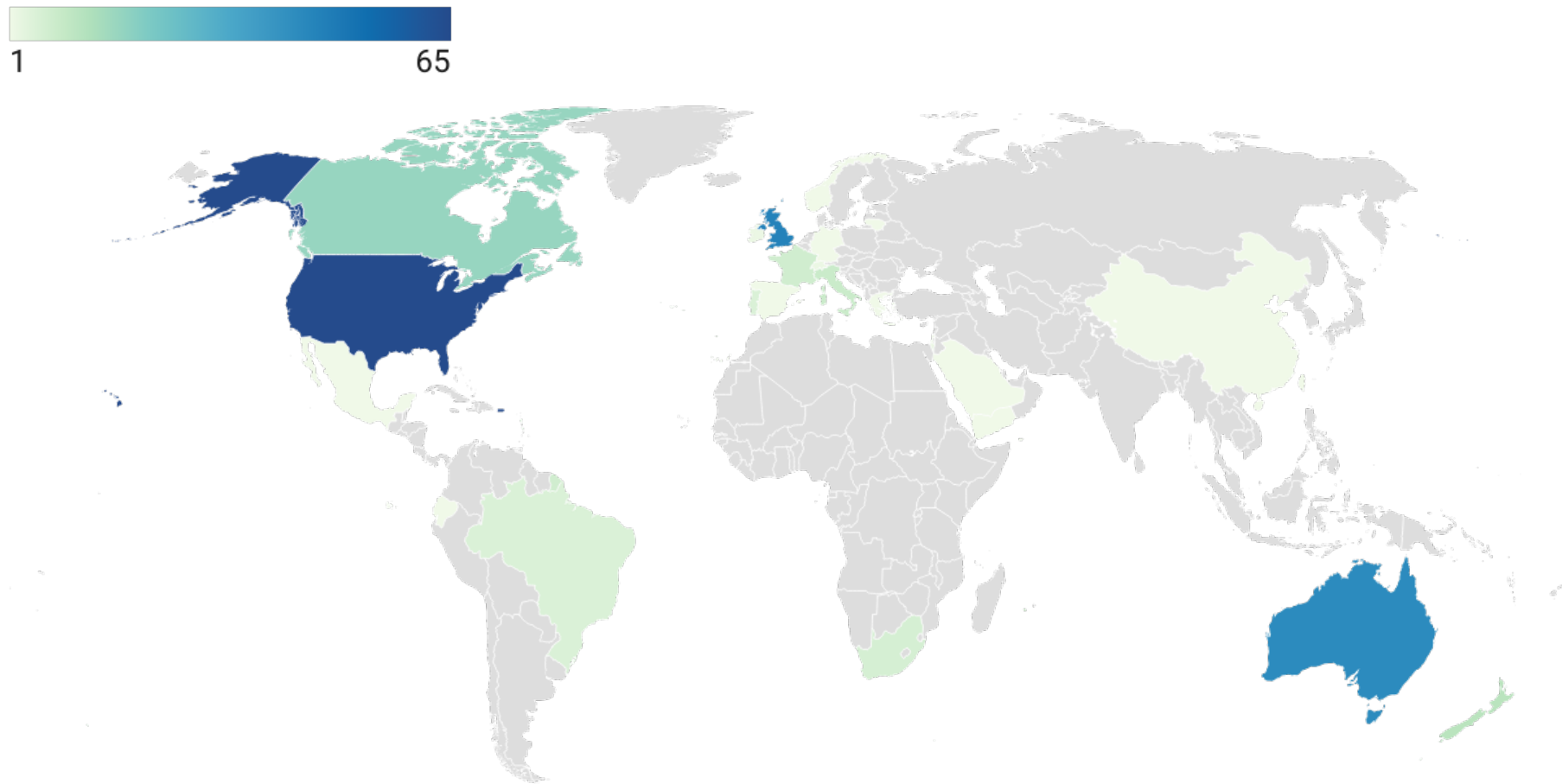


Figure A2. World heat map of 33 countries with relevant digital imagery studies employing quantification approaches (n = 258).

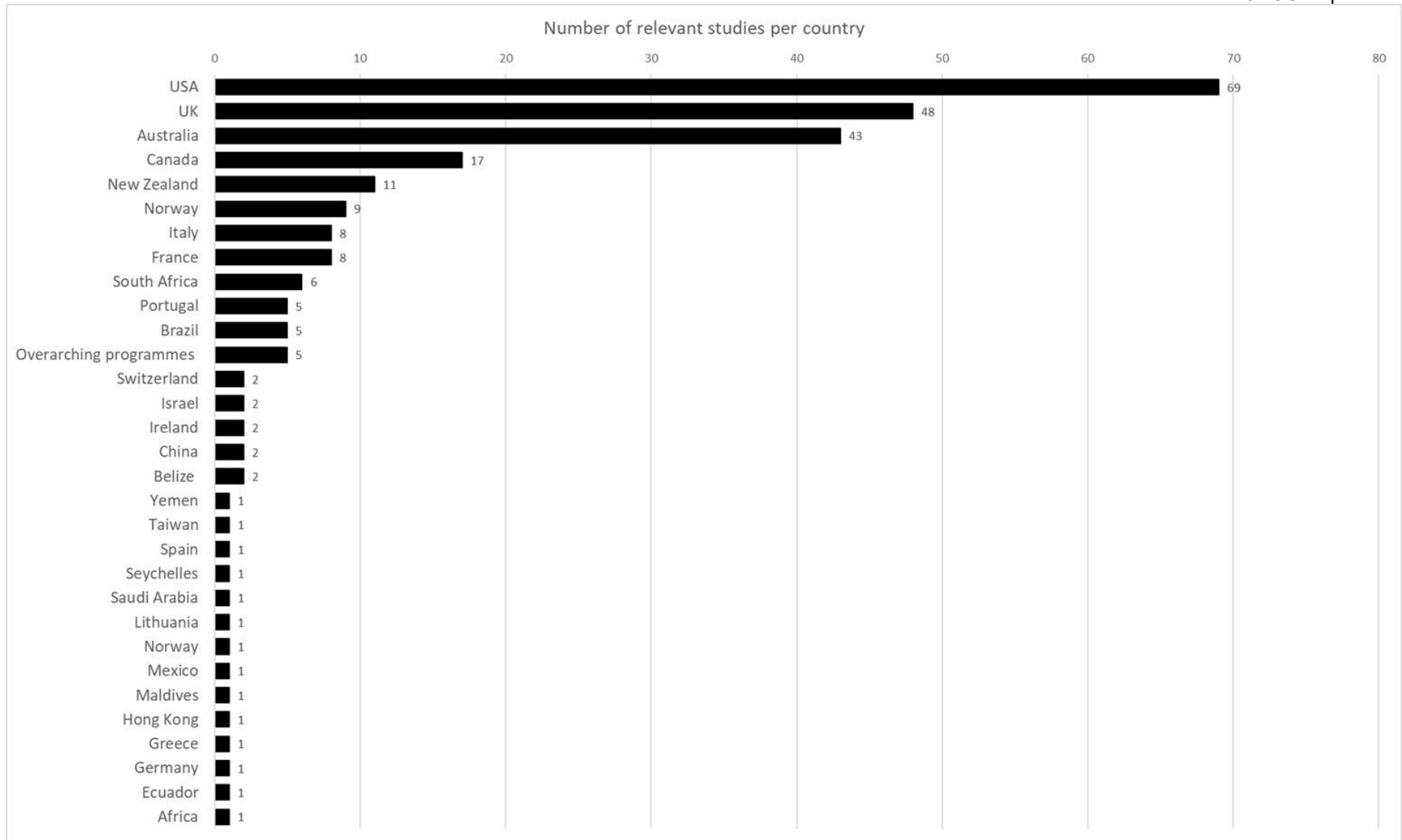


Figure A3. Relevant digital imagery studies employing quantification approaches (n = 258) by country.

Table A1. Use of Sampling platforms reported in literature listed by countries, in some instances more than one sampling platforms has been used per piece of literature. AUV - Autonomous Underwater Vehicle, BRUV - Baited Remote Underwater Video, MUV (HOV) - Manned Underwater Vehicle/Human Operated Vehicle, ROV - Remote Operated Vehicle, RUV - Remote Underwater Vehicle, UAV - Unmanned Aerial Vehicle.

Sampling platform	Category tot. all countries
ROV	84
SCUBA/Diver	67
Towed Camera	40
Video	36
AUV	23
BRUV	21
Photoquadrat/quadrat	18
Sled/	13
Drop Camera	9
Lander	9
Submersible	8
MUV (HOV)	6
Camera trap	5
Drop Frame	5
Observatory	4
Snorkelling	2
RUV	1
UAV	1
Tot. Platforms used for research	352

Table A2. Quantification method studies employed with digital imagery (n = 258 studies; with 342 reported uses of the various quantification methods) highlighting abundance being the most reported method and SACFOR being the least. Some studies (i.e. comparative) reported more than one quantification method.

Quantification Method	Subtotal
Abundance	208
Point Count	76
P/A - Presence/Absence	39
Frequency of Occurrence	12
SACFOR	7
Tot. quantification methods recorded	342

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