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MASTER THESIS

Comparative Study of Benthic Cover Estimation Methods from Macroalgal Photographic Samples

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Abstract

Coastal macroalgal communities serve as valuable bioindicators of marine ecosystem status, and their monitoring through standardized methods is essential for large-scale monitoring programs, such as those developed under the EU Water Framework Directive. The aim of this study was to cross-compare different methods for analysing the presence and cover of macroalgae in 100 photographic samples from Greek seas, to identify the most suitable in terms of cost-effectiveness and reliability of results. Specifically, the precision, accuracy, and time efficiency of stratified random point intercept cover estimations at three modes of analytical effort (50, 100, and 150 points) were evaluated against reference (REF) total cover measurements obtained using a digitally superimposed grid. Additionally, these techniques were compared in practical application scenarios assessing the ecological status using the Ecological Evaluation Index continuous formula (EEI-c [1]). The 150-point mode provided the closest mean Coverage Difference percentages to the REF method, while the 100-point mode yielded the best mean EEI-c Difference, and the 50-point mode required the shortest analysis time. Overall, the findings indicated that all three modes of the point intercept technique could effectively replace the REF methodology. The mode using 100 points offered the optimal balance between cost (in terms of analysis time) and reliability of results (in terms of precision and accuracy).

Keywords: phytobenthos, macroalgal cover, image analysis, method comparison, biotic indices

Περίληψη

Οι παράκτιες κοινωνίες μακροφυκών χρησιμεύουν ως πολύτιμοι βιοδείκτες της κατάστασης των θαλάσσιων οικοσυστημάτων και η παρακολούθησή τους μέσω τυποποιημένων μεθόδων είναι απαραίτητη για προγράμματα παρακολούθησης μεγάλης κλίμακας, όπως αυτά που αναπτύσσονται στο πλαίσιο της Οδηγίας-Πλαισίου για τα Ύδατα της ΕΕ. Σκοπός της παρούσας μελέτης ήταν η σύγκριση διαφορετικών μεθοδολογιών για την ανάλυση της παρουσίας και της κάλυψης των μακροφυκών σε 100 φωτογραφικά δείγματα από τις ελληνικές θάλασσες, ώστε να προσδιοριστεί η καταλληλότερη από πλευράς κόστους-αποτελεσματικότητας και αξιοπιστίας των αποτελεσμάτων. Συγκεκριμένα, αξιολογήθηκαν η ακρίβεια, η πιστότητα και η χρονική αποδοτικότητα των εκτιμήσεων κάλυψης με τη μέθοδο των στρωματοποιημένα τυχαίων σημείων σε τρία επίπεδα ανάλυσης (50, 100 και 150 σημεία) σε σύγκριση με τις μετρήσεις συνολικής κάλυψης αναφοράς (REF) που λαμβάνονται με τη χρήση ψηφιακού πλέγματος επικάλυψης. Επιπλέον, οι τεχνικές αυτές συγκρίθηκαν σε σενάρια πρακτικής εφαρμογής, αξιολογώντας την οικολογική κατάσταση με χρήση του συνεχή τύπου του Δείκτη Εκτίμησης Οικολογικής Ποιότητας ή Ecological Evaluation Index continuous formula (EEI-c [1]). Το επίπεδο ανάλυσης των 150 σημείων παρείχε τη μικρότερη μέση διαφορά κάλυψης σε σχέση με τη μέθοδο αναφοράς, το επίπεδο ανάλυσης των 100 σημείων έδωσε τη μικρότερη μέση διαφορά εκτίμησης EEI-c από τη μέθοδο αναφοράς, ενώ το επίπεδο ανάλυσης των 50 σημείων απαιτούσε τον μικρότερο χρόνο ανάλυσης. Συνολικά, τα ευρήματα έδειξαν ότι και τα τρία επίπεδα ανάλυσης της τεχνικής των σημείων μπορούν να αντικαταστήσουν αποτελεσματικά τη μέθοδο αναφοράς. Το επίπεδο ανάλυσης με τη χρήση 100 σημείων προσέφερε τη βέλτιστη ισορροπία μεταξύ του κόστους (όσον αφορά τον χρόνο ανάλυσης) και της αξιοπιστίας των αποτελεσμάτων (όσον αφορά την ακρίβεια και την ορθότητα).

Λέξεις- κλειδιά: φυτοβένθος, κάλυψη μακροφυκών, ανάλυση εικόνας, σύγκριση μεθόδων, φωτογραφικά δείγματα, βιοτικοί δείκτες

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A. Introduction

A.1. Macroalgae: Overview

Algae exhibit significant genetic diversity and can be categorized into macroalgae, which are larger than 50 μm and can grow up to several meters, and microalgae, which are typically smaller than 50 μm [2–4]. Marine macroalgae, commonly known as macroalgae, are macroscopic, multicellular, nonvascular eukaryotes that photosynthesize [5,6]. They include a vast variety of species (globally est. 12000-15000) which may grow in the intertidal and subtidal zones of the sea [7], and are broadly distributed along coastlines worldwide, ranging from tropical to polar regions [7,8].

Macroalgae are part of Plantae kingdom [7] and are classified into three major phyla based on their photosynthetic pigments and morphological characteristics: red algae (Rhodophyta), green algae (Chlorophyta), and brown algae (Ochrophyta) [3,9]. Each of these phyla possesses distinct properties and produces various bioactive compounds [3,10]. Therefore, macroalgae offer numerous advantages that benefit coastal communities, human well-being, and ecosystems [8].

A.1.1. Role of Macroalgae in Ecosystems

Macroalgae are a major driver of marine primary productivity, contributing significantly to the estimated net amount of global oxygen. They also play a crucial role in nutrient cycling and energy flow [11,12]. Macroalgae are important structural parts of the ecosystems and offer plenty ecosystem services [6,13]. For instance, their communities exhibit a three-dimensional structure that delivers habitat and shelter for other algae, invertebrates, and fish [14,15]. Furthermore, macroalgae form the foundation of complex food webs in aquatic ecosystems, supplying essential grazing and detrital food sources [14,16].

A.1.2. Rationale for Studying Macroalgae

Macroalgae abundance and productivity are determined by a range of abiotic and biotic factors, including light availability, hydrodynamism, tidal exposure [17], temperature changes, nutrient levels, substrate type and slope [8], space, salinity, UV radiation, environmental pollutants, grazing, and infections caused by bacteria, viruses, and fungi [5]. Additionally, macroalgae are known to respond fast to the prevailing environmental conditions across their distribution range, making the changes in their community structure and composition common and well-documented issues, along with biodiversity loss [18,19].

Several macroalgae contribute to bioaccumulation and bioremediation procedures and can act as bioindicators of water quality and marine ecosystem status [3,5]. Consequently, research on macroalgae, especially in shallow waters, has long been used to monitor and assess human impacts on coastal ecosystems [18,20]. Their monitoring through standardized methods within the European Union (EU), and consequently in Greece, is a fundamental requirement for the implementation of large-scale monitoring programs, such as those established under the scope of the EU

Water Framework Directive (WFD 2000/60/EC), the Marine Strategy Framework Directive (MSFD 2008/56/EC) [21,22], and the Habitats Directive (HD 92/43/EEC) [21].

A.1.3. Phytobenthic Ecological Quality Indices

In recent decades, numerous benthic macrophyte ecological quality indices have been created to enforce the WFD in the Mediterranean Sea [1,23,32–34,24–31]. Some are designed for rocky coastal waters (e.g., CARLIT [23]), while others target sedimentary transitional waters (e.g., MAQI [32]). The Ecological Evaluation Index (EEI [25]) is versatile, as it can be applied for both types of method [1].

The EEI has been successfully applied in coastal and transitional water ecosystems in Greece [25,35,36] and other Mediterranean countries, such as Cyprus [1,37], Slovenia [38], and Italy [1,39]. The Ecological Evaluation Index continuous formula (EEI-c), a new version of the EEI, has been developed to address the shortcomings of the EEI method [1].

A.2. Methods for Macroalgal Sampling

A.2.1. Destructive versus Non-Destructive Macroalgal Sampling Techniques

Macroalgal communities can be examined using destructive techniques [22,40]. Alternatively, non-destructive methods can be applied, including visual census methods [22,41] and photographic techniques [22].

The classic destructive sampling method involves scraping and collecting all organisms from an area that is defined, followed by laboratory identification and quantification [42]. This approach is widely regarded as suitable for characterizing assemblage structures of macroalgae and is essential for research on biodiversity and biomass [19,22]. Destructive sampling is also valuable for identifying cryptic species and obtaining voucher specimens for potential future use in taxonomic identification [22].

On the other hand, non-destructive sampling methods are preferred for use in vulnerable and protected habitats due to their minimal impact on biodiversity [22,43,44]. For example, they are appropriate for monitoring Marine Protected Areas (MPAs) to avoid additional damage to threatened habitats and species [45]. Non-destructive techniques are also suitable for studying canopy-forming algae because these organisms are susceptible to anthropogenic activities and are often displaced by faster-growing opportunistic algae, when subjected to continuous pressures [46]. In addition, there is an international concern over the extensive decline of canopy-forming algae along rocky coastal areas worldwide, including in the Mediterranean Sea (e.g., [47,48], as cited in [49]).

Non-destructive methods can be classified into direct and indirect. The direct techniques involve using quadrats of a defined area for species' cover percentage or frequency estimation. These estimates are conducted *in situ*, using sub-quadrats [42,50–52] or points [42,50,53,54]. Although these methods allow rapid data acquisition, their accuracy depends strongly on the diver’s prior taxonomic expertise [42]. While they are less precise than the destructive technique, they are also faster and enable sampling of larger areas with more replicates. These methods have been commonly employed in long-term monitoring due to their non-invasive nature, keeping the assemblages' structure intact [42,55]. However, more time in the field is necessary for these methods compared to the time needed for the indirect non-destructive techniques. Consequently, they are frequently used in intertidal habitats [56], but are less often applied in subtidal environments [22].

Indirect non-destructive methods, on the other hand, utilize photos or videos to evaluate the cover percentage or frequency of the visible species [42]. During the last decades, these methods have been significantly advanced by the recent development of digital photography [21]. Combining photographic techniques with the identification of primary taxa or morphological groups has proven to be an affordable and appropriate approach for assessing macroalgal communities, especially in monitoring programs and environmental impact assessments [22,57]. Furthermore, photographic sampling reduces underwater time requirements [22,42] and enables the collection of many samples, which is essential for assessments of ecosystems with high spatial diversity [21,22,58]. This method offers permanent archives of the studied communities, serving as proof of their ecological status for the public, among other benefits [59–64]. Nonetheless, the later frame analysis is time-consuming [42].

A.2.2. Indirect Non-Destructive Methods for Estimating Macroalgal Cover

A significant difficulty in photographic assessments of macroalgae assemblages lies in the efficient analysis of the visible macroalgal species that are present and the accurate estimation of the substrate area they cover [21]. To estimate the cover percentage of macroalgal species in photographic samples (also referred to as photosamples or photoquadrats hereafter), three main indirect non-destructive methods are used, depending on the study’s objectives, scale, available time and resources: the digital image segmentation, the grid, and the point intercept method.

In the digital image segmentation approach, species’ outlines are manually drawn in the photographic samples by researchers, and their coverage is assessed by quantifying these contours with image analysis software [65]. Although this technique is traditionally used in photogrammetry and provides the most precise evaluation of species' relative abundance in photosamples, it is also labor-intensive and inefficient for processing large numbers of photoquadrats from digital photography surveys [21].

In the grid methodology, sub-quadrats are employed for coverage data assessment [42,66,67]. In particular, the photosample is divided into smaller, uniformly sized grid cells, and the percentage cover of each species is determined by the number of cells it occupies compared to the total number of cells [50,53,59,67–69].

The point intercept method involves estimating the percentage of points that are overlaid on the substrate or organisms [21]. The number of points attributed to a species is then expressed as a percentage of the overall number of points [54,59,70–72]. This methodology can be applied with different point distributions (e.g., uniform, random, or stratified random) depending on specific criteria, such as the dimensions of the photoquadrats and the spatial variability of the community structure under study. Among these point distributions, stratified random points deliver the most reliable and accurate results [21,73]. The point intercept technique is also considered a more effective approach than digital image segmentation for calculating the proportion of area covered by organisms [21].

Nevertheless, thorough post-survey processing in the laboratory is demanded for deriving outcomes from photographic samples and there is a lack of software platforms to support these analysis methods of benthic photoquadrats [64,74,75]. Some tools are currently available, including PhotoGrid, which is used for random point counts and other broadly applicable image processing procedures; photoQuad, a software system dedicated to photosample image analysis in the field of ecology [59]; and Coral Point Count with Excel extensions (CPCe [76]), which enables random point counts and planar area estimations. CPCe, in particular, simplifies the point intercept technique for photoquadrats more than other software solutions, due to its user-friendly interface [66,76]. Despite the fact that it was initially designed for coral reef research, CPCe is readily adjustable to other assemblages [66,76], such as artificial habitats and rocky intertidal assemblages [77,78].

In recent years, relatively few studies have compared destructive and non-destructive methods for estimating benthic cover (but see [21,42,67]). On the contrary, most studies, in which different methods of benthic cover assessment are compared, involve direct (*in situ*) and indirect non-destructive techniques (usually digital image segmentation, grid and point intercept methods using photosamples) [42,64,72,75,79–84]. The literature is abundant with studies that analyze photoquadrats using indirect non-destructive techniques, including the digital image segmentation method (e.g., [85,86]), the grid method (e.g., [17,60,68,87]) or the point intercept method (e.g., [88–90]), to study various subjects. Nevertheless, fewer studies have directly compared these techniques, particularly when evaluating different effort intensities (e.g., varying the number of sub-quadrats or points).

As far as the digital image segmentation approach is concerned, some studies compare it to the grid or point intercept methods for analyzing photo samples [21,61,75] and another study compares different techniques of the digital image segmentation method [91]. Certain comparative studies involving the grid and point intercept methods focus on photographic samples of invertebrates, particularly corals [59,66,92,93]. There are fewer studies that compare the grid and point intercept techniques or their varying effort intensities for macroalgal photoquadrats, and these studies are sometimes combined with research on sessile invertebrates [21,94].

To the best of our knowledge, there is a gap in research comparing the most commonly used indirect non-destructive methods, particularly regarding varying sampling intensity (e.g., number of sub-quadrats or points) for macroalgal photosamples. Despite the aforementioned efforts, more concrete evaluations between these techniques are essential. Therefore, a key challenge remains in determining which percentage cover estimation methods in macroalgal photoquadrats are most efficient at balancing cost (in terms of analysis time) and the reliability of results (in terms of precision and accuracy), while meeting statistical requirements.

A.3. Aim of the Study

This study aims to cross-compare different approaches in the analysis of the presence and cover of macroalgae in photographic samples from various areas of Greece, to identify the optimal in terms of cost-effectiveness and reliability of results. Specifically, stratified random point intercept cover assessments are conducted at three modes of analysis effort intensity (50, 100, and 150 points) for a dataset of macroalgal photographic samples. Two key questions are addressed statistically: (i) how precise and accurate are the point intercept cover assessments in regards to the total cover estimations, which are considered as reference (REF) measurements and are obtained through a digitally superimposed grid, and (ii) which level of analysis effort is more time-, and thus cost-efficient. Moreover, all these analysis methods are compared in realistic application scenarios for the assessment of the ecological status with the Ecological Evaluation Index continuous formula (EEI-c [1]).

B. Methods and Materials

B.1. Dataset and photo system

A dataset of 100 macroalgal photographic samples of varying taxonomic diversity and cover was selected for this study. They were acquired through standardized monitoring activities of the Hellenic Centre for Marine Research (HCMR) across Greece during 2020-2024, in the context of national projects (Gyaros MPA, Dressage, KELTH-KELPS) and European Directives, i.e., the WFD and the HD. This dataset was specifically selected to reflect the high heterogeneity across the studied areas and natural reef habitats.

All photosamples (21 cm × 30 cm) were obtained in shallow rocky reefs along the upper infralittoral zone (0-5 m) by a photo system that includes an Olympus Tough TG-6 camera, attached to a fixed quadrat constructed with PVC pipes and fittings (Figure 1a). The photosampling procedure employed in all study areas involved collecting six systematic randomly positioned photographic samples at equal distances along a 25-meter transect line at each location, following the fieldwork protocol for the ecological status assessment of shallow rocky reefs in Salomidi *et al.* [87] (Figure 1a).

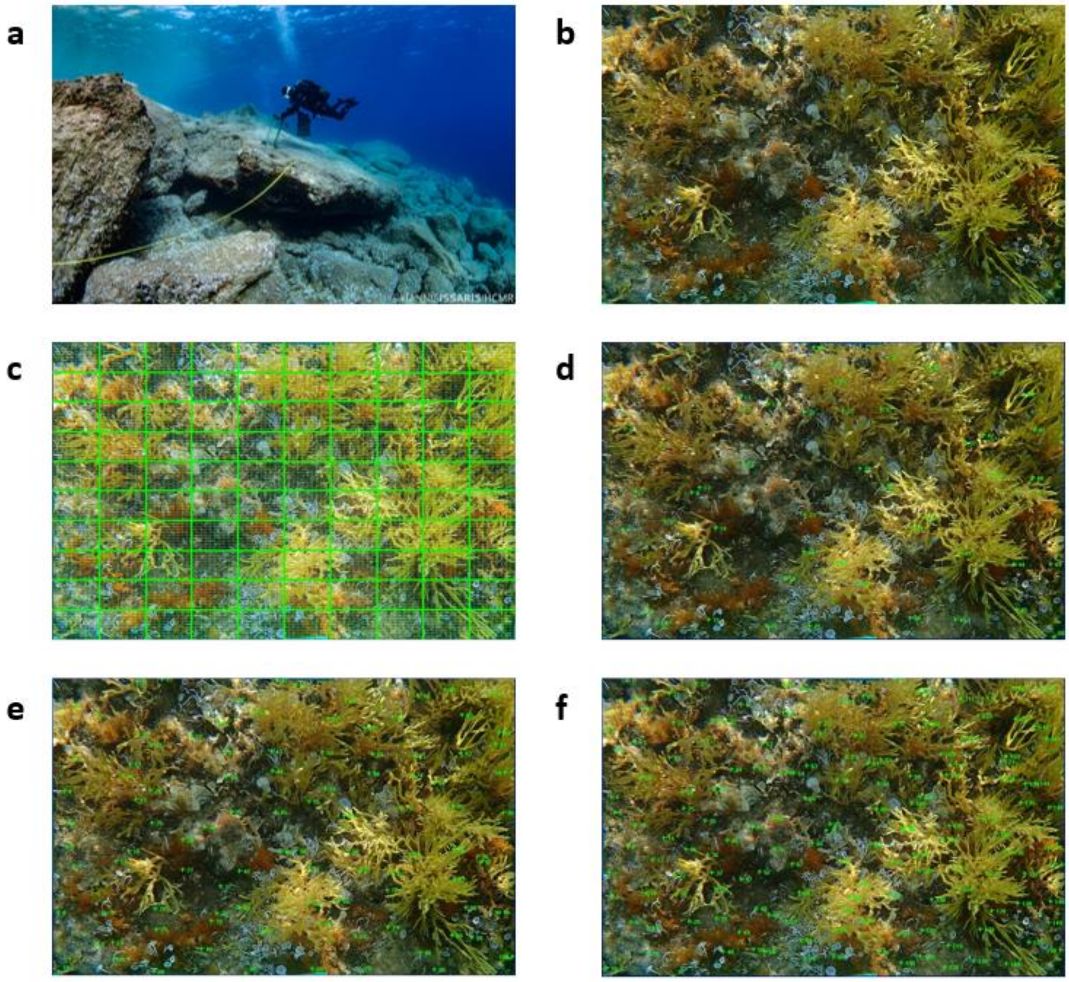

Figure 1: **The flow of sampling and analysis of macroalgal photoquadrats.** (a) Photoquadrat sampling in the field. (b) Photographic sample before image analysis. (c) Sample analyzed with REF total cover method (the grid contains 100 cells). (d) Random point intercept cover using 50 points. (e) Random point intercept cover using 100 points. (f) Random point intercept cover using 150 points.

B.2. Benthic Cover Image Analysis

The precision, accuracy, and time performance dynamics of the point intercept cover method under three modes of analysis effort intensity (50, 100, and 150 stratified random points) were assessed. This was achieved using the ‘point overlay’ tool of the ad hoc image analysis software Coral Point Count with Excel extensions – Version 4.1 (CPCe) (Figure 1d, e and f). The approach used in this methodology treated the benthic multilayered system as essentially two-dimensional, with a maximum possible cover of 100%. Accordingly, the first visible individual intercepted beneath each point was identified. All photosamples were cropped prior to image analysis to minimize the presence of the PVC frame in the photoquadrat under processing. During the image processing, the maximum zoom applied was 300%. Additionally, the time required for each analysis was recorded.

This technique was compared against reference total cover estimations (REF) obtained through the application of a detailed digitally superimposed grid (100 equal squares) in the Adobe Photoshop CS5 image editing environment (Figure 1c). The REF method, based on the Rapid Assessment of Coastal Ecological Status (RACES) methodology described by Salomidi [95], has been applied in several studies of Greek shallow rocky reefs, including Salomidi *et al.* [87]. This approach is currently the standard method for estimating the ecological quality status of shallow rocky reefs in Greece's coastal waters, as part of HCMR's monitoring activities.

As the analysis time for the REF assessments was not previously recorded, a similar technique was employed to estimate the mean analysis time for the REF methodology. Using this REF-like technique, the percentage of macroalgal cover for six photoquadrats of the study’s dataset was assessed. A detailed digitally superimposed grid consisting of 400 equal squares was applied using the photoQuad software [59].

In all methods, macroalgae were identified to the lowest feasible taxonomic level based on the observable species- or genus-specific morphological characteristics. A list of cover categories similar to that used in the REF methodology was developed for cover category identification using both the point intercept and REF-like techniques (Figure 2). It is acknowledged that the choice of methods for estimating macroalgal cover depends on the specific hypotheses being tested, and this study serves only as a guide.

B.3. EEI-c application

The analysis methods are compared in realistic application scenarios for the assessment of the ecological status with the Ecological Evaluation Index continuous formula (EEI-c [1]). Values of the Ecological Quality Ratios of the EEI-c ($\text{EEI-c}_{(EQR)}$, hereafter referred to as EEI-c) range from 0 to 1. In Greek coastal waters, EEI-c values above 0.48 (±0.09 SD) signify sustainable ecosystems of good or high Ecological Status Class (ESC). Conversely, EEI-c values below 0.48 suggest that the ecosystems require restoration to achieve a higher ESC [1].

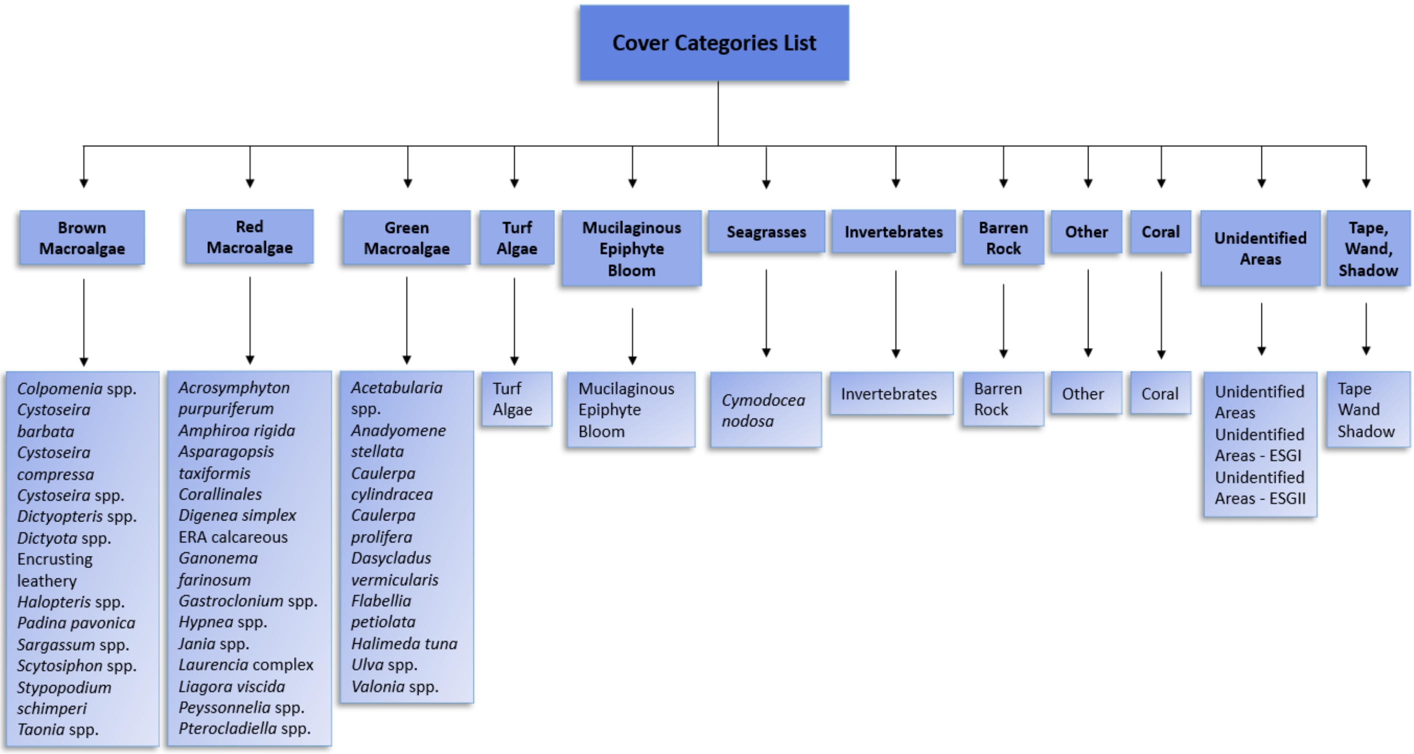

Figure 2: List of Cover Categories used for analyzing the macroalgal photoquadrat dataset with the point intercept and REF-like cover estimation methods. The subcategory "ERA calcareous" indicates calcareous Encrusting Red Algae.

In this study, all filamentous turf-forming macroalgae ("turf algae") were grouped together into Ecological Status Group (ESG) II for EEI-c estimation, following the approach of Orfanidis *et al.* [96]. Although Orfanidis *et al.* [1] classify turf algae into two ESGs for EEI-c estimation (e.g., brown turf algae of the genus *Sphacelaria* in ESG IIA, red turf algae of the genus *Ceramium* as well as green turf algae of the genera *Chaetomorpha* and *Cladophora* in ESG IIB), this simplification was necessary due to the difficulty of visually distinguishing these subcategories without notes from the photosampling procedure or samples obtained via destructive sampling for laboratory analysis.

B.4. Data Analysis

Data analysis was performed using Microsoft Excel. The mean and 95% Confidence Intervals (CIs) for each cover category were calculated using the descriptive statistics tool in Microsoft Excel and compared across the different optical cover estimation methods. All differences were considered statistically significant at $p \leq 0.05$.

In grid and point intercept cover estimation methods, the collected data generally follows a normal distribution. For these normally distributed data, statistically significant differences between two sample means can be assessed by examining whether their respective 95% Confidence Intervals overlap [97]. Confidence Intervals were used to assess whether the means of two sample groups could reasonably be attributed to the same population, assuming normal distribution of the datasets. When the 95% CIs of two means overlap, we infer that there is no statistically significant difference between the values, based on the confidence level corresponding to the t-value used [98,99].

The accuracy of an estimation method reflects the average proximity of the measured value to its true value [100]. In contrast, bias measures the difference between the estimated value and the true, typically unknown value. In this study, the 95% Confidence Intervals for the mean values were calculated to provide a statistical estimate of the “maximum bias” that each method might produce [61]. The precision of an estimated value reflects the degree of closeness in repeated measurements of the same quantity [100]. On the other hand, variance quantifies the average spread of repeated estimates around their mean [61]. This study focuses on evaluating the relative accuracy and precision of different levels of analysis effort intensity in point intercept cover assessments.

C. Results

In this section, we present the results of comparing mean % Benthic Coverage Differences (DCov) and mean EEI-c Differences (DEEI-c) between the three point modes and the REF method. Additionally, we assess the mean analysis time for each of the point modes in comparison to the REF-like method. Our aim is to evaluate how close each point mode's results are to the REF measurements, focusing on identifying any statistically significant differences that would indicate substantial deviations from the reference values. Statistically significant differences are those whose 95% Confidence Intervals cross the y=0 axis in red (Figures 3, 4, and 5).

The majority of mean % Benthic Coverage Differences (DCov) from REF, along with their 95% Confidence Intervals, are low and not statistically significant (p-value > 0.05) for all modes of points (43 out of 60 cases, Figure 3). The mean values and 95% CIs, when available, range from -10.93 (lower 95% CI for Mucilaginous Epiphyte Bloom, 100 points) to 11.8 (mean for *Digenea simplex*, 50 and 100 points), except for *Halopteris* spp., which shows a higher 95% CI value (upper 95% CI = 19.53, 150 points). The statistically significant mean % DCov values from REF and the 95% CIs (p < 0.05) are too low to have practical significance. For example, *Cystoseira* spp. shows a mean % DCov of 5.40 for 50 points and 3.54 for 100 points, *Jania* spp. has means of -7.16, -7.24, and -3.21 for 50, 100, and 150 points, respectively, and ERA calcareous shows a mean of 2.28 for both 100 and 150 points.

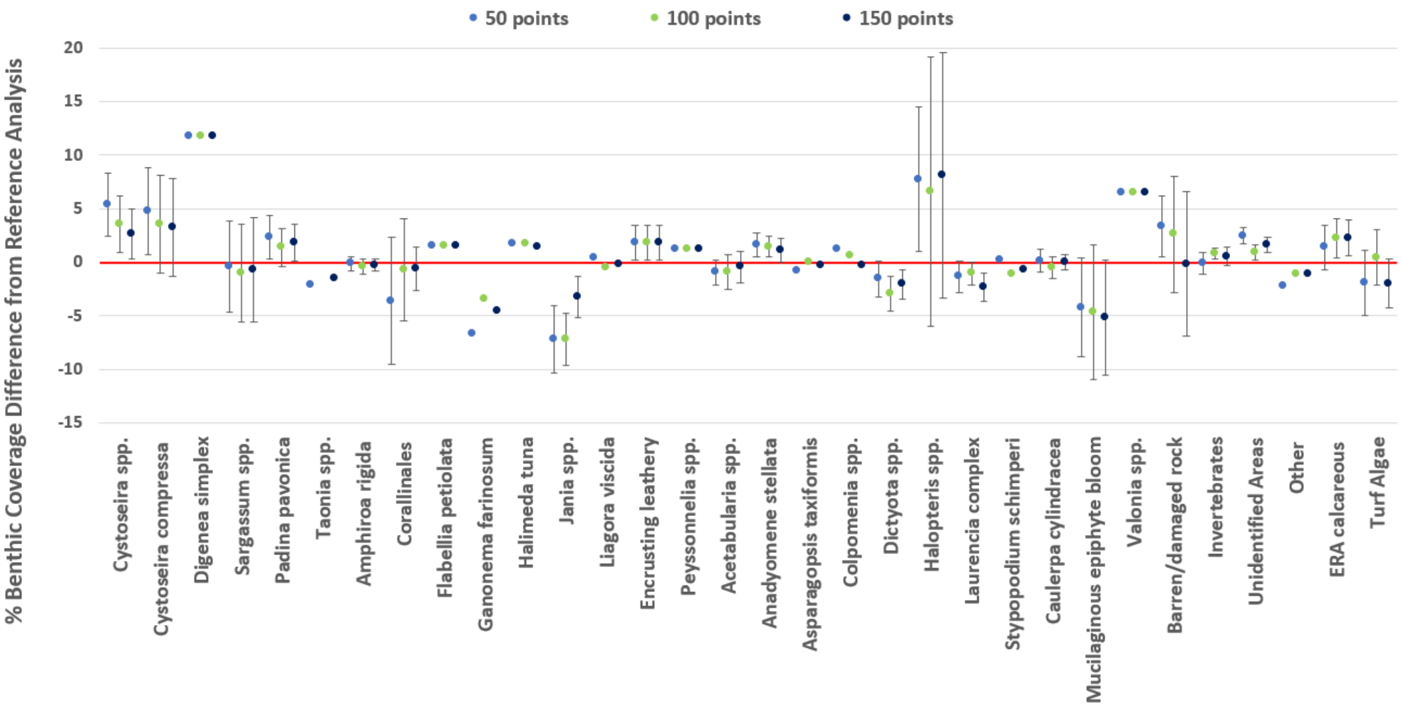

Figure 3: Mean Differences of % Benthic Coverage estimations based on point intercept method with 50, 100, 150 points from REF assessments. Error bars denote 95% Confidence Intervals.

Among the 20 cover categories with the highest occurrence counts (i.e., the number of photoquadrats in which a cover category is recorded) for all modes of the point method, the lowest mean % DCov is most frequently observed in the case of the 150-point mode (8 out of 20 cover categories, Table 1). For the remaining categories, the lowest mean % DCov is found using the 50- and 100-point modes, with minimal differences between them (6 and 5 out of 20 cover categories, respectively).* The highest 95% CI values are observed for cover categories recorded in only a few photosamples (Figure 3). For instance, *Halopteris* spp., with the lowest occurrence counts (5), has the highest 95% CI value (12.57, 100 points). The calculation of 95% CIs for cover categories observed in only 1 to 4 photoquadrats has no practical significance; thus, error bars for these cover categories were removed from Figure 3 and excluded from comparisons of statistically significant differences between methods.

Table 1: Most common cover categories observed in macroalgal photoquadrats, based on the number of photoquadrats in which each category is recorded, using point intercept cover estimates at three levels of analytical effort (50, 100, and 150 points), listed alphabetically.

Cover categories with the highest occurrence counts	
Acetabularia spp.	ERA calcareous
Amphiroa rigida	*Halopteris* spp.
Anadyomene stellata	Invertebrates
Barren/damaged rock	*Jania* spp.
Caulerpa cylindracea	*Laurencia* complex
Corallinales	Mucilaginous Epiphyte Bloom
Cystoseira compressa	*Padina pavonica*
Cystoseira spp.	*Sargassum* spp.
Dictyota spp.	Turf Algae
Encrusting leathery	Unidentified Areas

For rare cover categories with absolute mean % DCov values of 5% or higher (e.g., *Digenea simplex*, *Halopteris* spp., *Valonia* spp.), underestimation is frequently observed, except for *Ganonema farinosum* (with 50 points), which is overestimated. Another cover category with absolute mean % DCov values above 5% (with 50 and 100 points), *Jania* spp., is also overestimated but is commonly observed in the photoquadrats. Mean % Coverage Differences for Mucilaginous Epiphyte Bloom, another frequently observed cover categories, are similarly overestimated across all point methods, with absolute values approaching 5%. Mean % DCov values for *Cystoseira* spp. and *Cystoseira compressa*, both commonly observed cover categories,

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* For one cover category (Encrusting leathery), all point modes exhibit the same mean % DCov values.

are slightly underestimated with all point methods, with absolute values ranging from 2.62% to 5.40%.

Furthermore, all mean Differences of EEI-c (DEEI-c) from REF assessments are low (Figure 4). The 95% Confidence Intervals are also low and show similar values across all point modes: 0.037 for 50 points, 0.034 for 100 points, and 0.030 for 150 points. The 50- and 100-point modes have non-significant 95% CIs and mean EEI-c Differences from REF ($p > 0.05$). On the other hand, the mean DEEI-c and 95% CI for the 150-point method are statistically significant ($p < 0.05$), but these differences are too low to have any practical implications (mean DEEI-c = 0.037, lower 95% CI = 0.007, upper 95% CI = 0.067). A gradual decrease in mean DEEI-c from REF method is observed between the 50- and 100-point modes, followed by an increase when using 150 points, which exceeds the mean DEEI-c of the other two modes. However, these differences are not statistically significant ($p > 0.05$).

The mean analysis time for all modes of points is lower than that of the REF-like method (Figure 5; 50 points: 10 min, 100 points: 18 min, 150 points: 27 min, REF-like method: 30 min). A gradual increase in mean analysis time is observed as the number of points increases from 50 to 100 and 150 points.

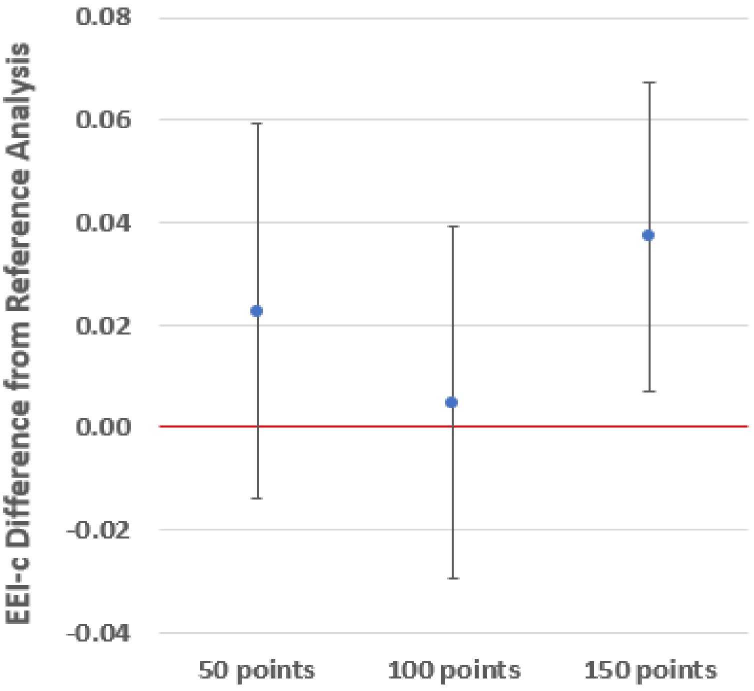

Figure 4: Mean Differences of EEI-c estimations based on point intercept method with 50, 100, 150 points from REF assessments. Error bars denote 95% Confidence Intervals.

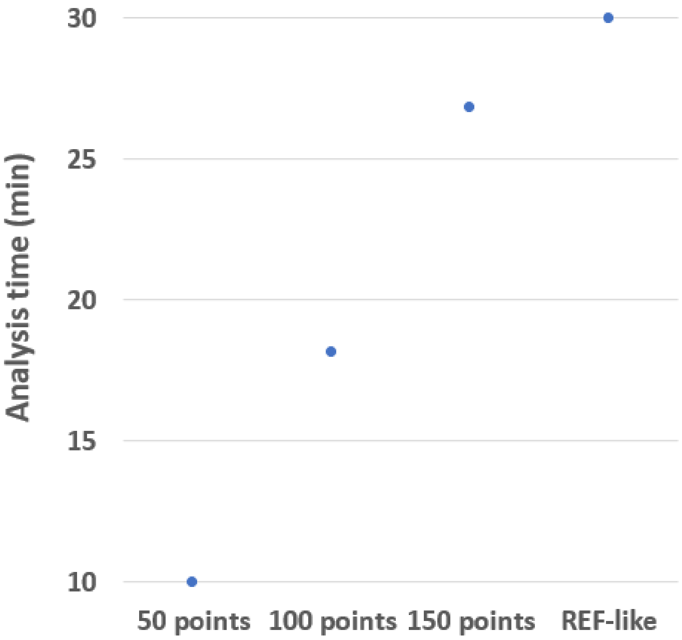

Figure 5: Mean analysis time needed based on point intercept method with 50, 100, 150 points and REF-like method.

D. Discussion

Comparative evaluations of benthic cover assessment methods reveal distinct trade-offs between cost-effectiveness and results' reliability [42,54,61,72,92] — both crucial for monitoring programs within coastal ecosystems [101,102]. In this study, we aimed to identify the most precise, accurate, and time-efficient approach for assessing macroalgal cover in photographic samples from Greek regions. This was achieved by comparing three levels of analysis intensity (50, 100, and 150 points) of the stratified random point intercept cover estimation method against reference measurements derived from the grid cover estimation method. Additionally, we tested each technique's application for ecological status assessments using the EEI-c.

Given that all values of the mean Coverage Differences from the reference analysis method (REF) and 95% Confidence Intervals are low and practically non-significant, the three studied modes of the point intercept method can effectively replace the REF method in terms of % Coverage Differences (Figure 3). For cover categories that appear in most of the analyzed photoquadrats, the 150-point mode gave mean % Cov values closest to those of the REF method, though the other two modes were not too far apart (Figure 3). In terms of the EEI-c values, all point modes seem suitable for use, as their mean DEEI-c values and 95% CIs are practically non-significant. However, the 100-point method demonstrated the best performance among the tested point intercept methods. In terms of analysis time, the optimal choice would be the 50-point mode, followed by the 100-point mode, with the 150-point mode being the least efficient in terms of analysis time. Overall, the point intercept method with 100 points appears to be the most appropriate offering a good balance between cost-effectiveness and results' reliability.

The selection of 100 points per image in this study corresponds to 1,587 points per m². This represents an order of magnitude difference compared to the recommendation by Berov *et al.* [21], where or 158 points per m² are suggested. There is a significant difference in photoquadrat sizes between the two studies, with our study using 21 x 30 cm photoquadrats, whereas Berov *et al.* [21] used 60 x 90 cm.

In the present study, the underestimation of mean % Cov for rare cover categories is a drawback of the point intercept method compared to the REF methodology, which provides more detailed % Coverage estimates (Figure 3). Besides, species abundance is a known variable that significantly influences probabilistic methods, such as the random point intercept methodology [103], but does not impact the grid technique [59]. However, since rare cover categories do not significantly influence EEI-c values in this study, this limitation does not affect the choice of the optimal method. As far as the mean % Cov of *Jania* spp. is concerned, its overestimation may be due to misidentification, potentially confusing it with Turf Algae. The underestimation of mean % Coverage for *Cystoseira* spp. and *Cystoseira compressa* could arise from transforming their complex, habitat-forming three-dimensional structures to two-dimensional image projections, as noted in the study by Berov *et al.* [21].

One factor that may have contributed to the similarity in mean % DCov values across the different point modes is the selection of stratified random point distribution (Figure 3). The greater accuracy and precision observed with 150 points, compared to 50 and 100 points, is partially in agreement with the findings of Berov *et al.* [21]. They demonstrated that increasing the number of sampling points improves accuracy, precision, and repeatability when comparing cover estimates derived from applying different numbers of stratified random points to those obtained using the contour outline cover (digital image segmentation) method.

Some relatively high 95% CIs and mean % Coverage Differences from the REF method in Figure 3 can be explained by the following factors. Firstly, absolute accuracy, defined as the deviation of an estimate from its true value, is seldom measurable in ecological studies [94]. In this study, no true measure of percentage cover was possible, and even the REF method may contain significant errors. Moreover, macroalgae identification is particularly demanding without field notes or samples collected via destructive methods. This is primarily due to the difficulty in detecting conspicuous species that live in the canopy of habitat-forming species or on the substrate beneath them [21,64,86]. Consequently, bias may occur in both methods, especially in the point intercept method, which was applied without any of these supportive resources.

Additionally, it is generally challenging to identify which cover category is beneath each point in multilayered systems, especially when different cover categories are particularly blended. Some photosamples were occasionally blurry or poorly lit, complicating identification, a challenge also reported by Berov *et al.* [21]. The high heterogeneity of areas and habitats where the photoquadrats were taken for our study results in varying taxonomic diversity and cover, leading to increased variance and wider 95% Confidence Intervals. Also, the lower the number of cover categories occurrence counts in the total 100 samples analysed, the higher their 95% CI values.

Furthermore, bias may have arisen from the dataset being analyzed by different operators. This discrepancy in analysis methods, with the REF method applied by multiple researchers and the point method conducted by a single person, could introduce bias and affect the overall reliability of the results. So, the subjectivity of each operator likely played a significant role in the differing findings between the methods. Other factors, such as varying operators' experience in both image analysis and fieldwork, differences in screen quality, inconsistent availability of field notes, and whether or not the same person took and analyzed the photos, also contributed to these variations. The results of the point method may also have been influenced by the operator's limited experience in macroalgal taxonomy, especially early in the study. Further bias may have occurred when the same photoquadrat was analyzed with different point modes by the same person, as the operator's experience increased during the course of the study. To restrict this bias, the same photoquadrats were typically reanalyzed using a different point mode after a minimum interval of three weeks.

Minor biases may derive from variations in how different operators cropped the photoquadrats before image analysis, which could influence the comparison between the point and the REF method. Additional factors contributing to these biases are related to the REF methodology itself. For example, no restrictions were applied to zoom levels, and identifying the first visible individual beneath each point was not always consistent, as it is in the point intercept method.

The 150-point mode leads to the lowest mean % DCov values, while DEEI-c values indicate better performance with the 50- and 100-point modes, which seems contradictory. This discrepancy can likely be attributed to the mean % DCov of Turf Algae and Mucilaginous Epiphyte Bloom. These two cover categories yield better results with 50 and 100 points compared to 150 points and have a greater influence on EEI-c values than other cover categories. In addition, four of the five cover categories with the highest occurrence counts across all point methods show the best EEI-c results with 50 or 100 points (two cover categories with 50 points and two with 100 points). In terms of analysis time, the point intercept method shows a gradual increase in mean analysis time as the number of points rises from 50 to 100 and 150, which is expected since more points require additional processing time.

E. Conclusions and Future Work

This study provides a practical framework for optimizing macroalgal cover assessment methods, highlighting that total cover estimations (REF) can effectively be replaced with stratified random point intercept cover assessments. Specifically, the 100-point mode of analysis effort intensity is suggested as the optimal one for the selected dataset in terms of cost-effectiveness and reliability of results. Although the 150-point mode yielded the best mean % DCov results and the 50-point mode was the quickest, the 100-point mode offered the best overall balance. It provided the closest mean EEI-c Difference (DEEI-c) to the REF method and effectively combined accuracy, precision, and time efficiency. Moreover, it is straightforward to implement and user-friendly.

The current results could serve as a foundation for future efforts to develop a guide that recommends optimal analysis intensity for stratified random point intercept macroalgae cover assessments, tailored to the specific characteristics of the algal community that is being sampled every time, such as species distribution patterns (e.g., patchy or uniform), and diversity.

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