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Abstract: Ecosystem engineers that increase habitat complexity are keystone species in marine systems, increasing shelter and niche availability, and therefore biodiversity. For example, kelp holdfasts form intricate structures and host the largest number of organisms in kelp ecosystems. However, methods that quantify 3D habitat complexity have only seldom been used in marine habitats, and never in kelp holdfast communities. This study investigated the role of kelp holdfasts (Laminaria hyperborea) in supporting benthic faunal biodiversity. Computer-aided tomography scanning (CT-) was used to quantify the three-dimensional geometrical complexity of holdfasts, including volume, surface area and surface fractal dimension (FD). Additionally, the number of haptera, number of haptera per unit of volume, and age of kelps were estimated. These measurements were compared to faunal biodiversity and community structure, using partial least-squares regression and multivariate ordination. Holdfast volume explained most of the variance observed in biodiversity indices, however all other complexity measures also strongly contributed to the variance observed. Multivariate ordinations further revealed that surface area and haptera per unit of volume accounted for the patterns observed in faunal community structure. Using 3D image analysis, this study makes a strong contribution to elucidate quantitative mechanisms underlying the observed relationship between biodiversity and habitat complexity. Furthermore, the potential of CT-scanning as an ecological tool is demonstrated, and a methodology for its use in future similar studies is established. Such spatially resolved imager analysis could help identify structurally complex areas as biodiversity hotspots, and may support the prioritization of areas for conservation.

11th December 2015

Dear Dr Shumway,

We have submitted the manuscript *"Application of computer-aided tomography techniques to visualise kelp holdfast structure reveals the importance of habitat complexity for supporting marine biodiversity"*, which we believe to be of high relevance to your readership. We introduce a novel approach to quantifying three-dimensional complexity in kelp habitats, an attribute of marine systems widely recognized as having a fundamental role in the sustenance of marine biodiversity, but seldom quantitatively assessed. This work helps to elucidate, in a quantitative manner, the mechanisms underlying biodiversity and kelp 3D complexity. As such, this study is of great relevance to ecologists concerned with the conservation of biodiversity, particularly given the recent interest in the role of kelp beds as hot-spots for marine life.

We introduce computer aided tomography (CT-scanning) as an ecological tool with great potential for development in the context of quantifying 3D complexity in kelp habitats, having recently been used with success in sedimentary habitats (Mazik et al. 2008). Further, we provide the mathematical protocol to quantify 3D complexity of holdfasts, by using fractal dimensions and borrowing 3D autocorrelation indices more commonly used in medicine. CT-scanning provides highly valuable information, and emerges as a unique tool to analyse habitat complexity quantitatively.

We present a study rich in innovative techniques, both in data acquisition and processing, and successfully demonstrate its wider potential for application in marine community and ecosystem research. We therefore believe that this study is a fitting contribution to JEMBE.

For the authors, with our kindest regards,

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***Detailed Response to Reviewers**

Cambridge, January 3rd 2016

Dear Mrs. Shumway,

I have modified the 5 figures according to your recommendations. I hope the manuscript is now fit for publication.

Sincerely yours,
Chloé Orland

Highlights

- CT-scanning was used for fine scale quantification of the 3D geometrical complexity of kelp holdfasts.
- Biodiversity was strongly affected by all measures of holdfast complexity.
- CT-scanning is an emerging and potentially powerful tool for ecological research.

Application of computer-aided tomography techniques to visualize kelp holdfast structure reveals the importance of habitat complexity for supporting marine biodiversity

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Author contributions: SW originally formulated the idea, CO and AMQ collaborated in developing methodology, CO conducted fieldwork, SH, SG, TZ and LA generated scanned images, CO and CLM generated biodiversity data, CO analyzed the images, developed the complexity indices and performed statistical analyses in collaboration with AMQ, and CO, AMQ, SW and JIS wrote the manuscript.

Abbreviations: CT = computer-aided tomography, FD = fractal dimension

Abstract Ecosystem engineers that increase habitat complexity are keystone species in marine systems, increasing shelter and niche availability, and therefore biodiversity. For example, kelp holdfasts form intricate structures and host the largest number of organisms in kelp ecosystems. However, methods that quantify 3D habitat complexity have only seldom been used in marine habitats, and never in kelp holdfast communities. This study investigated the role of kelp holdfasts (*Laminaria hyperborea*) in supporting benthic faunal biodiversity. Computer-aided tomography scanning (CT-) was used to quantify the three-dimensional geometrical complexity of holdfasts, including volume, surface area and surface fractal dimension (FD). Additionally, the number of haptera, number of haptera per unit of volume, and age of kelps were estimated. These measurements were compared to faunal biodiversity and community structure, using partial least-squares regression and multivariate ordination. Holdfast volume explained most of the variance observed in biodiversity indices, however all other complexity measures also strongly contributed to the variance observed. Multivariate ordinations further revealed that surface area and haptera per unit of volume accounted for the patterns observed in faunal community structure. Using 3D image analysis, this study makes a strong contribution to elucidate quantitative mechanisms underlying the observed relationship between biodiversity and habitat complexity. Furthermore, the potential of CT-scanning as an ecological tool is demonstrated, and a methodology for its use in future similar studies is established. Such spatially resolved imager analysis could help identify structurally complex areas as biodiversity hotspots, and may support the prioritization of areas for conservation.

Keywords Ecosystem engineer · Fractal · Image analysis · Kelp holdfasts · Benthic fauna · *Laminaria hyperborea*

1. Introduction

The idea that habitat structure is a major influence on biodiversity (Simpson, 1949) is now well accepted, and an increase in available surface area is considered to be the main explanation for the positive correlations often observed between habitat structure and biodiversity (Connor and McCoy,

1979). This idea follows the classical theory of island biogeography (MacArthur and Wilson, 1967), whereby they explained the effects of distance and area on biodiversity. Underpinning this relationship is the understanding that the structural complexity of habitats regulates the distribution of refuges available for protection, nesting, nurseries, mating and resting (Steneck et al., 2002), food resources and foraging space, thus reducing competition and enabling the coexistence of a wide range of species (Bell et al., 1991). Given that space is a limiting resource in many shallow marine systems, large organisms which provide additional three-dimensional habitats, e.g. macrophytes (Blight and Thompson, 2008), are typically seen as key or critical species in marine systems (Jones et al., 1994; Wright and Jones, 2006; Hastings et al., 2007). As these large structure-forming organisms modify the physical and chemical structure of habitats, they can be considered as ecosystem engineers (Jones et al., 1994).

Kelps were one of the first groups of species identified as structurally enriching engineers, with the loss of kelp forest due to grazing being recognized as a simplification of trophic complexity in near shore systems (Estes et al., 1998). The physical presence of kelp has numerous positive effects on species diversity by enhancing ecosystem stability spatially and temporally, modifying the distribution and abundance of resources, as well as altering current speed, light availability and nutrient cycles (Jones et al., 1997; Hastings et al., 2007). Therefore, identifying the physical attributes of macroalgae that contribute to the maintenance of the kelp-associated community diversity will help to elucidate the importance of keystone structures in marine habitats (Tews et al., 2004) and support conservation measurements.

Within kelp habitats, it is the holdfast which provides the majority of intricate structures and hosts a vast number of associated faunal species (Christie et al., 2003; Arroyo et al., 2004). The holdfast is composed of numerous root-like haptera structures, entangling and creating spaces and gaps (Moore, 1972), with new, outer hapteron layers adding to the structure as the kelp ages (Smith et al., 1996). Numerous studies have identified a relationship between holdfast size or volume and benthic faunal communities (Jones, 1971; Ojeda and Santelices, 1984; Dean and Connell, 1987; Smith et al., 1996; Torres et al., 2015). Habitat complexity has been shown to have a powerful influence on algal meiofaunal communities, as seaweed of simple structure, with more planar surfaces, attract a lesser

number of species and in lower abundance (Hicks, 1985). Additionally, it is thought that by creating microhabitats, increased complexity in kelp holdfasts augments the number of niches available, reducing in this way competition, and providing protection from physical stress (Gibbons, 1988). Species that are less dependent on drifting may therefore choose to inhabit the holdfast rather than the frond, in order to benefit from the structure's protection against currents and potential predators (Christie et al., 2007). Furthermore, elevated complexity enhances silt accumulation, which becomes trapped in holdfasts (Moore, 1972). This may explain why both psammic and phytal organisms are found within holdfasts, adding diversity to the specialist fauna already present (Arroyo et al., 2004). Spatial complexity created by holdfast branching may cause competition for hapteron attachment sites and encourage sessile species to occupy voids created between haptera (Moore, 1986). Tubiculous and sessile fauna, like polychaetes, bivalves and sponges, also create habitats for other organisms, which can inhabit vacated tubes and benefit from increased habitable surface (Smith et al., 1996).

Despite numerous studies on this topic, the general lack of quantitative ecological data has been highlighted at multiple times (Arroyo et al., 2004; Anderson et al., 2005): the relationship between habitat complexity and biodiversity can neither be fully explained by surface-area alone nor by holdfast weight or volume (Hicks, 1985; Smith et al., 1996; Norderhaug et al., 2002; Arroyo et al., 2004; Hauser et al., 2006; Norderhaug et al., 2007). Current theories on habitat complexity are often based on these variables, and rarely account for other factors that influence the structure. They may thus dismiss important architectural features of the habitat. Additionally, previous studies that have established the importance of structural complexity in explaining patterns of diversity have only addressed this issue using artificial mimics (Hauser et al., 2006; Christie et al., 2007; Norderhaug et al., 2007). The fact that chemicals released by the structure may influence the species which choose to inhabit it (i.e. amount of dissolved oxygen, chemical cues and nutrient flow) and that communities may evolve over time (Bell et al., 1991) should be accounted for.

Several methods have been applied in ecology to quantify complexity. Most commonly, the analysis of fractal geometry has been used in marine ecological contexts (Jeffries, 1993; Gee and Warwick, 1994a,b, McAbendroth et al., 2005; Torres et al., 2015). Fractal objects are those whose size increases as their unit of measurement decreases (Mandelbrot, 1967), and fractal geometric patterns

are observable in various naturally occurring objects like coastlines, coral reefs and leaf vegetation (Sugihara and May, 1990). The fractal dimension (FD) – the rate by which the object size increases with decreasing units – provides a quantifiable measure of complexity, independent of the nature of the structure, and accounts for habitat scale and what it represents for organisms of different sizes (Gee and Warwick, 1994b). Less explored methods include the use of indices of spatial auto-correlation, i.e. a quantification of the similarity between objects considering the proximity between them, such as Moran's index and Geary's coefficient (Legendre and Fortin, 1989; Corrêa da Silva et al., 2008). Similarly to fractal dimensions, spatial autocorrelation methods are multi-dimensional: so long as the factors are weighted correctly, the latter can be applied to three-dimensional spaces, such as kelp (Chen, 2013).

The primary aim of the present study was to investigate the relationship between habitat complexity and faunal biodiversity in kelp holdfast communities. The application of a novel method to quantify a complex biological structure is presented. Computer-aided tomography (CT-) scanning is a technique typically applied for medical uses that has more recently been used in an ecological context to portray aspects of 3-dimensional structure and complexity (Mazik et al., 2008; Naumann et al., 2009; Faulwetter et al., 2013). In the present study, CT-scanning was used for the first time to acquire detailed 3D data from holdfasts of the kelp *Laminaria hyperborea* and establish whether structural complexity of holdfasts relates to holdfast fauna community structure. This species of brown alga is an ideal model for studies on habitat complexity due to the intricate and ramified nature of its holdfast as well as its importance as a benthic habitat (Arroyo et al., 2004; Norderhaug et al., 2007; Christie et al., 2007; Blight and Thompson, 2008). By offering 3D imagery of the habitat, CT-scanning enabled *in vivo* visualization of both the internal and external rhizoid assemblage – spatial information otherwise difficult to acquire (Dutilleul et al., 2005). Indeed, the main advantage of CT-scanning is that it allows access to quantitative data that are extremely relevant to architectural properties of the habitat (e.g. surface, volume, fractality). The benthic fauna inhabit the holdfast's surface and internal spaces, and thus the information acquired through this method is a reflection of the provision of substrate for fauna and associated protection from currents and predators. As the kelp holdfasts used in this experiment have geometrical patterns that lend themselves well to fractal analysis, and as the distribution of voxel

intensities in the scans is associated to some form of spatial variability (Corrêa da Silva et al., 2008), fractal dimensions and spatial autocorrelation indices were computed from the CT-scan data in order to quantify the spatial complexity of the holdfast habitat. We hypothesized that increased habitat complexity leads to increased faunal biodiversity.

2. Materials and methods

2.1 Sample collection

Seventeen individual *Laminaria hyperborea* holdfasts of different sizes (5.19 - 240.42 cm^3) were collected sub-tidally by SCUBA divers, at random positions within two kelp beds in the Plymouth Sound, UK: Andurn Point (50°19.235' N, 004°07.820' W) and Ramscliff Point (50°19.558' N, 004°07.820' W) on the 22nd of February 2013. Both sites are characterized by a similar degree of wave exposure and water depths (7-11m). The stipe of each individual was cut 5 cm above the holdfast with a diver's knife and each holdfast was levered carefully from the substrate before being placed quickly into a plastic bag, which was immediately sealed with a cable-tie. Great care was taken to avoid damaging the structure of the holdfasts during collection. Each bag containing a holdfast was then placed into another bag to reduce the risk of losing the mobile fauna if the first bag was damaged. To exclude air from entering the holdfast structure, as this could affect CT-scanning results, each bag containing a holdfast was immediately placed in a bucket of seawater on retrieval to the dive boat. The holdfasts were transported in individual buckets to Plymouth Marine Laboratory, UK.

2.2 Pre-scanning procedure

During CT-scanning, the absorption of X-rays is sensitive to differences in the density of the materials scanned (Mazik et al., 2008; Ketcham and Carlson, 2011). The CT-value obtained is an average of the properties of the different materials, meaning the material boundaries may be blurred, and leading to what is referred to as partial-volume effects (Ketcham and Carlson, 2011). In order to limit such artifacts, which may affect the resolution of the imagery, as much of the fauna as possible was removed from the holdfasts prior to scanning. The best method to do so in a non-destructive way was

sonication. Without removing them from their plastic bags, each holdfast was placed in an ultra-sound bath for 15 min. Once the samples had been sonicated, in order to ensure the holdfasts were immersed at all times and to avoid exposure to air, they were transferred into buckets of locally collected seawater in which the rest of the procedure was carried out. The plastic bags were then cut open and the holdfasts were left to sit for 10 min, to allow fauna to escape. The fauna extracted during this procedure was sieved over a 250 μm mesh and fixed in 10% buffered formaldehyde. Each holdfast was then immersed into the same formaldehyde solution within a standard plastic pot for scanning, with particular care not to introduce air bubbles into the holdfast structure. The pots were selected for their low X-ray absorption levels, to maximize the quality of the scan (Lontoc-Roy et al., 2006). Fauna samples were preserved in 75% industrial methylated spirit after 48 hours until analysis.

2.3 CT-scan data acquisition and post-processing

Each holdfast was scanned at the CT Suite of the Radiology Department located at Torbay Hospital, in a helical, medical high resolution CT-scanner (Discovery CT750HD, GE Healthcare). CT-scanning generates two-dimensional cross sectional images called slices using a single x-ray tube and an array of detectors that rotate around the object of study. Reconstructed slices are then used to recreate the 3 dimensional structures as a composite (Dufour et al., 2005; Ketcham and Carlson, 2011). The CT-scan configuration parameters were set at 80 kV for the X-ray tube voltage and 300 mA for the X-ray tube current. Rotation speed was set at 0.7 s, the table moved at 15.1 mm/s and exposure time was 10-12 s. The image reconstruction interval was 0.625 mm, giving a voxel size of 0.244 mm^3 (a voxel is a "volume pixel" – a unit of volume in three-dimensional space; the smaller a voxel, the higher the resolution of the image). 2D slice size was 512 x 512 pixels. Images were saved in DICOM format in, 16-bits and 3D composites reconstructed using the freeware OsiriX version (Rosset et al., 2004; Fig. 1a and b).

The raw CT-scan images were transformed into a set of raster files, each one encoding a 2 dimensional cut through the object. In other words, the images were stored as a succession of voxels, each of which was specified by its position (x,y,z) and its brightness I(x,y,z), normalized between 0 and 1. As the interest hereby lies in extracting geometrical properties of the images, the first step was

to segment the image in order to: i) remove the background noise inherent to the CT-scan; and ii) remove the image of the pot housing the kelp. Since the noise and the container correspond to low values of the brightness intensity, a threshold θ was defined such that any intensity lower than θ was set to 0, and any intensity larger was set to 1. By doing so, all the background noise was removed as well as the image of the container and all the significant voxels were set to the maximal brightness 1. Each kelp was hereafter represented by a filtered image, defined by voxels with I(x,y,z) = 0 or 1 (Fig. 2).

2.4 Processing of fauna and kelp samples

Following scanning, the holdfasts were manually broken up and thoroughly washed over a 250 μm sieve to retain the remaining fauna, which was fixed and preserved as before. All collected individuals were pooled per kelp, and all individuals were counted and identified under low power magnification (x500), to the lowest taxonomic level possible. Finally, the age of each kelp plant was determined using the method of Kain (1963), which consists of splitting the stipe in half longitudinally just above the holdfast and counting the number of growth rings present. The number of separate haptera branching out from the stipe was also recorded.

2.5 Image analysis and calculation of complexity indices

2.5.1 Volume

Calculating the volumes of the holdfast presents an adequate measure of inhabitable space within the kelp. The volumes of the kelps were computed by drawing regions of interest (ROIs) as precisely as possible onto each 2D slice of the scanned holdfast at maximum resolution, using the image software OsiriX. In principle, these measurements could also be obtained by counting the total number of voxels with brightness 1 on the filtered image, and multiplying it by the volume of a voxel $v = a^3$, where a is the spatial resolution of the scanner in the x, y and z direction. Indeed, the filtered encoding of the images allows for the computation of all kind of geometrical properties of the kelp.

2.5.2 Surface

Since a number of organisms live on the surface of the kelp, it is interesting to retrieve information from the scans pertaining to the surface area. Using the filtered data, the surface area was extracted using two algorithms defining the surface of the kelp:

1. *Translation*: consider the image of a kelp after threshold. This image can be translated by one voxel in a given direction (for example the z-direction, perpendicular to the planes of the CT-scan). The voxels that are different in the original and in the translated image belong to the surface of the kelp (this is true only after restricting the brightness intensity to 0 or 1).
2. *Neighbours*: since the image is 3-dimensional, each point of the grid has 6 neighbours. A voxel belongs to the bulk of the kelp if it has exactly 6 direct neighbours with an equal brightness of 1, and belongs to its surface if its number of neighbours is smaller than 6.

Both methods were tested, and these gave results that were consistent with each other. Throughout this paper, the surface areas were computed by the translation method. The total surface area of the kelp is thus equal to the number of voxels of the surface, times the area of a face of the voxel, a^2.

2.5.3 Fractal dimension

An important characteristic of a complex geometry is its fractal dimension (Mandelbrot, 1983). It quantifies the degree of branching and embedded small structures of the object. It is defined mathematically in the following way: given a geometrical object, in order to cover it with voxels of size ε^3, one needs $N(\varepsilon)$ such voxels. When the linear size ε gets smaller (i.e. the resolution increases), a larger number of voxels $N(\varepsilon)$ is needed to cover the object. The fractal dimension, d_F, characterizes how the number of voxel increases when the resolution increases and is defined mathematically as the limit:

$$d_F = -\lim_{\varepsilon \to 0} \frac{Log\ N(\varepsilon)}{Log\ \varepsilon}$$

where Log denotes the natural logarithm function.

The fractal dimension can be computed by using the "box counting" method (Mandelbrot, 1983). Starting at the highest resolution (i.e. smallest voxel size a), a voxel is said to be occupied (by kelp) if its value is 1, otherwise it is said to be empty. The total number N(a) of occupied voxels at this resolution is calculated. Now consider double sized voxels with linear size 2a. Each of these new voxels comprise 2x2x2=8 smaller original voxels. Each of these is occupied if it contains at least 4 occupied smaller voxels, and empty otherwise. The number N(2a) of occupied voxels of size 2a can now be computed. This procedure is iterated by doubling the size of the voxels and computing each time the corresponding number of occupied new voxels. A log-log plot of $N(\varepsilon)$ as a function of the resolution ε can then be plotted. If the object is fractal, this plot is expected to look like a straight line with a negative slope, the slope being the fractal dimension.

It is interesting to note here the relevance of the fractal dimension of the surface. Indeed, the concept of fractal dimension can be applied to the volume voxels – all the voxels of the image – or to the surface voxels, obtained by either method described above. The small hierarchical structures constituting the surface of the holdfast provide suitable habitats for small organisms, and thus it is logical to try to correlate the biological diversity with the surface fractal dimension of the kelp. The surface fractal dimension of the kelp is calculated by the box-counting method, exactly as depicted previously, except that the voxels used are those of the surface, computed by the translation algorithm described above. According to the above definition, the fractal dimension is just the opposite of the slope of the best-fit straight line. Practically, the fractal dimension was computed by doing a best linear fit for $\log N(\varepsilon)$ as a function of Log ε, where ε takes the value of the resolution at each of five resolutions.

2.5.4 Spatial autocorrelation

Holdfast complexity was also measured using 3D extensions of 2D spatial autocorrelation indices. 3D extensions of the spatial autocorrelation indices were calculated with Moran's I (Moran, 1950) and Geary's C (Geary, 1954) using a custom made program in Fortran, based on equations from Marwan et al. (2012) and Corrêa da Silva et al. (2008). The Moran index I and the Geary coefficient C were computed as:

$$I = \frac{N}{\sum_i \sum_j \omega_{ij}} \frac{\sum_i \sum_j \omega_{ij}(X_i - \bar{X})(X_j - \bar{X})}{\sum_i (X_i - \bar{X})^2}$$

$$C = \frac{(N-1)}{2\sum_i \sum_j \omega_{ij}} \frac{\sum_i \sum_j \omega_{ij}(X_i - X_j)^2}{\sum_i (X_i - \bar{X})^2}$$

where $\overline{X}$ is the mean value of all the voxel values, N is the total number of voxels, X_i is the voxel value at particular point i, X_j is the voxel value at a particular point j ($i \neq j$) and ω_{ij} is the neighbourhood matrix, equal to 1 when i and j are neighbours, and to 0 if else.

2.6 Statistical analysis

Diversity indices capture different aspects of biodiversity, and it is therefore customary to calculate various indices in parallel to capture those different aspects (Magurran, 2004). The total number of species (S), number of individuals – abundance – (N), species richness (Margalef's d), species evenness (Pielou's J) and a diversity index representing a balance between both richness and evenness (Shannon-Wiener's H') were calculated using the faunal abundance data. All possible pairwise correlations between explanatory and response variables were identified by carrying out a Spearman's rank correlation (ρ) test (Fig. 3). All of the 15 possible pairwise correlations between the 6 complexity explanatory variables were significant (Fig. 3). The variable "site" from which the holdfasts were collected was not correlated to any of the explanatory variables (Fig. 3). Since the holdfasts from the two sites did not differ significantly in terms of their age or structural attributes, "site" was not included as a factor in the diversity indices analyses. Univariate measures of community assemblage do not account for the identity of species present and therefore they may be less sensitive than multivariate methods (Warwick and Clarke, 1991). As their output is under numerical form though, they can more directly be related to the indices of complexity developed here (Gee and Warwick, 1994a).

2.6.1 Partial least squares regressions

Partial least squares regression analyses (PLSRs) were carried out to quantify how much each of the explanatory variables (holdfast complexity, i.e. number of haptera per unit volume and fractal dimension of the surface, as well as kelp age, number of haptera, surface area and volume of the holdfasts) explained the variance structure of each diversity measure, separately. The PLSR algorithm finds a reduced number of components (latent variables) maximizing the percentage of variance structure of a response variable (in this case, each of the diversity indices) explained by the matrix of potential predictors (i.e. complexity proxies, Carrascal et al., 2009). This method is an extension of multiple regression methods, and is particularly suited as a tool for small sample sizes and correlated predictor variables (Carrascal et al., 2009), as is the case here. The correlations between all possible pairwise correlations suggested a PLSR analysis was appropriate, due to the large number of correlated explanatory variables and small sample size (i.e. 17 observations (individual holdfasts) and correlations identified between all predictor variables, Fig. 3). Individual PLSRs were carried out in order to determine the percentage contribution of holdfast physical attributes and indices of complexity in explaining the variance structure of each response variable. The PLSR were performed using the "pls" package (Mevik and Wehrens, 2007) in the open source statistical software R (R Core Team, 2012).

2.6.2 Multivariate analysis

Multivariate ordination and clustering methods were undertaken to further highlight potential differences in faunal assemblage structure between kelp holdfasts of varying complexity, using the statistical analysis software Plymouth Routines In Multivariate Ecological Research 6+ (PRIMER, Clarke and Gorley, 2006). The species abundance data was 4^{th}-root transformed to down-weight the influence of heavily abundant taxa (Clarke, 1993) and the rest of the analysis was carried out on both datasets (hereafter "transformed" and "untransformed data"). Prior to further analysis, the effect of site on fauna community structure was examined again, as holdfast collection had been carried out at two different sites. Using the PERMANOVA+ routines add-in to PRIMER 6, a preliminary non-

parametric permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was carried out using "site" as a fixed factor and the community matrix as the response variables. This analysis showed that although the faunal assemblages differed significantly between sites, no significant interaction was found between the effect of site and the other independent variables listed in Table 1 on assemblage structure. This indicated that, despite being different, the kelp holdfast communities at each site responded similarly to the complexity measures used. As such, "site" was not considered in the subsequent multivariate analysis. To assess the similarity of the holdfast faunal communities, a non-metric multidimensional scaling (nMDS) was conducted using the Bray-Curtis similarity index (Clark and Warwick, 2001). To visualize similarities in assemblages between holdfasts, an nMDS was plotted in a two-dimensional space preserving the multidimensional distance between kelp communities based on similarity. Individual communities were labeled on the plot using circles with a diameter proportional to the values of each explanatory variable (i.e. bubble plot) to investigate possible similarity between holdfast communities and holdfast complexity attributes. Finally, a BEST analysis was carried out on the transformed data, to identify which variable, or combination of variables, best explains the patterns of faunal assemblage similarity (Clarke and Gorley, 2006).

3. Results

3.1 Complexity of holdfasts

3.1.1 Fractal dimension

Fractal dimensions of objects embedded in 3-dimensional space are comprised between 0 and 3 (Mandelbrot, 1983; Russ, 2013). The values for the fractal dimension of the surface fell within this interval (Table 1). This method is a promising avenue as the use of CT-scanner does allow for a fractal analysis in full 3 dimensions whereas traditional techniques, more commonly described in the literature, are reduced to 2 dimensions.

3.1.2 Spatial autocorrelation

The values for Moran's I and Geary's C fell within their expected values (I=[-1;1], C=[0;2]), however they were not correlated to any of the diversity indices and did not seem to be appropriate indicators of complexity in this study. They were therefore not considered further.

3.2 Holdfast communities

A total of 7206 individuals from 159 taxa were identified. Juvenile Nereididae (Annelida) was the most numerically dominant group, constituting 23.7% of the total abundance. *Verruca stroemia* (Arhtropoda) were also well represented in the assemblages (19% of the total abundance). *Anomioidea* (Mollusca), *Hiatella arctica* (Mollusca), and Nematoda formed another 22.7% of the number of individuals. Polychaete (Annelida) species (67 in total) constituted 42.1% of total species and amphipods another 20.1% with 32 different species.

The number of species (S) within a holdfast ranged between 18 and 82 and abundance (N) between 51 and 1082, thus exhibiting important variations between holdfasts in terms of species richness (d), which ranged between 2.52 and 11.89 (Table 1). Shannon-Wienner's species richness and evenness values were comprised between 0.72 and 3.33 and Pielou's evenness J between 0.21 and 0.90.

On closer analysis of the raw abundance data, one of the holdfasts (sample 7) exhibited a surprisingly high total number of individuals (N=706, 618 of which are juvenile nereids) compared to its volume (48.27 cm^3), haptera.cm^{-3} (0.50) and age (4 years old). As Shannon-Wiener's H' and Pielou's J are indices of diversity that take into account evenness, they are especially sensitive to outliers in the abundance data and are strongly influenced by dominant species. Consequently, this individual holdfast showed very low values for H' (0.72) and J (0.21) (Table 1), indicating low heterogeneity in the assemblage and a highly uneven distribution of species. The correlation plot (Fig. 3) confirmed this with strongly skewed graphs and a clear outlier point in both H' and J. Thus, this holdfast was removed in further analyses in order to avoid observing unexpected variance structures in H' and J.

3.3 Statistical analysis

3.3.1 Partial least squares regressions

The loading plots of the PLSR analyses for each response variable revealed that the majority of the variance structure in each biodiversity index was best explained by components for which volume had the highest loading among all potential explanatory variables (Fig. 4). However, the contribution of the other 5 variables for the best component– age, surface area, fractal dimension, haptera number and haptera.cm^{-3} – closely followed that of volume, with approximately equal loading values, indicating they all played an important role in explaining the variance observed in each response variable explained by that component.

The most important findings of the PLSR analyses are presented in Table 2. The same relationships were observed for S and d, as these indices were strongly correlated to each other (Fig. 3). Despite yielding slightly lower values of variance explained by the PLSR on S, N and d, the variance structures for both H' and J showed comparable patterns to that of the other biodiversity indices.

In sum, volume influenced biodiversity the most, followed by age, and the fractal dimension of the surface (Fig. 4; Table 2). It is interesting to note that the full models with 5 components explained between 37.17% and 81.19% of the variance structure, meaning that other factors not included in this analysis must account for the remaining variance.

3.3.2 Multivariate analysis

The BEST analysis suggested that the combination of surface area and number of haptera per unit of volume of holdfast explained most of the similarity structure in the community matrix, and this effect was more evident in the less abundant species (4th root transformed data, $\rho_{Spearman}$ = 62.1% and $p<0.05$) than in the numerically dominant species (untransformed data, $\rho_{Spearman}$ = 38.0% and $p<0.05$). This pattern was evident in the nMDS plots (Fig. 5), suggesting that number of haptera per unit of volume (Fig. 5a and c) and surface area (Fig. 5b and d) have opposite effects on community structure. This observation is evidenced by the results from the correlation analysis (Fig. 3): high surface area coincides with low haptera.cm^{-3}, and is negatively correlated to all the other explanatory variables too.

The nMDS plots yielded stress values between 0.08 and 0.13, indicating the data were well represented by the two-dimensional plots and that reliable interpretations could be made from them (Clarke, 1993).

4. Discussion

Using indices of complexity that had never been applied in this context previously, the relationship between habitat complexity and marine biodiversity was demonstrated while investigating structural features of *Laminaria hyperborea* holdfasts. This study also demonstrated the great potential of CT-scanning as a tool for ecological studies concerned with habitat complexity, particularly in the study of kelp systems. The 3D imaging post-processing techniques described here represent a first approach to examine a quantitative relationship between faunal diversity and kelp holdfast 3D complexity. In particular, the surface area of the kelp holdfasts could be easily calculated – a measurement otherwise extremely difficult to obtain – and rarer indices of complexity like fractal dimensions (volume or surface) and spatial autocorrelation indices could be computed. Albeit frequently overlooked in ecological studies, partial least squares regression (PLSR) analysis were shown to be an appropriate and promising statistical tool for spatial analyses like this one, which often require using numerous, correlated variables (Rossi and Van Halder, 2010) – in this case, by enabling the identification of those spatial attributes that were most consistently linked to diversity attributes. The PLSR analyses showed that volume explained the majority of the variance structure observed in all the fauna biodiversity indices. Community structure was, however, best explained by surface area and haptera per unit volume of the holdfast.

The fact that volume emerged as the most important explanatory variable agreed with previous studies (Jones, 1973; Ojeda and Santelices, 1984; Smith et al., 1996; Blight and Thompson 2008; Torres et al., 2015). Volume constitutes a good measure of the amount of living space available for organisms; an increase in volume implies there are more niches to colonize, and is likely to reflect also an increase in resources. Rather than indicating how much living space is available, surface area reveals how much attachment space is provided for individuals and haptera per unit volume represents

the amount of inter-haptera space created by the structure. These measures are therefore good indices of complexity as they account for the multiplicity of microhabitats created by the branching of the haptera, and these indices were expected to be important drivers of community structure. Our multivariate analysis revealed this to be true, with different faunal assemblages responding differently to varying levels of complexity. The fact that less abundant species were more responsive to the effect of surface area and haptera.cm^{-3} than dominant ones could have been expected because these species will benefit the most from increases in attachment space complexity, whilst dominant species will occupy the majority of the attachment space available.

The strong correlation observed between kelp age and volume, surface area, haptera number and haptera.cm^{-3} underlines the importance of determining the age structure of kelp forests in order to define adequate conservation measures in this type of habitat. Indeed, since knowing the age of kelp can help estimate its level of complexity, which itself has been shown in the current study to be linked to biodiversity levels, age should be a determinant factor when restoring previously damaged kelp beds, and when electing which beds to prioritize for management or fishing (Bell et al., 1991). Additionally, as volume and surface area were negatively correlated to haptera per unit volume, we hypothesized that as the kelps grows, it adds volume and surface more quickly than it adds new haptera at the stipe. This would suggest that the complexity of the holdfast arises from the multiplicity of haptera emerging from extant haptera, rather than from the addition of new haptera at the stipe.

The composition of the faunal communities recorded here is similar to that reported by Moore (1973), in which Syllidae, Nereididae, Terebellidae, Corophiidae and Ischyroceridae were identified as the families with the highest abundance in *Laminaria hyperborea* holdfasts. The holdfast is an ideal environment for amphipods which feed on sediment particles rich in organic matter and for gastropods that graze on the algae or filter-feed passively (Moore, 1973). Numerous juvenile individuals, mainly from the Nereididae family and the Mollusca, as well as juvenile crabs and ophiuroids, were identified in the holdfasts. Kelp holdfasts thus seem to provide ideal nursing grounds for benthic organisms. This is not surprising given that holdfasts offer physical protection from waves and predators, and trap sediment rich in nutrients (Dean and Connell, 1987; Steneck et al., 2002). Similarly to the findings of Smith et al. (1996) on *Ecklonia radiata* holdfasts, the apparent increased abundance of serpulids (e.g.

Spirobranchus species) and barnacles in holdfasts of higher complexity may further facilitate increase in other species (e.g. syllids), which are able to colonize empty calcareous tubes provided by these organisms.

Further investigation of the links between taxonomic groups, dispersal modes and feeding strategies with complexity of the holdfast might shed light on the dynamics of colonization and community succession in this habitat (Smith et al., 1996). A number of holdfasts investigated here exhibited particularly high abundance of certain taxa – for instance, hundreds of juvenile nereids but much fewer other species of polychaetes. This finding may be indicative of founder effects, by which the multiplication and predominance of certain species of early colonizers is facilitated. Since a large number of species were found in both simple and complex holdfasts, yet more complex holdfasts hosted a larger number of species, it is reasonable to suggest that succession occurred through the addition of species over time rather than species replacement, as suggested by Smith et al. (1996) and Ojeda and Santelices (1984).

The CT-scanning data significantly improved our ability to quantify habitat complexity in this study, and to predict changes in biodiversity with spatial heterogeneity. The usefulness of CT data was clearly maximized by the computation of otherwise unavailable, or very hardly achievable measurements. Indeed, surface area and the fractal dimension of the surface are both strongly positively correlated to species richness, abundance and diversity, and together contribute to about 20% of the variance structure observed in the response variables. Both spatial autocorrelation coefficients showed high positive autocorrelation within holdfasts and very little variance between holdfasts of differing size, suggesting the existence of a “typical” holdfasts structure, that was fairly homogenous between individuals, with low variation at a global and local level. Unfortunately, these values could not provide explanation towards the biological diversity investigated, and are possible not promising avenues for future studies with aims similar to ours. Conversely, fractal dimensions gave biologically meaningful information. By providing an interpretation of habitat size in relation to the scale of measurement, these measures have also been found to be good predictors of body size in fauna assemblages in other studies (Gee and Warwick, 1994a,b). While larger volumes may provide more occasions to find resources and partners to mate with, more fractal surfaces suggest more

opportunities to avoid stress and predators, through increased intricacy and hiding areas. The fact that the biodiversity hereby responded to the surface fractal dimension of the holdfasts suggests that this variable has ecological implications in benthic communities. Additionally, a study on coralline algal turf gastropod assemblages found that there may exist an upper threshold of habitat complexity beyond which species diversity does not increase (Kelaher, 2003). Reduced interstitial space associated with architecturally more complex habitats, leading to decreased amount of light, food and space available for organisms, may be particularly important for larger sized fauna, and there may be an optimal level between the provision of attachment space for fauna, and the complexity of that habitat space.

A future avenue that deserves attention should be to fully develop the potential of data extracted from CT-scanning. A method that could provide additional insightful information in terms of faunal diversity appears to be the one described by Mazik et al. (2008). In their study of scanned sediment cores, they extracted information on the burrow volumes and surface areas, as well as on the mean burrow diameter, mean number of burrows and mean density. It would be interesting to obtain similar data on the 3D geometry of inter-haptera space, as this would provide a robust measure of the properties of the holdfast and of the amount of living space available for the fauna (i.e. volumes of the spaces created by the structure, mean number of spaces and their dimensions which relate to the probability of having a certain number of spaces of a certain size). Such parameters could provide another set of indices of complexity, and could be linked more directly to body size distribution of the fauna, offering considerably more information than that available without scanning. Marwan et al. (2009; 2012) investigated structural complexity using CT-scanning in a medical context and developed a method of differentiating shapes with the same volume but different surface areas. In the present study, quantifying the shapes of the inter-haptera spaces, as well as the variety of these shapes in relation to the volume, could supplement the analysis well, by providing data relatable to the morphological diversity of the faunal samples.

Despite explaining up to 81% of the variance structure observed, the complexity measures investigated did not fully explain the variance structure observed in the biodiversity indices, with lowest correspondence between complexity and community evenness. This finding would suggest that

dominance of holdfast communities is not related to holdfast structural complexity. Future work could also investigate measurements of complexity in the faunal community that were not used here. For instance, recording the biomass and size of the faunal samples should provide precious information on the type of fauna expected to be found in habitats of a certain complexity, as suggested by Warwick and Clarke (1984), who showed that specific traits in animals, and more precisely in benthic fauna, can be favoured according to their body size. The presence of epiphytes on the stipe of the kelp was not recorded but numerous studies have previously shown it had a negligible effect on the faunal community in the holdfast (Dean and Connell, 1987; Norderhaug, 2002; Hauser et al., 2006).

Interestingly, encrusting organisms were easily identified on the scanned images. Indeed, due to the distinct density of fauna shells and skeletons, one could localize organisms like echinoderms and sessile mollusks on the holdfast scans alone. Such data could prove to be particularly interesting in the frame of ocean acidification, a chemical process by which the reduction in pH and consequent decrease in carbonate ions strongly diminishes the ability for organisms to sustain carbonated skeletons (Fabry et al., 2008). Recent research has already began to make use of this approach (Queirós et al., 2015), and the present study shows that this could be extended to kelp holdfast communities.

In conclusion, there are many promising extensions for the techniques and approaches presented here. Understanding community structure in keystone habitats such as kelp forests will be critical when trying to preserve and restore ecosystems and their associated ecological processes (Steneck et al., 2002). Through the modulation of resources and their strong interactions with faunal communities, the loss of ecosystem engineers like kelp, can have cascading effects on biodiversity (Coleman and Williams, 2002; Hastings et al., 2007). By reducing the amount of time that would otherwise be needed to manually slice kelp holdfasts, and by providing information of much higher resolution, CT-scanning offers a rapid and powerful technique for faunal community monitoring (Mazik et al., 2008) and a suitable way to assess anthropogenic impacts on macrophyte systems. In particular, it allows detailed visualization of complex systems in their natural state and with minimal disturbance (Dufour et al., 2005), an impossible task when using artificial structures as proxies of holdfast complexity. Indeed, it makes it feasible to study kelp populations that have matured over time, which is

particularly meaningful in perennial species. It also accounts for the chemicals released by the kelp, some of which are responsible for the growth of biofilm which facilitate faunal attachment (Hellio et al., 2000), and others which influence the nutritional value of the kelp (Norderhaug et al., 2003; Toth et al., 2005). Importantly, it is the only way to estimate the holdfast surface area precisely. Therefore, CT-scanning provides highly valuable information, and emerges as a unique tool to analyze habitat complexity. Further research could focus on the use of higher resolution devices, improving the extant methods of calculating complexity and developing new ones. The scanning procedure developed here will undeniably prove useful in future ecological research, by providing valuable data for conservation purposes, though the identification of structurally complex communities as biodiversity hotspots, which can help to support efficient design of marine protected areas. Appreciating how the spatial distribution of organisms relates to structural complexity of habitats has great relevance in ecological modeling and can support the projection of the potential consequences of environmental change for the benthos (Tilman, 1994).

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Table 1 Summary of data, including holdfast number, age, volume, surface area, haptera number, indices of complexity (haptera.cm^{-3}, fractal dimension,) and biodiversity indices (S – number of species, N – abundance, d – Margalef's species richness, H' – Shannon-Wienner's species evenness and richness, J – Pielou's species evenness).

Holdfast	Age (years)	Surface (cm^2)	Volume (cm^3)	Number of haptera	Haptera per cm^3	Fractal dimension of surface	S	N	d	J	H'
1	2	25.99	10.01	18	1.80	1.89	13	116	2.52	0.77	1.98
2	4	90.81	32.94	21	0.64	1.93	22	125	4.35	0.73	2.25
3	4	43.84	16.44	20	1.22	2.01	22	74	4.88	0.78	2.42
4	5	99.22	18.76	22	1.17	1.69	20	113	4.02	0.79	2.36
5	7	224.23	105.82	22	0.21	1.98	43	240	7.66	0.74	2.76
6	7	104.46	35.36	26	0.74	1.92	27	184	4.99	0.65	2.15
7	4	108.09	48.27	24	0.50	1.96	31	706	4.57	0.21	0.72
8	7	252.42	118.89	32	0.27	1.94	54	275	9.44	0.83	3.33
9	5	95.11	41.81	22	0.53	2.05	18	51	4.32	0.86	2.48
10	6	473.39	201.10	39	0.19	2.04	48	269	8.40	0.79	3.05
11	6	322.49	110.97	30	0.27	2.08	49	437	7.90	0.78	3.04
12	5	256.32	98.72	22	0.22	2.02	62	734	9.25	0.62	2.54
13	5	344.40	132.46	32	0.24	2.08	67	1082	9.45	0.53	2.23
14	5	196.18	72.91	28	0.38	2.02	45	543	6.99	0.61	2.32
15	1	22.60	11.41	11	0.96	1.84	24	74	5.34	0.85	2.70
16	5	329.36	137.78	24	0.17	2.02	53	437	8.55	0.76	2.30
17	1	58.32	5.20	12	2.31	1.72	19	53	4.53	0.90	2.65

Table 2 Percentage of the variance of each response variable explained by each partial least squares analyses component (numbers) given with the associated explanatory variable (volume, haptera number, age, haptera.cm^{-3}, surface area and the fractal dimension of the surface) which had the highest loading for that component.

	S	**N**	**d**	**H'**	**J**
Volume	68.52	38.51	69.34	26.14	20.95
Haptera Number	0.34	4.79	N/A	4.03	5.08
Age	7.81	7.96	9.91	1.13	N/A
Haptera.cm^{-3}	N/A	N/A	1.92	2.28	2.64
Surface area	0.94	N/A	N/A	N/A	N/A
FD Surface	1.36	5.83	0.02	17.18	5.58
Variance explained	78.97	58.55	81.19	50.76	37.17

Fig. 1 3D reconstruction of a scanned kelp using OsiriX (using a blood-vessel and bone mask to aid visualization - red-brown: kelp; white-gray: calcifying fauna). A) Lateral view of the holdfast. B) View of the base of the holdfast where calcifying fauna can be seen in white, exhibiting densities similar to human bone.

Fig. 2 A) One of the 141 slices constituting the outcome of the CT-scanning of an individual kelp holdfast. B) The similar slice following the cropping of the stipe and bucket after setting a threshold θ. Only the holdfast remains. Non-black voxels were considered as the foreground; black voxels were considered as background and therefore excluded from the surface and fractal dimension calculations.

Fig. 3 Correlations between the variables used in the statistical analysis (both explanatory and response variables). The pie charts depict the strength of the correlations between variables, solid pies indicating a positive correlation and striped pies indicating a negative correlation.

Fig. 4 Loading plots showing the loading value of each variable for the first three main components (values written inside each graph, see table 2 for the percentage contribution of each component). Variables: 1 = surface area, 2 = FD of surface, 3 = age, 4 = number of haptera, 5 = volume, 6 = haptera.cm^{-3}.

Fig. 5 Two-dimensional nMDS ordination (Bray-Curtis similarity index) on holdfast assemblages (N=17), after transformation (A and B) and before transformation (C and D), on haptera per unit volume (A and C) and on surface area (B and D).

Figure 1

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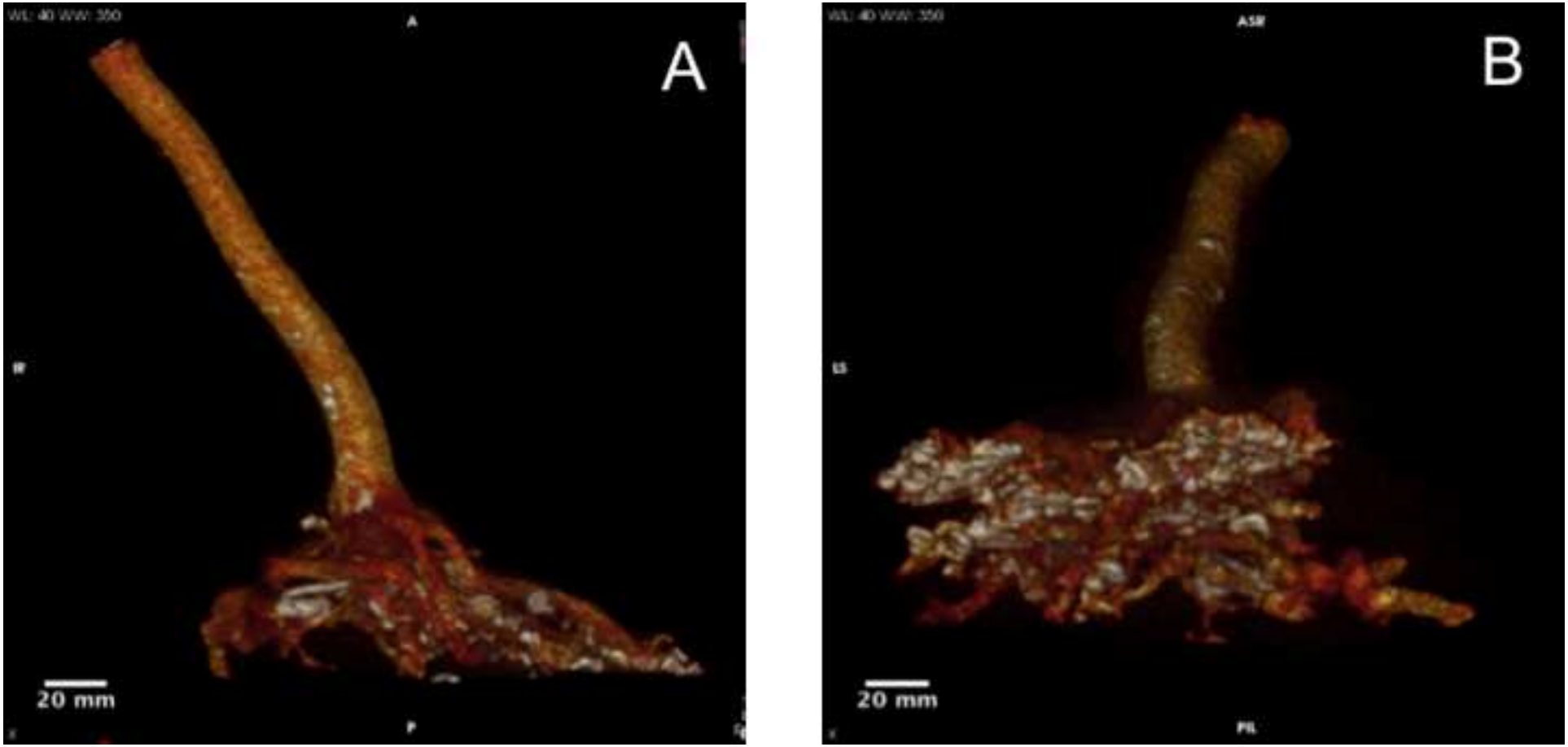

Figure 2

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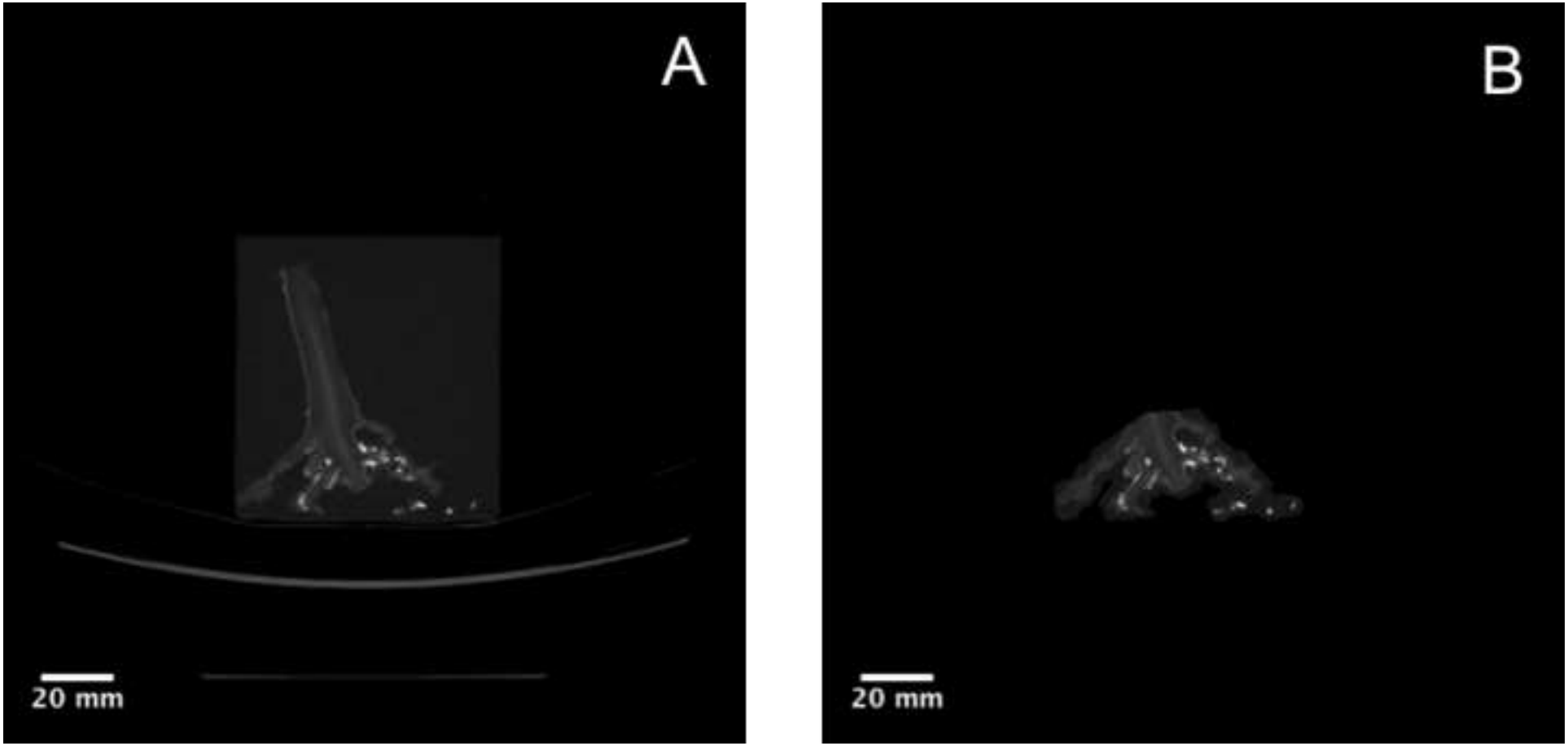

Figure 3

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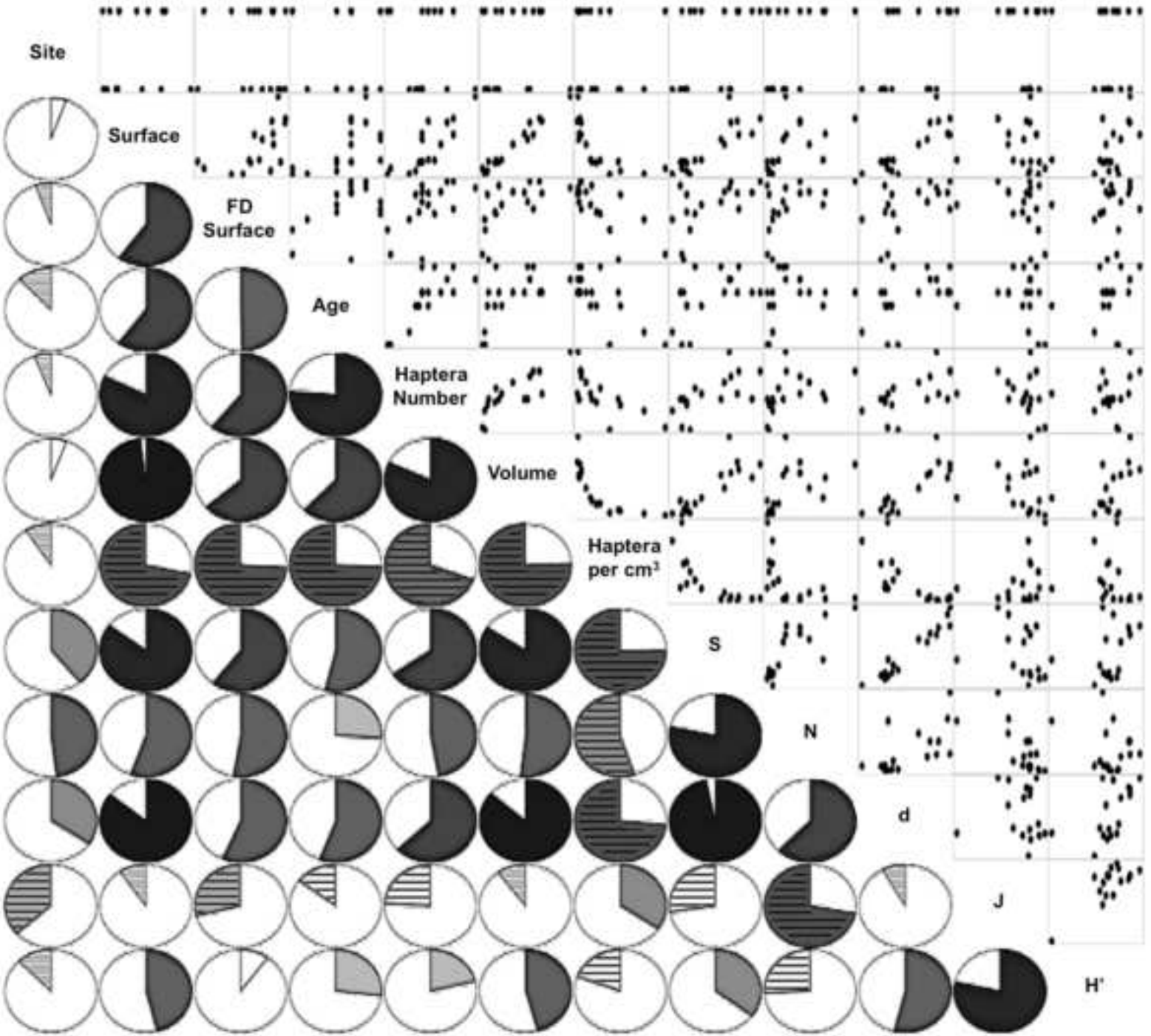

Figure 4

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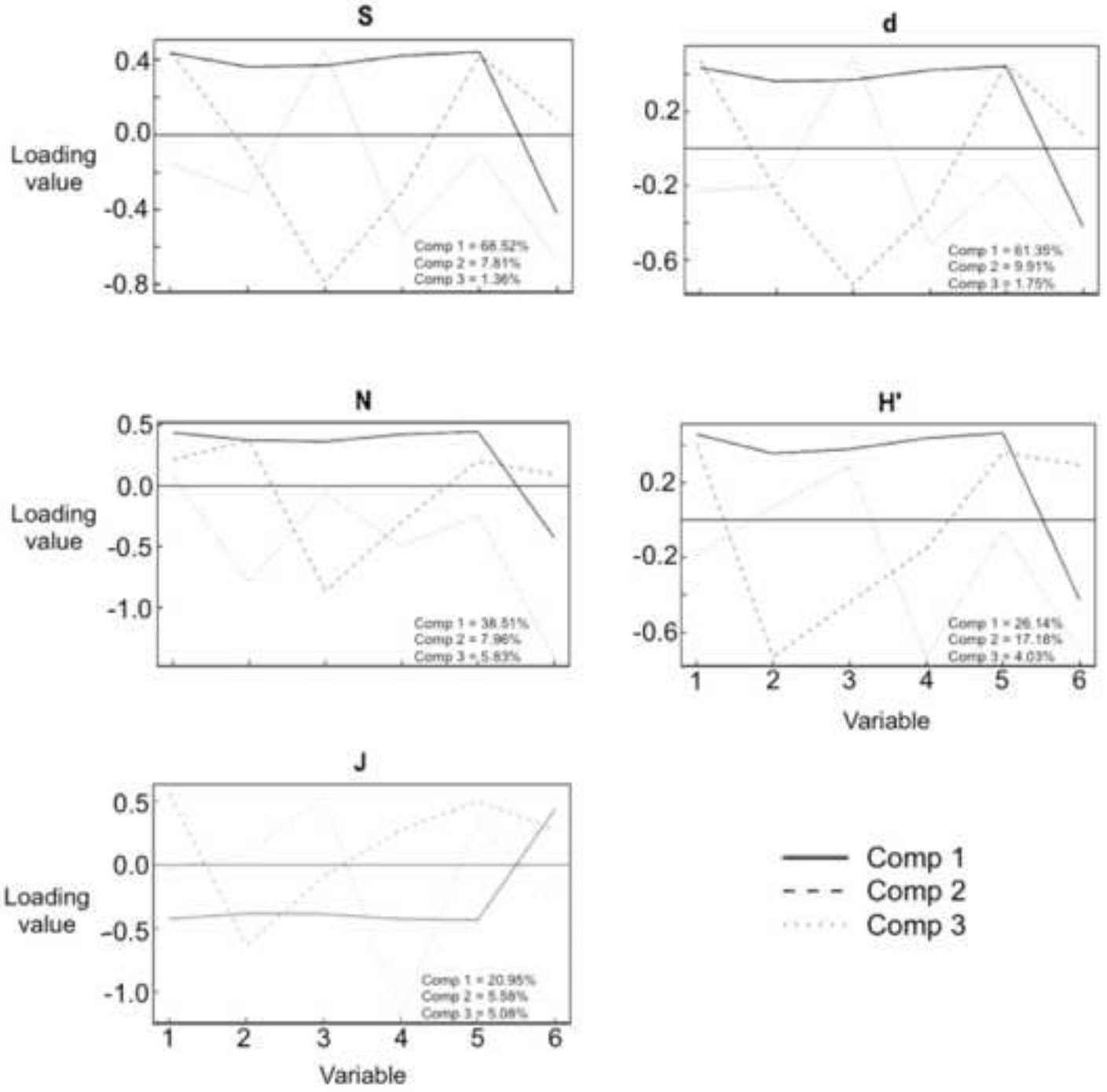

Figure 5

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