Marine Environmental Research 182 (2022) 105772

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Could the annual *Saccorhiza polyschides* replace a sympatric perennial kelp (*Laminaria ochroleuca*) when it comes to supporting the holdfast-associated fauna?

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ARTICLE INFO

Keywords:
Holdfast
Kelp decline
Laminaria ochroleuca
Saccorhiza polyschides
Surrogate
Epifauna

ABSTRACT

Kelp forests dominate temperate rocky shores worldwide but are declining globally with consequences for organisms that depend on them. In NW Iberia, the golden kelp (*Laminaria ochroleuca*) commonly occurs alongside a fast-growing annual that, unlike the golden kelp, does not seem to have receded in recent times (*Saccorhiza polyschides*). Here, we assessed whether the bulbous holdfast of *S. polyschides* could replace the intricate holdfast of *L. ochroleuca* as epifaunal habitat provider. Richness, diversity and total abundance of epifauna was similar in both seaweeds, while colonial/encrusting fauna was more abundant in *L. ochroleuca*. More importantly, each host supported a distinctive assemblage structure, indicating that *S. polyschides* seems an unsuitable replacement for *L. ochroleuca* as habitat provider for holdfast epifauna. Therefore, while *S. polyschides* may contribute substantially to the kelp forest canopy in some seasons, a regional decline of *L. ochroleuca* will likely alter the patterns of biodiversity within kelp stands.

1. Introduction

Kelp forests dominate shallow rocky shores in temperate and subpolar latitudes worldwide, being found along 25% of the world's coastlines (Krumhansl et al., 2016; Smale, 2020; Steneck et al., 2002). Widely regarded as one of the most productive ecosystems in the world, most of the production of this biogenic habitat enters the food chain as detritus that may end up in the deep ocean, thus contributing to carbon storage (Krumhansl and Scheibling, 2012; Wernberg et al., 2019). They also support high levels of biodiversity because kelps are exemplary ecosystem engineers that create habitat for other species by modifying the environment (Dayton, 1985) and by providing new biogenic substrata for colonizing organisms (Teagle et al., 2017). The latter is particularly relevant in terms of biodiversity. Unlike many other seaweeds, kelps are structurally complex and provide three distinct microhabitats (stipe, blade, holdfast) which differ considerably in their morphology and in the composition of their associated assemblages (Blight and Thompson, 2008; Teagle et al., 2017). Among the three biogenic microhabitats, holdfasts have received most attention with several studies concluding that they support the greatest biodiversity (Schoenrock et al., 2021; Teagle et al., 2017, 2018), possibly because holdfasts not only increase the amount of habitable space but also provide greater shelter from predators and adverse environmental conditions (Orland et al., 2016; Velasco-Charpentier et al., 2021).

Holdfast assemblages include both mobile (copepods, polychaetes, gastropods, amphipods, echinoderms) and sessile (bryozoans, bivalves, sponges) fauna. Their composition responds to factors such as local environmental conditions (e.g. wave exposure, turbidity, pollution), propagule supply from surrounding areas, seasonality and even holdfasts ageing (Akita et al., 2019; Ojeda and Santelices, 1984; Ríos et al., 2007; Salland and Smale, 2021; Teagle et al., 2017; Winkler et al., 2017). Despite this variability, some studies have reported consistencies across large spatial scales in the structure and richness of holdfast assemblages in a given kelp species (Anderson et al., 2005), although this may not apply to kelps whose range spans over more than one biogeographic region (Ojeda and Santelices, 1984). Beyond contributing to local biodiversity, the holdfast fauna can also be an important food source for other inhabitants of the kelp forest and nearby habitats. In particular, highly mobile components such as amphipods, isopods and gastropods have been shown to move through kelp forest in high numbers, preferably by crawling close to the bottom (Jørgensen and Christie, 2003; Moreno and Jara, 1984; Norderhaug et al., 2005).

Global change is redistributing species and communities on a global scale. This includes kelp forests which, regional variability aside, have

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https://doi.org/10.1016/j.marenvres.2022.105772
Received 13 May 2022; Received in revised form 20 September 2022; Accepted 9 October 2022

globally declined ~2% per year due to a combination of direct and indirect impacts on kelp performance (Krumhansl et al., 2016; Smale, 2020; Steneck et al., 2002; Wernberg et al., 2019). International organizations such as the OSPAR Convention recently added kelp forests to its list of threatened and/or declining habitats, recommending its contracting parties to take measures to further their protection and conservation (OSPAR Commision, 2021). In doing so, OSPAR adopted the increasingly accepted practice of not restricting the definition of kelp forests to members of the order Laminariales (sometimes known as "true" kelps). Instead, OSPAR broadened it to include dense stands of other large canopy-forming brown algae that perform similar functions (Bolton, 2016; Fraser, 2012). Therefore, OSPAR also listed forest habitat dominated by a species of order Tilopteridales (*Saccorhiza polyschides* (Lightfoot) Batters 1902) (de Bettignies et al., 2021; OSPAR Commision, 2021). Interestingly, while the rest of OSPAR list are all multi-year perennial "true" kelps, *S. polyschides* is an annual or pseudo-annual whose macroscopic sporophyte lasts only 12–18 months depending on latitude (Ardré, 1971; Norton and Burrows, 1969).

Designating habitats as biological units under protection has obvious benefits, especially when they support high levels of biodiversity, as in the case of kelp forests. Nevertheless, these benefits can be compromised when more than one species provides the designated habitat if these species respond differently to human impact and environmental managers/regulators assume that the less sensitive habitat builders may take over the role of the more sensitive ones with little disturbance to the rest of the community. Recent evidence suggests that the opposite may be more likely for kelp forests, even when closely related and structurally similar kelps are involved. For example, the substitution of a cold-temperate kelp (*Laminaria hyperborea* (Gunnerus) Foslie 1885) by a warm-temperate one (*Laminaria ochroleuca* Bachelot Pylaie, 1824) have been shown to shift core ecological functions such as the cycling of organic matter (Pessarrodona et al., 2019), or the ability of kelp canopies to withstand storm disturbances (Smale and Vance, 2016). Similarly, the replacement of the cold-temperate kelp by the warm-temperate one has led to a loss of stipe-associated epiphytic algae and mobile invertebrates, therefore affecting local food webs (Smale et al., 2022). Conceivably, the same also applies to other equally important functions, such as habitat provision. Holdfast assemblages, for example, are sensitive to holdfast complexity and size, two attributes that sometimes vary considerably among species. Studies that investigated the interspecific variability in holdfast assemblage structure frequently found differences, although there are exceptions that suggest that the biogeographic context may also be important (McKenzie and Moore, 1981; Norton, 1971; Teagle et al., 2017; Teagle and Smale, 2018; Tuya et al., 2011).

Here, we investigated whether the "pseudo-kelp" *S. polyschides* might be a suitable replacement for a true kelp (*L. ochroleuca*) as habitat provider. The ranges of *L. ochroleuca* and *S. polyschides* overlap from Morocco to the south-west of the British Isles, although *S. polyschides* tolerates cooler conditions and extends poleward to Norway and the Faeroes (Dangeard, 1949; Miranda, 1931; Norton and Burrows, 1969; Seoane-Camba, 1957). In the last decades, several independent studies reported declines and range contractions of these two brown seaweeds at their warm range edge in the Iberian Peninsula. The westward retreat along the north coast of Iberia (Díez et al., 2012; Fernández, 2011; Voerman et al., 2013) together with population declines in southern Portugal (Assis et al., 2013) suggested that northwestern Iberia might be a potential climatic refugium from further warming, and model projections based on climatic predictors supported this notion (Assis et al., 2018). However, the recent discovery that *L. ochroleuca* forests are declining in northwestern Iberia due to excessive fish herbivory highlights the limitations of projections based solely on climatic predictors and suggests that northwestern Iberia might not be the safe haven for true kelps (Barrientos et al., 2022a, 2022b). Instead, *L. ochroleuca* might be gradually replaced by *S. polyschides* as there is no evidence that the latter may be decreasing in the region and this fast-growing brown seaweed is an opportunistic pioneer that can rapidly colonize disturbed areas. Our study focused on holdfast assemblages, and we anticipated that the transient nature of *S. polyschides* holdfasts, together with their distinct morphology (bulbous and hollow, while laminarian holdfasts are typically intricate) would likely result in a markedly different assemblage composition from that of *L. ochroleuca* holdfasts.

2. Materials and methods

2.1. Sample collection and processing

Holdfast samples were collected from eight sites in a large ria (drowned river valley) in northwestern Iberia (Ría de Vigo). Four sites were within the limits of the Atlantic Islands National Park, while the other four are located close to the park limits, both on the northern and southern shores of the ria (Fig. 1). These sites were selected based on previous knowledge of the conservation status of kelp forests in the area and had similar conditions in terms of depth (8 m on average), wave exposure (semi-sheltered), substrate (rocky or rock with sand), temperature (13–17 °C depending on season), and salinity (29–36 S/m). *S. polyschides* and *L. ochroleuca* occur intermingled at all eight sites. Two other kelps of the order Laminariales are occasionally found in some of the study sites but they always occur in very low abundances, the native *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders 2006 and the introduced *Undaria pinnatifida* (Harvey) Suringar 1873. Tall canopy-forming sporophytes of *S. polyschides* are common both inside and outside the park. However, mature canopy-forming sporophytes of *L. ochroleuca* only occur outside the park as *L. ochroleuca* populations inside the park have been reduced in recent years to small young or newly recruited plants by excessive fish herbivory (Barrientos et al., 2022a, 2022b). To capture the variability of host-specific holdfast assemblages in the study area, 18 individuals of *S. polyschides* and 18 of *L. ochroleuca* covering a wide range of holdfast sizes were haphazardly sampled across the eight sites in summer 2021 by scuba divers. After cutting off the plant stipe at its base, each holdfast was carefully removed with the help of a dive knife, covered with a fine mesh nylon bag, and the bag sealed underwater with a drawstring to retain all attached organisms. Samples were immediately returned to the laboratory where they were preserved in 96% ethanol until analysis.

All organisms were identified to the finest level of taxonomic resolution possible. The abundance of individual fauna was counted and recorded, while encrusting or colonial fauna was given an ordinal score from 0 to 3 according to their relative coverage on the holdfast as in Anderson et al. (2005). Holdfast volume was determined using water displacement. The entire holdfast was vacuum packaged in a vacuum sealer bag before being submerged in freshwater to measure the total volume of the holdfast. The holdfast was then removed from the bag and submerged again in freshwater to measure the volume of solid holdfast tissue. The latter was subsequently subtracted from the total holdfast volume to give the volume of potentially habitable space (interstices volume). Each measurement was repeated three times, using the average as the final estimate.

2.2. Statistical analysis

The effect of the volume of holdfast interstices (*Vh*, fixed continuous factor) on the diversity and abundance of the epifaunal assemblages in each host seaweed (*Host,* fixed categorical factor with two levels: *L. ochroleuca* and *S. polyschides*) was assessed by fitting generalized linear mixed-effects models (GLMM) with package "glmmTMB" of R 4.1.2 software with the help of the RStudio interface (R Core Team, 2021). Several error distributions and models with and without sampling site (*Site*) as a random effect were fitted and the best-fit model determined by the Akaike Information Criterion (AIC). The best-fit model was validated by plotting residuals *vs*. fitted values, *vs*. each covariate, and with simulation-based approaches to test usual adjustment

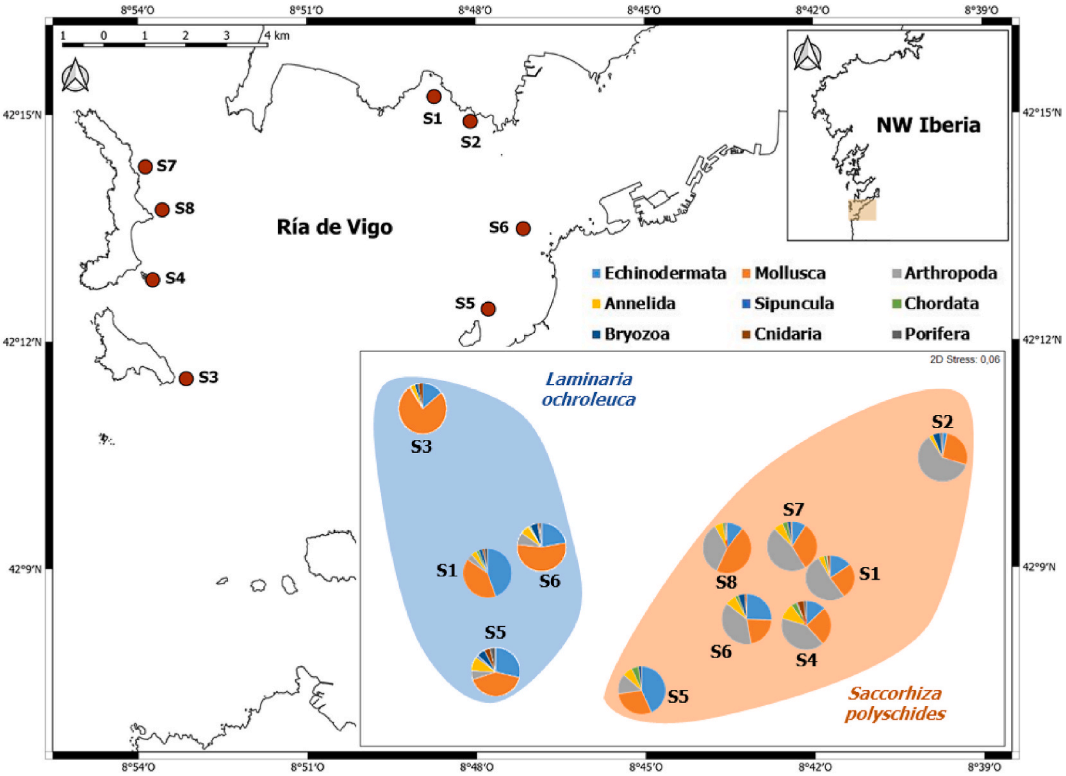

Fig. 1. Map showing the location of the eight kelp reefs in Ría de Vigo, NW Iberia, used in the study. **Inset:** two-dimensional non-metric multidimensional scaling (nMDS) showing dissimilarities (Bray-Curtis for square-root transformed relative abundance of phyla) between epifaunal assemblages in *L. ochroleuca* and *S. polyschides*. Each pie chart is the centroid of several holdfasts collected at a single sampling site.

errors in mixed models available in "DHARMa" package. Wald X^2 statistics were calculated with package "car" to test for the significance of the fixed factors and their interaction. Interpretation of the best-fit model was done with the help of packages "effects" and "emmeans" to estimate the influence of each level of factor *Host*, taking into account the effect of *Vh*.

The structure of the holdfast assemblage in each host was compared using permutational multivariate analysis of variance (PERMANOVA) with seaweed *Host* as fixed factor, sampling *Site* as random factor nested in *Host*, and *Vh* as a covariate to account for any potential influence of holdfast volume on the faunal composition. Since PERMANOVA is sensitive to differences in multivariate dispersions, the homogeneity of multivariate dispersions between hosts was tested with a permutational procedure based on distances to group centroids (PERMDISP). Principal coordinate analysis (PCO) was used to elucidate multivariate patterns in the assemblages, with vector overlays added to the PCO plot to visualize potential relations between the ordination axes and taxa whose standardized abundances had a Pearson correlation >0.5 with any PCO axis. The above multivariate analyses were done on the basis of a Bray-Curtis dissimilarity matrix calculated from square-root transformed relative abundance data (species abundances in each sample standardized by total abundance in that sample). These analyses were subsequently repeated with (i) species grouped by phylum and (ii) abundances transformed to presence/absence (P/A) data to facilitate comparison with previous studies using lower taxonomic or numerical resolutions (Bray-Curtis dissimilarities calculated on P/A data are equivalent to Sørensen distances). Relative species abundances were also used to calculate the contribution of each taxon to the average Bray-Curtis dissimilarity between *L. ochroleuca* and *S. polyschides* holdfast assemblages. Finally, phylum abundance data was used to calculate the distance among the centroids for each combination of host x sampling site, and the resulting resemblance matrix was visualized with non-metric multidimensional scaling (nMDS). All multivariate analyses were done using the PERMANOVA + add-on for PRIMER v7 software (for detailed references on the methods see Anderson et al., 2008; Clarke and Gorley, 2015).

To examine the possible effect of bivalves and/or colonial/encrusting fauna as potential secondary habitat formers facilitating other mobile invertebrates, we used a distance-based linear models (DISTLM) to quantify the relative contribution of their abundances to the composition of epifauna assemblages (individual taxa only, bivalves excluded). The volume of holdfast interstices (*Vh)* was also included in these models. Prior to DISTLM, the distribution of these three predictor variables and relationships with one and other were examined in PRIMER v7 to verify that they were reasonably evenly distributed and not strongly correlated. For comparative purposes, the whole procedure was repeated replacing the abundance of bivalves by the abundance of other groups equally important in the epifauna but not possessing the attributes of secondary habitat formers (arthropods, echinoderms, annelids).

3. Results

Holdfast volume ranges and medians were similar in both seaweed hosts regardless of the method used to assess size (volume of interstices, volume of holdfast tissue, total volume), even though the interquartile range tended to be wider in *L. ochroleuca* than in *S. polyschides* (Fig. 2). As expected from the markedly different holdfast morphology of each host, the largest holdfasts in terms of total volume and/or volume of solid tissue were those of *S. polyschides*, but the largest volumes of space potentially available for colonization were recorded in *L. ochroleuca*.

From the 36 holdfasts, we identified 104 taxa from 9 phyla. All 9 phyla were found in *L. ochroleuca* while only 8 phyla were detected in *S. polyschides* (no Porifera detected). With samples pooled by seaweed host, *L. ochroleuca* assemblages were equally dominated by Mollusca (mostly Bivalvia) and Echinodermata (mostly Holothuroidea), while all other phyla accounted for only <25% of the total abundance (Fig. 3). On

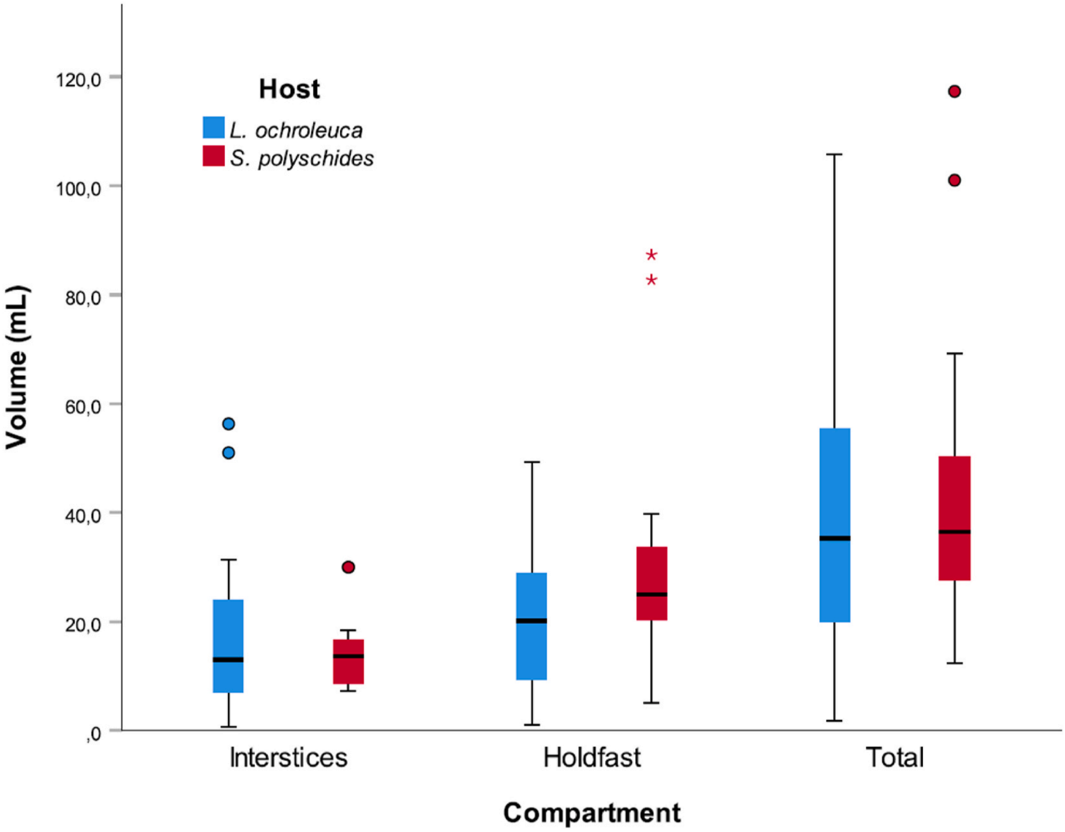

Fig. 2. Volume estimates for holdfast samples of *L. ochroleuca* and *S. polyschides*. "Interstices" is the space potentially available for colonization between haptera (*L. ochroleuca*) or protuberances (*S. polyschides*), "Holdfast" is the volume of solid holdfast tissue, and "Total" is the sum of both. From bottom to top, boxplots show minimum, first quartile, median, third quartile, and maximum. Outside (>1.5 times the interquartile range above or below the box) and far outside (>3 times the interquartile range) values displayed as dots and asterisks, respectively.

the other hand, *S. polyschides* assemblages were clearly dominated by Arthropoda (mostly Amphipoda), with almost 50% of the total abundance, followed in decreasing order by Mollusca (30%) and Echinodermata (11%)Table 1.

The volume of space potentially available for colonization had a significant effect on most of the univariate attributes of the holdfast assemblage, except for Shannon diversity (Table 1 and Fig. S2). This effect appeared to be largely similar in both seaweeds, as the interaction between holdfast volume and host identity was statistically non-significant across all attributes. The epifauna assemblage of both hosts was very similar in terms of total abundance, with average counts per holdfast of 74.9 ± 8.39 in *L. ochroleuca* and 67.2 ± 7.73 in *S. polyschides* (values are marginal means ± SE estimated from the best-fit model in Table 1), taxon richness (17.1 ± 1.00 taxa per holdfast in *L. ochroleuca*, 16.8 ± 0.99 in *S. polyschides*), and Shannon diversity (2.2 ± 0.09 in *L. ochroleuca* and 2.1 ± 0.09 in *S. polyschides*) (Fig. 3b). However, colonial/encrusting fauna were significantly less abundant in *S. polyschides*, with a mean abundance per holdfast that was almost half that in *L. ochroleuca* (4.2 ± 0.50 *vs.* 8.2 ± 0.69). Similarly, the mean number of phyla per holdfast was slightly, but significantly, smaller in *S. polyschides* (5.9 ± 0.24) than in *L. ochroleuca* (7.0 ± 0.23).

Holdfast assemblage composition showed a significant relationship with volume of the holdfast when the data were analyzed at the finest taxonomic and numerical resolution possible (Table 2). Otherwise, the relationship became marginally significant ($P = 0.0539$) when numerical resolution was decreased to presence/absence, and non-significant when taxa were grouped by phylum or the dataset was narrowed to colonial taxa. More importantly, PERMANOVA revealed that the epifaunal assemblages had compositions significantly different between seaweed hosts, regardless of taxonomic and/or numerical resolution of the dataset ($P < 0.001$ for factor Host in every dataset except for the colonial taxa subset where $P = 0.0243$), and despite a significant variability between sampling sites for each host (P for factor Site(Host) ranged from <0.001 to 0.0155 depending on the dataset). PERMDISP tests were consistently non-significant for factor Host, corroborating that the significant variability detected by PERMANOVA was due to differences in assemblage composition rather than to heterogeneity in the multivariate dispersions of holdfast samples for each host.

The first two axes of the PCO ordinations explained 73% of the variation when the assemblage was described at phylum level dropping to 38% for taxon-level data (Fig. 4 and S1). This indicated that the 2-dimensional ordination likely captured the salient patterns of variability in the simpler phylum-level data set, while a substantial portion of the variation went unexplained by the ordination when the assemblage was described with the more informative taxon-level data. Nonetheless, and in agreement with PERMANOVA, it was readily apparent that the identity of the seaweed host was a major source of variation in the composition of the holdfast assemblages (Fig. 4). Regardless of taxonomic/numerical resolution, the first PCO axis was always clearly associated with the separation of *S. polyschides* assemblages from *L. ochroleuca* assemblages, explaining half of the total variation for phylum-level data (52%, Fig. S1a) and about a quarter when the assemblage was described with the more variable taxon-level data (28.2% for taxa abundance data, 25.1% for taxa presence/absence data; Fig. 4 and S1b). No obvious pattern was discernible along the second PCO axis, although the observation that samples collected at the same site tended to cluster along it in some of ordinations (e.g. Fig. 4) suggests that PCO2 might partly reflect the significant inter-site variation revealed by PERMANOVA. Nevertheless, PCO2 explained only one-third to one-half of the percentage of variation explained by the first

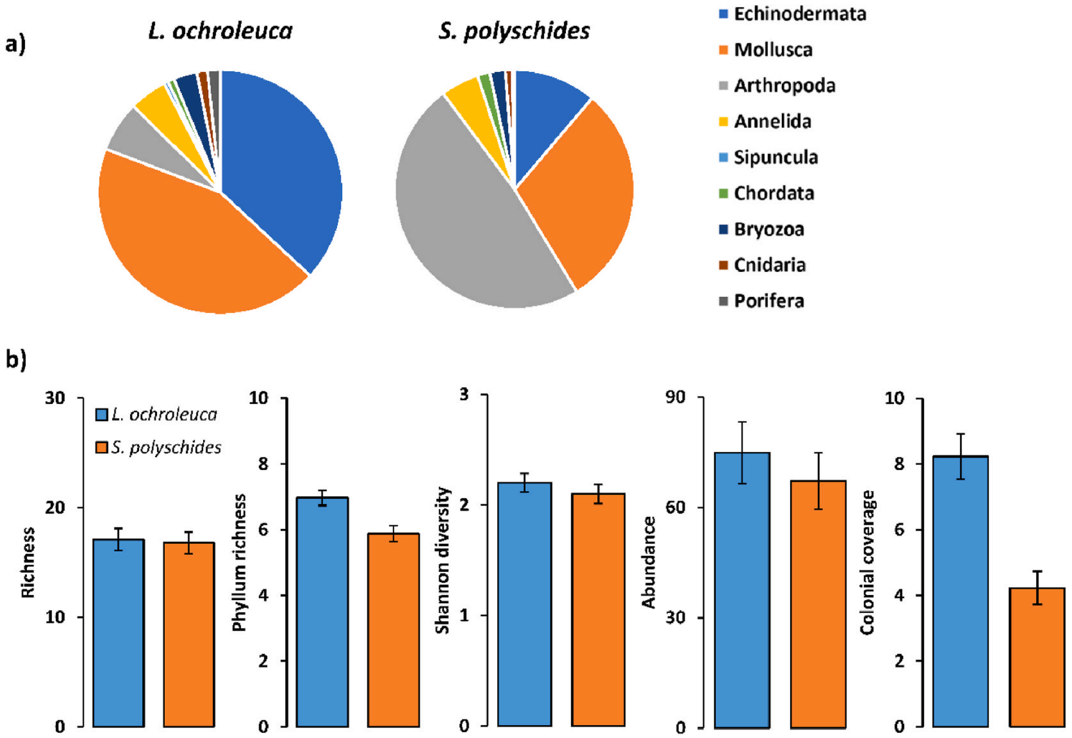

Fig. 3. Epifauna diversity and abundance in *L. ochroleuca* and *S. polyschides* holdfasts. **(a)** Relative abundances of phyla found in the *L. ochroleuca* and *S. polyschides* holdfasts (n = 18 for each seaweed). **(b)** Species richness ("Richness"), phylum richness ("Phylum Richness"), Shannon diversity index ("Shannon diversity"), total abundance of epifauna ("Abundance"), and total coverage of colonial and/or encrusting fauna ("Colonial coverage"). Values are least-squares means (±SE) from a generalized linear mixed model GLMM. A detailed summary of each GLMM can be found in the.

Table 1

Best-fit generalized linear models evaluating the effects of host ("Host"), volume of holdfast interstices ("Vh") and their interaction ("Host * Vh") on several univariate attributes of holdfast assemblages. "Colonial coverage" is the total coverage of colonial/encrusting fauna per holdfast. The effect of sampling site (Site) as a random covariate was also tested but never yielded the best-fit. *** <0.001 ** < 0.01 * <0.05. In bold, P-value < 0.05. AIC = Akaike Information Criterion, χ^2 = chi square of Wald, Log-Likelihood = verisimilitude log.

		Richness		Phylum Richness		Shannon		Abundance		Colonial coverage	
Fixed effects	*df*	χ^2	*P-value*	χ^2	*P-value*	χ^2	*P-value*	χ^2	*P-value*	χ^2	*P-value*
Host	1	0.050	0.8223	9.733	**< 0.001*****	1.186	0.2762	0.242	0.6224	21.364	**< 0.0001*****
Vh	1	19.679	**< 0.0001*****	7.232	**0.0072 ****	1.625	0.2024	17.059	**< 0.0001*****	4.897	**0.0269 ***
Host * Vh	1	0.286	0.5926	1.581	0.2085	3.095	0.0786	2.169	0.1408	0.010	0.9188
Log-Likelihood		−106.3		−50.2		−14.2		−175.7		−79.8	
AIC		220.6		110.4		38.4		361.3		167.6	
Model		Poisson		Gaussian		Gaussian		Negative binomial		Poisson	

Table 2

Results of permutational multivariate analyses of variance (PERMANOVA) and tests of homogeneity of multivariate dispersions (PERMDISP) for differences in holdfast assemblage between *L. ochroleuca* and *S. polyschides* ("Host", fixed). Analyses based on the Bray-Curtis dissimilarity measure for square-root transformed relative abundances of all taxa ("Species"), and for other levels of taxonomic/numeric resolution: taxa grouped by "Phylum", abundances transformed to "Presence/Absence" data, relative abundance (counts) of "Individual taxa", and relative abundance (coverage) of "Colonial taxa". Sampling "Site" treated as a random factor nested in seaweed host; the volume of holdfast available for colonization ("Vh") included as covariate in PERMANOVA. Significance tested with 9999 permutations in all cases. *** <0.001 ** < 0.01 * <0.05. In bold, *P* < 0.05.

PERMANOVA	*df*	Species		Phylum		Presence/Absence		Individual taxa		Colonial taxa	
Source		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Vh	1	1.842	**0.0264 ***	0.490	0.7434	1.777	0.0539	2.029	**0.0197 ***	0.859	0.5269
Host	1	4.735	**<0.0001*****	10.921	**<0.0001*****	4.290	**<0.0001*****	5.195	**<0.0001*****	3.516	**0.0243 ***
Site (Host)	9	2.131	**<0.0001*****	1.827	**0.0155 ***	1.880	**<0.0001*****	2.125	**<0.0001*****	1.768	**0.0068 ****
PERMDISP		**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Host		3.107	0.1913	0.002	0.9529	1.223	0.4140	3.309	0.1830	1.498	0.3335
Site (Host)		7.818	0.2584	2.499	0.5024	4.405	0.5933	8.687	0.1063	8.847	**0.0033 ****

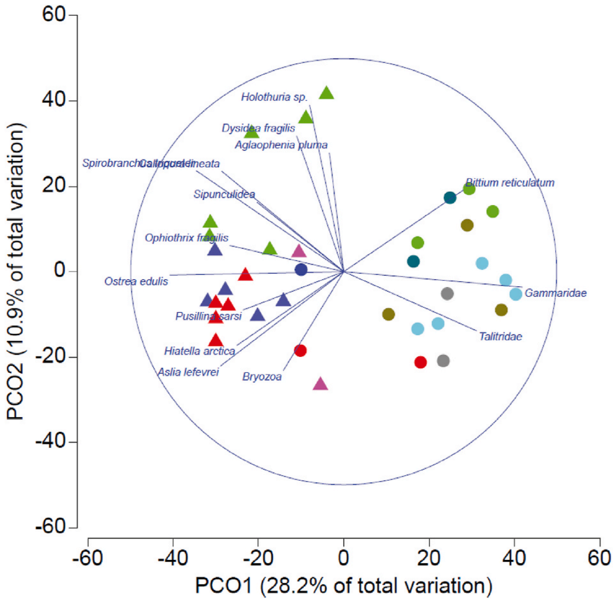

Fig. 4. Principal coordinate analysis (PCO) ordinations comparing holdfast-associated epifauna in L. ochroleuca (triangles) and S. polyschides (circles). Symbols colors identify sampling site. Overlapping vectors show taxa with Pearson correlations >0.5 with any PCO axis. The circle has a radius equivalent to a Person correlation = 1.

axis, indicating that inter-site and inter-sample differences were far less important than host identity as a source of variation.

Four taxa explained 24.8% of the mean dissimilarity between the holdfast epifauna of *L. ochroleuca* and *S. polyschides,* with up to 26 taxa needed to account for 70.6%. The family Gammaridae was particularly distinctive, with a mean relative abundance of 21.1% in *S. polyschides,* but absent from *L. ochroleuca* holdfasts, and thereby explaining 9.5% of the dissimilarity between hosts. Other discriminating taxa tended to be more abundant in *L. ochroleuca*: the bivalve *Ostrea edulis* (6.0% of inter-host dissimilarity; mean relative abundance: 9.7% in *L. ochroleuca,* 0.1% in *S. polyschides*), the sea cucumber *Aslia lefevrei* (5.3% of inter-host dissimilarity; 9.7% in *L. ochroleuca,* 1.3% in *S. polyschides*), and the brittle star *Ophiothrix fragilis* (4.0% of inter-host dissimilarity; 8.6% in *L. ochroleuca,* 2.4% in *S. polyschides*). The association between these and other taxa with the separation of the two hosts was likewise evident in the ordination plot as taxon vectors ran parallel or nearly parallel to PCO1, even though the latter were calculated with an entirely different approach (Fig. 4). Furthermore, the same set of taxa showed a similar association with PCO1 in the ordination based on presence/absence data, indicating that host separation was not only due to the relative abundances of taxa in each host but also but also to the identity of those taxa (Fig. S1b). The fact that the taxa that contributed most to discriminate between hosts belong to different phyla also explains the strong association between Arthropoda and PCO1 in the phylum-based ordination (Fig. S1a). The nMDS calculated for the centroids of the holdfasts collected at each sampling site shows that the discrimination between hosts in terms of the relative abundance of phyla in their assemblages was quite consistent across the study area (Fig. 1). Arthropoda were the least common in *L. ochroleuca* assemblages across all sites, while they dominated *S. polyschides* assemblages almost everywhere. Nevertheless, the substantial inter-site variability detected by PERMANOVA was equally discernible in the nMDS, especially for *S. polyschides*.

The marginal test in the DISTLM analysis report the proportion of the variation in the composition of epifauna assemblages (individual taxa only) explained by each predictor variable, independent of other explanatory variables. The abundance of bivalves and colonial/encrusting fauna explained much more of the total variation (16.1% and 12.7%, respectively) than the volume of holdfast interstices (3.7%) (Table S1). When DISTLM was refitted in combination with a procedure that compares the performance of all possible combination of predictor variables using Corrected Akaike Information Criterion (AICc) for small samples, the model that produced the best fit was the one that used bivalve abundance as the only predictor variable (Table S2). Therefore, the PERMANOVA was repeated for the assemblage of individual taxa with the abundance of bivalves as covariate to test whether taken into account this covariate has a significant impact on the differences in holdfast assemblage between *L. ochroleuca* and *S. polyschides*. PERMANOVA results indicated otherwise, as differences between seaweeds host remained highly significant ($P < 0.0001$, for factor *Host*, Table S3). Rerunning DISTLM for other groups equally important in the epifauna but not possessing the attributes of secondary habitat formers (arthropods, echinoderms, annelids), showed that their abundances explained only half or less than half of the proportion of the variation in the composition of epifauna assemblages (abundance of the contributing group excluded) explained by the abundance of bivalves (Table S4).

4. Discussion

Many sites where *L. ochroleuca* is no longer able to consolidate a canopy forest in northwestern Iberia due to excessive fish herbivory still display a canopy of *S. polyschides* in spring-summer (Barrientos et al., 2022a, 2022b). This complicates the task of conveying the seriousness of the collapse of *L. ochroleuca* to managers/regulators, as the widespread occurrence of *S. polyschides* in the region, along with the rather similar appearance of its mature stands to those of *L. ochroleuca*, masks the decline of the true kelp. Moreover, both *L. ochroleuca* and *S. polyschides* forests are listed as threatened habitats under OSPAR, and there is a risk that managers/regulators assume that they are equivalent. Our results indicate otherwise and, at least in terms of the epifauna of their holdfasts, the pseudo-kelp *S. polyschides* should not be seen as a substitute for the true kelp *L. ochroleuca* as a habitat provider for holdfast epifauna. Although both kelp harbored comparable epifaunal assemblages in terms of richness, diversity and total abundance of taxa per holdfast, their species composition was markedly different. The differences did not arise from small variations in the relative abundance of some species. Instead, they were evident even when the assemblages were described very coarsely (relative abundance of phyla, presence/absence of species), indicating that each kelp supported a distinct epifaunal assemblage. Therefore, our results agreed with earlier studies that noted a contrast between the fauna associated with *S. polyschides* holdfasts and that of other true kelps closely related to *L. ochroleuca* (McKenzie and Moore, 1981; Norton, 1971), as well as with studies that compared another large annual brown seaweed (*Undaria pinnatifida*) to perennial kelps (Arnold et al., 2016; Raffo et al., 2009). On the other hand, while we found no evidence of the loss of species reported in other studies that compared annual hosts to perennial kelps (Arnold et al., 2016; Raffo et al., 2009), the colonial/encrusting fauna was considerably less abundant in the pseudo-kelp. This seems a predictable consequence of the shorter life span of *S. polyschides,* and may partly explain the lower phylum richness of its assemblages.

Despite substantial intra-host variability among sites, the separation between our two kelp hosts was clearly discernible. This is consistent with the notion that holdfast assemblages on a given kelp host exhibit fairly uniform composition even at broad spatial scales, particularly when the taxonomic resolution is decreased to phylum level (Anderson et al., 2005). Large spatial variability in the range of cm to a few km is characteristic of many coastal systems (Anderson et al., 2005; Barrientos et al., 2019; Piñeiro-Corbeira et al., 2018; Underwood and Chapman, 1996), and may be exacerbated by the known responsiveness of holdfast epifauna to local environmental conditions (Ríos et al., 2007; Salland and Smale, 2021; Teagle et al., 2017). In this regard, the broader spatial scale of our study might partly explain why a previous attempt to compare the epifaunal assemblage of the pseudo-kelp to that of another true kelp (*L. hyperborea*) found no significant difference when both kelps were sampled at a single site (Tuya et al., 2011). Although, the fact that Tuya et al. (2011) focused their study to mobile fauna may also be partly responsible for the lack of differences between seaweed hosts. Our results indicate that a gradual shift towards *S. polyschides* dominated stands in northwestern Iberia in coming years could be detrimental for organisms that seem to prefer the longer-lasting and more intricate holdfasts of *L. ochroleuca* (e.g. some bivalves, echinoderms, colonial/encrusting organisms), while favoring others (e.g. gammarids). Large relative abundances of gammarids in *S. polyschides* compared to other kelps have been reported before (Tuya et al., 2011), and might be due to greater food availability in its bulbous holdfast (sediment traped in invaginations on the holdfast surface) and/or to amphipods been better equiped to rapidly colonize a transient substrate. Whatever the cause, their large abundance in *S. polyschides* may have consequences for other kelp forest inhabitants, as amphipods is one of the most mobile and most heavily preyed upon groups in the epifauna (Jørgensen and Christie, 2003; Moreno and Jara, 1984; Norderhaug et al., 2005). Therefore, further work comparing the dispersal of kelp fauna from stands of each kelp host would seem warranted to properly assess the implications of these changes.

The higher abundance of bivalves in *L. ochroleuca* prompted us to test whether they might play a role as secondary habitat formers facilitating the presence of other organisms, as seen in other examples in marine environment (Smale et al., 2022; Thomsen et al., 2018; Gribben et al., 2019). In accordance with this hypothesis, bivalves accounted for a larger portion of the total variability in epifaunal composition than other equally abundant groups, or than holdfast size. Nevertheless, bivalve abundance explained only 16% of the total variation, suggesting a moderate effect at best. Therefore, manipulative experiments controlling for the effect of other variables seem warranted to more adequately test the actual influence of bivalves as secondary habitat formers (Smale et al., 2022; Thomsen et al., 2018; Gribben et al., 2019).

Like other studies of kelp epibiota, our data are limited to organisms living on biogenic substrates. Again, additional work would be needed to quantify their presence in other compartments of the kelp forest habitat to more accurately anticipate the magnitude of the changes caused by an eventual disappearance of *L. ochroleuca* (Arnold et al., 2016). Meanwhile, our study suggests that a replacement of *L. ochroleuca* by *S. polyschides* would alter the biodiversity patterns and the structure of the invertebrate assemblage in kelp forests. Actually, the consequences could be stronger than our study suggests, as the annual character of *S. polyschides,* compared to the perennial *L. ochroleuca,* would imply that the biogenic substrate provided by its holdfasts would only be available for part of the year.

CRediT authorship contribution statement

Cristina Fernández: Formal analysis, Methodology, Writing – original draft. **Cristina Piñeiro-Corbeira:** Conceptualization, Investigation, Sampling. **Sara Barrientos:** Conceptualization, Methodology, Software. **Rodolfo Barreiro:** Lozano, Conceptualization, Supervision, Funding acquisition, Writing – review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research was funded by the “Fundación Biodiversidad y el Ministerio de Transición Ecológica y el Reto Demográfico.” BioCost Research Group acknowledges funding from Xunta de Galicia (grant ED431B 2021/32 of “Axudas para a consolidación e estructuración de unidades de investigación competitivas do SUG” program). SB received financial support from the Xunta de Galicia and the European Union (European Social Found – ESF) (grant ED481A2018/101). CP-C received financial support from Xunta de Galicia “Axudas de apoio á etapa de formación posdoutoral “(grant ED481B-2021/095)”.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2022.105772.

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