



Genus-specific response of kelp photosynthetic pigments to decomposition

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Abstract

Detritus is widely considered to be dead organic matter. However, recent studies have shown that kelp detritus can be functionally equivalent to the attached plant. This is significant, because detritus makes up ~ 42% of all plant biomass. In the Northeast Atlantic, studies on detrital photobiology have been restricted to the genus *Laminaria*. Here, we present data from a 46 d in situ experiment investigating the effect of decomposition on detrital photosynthetic pigment concentrations and stoichiometry in four Northeast Atlantic kelp species from three genera. We corroborate the lack of a decomposition effect on pigments of cold temperate *Laminaria* species as identified by previous studies but show that the photophysiology of other kelp genera responds differently. While *Saccharina latissima* displays an idiosyncratic parabolic response, the annual and thermally plastic kelp *Saccorhiza polyschides* seems to become less pigmented with increasing detrital age. If *S. polyschides* becomes increasingly dominant in future parts of a warmer Northeast Atlantic, this may translate to lower detrital photosynthetic potential at the forest scale. However, given our somewhat ambiguous data on this species, this conjecture requires further investigation into detrital photosynthesis.

Keywords Accessory pigments · Carbon cycle · Chlorophyll · Detached photosynthesis · Fucoxanthin · Marine forest

Introduction

Biomass produced by plants (sensu Bolton 2016) largely turns into detritus. Detrital production is estimated to account for 85% (median, range: 0.44–100%, $n = 293$, data from Cebrián and Lartigue 2004) of primary production. Conservatively considering ~ 17% faster detrital than plant turnover (Cebrián and Lartigue 2004), this means that at any given moment in time 42% (0.37–46%, $n = 293$) of all plant biomass is detritus. This significant fraction was long excluded from trophic ecological theory (Moore et al. 2004), and is still largely overlooked by physiologists on the grounds of its traditional definition. In its broadest sense, plant detritus is any type of organic matter that has

come to be released by exudation, erosion, abscission or dislodgement and, therefore, no longer constitutes (part of) a plant. Traditionally, detritus is more narrowly defined as dead organic matter which, as a logical consequence of its definition as a separate entity, is detached from the living organism (Moore et al. 2004). This definition assumes that the “ontogeny of detritus” (Moore et al. 2004) is initiated by simultaneous release and death and that these processes are thus equivalent. However, these assumptions are not entirely warranted.

There is growing evidence from research on macroalgal decomposition that some plants produce living detritus (e.g., Frontier et al. 2021a; Wright et al. 2022). This is backed by dispersal studies (e.g., Tala et al. 2013; Rothäusler et al. 2018) and has been confirmed using a variety of measures: oxygen production (Frontier et al. 2021a; Wright et al. 2022), chlorophyll fluorescence (e.g., Tala et al. 2013; Rothäusler et al. 2018; Frontier et al. 2021a, b), photosynthetic pigments (e.g., Tala et al. 2013; Rothäusler et al. 2018; Wright and Foggo 2021), growth (e.g., Frontier et al. 2021a, b; Wright et al. 2022) and fertility (e.g., Tala et al. 2013; Rothäusler et al. 2018; Frontier et al. 2021a). The phenomenon of detrital photosynthesis (sensu Frontier et al. 2021a;

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Wright et al. 2022) in macroalgae is readily explained by their lesser tissue differentiation compared to land plants and their descendants. All macroalgal tissues photosynthesise (Dominik and Zimmerman 2006) and assimilate nutrients (Stephens and Hepburn 2016), albeit to different extents.

Plants produce a considerable amount of detrital carbon, around $0.19 \text{ kg C m}^{-2} \text{ yr}^{-1}$ (5×10^{-5} – $2.92 \text{ kg C m}^{-2} \text{ yr}^{-1}$, $n = 296$, data from Cebrián and Lartigue 2004). Whether that detritus is living, as in many macroalgae, or not, likely has major implications for the magnitude and fate of this carbon pool. Photosynthetically active detritus can maintain carbon uptake in the right photoenvironment, thereby being functionally equivalent to the attached and/or intact plant. This could enhance detrital longevity and thus contribution to detritivore diets and carbon sinks (Wright et al. 2022). Understanding variability in detrital photophysiology is, therefore, essential to understanding carbon flow through plant ecosystems. Our knowledge of detrital photophysiology in the Northeast Atlantic is restricted to the most speciose genus *Laminaria*, which does not allow prediction for kelps more broadly.

Northeast Atlantic kelp forests are made up of diverse genera of which *Laminaria* is not necessarily representative. While *Laminaria digitata*, *L. hyperborea* and *Saccharina latissima* (Laminariales) are perennial (Parke 1948; Kain 1963; Lüning 1969; Schaffelke and Lüning 1994) and cold temperate (Bolton and Lüning 1982), *Saccorhiza polyschides* (Tilopteridales) is an annual plant (Phillips 1896; Norton and Burrows 1969) with a wide temperature tolerance (Norton 1977; Pereira et al. 2011; Biskup et al. 2014). Consequently, the whole sporophyte of *S. polyschides* is shed as detritus every winter, leaving behind only the bulb that forms its holdfast (Norton and Burrows 1969), while distal frond erosion (Parke 1948; Hereward et al. 2018; Pedersen et al. 2020; Wright et al. 2022) and storm-induced sporophyte dislodgement (Parke 1948; Brady-Campbell et al. 1984; Hereward et al. 2018; Pedersen et al. 2020) are the main mechanisms of detrital production in the representatives of Laminariales. However, *S. polyschides* is comparable to the other kelps in terms of its laminose morphology (Norton and Burrows 1969) and high capacity to use HCO_3^- as a carbon source for photosynthesis (Forster and Dring 1992; Olischläger et al. 2012, 2017; García-Sánchez et al. 2016).

Photosynthetic pigment concentrations provide substantial insight into detrital photobiology but are comparatively understudied in this context. Chlorophyll *a* correlates with photosynthetic capacity (Wheeler 1980) and efficiency (Blain and Shears 2019; Blain et al. 2020) in kelps and marine macrophytes more generally (Enriquez et al. 1995), which is also supported by seminal work on microalgal model species (Fleischer 1935). Greater photosynthetic potential can therefore be inferred from increased chlorophyll *a* while acknowledging that pigment content can

respond inversely to irradiance in kelps (Wheeler 1980; Roleda and Dethleff 2011; Blain and Shears 2019; Blain et al. 2020), may consequently show the opposite trend to photosynthetic rate (Blain and Shears 2019; Blain et al. 2020) and is hence an indirect measure of photosynthesis. Nonetheless, accessory pigments provide further insight into the capacity of the light-harvesting complex (Wright and Foggo 2021).

Here we aim to expand previous research on detrital pigmentation of *Laminaria* species which found no effect of decomposition over 32 d (Wright and Foggo 2021) by conducting an in situ litter bag experiment at a higher latitude and later season over a 14-d longer period. We compare the response of *Laminaria* with that of the other kelp (sensu Fraser 2012) genera *Saccharina* and *Saccorhiza* and test the null hypothesis that the photosynthetic pigment concentrations of these kelps are similarly unaffected by decomposition (here defined as combined saprotrophy, detritivory, herbivory and physical degradation). In doing so, we present the first multi-genus comparison of detrital photophysiology and concurrently a high-throughput microplate improvement of the previously used pigment quantification method (Wright and Foggo 2021).

Methods

Model system

Sampling and experimentation were carried out in the Walter Rocks kelp forest (54.384239°N , 5.557793°W), situated in the narrows of Strangford Lough, Northern Ireland, ~ 650 m from the Queen's University Marine Laboratory. This kelp forest is comparatively sheltered from tidal currents and supports the four most common Northeast Atlantic kelp species *Laminaria digitata* (Laminariales), *Laminaria hyperborea* (Laminariales), *Saccharina latissima* (Laminariales) and *Saccorhiza polyschides* (Tilopteridales). Here, *L. digitata* occurs in an exceptionally wide (~ 15 m) upper vertical band due to a shallowly sloping pebble reef platform which then drops off and provides deeper substratum for a mixed forest of *L. hyperborea*, *S. latissima* and *S. polyschides* interspersed by sediment.

As mentioned above, *S. polyschides* is an annual plant that sheds its entire sporophyte throughout winter starting in October (Norton and Burrows 1969). Distal frond erosion is somewhat elevated during autumn in the representatives of Laminariales (Parke 1948; Hereward et al. 2018; Pedersen et al. 2020; Wright et al. 2022) and in winter storm-induced sporophyte dislodgement is more likely (Parke 1948; Brady-Campbell et al. 1984; Hereward et al. 2018; Pedersen et al. 2020). At the forest scale, this period leading into winter therefore seems to be one of the periods of highest detrital

production and we chose October as the natural start date of our in situ decomposition experiment. It must nevertheless be noted that *L. hyperborea* produces most of its detritus by abscission, a process which exclusively takes place in spring, outside of our experimental period, and is known as the May cast (Lüning 1969; Schaffelke and Lüning 1994; Pedersen et al. 2020; Wright et al. 2022).

Decomposition experiment

We carried out a litter bag experiment within the Walter Rocks kelp forest between 7th October and 22nd November 2021 (46 d). On the day before the start of the experiment, ten mature sporophytes each of the kelp species *L. digitata*, *L. hyperborea*, *S. latissima* and *S. polyschides* were sampled from their respective vertical zones at Walter Rocks down to ~ 1 m below lowest astronomical tide. Before and during the experiment, $n = 17$ baseline samples from each species were similarly collected on 24th September ($n = 10$), 29th September ($n = 3$) and 22nd October ($n = 4$) to ensure potential changes in detrital pigmentation were in response to decomposition and not merely an artefact of temporal changes in attached sporophytes (Schmid et al. 2017; Blain and Shears 2019). Plants were transported to Queen's University Marine Laboratory within 20 min of collection, where they were placed in flowing, filtered seawater at 12 °C and immediately processed.

Froned segments (~ 15 cm length) were cut from each sporophyte using a scalpel, avoiding the growing (meristematic) basal and eroding distal regions of the thallus (cf. Wright and Foggo 2021; Wright et al. 2022), since the experiment was intended to capture the effect of decomposition from its onset. Eight samples per species were set aside for reference measurement. Added to the $n = 17$ initial samples, this raises the sample size for t_0 to $n = 25$ per species. The remaining segments were weighed to 1-mg accuracy (Excellence Plus XP603SDR Precision Balance, Mettler Toledo, Greifensee, Switzerland) in a glass beaker with ~ 200 ml of filtered seawater to obtain initial buoyant mass and placed in individually labelled mesh bags with 2 mm (1-mm hexagon) and 15 mm (10 × 10 mm square) mesh diameter. These mesh diameters were chosen to variably exclude (2 mm) or allow (15 mm) grazing by the important macrofaunal detritivores *Gammarus locusta* (20–33 mm), *Idotea balthica* (30 × 10 mm), *Steromphala cineraria* (16 × 15 mm), *Lacuna vineta* (10 × 5 mm) and *Rissoia parva* (5 × 3 mm) (Krumhansl and Scheibling 2011; de Bettignies et al. 2020; Horton et al. 2022).

Using cable ties, all mesh bags were tied to the same metal frame equipped with two integrating light loggers (Odyssey, Dataflow Systems Pty Ltd, Christchurch, New Zealand) and one temperature logger (Odyssey, Dataflow Systems Pty Ltd, Christchurch, New Zealand), recording at

10-min intervals. The frame was kept in an outdoor mesocosm with ambient flow-through seawater overnight and deployed on a sediment flat within the Walter Rocks kelp forest at 1.2 m below lowest astronomical tide at 8:00 on the following day. Mesh bags were retrieved at low tide after 20, 32, 39 and 46 d in situ and samples reweighed. A total of $n = 17$ and 8, 18 and 7, 13 and 4, and 10 and 3 2- and 15-mm diameter mesh bags were retrieved for *L. digitata*, *L. hyperborea*, *S. latissima* and *S. polyschides*, respectively. Buoyant masses were converted to dry masses using species-specific buoyant-blotted and blotted-lyophilised mass relationships.

Pigment extraction

The pigment extraction method described by Wright and Foggo (2021) was modified to suit a smaller sample mass. Kelp tissue was lyophilised (Alpha 2–4 LD plus, Christ, Osterode am Harz, Germany), ground with a pestle and mortar, sieved to 355 µm (Endecotts Ltd., London, United Kingdom) and stored at – 20 °C. Of the resulting powder, 36.6–206.2 mg was weighed to 0.1-mg accuracy (AB104 Analytical Balance, Mettler Toledo, Greifensee, Switzerland) in 5-ml glass test tubes (PYREX[®], Stoke-on-Trent, United Kingdom) and sample mass noted for correction during spectral deconvolution.

Pigments were then extracted by eluting (vortexing at 2500 rpm for 10 s; SA8, Stuart[®], St Neots, United Kingdom) the powder five times with 2 ml of 100% acetone (32,201, Sigma-Aldrich[®], Burlington, United States) and collecting the resulting pigment solution in 10-ml volumetric flasks (PYREX[®], Stoke-on-Trent, United Kingdom). Acetone was chosen as the solvent, because it is most widely used for pigment extraction (Jeffrey et al. 1997) and thus has well-known absorbance characteristics (Table S2 in Wright and Foggo 2021). Rapid evaporation caused by the high volatility of acetone (Thrane et al. 2015) was combated by topping up each volumetric flask to the 10-ml mark with acetone at the end of the serial elution. Finally, the solution was homogenised, decanted into labelled 1.5-ml polypropylene microtubes (616273, Greiner Bio-One GmbH, Frickenhausen, Germany) and stored in the dark at – 20 °C.

Spectrophotometry

The previously described spectrophotometry method (Wright and Foggo 2021) was also adapted to support our high-throughput requirements using a microplate reader rather than a traditional spectrophotometer. Samples were transported to the microplate reader laboratory at Queen's University Belfast within 2 h, where they were centrifuged at 14,000 × g for 5 min (1–14, Sigma, Osterode am Harz, Germany). The supernatant of each microtube was then pipetted into clear V-bottom polypropylene screening microplates (651201, Greiner Bio-One

GmbH, Frickenhausen, Germany) in technical triplicates or five technical replicates of 200 µl. We also included 200-µl technical triplicates or five technical replicates of 100% acetone blanks in each microplate plate for good practice. Absorbance was immediately read between 400 and 750 nm at 1 nm resolution on a multi-mode microplate reader (CLARIOstar Plus, BMG Labtech Ltd., Aylesbury, United Kingdom). The mean measurement error (mean standard error of the technical mean) was 15.74, 9.97, 1.76 and 9.55 µg g⁻¹ for total pigment, chlorophyll a, c and fucoxanthin, respectively, and 0.009 for the antenna pigment to chlorophyll *a* ratio (see below).

Potential drawbacks in the use of acetone in microplate assays are its previously mentioned high vapour pressure and its ability to dissolve common polystyrene microplates (Thrane et al. 2015). We nonetheless prefer acetone elution (Wright and Foggo 2021) over an overnight ethanol extraction (Thrane et al. 2015), because its comparatively short extraction time (~ 5 min per sample) is more in-line with our high-throughput requirements (technical $n = 636$) and acetone is the traditional pigment solvent (see above). The polystyrene dissolution problem posed by acetone solutions is easily solved using polypropylene microtubes and -plates, but its volatility required some more thought. The time taken for absorbance reading is negligible (< 1 min per microplate). To understand how evaporation during the short pipetting step was affecting our measurements, we empirically determined that evaporation is linearly reducing the pathlength by 0.15 ± 0.003 (mean \pm s.e.m., $n = 48$) mm min⁻¹ over 5 min using the built-in fluorescence focal height measurement feature of our microplate reader. The pipetting step including microplate loading for all well number variations was then timed (3.06 ± 0.24 min, $n = 17$) and the pathlength corrected (see below).

Spectral deconvolution

We used the R (R Core Team 2023) pipeline provided by Thrane et al. (2015) to deconvolute our absorbance spectra into individual pigment spectra (Fig. S2 in Wright and Foggo 2021) and subsequently calculate pigment concentrations. This validated method provides a cost-effective open-source alternative to high performance liquid chromatography. We reused the maximum absorbances (λ_{\max}) and absorption coefficients (α) previously collated for pigments dissolved in acetone (Table S2 in Wright and Foggo 2021). The pathlength was empirically determined to be 0.61 ± 0.001 cm ($n = 283$) with ultrapure water using the water-peak pathlength equation given as

$$P = \frac{A_{977} - A_{900}}{0.18}$$

where P is the pathlength (cm), A is absorbance and the subscript denotes the wavelength (nm). We calculated a

corrected pathlength ($P_C = 0.59$ cm) by accounting for evaporation during pipetting (see above). Unfortunately, potential alterations to pigment concentration caused by evaporation are impossible to correct but we are confident that the small reduction in volume had little to no effect. Since the microplate plus solvent blanks are modelled as part of the background spectrum (Thrane et al. 2015), there is no necessity to manually correct for blank absorbance by subtracting it from the sample spectra. We trust this modelling approach, since it was validated for polypropylene microplates of the same make as ours (cf. 655201, Greiner Bio-One GmbH, Frickenhausen, Germany in Fig. S2 in Thrane et al. 2015). After calculating volume-based pigment concentrations (c_V , µg ml⁻¹) using the spectral deconvolution pipeline, they were converted to mass-based pigment concentrations (c_M , µg g⁻¹ dry mass) as

$$c_M = c_V \times \frac{V}{M}$$

where V is the sample solution volume (10 ml) and M is the unique sample mass (g dry mass) recorded during the extraction step. The modified spectral deconvolution pipeline is made available at github.com/lukaseamus/detriral-pigments/Deconvolution. In addition to concentrations of the photosynthetic pigments, relative antenna size is reported as the ratio of antenna (i.e., accessory) pigments (chlorophyll *c* and fucoxanthin) to chlorophyll *a* (Wright and Foggo 2021).

In summary, our new method retains its cost-effectiveness compared to high-performance liquid chromatography while enabling higher throughput than the previous method (Wright and Foggo 2021). In comparison, the pros of the present method are (1) a 15-ml reduction in elution volume, (2) a 1.8-ml reduction in assay volume, (3) an up to 96-fold reduction in assay duration and consequently (4) the possibility to account for measurement error using ample technical replication. The only con is having to account for the volatility of acetone by working faster and, if necessary, correcting for evaporation. While to the best of our knowledge our method is novel for macroalgal pigment extraction, the original method for microalgae (Thrane et al. 2015) already employs microplates. The original method provides a workaround for the identified con of our method using less volatile solvents, such as ethanol (Thrane et al. 2015). However, this leads to a substantial extension of required extraction time and thereby partly counteracts the time saved during spectrophotometry. Due to this trade-off, the ideal method must be considered on a case-by-case basis.

Data analysis and visualisation

Analysis and visualisation were carried out in R v4.1.2 (R Core Team 2023) with the tidyverse package collection v2.0.0 (Wickham et al. 2019) within the integrated

development environment RStudio v2022.07.2 (RStudio Team 2022). All R scripts and data are made available at github.com/lukaseamus/detrital-pigments. Technical replicates were retained for analysis rather than averaged to account for full within-sample variance which allows for calculation of measurement error and provides the model with maximal information. To avoid inflation of test statistics caused by artificially increased sample size (technical $n = 636$ vs. statistical $n = 180$), we employed multilevel modelling with the *lme* function of nlme v3.1–153 (Pinheiro et al. 2021). Each model includes a secondary model for the intercept of each sample.

To test our null hypothesis on the lack of a decomposition effect on pigment concentrations across species, we built several models with pigment concentrations or antenna size as the response variable, detrital age as the continuous explanatory variable, species as the categorical explanatory variable and sample as the random variable. Visual data exploration revealed that in the case of *S. latissima* all response variables followed a parabola relationship with detrital age. Since responses of other kelps could on the other hand be satisfactorily described linearly and linear regression is more extrapolatable and interpretable than polynomial regression, we decided to build one linear regression for three species and a separate second-order polynomial regression for *S. latissima*. Both were implemented in nlme v3.1–153. Graphical model validation revealed that residual variance was heterogenous despite normality of residuals for all linearly modelled pigment concentrations. This was solved by weighting residual variance by the covariates detrital age and species with the *weights* argument of the *lme* function in nlme v3.1–153.

Mesh size is interesting in terms of trophic ecology but is not needed to test our null hypothesis in this short note. Nonetheless, differences in shading caused by the mesh may

have affected pigment concentrations. Mesh sizes are unfortunately unevenly represented across species (see varying sample sizes in [Decomposition experiment](#)) and 15-mm diameter mesh is only represented at t_2 (32 d). Since we, therefore, could not test for an interaction between mesh size and detrital age, we conservatively tested for an effect of mesh size by species on various pigment concentrations at t_2 to determine if we could safely remove this factor from our analyses, the rationale being that if mesh size does not affect pigment concentrations it is unlikely to affect their response to decomposition. Mesh size was not found to affect total pigment, chlorophyll *a*, chlorophyll *c*, fucoxanthin or antenna size in *Laminaria* spp., but 15-mm diameter mesh had a positive effect on all pigment concentrations and a negative effect on antenna size in *S. latissima*, and positive effects on chlorophyll *c* and antenna size in *S. polyschides* (Table S1). Due to this interspecific variability, we also analysed the data excluding 15-mm diameter mesh samples (Fig. S1, Table S2a) to show that including them has negligible influence on the decomposition effect while being desirable with regard to increased sample size (cf. Figure 2, Table S2b). Samples from both mesh diameters were therefore retained and pooled for the main analysis.

Results and discussion

According to season, ambient light (Fig. 1a) and temperature (Fig. 1b) declined during our 46-day field experiment. Daily average daytime and 24-h photon flux densities peaked on day 9 at 1236.79 ± 109.68 and 575.45 ± 62.61 (mean \pm s.e.m.) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and troughed on day 39 at 31.19 ± 2.21 and 11.59 ± 1.21 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively (Fig. 1a). Temperature ranged from 12.96 ± 0.005 °C on day 3 to 11.13 ± 0.02 °C on day 46

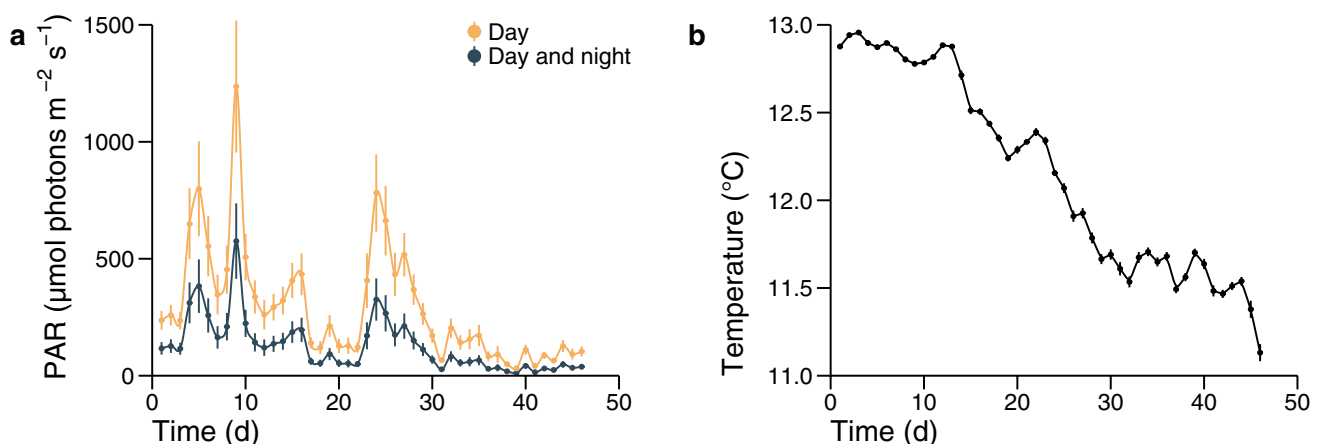


Fig. 1 Daily kelp forest daytime and 24-h photon flux density (a) and temperature (b) during the decomposition experiment. Point ranges are means and 99% confidence intervals. Means are joined by splines

(Fig. 1b). During this time, *L. digitata*, *L. hyperborea*, *S. latissima* and *S. polyschides* lamina tissue decomposed at rates of 3.98 ± 0.76 , 24.01 ± 4.13 , 37.39 ± 11.58 and 37.17 ± 6.79 mg dry mass d^{-1} . In contrast, *L. digitata* was found to decompose at a statistically similar rate but somewhat faster than *L. hyperborea* in the southern British Isles (Wright and Foggo 2021; Wright et al. 2022), suggesting latitudinal (Plymouth Sound vs. Strangford Lough) and/or seasonal (summer vs. autumn) variation in the relative decomposition rates of these two boreal kelps.

According to our assay data, detrital photosynthetic pigment concentrations of both *Laminaria* species remain unaffected by decomposition (Table 1, Fig. 2a–c) but antenna size increases in *L. digitata* as opposed to *L. hyperborea* (Table 1, Fig. 2d). These results are directly in accordance with previous findings (cf. Table S4 in Wright and Foggo 2021). Even the positive slope of *L. digitata* antenna size over detrital age ($y = 0.005d + 0.82$) is approximately the same as that previously determined for this kelp (cf. $y = 0.008d + 0.6$ in Fig. S5 in Wright and Foggo 2021). Our novel data from an autumnal kelp forest in the northern British Isles therefore corroborate previous data from a southern spring equivalent (Wright and Foggo 2021). This suggests that regardless of season and latitude, i.e., light and temperature environment, photosynthetic potential of *Laminaria* detritus is unaffected by decomposition over periods at least 46 d. For these two species our indirect evidence from pigments is corroborated by direct evidence for maintenance of photosynthesis as shown by continued oxygen production (Frontier et al. 2021a; Wright et al. 2022). Finally, *L. digitata* has 56% higher chlorophyll *a* (pairwise *t* test, $t = 3.16$, $p = 0.002$, $n = 100$) and 47% higher fucoxanthin (pairwise *t* test, $t = 2.8$, $p = 0.006$, $n = 100$) concentrations than *L. hyperborea*, which is supported by previous findings (Schmid and Stengel 2015; Shannon and Abu-Ghannam 2017; Wright and Foggo 2021).

In the case of *S. latissima*, pigment concentrations and stoichiometry respond idiosyncratically to decomposition (Table 1, Fig. 2). Chlorophyll *a*, *c*, fucoxanthin and total pigment in lamina detritus of *S. latissima* display a parabola shape (Table 1, Fig. 2a–c), peaking on day 20 at 1.9 ± 0.26 , 0.11 ± 0.01 , 1.65 ± 0.19 and 3.74 ± 0.49 mg g^{-1} dry mass, respectively, while antenna size remains unchanged (Table 1, Fig. 2d). This approximately opposes observed trends in irradiance, which troughed around day 20 (Fig. 1a), suggesting an implication of light (Wheeler 1980; Roleda and Dethleff 2011; Blain and Shears 2019; Blain et al. 2020). However, the fact that this trend was not evident in any of the other kelps (Fig. 1) alongside the relatively weak irradiance effect on pigmentation of *S. latissima* (Roleda and Dethleff 2011; Boderskov et al. 2016) suggest that idiosyncrasy is more likely. Within the temporal confines of our study period, the parabola shape translates to de facto unaffected detrital pigment concentrations, since the final mean is always close to the initial (Fig. 2a–c). Therefore, our conclusion is ultimately equivalent to that for *Laminaria* species and we cannot reject the null hypothesis for *S. latissima*. There is currently no direct evidence for detrital oxygen production in this species to back our findings. Finally, our data (cf. Shannon and Abu-Ghannam 2017) do not support a previous report of exceptionally low pigment concentrations in *S. latissima* relative to other kelps (Schmid and Stengel 2015).

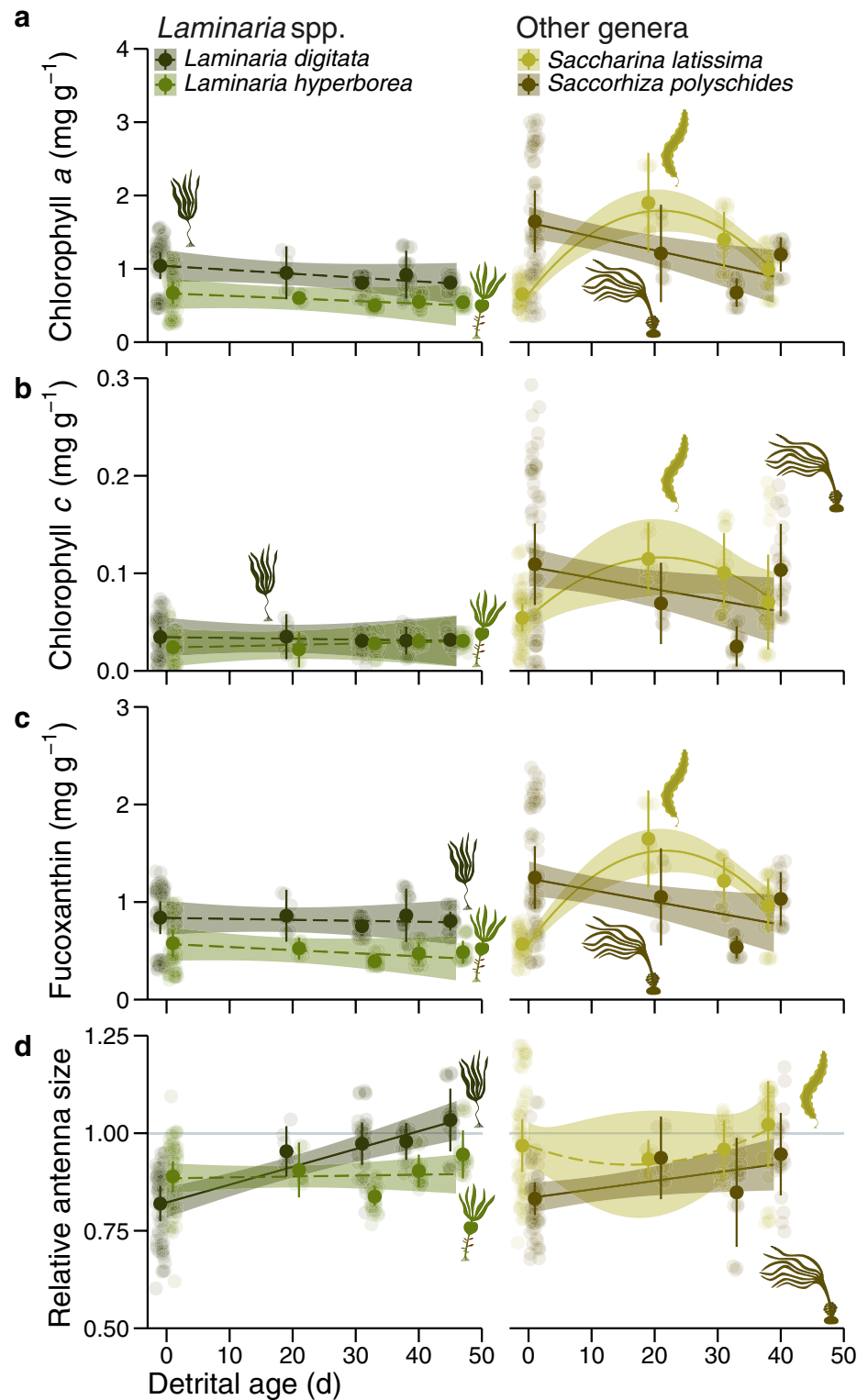
In the case of *S. polyschides*, photosynthetic pigments decline with detrital age as antenna size increases (Table 1, Fig. 2) which supports rejection of the null hypothesis for this kelp. However, the baseline pigment concentrations for this species were extremely variable compared to the other kelps (range = 4.62, 2.65, 0.25 and 1.95 mg g^{-1} for total pigment, chlorophyll *a*, *c* and fucoxanthin) and the detrital minima were always within this range (Fig. 2a–c). Despite statistical significance and due to lack of direct evidence for detrital oxygen production,

Table 1 Linear and polynomial multilevel model chi-square (χ^2) test results for detrital photosynthetic pigment concentrations (mg g^{-1}) and stoichiometry (ratio) given as *y* in relation to detrital age (*d*) (cf. Table S2b)

	<i>Laminaria digitata</i> (<i>n</i> = 50)	<i>Laminaria hyperborea</i> (<i>n</i> = 50)	<i>Saccharina latissima</i> (<i>n</i> = 42)	<i>Saccorhiza polyschides</i> (<i>n</i> = 38)
Total pigment	$y = -0.006d + 1.92$ $\chi^2_{1,138} = 0.93$	$y = -0.006d + 1.27$ $\chi^2_{1,138} = 0.97$	$y = 0.2d - 0.005d^2 + 1.42$ $\chi^2_{2,42} = 80.8^{***}$	$y = -0.03d + 3.11$ $\chi^2_{1,138} = 14.26^{***}$
Chlorophyll <i>a</i>	$y = -0.005d + 1.04$ $\chi^2_{1,138} = 2.61$	$y = -0.004d + 0.67$ $\chi^2_{1,138} = 1.18$	$y = 0.11d - 0.003d^2 + 0.66$ $\chi^2_{2,42} = 98.36^{***}$	$y = -0.02d + 1.63$ $\chi^2_{1,138} = 18.5^{***}$
Chlorophyll <i>c</i>	$y = -8.31 \times 10^{-5}d + 0.03$ $\chi^2_{1,138} = 0.08$	$y = 0.0001d + 0.02$ $\chi^2_{1,138} = 0.22$	$y = 0.006d - 0.0001d^2 + 0.05$ $\chi^2_{2,42} = 16.12^{***}$	$y = -0.001d + 0.11$ $\chi^2_{1,138} = 7.95^{**}$
Fucoxanthin	$y = -0.001d + 0.84$ $\chi^2_{1,138} = 0.14$	$y = -0.003d + 0.57$ $\chi^2_{1,138} = 1.48$	$y = 0.09d - 0.002d^2 + 0.57$ $\chi^2_{2,42} = 122.39^{***}$	$y = -0.01d + 1.24$ $\chi^2_{1,138} = 10.99^{***}$
Antenna size	$y = 0.005d + 0.82$ $\chi^2_{1,138} = 61.79^{***}$	$y = 0.0002d + 0.88$ $\chi^2_{1,138} = 0.16$	$y = -0.006d + 0.0002d^2 + 0.97$ $\chi^2_{2,42} = 1.65$	$y = 0.002d + 0.83$ $\chi^2_{1,138} = 7.62^{**}$

Significant relationships at $\alpha = 0.05$ are highlighted in bold. Asterisks indicate $p < 0.05$ *, $p < 0.01$ ** and $p < 0.001$ ***

Fig. 2 Chlorophyll *a* (a), *c* (b) and fucoxanthin (c) concentration per unit dry mass as well as antenna pigment (chlorophyll *c* + fucoxanthin) to chlorophyll *a* ratio (d) in shallow benthic detritus of four kelps across three genera in relation to detrital age (technical $n = 636$, statistical $n = 180$). Point ranges are means and 99% confidence intervals, lines are model fits and shaded areas are 99% confidence intervals. Solid lines indicate a significant relationship between pigment content and detrital age at $\alpha = 0.05$. The horizontal grey line in d marks the ratio above which accessory pigments exceed chlorophyll *a*. Note that datapoints were jittered and x-shifted and point ranges x-shifted relative to one another to avoid overplotting



no safe conclusion can therefore be drawn for this species without further investigation. It is strictly necessary to expand research on photosynthetic rates of this species, which currently is limited to only two studies (Biskup et al. 2014; García-Sánchez et al. 2016). This data scarcity

is surprising given the important forest-forming role *S. polyschides* will play in a warmer North Atlantic (Norton 1977; Pereira et al. 2011; Biskup et al. 2014). Similarly, only three studies (Schmid and Stengel 2015; Fernandes et al. 2016; Shannon and Abu-Ghannam 2017) have

previously analysed the pigments of intact *S. polyschides*. Unlike these studies (Schmid and Stengel 2015; Shannon and Abu-Ghannam 2017), we report 57% (pairwise *t* test, $t = 5.01$, $p < 0.001$, $n = 88$) and 145% (pairwise *t* test, $t = 8.16$, $p < 0.001$, $n = 88$) higher chlorophyll *a* and 48% (pairwise *t* test, $t = 4.21$, $p < 0.001$, $n = 88$) and 118% (pairwise *t* test, $t = 6.99$, $p < 0.001$, $n = 88$) higher fucoxanthin than in *L. digitata* and *L. hyperborea*, respectively.

Our results show conclusively that photosynthetic pigment concentrations in detritus of Northeast Atlantic *Laminaria* species are unaffected by decomposition over periods longer than a month. Consequently, recently detached lamina tissue of these species appears to be functionally equivalent to the respective living plants. However, the photophysiology of kelps from other genera responds differently, resulting in a potentially divergent functional consequence for *S. polyschides*. This annual kelp with a high thermal plasticity seems to be unable to maintain photosynthetic potential into the detrital phase, which may partially be explained by its faster decomposition rate. However, *S. latissima* also loses biomass at a faster rate without the concomitant reduced pigmentation. Whether the observed effect, if it is not just a product of the larger variability, is therefore unique to annual kelps and what mechanistic driver underlies it remains to be determined. Extrapolating to a biogeographical and climate change context, decline of photosynthetic potential in the detrital phase of kelps that are tolerant to warm temperate conditions, such as *S. polyschides*, could result in reduced longevity of the forest-scale detrital pool with increased ocean warming. However, given our somewhat ambiguous data on this species, this conjecture requires further investigation into the photobiology of *S. polyschides*.

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Author contributions LSW designed the experiment, collected, analysed and visualised all data and wrote the manuscript. LK supported the experimental design, organised funding and logistics, set up equipment, provided local insight and edited the manuscript.

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Data availability All data and code are available with no restrictions at github.com/lukaseamus/detrital-pigments.

Declarations

Conflict of interest We have no conflict of interest to disclose.

Ethical approval Our work requires no ethics approval, since no work on animals was conducted.

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