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Abstract

Macroalgae are foundational to the health of many Indigenous social-ecological systems, and their production of dissolved organic carbon (DOC) serves various biogeochemical roles. Improved understanding of seasonal variations in DOC release as an ecophysiological response could therefore help Indigenous stewards balance these implications. However, multi-year seasonal studies of macroalgal DOC release are few and the underlying roles of passive and active DOC diffusion need clarifying. This study focuses on the kelp *Saccharina japonica* var. *religiosa* (class Phaeophyceae) from Oshoro Bay, Ainu Mosir (Hokkaido). The conclusions are supported by three years (2020–2022) of data, including 1091 DOC samples from 16 incubation experiments ($t = 4–9$ days) comparing individual kelp ($n = 88$) to in situ seawater control tanks ($n = 31$) under different photosynthetically active radiation (PAR) treatments (200, 400, 1200, or 1500 μmol photons · m$^{-2}$ · s$^{-1}$). Differences in PAR, dry weight biomass, sea surface temperature, or salinity could not explain DOC release rate variability, which was high between individual kelp. Instead, there were significant intra-annual differences, with mean DOC release rates (mg C · g DW$^{-1}$ · d$^{-1}$) ($±$ standard error between $n$ kelp) higher ($p < 0.05$) during the autumn “late decay” period (0.82 ± 0.12, $n = 27$) compared to the winter “early growth” period (0.20 ± 0.028, $n = 10$) and summer “early decay” period (0.34 ± 0.066, $n = 24$). Monitoring this relationship between seasonal decay and macroalgal DOC release may therefore help inform Indigenous stewardship strategies.
Seasonal variability of kelp dissolved organic carbon release driven by decay not growth: a key relationship for Indigenous stewards to monitor

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Seasonal variability of kelp dissolved organic carbon release driven by decay not growth: a key relationship for Indigenous stewards to monitor¹

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Abstract

Macroalgae are foundational to the health of many Indigenous social-ecological systems, and their production of dissolved organic carbon (DOC) serves various biogeochemical roles. Improved understanding of seasonal variations in DOC release as an ecophysiological response could therefore help Indigenous stewards balance these implications. However, multi-year seasonal studies of macroagal DOC release are few and the underlying roles of passive and active DOC diffusion need clarifying. This study focuses on the kelp *Saccharina japonica* var. *religiosa* (class Phaeophyceae) from Oshoro Bay, Ainu Mosir (Hokkaido). The conclusions are supported by three years (2020–2022) of data, including 1091 DOC samples from 16 incubation experiments (*t* = 4–9 days) comparing individual kelp (*n* = 88) to in situ seawater control tanks (*n* = 31) under different photosynthetically active radiation (PAR) treatments (200, 400, 1200, or 1500 µmol photons · m⁻² · s⁻¹). Differences in PAR, dry weight biomass, sea surface temperature, or salinity could not explain DOC release rate variability, which was high between individual kelp. Instead, there were significant intra-annual differences, with mean DOC release rates (mg C · g DW⁻¹ · d⁻¹) (± standard error between *n* kelp) higher (*p* < 0.05) during the autumn “late decay” period (0.82 ± 0.12, *n* = 27) compared to the winter “early growth” period (0.20 ± 0.028, *n* = 10) and summer “early decay” period (0.34 ± 0.066, *n* = 24). Monitoring this relationship between seasonal decay and macroagal DOC release may therefore help inform Indigenous stewardship strategies.

Key index words: Blue Carbon; dissolved organic carbon; Indigenous methodologies; kelp; light; macroalgae; seasonal variability

Abbreviations: DOC, dissolved organic carbon; PAR, photosynthetically active radiation
Introduction

The primary objective of this study is to provide insights into the mechanisms controlling seasonal macroalgal dissolved organic carbon (DOC) release variability to help Indigenous coastal stewards anticipate variable local DOC accumulation and plan for ecological implications. Specifically, macroalgal DOC release is an ecophysiological response (Hurd et al., 2014) and serves many biogeochemical roles that can impact microbial activity, oxygen concentrations, pH values, or Blue Carbon sequestration (Carlson and Carlson, 1984; Wada and Hama, 2013; Carlson and Hansell, 2015; Edworthy et al., 2023). Understanding trends in macroalgal DOC release may therefore be useful to Indigenous stewards when considering selective harvesting strategies or adjusting engineering controls. For example, Indigenous integrated multi-trophic aquaculture such as loko iʻa (fishponds that integrate seaweed cultivation) in Hawaiʻi are engineered to adjust water exchange (Keala et al., 2007) within the context of broader systems of social-ecological resource governance (Winter et al., 2018). Therefore, monitoring the relationship between macroalgal DOC release and factors such as environmental parameters or seasonal growth stage may be one relevant metric for decision making.

The orientation of this macroalgal ecophysiology and biogeochemistry experiment toward empowering sovereign Indigenous seaweed cultivation is based on the lead author’s research methodology (Carlson, 2024) as a diasporic Kanaka ʻŌiwi (Native Hawaiian) in Ainu Mosir (Hokkaido) and is therefore an intrinsic part of this research. This application is consistent with the broader obligations of geoscience to advance decolonization (i.e., Black and Indigenous sovereign liberation) (Yusoff, 2018; Liboiron, 2021; Sultana, 2022; Carlson, 2024). This discussion is also critically relevant to this field of research because Indigenous coastal
stewardship and biocultural restoration are essential for social-ecological health (Morishige et al., 2018; Bennet et al., 2021; Jacobs et al., 2022). To be clear, this also applies to Ainu People throughout Ainu Mosir (Grunow et al., 2019; Ishihara, 2020; Uzawa, 2020), inclusive of this study’s sampling location of Oshoro Bay. However, Hokkaido University’s Oshoro Marine Biological Station currently manages access to the sampling location and there are no established mechanisms for Indigenous community peer-review, consent, or community-researcher partnerships as exist elsewhere for geoscience research (Liboiron et al., 2018; Alegado et al., 2023).

Since this study is focused on macroalgal DOC release as an ecophysiological mechanism (and how these insights might be relevant to Indigenous monitoring and stewardship), Indigenous Knowledge of kelp is not the subject of investigation and is not discussed here in detail. However, it is important to briefly comment on the cultural relevance of kelp to Ainu People. First, the historical cultivation of kelp by Ainu People is not disputed (Kawai et al., 2012). Moreover, the Japanese word konbu derives from the (southern regional) Ainu word kompu, giving some indication of its prominent societal role. Currently, kelp continues to be culturally relevant, for example, in cuisine such as kompusito (Kaminaga, 2018).

While settler-colonialism continues to disrupt modern Ainu kelp cultivation from being practiced at larger scales, Ainu People and culture persist. Therefore, Ainu People interested in kelp cultivation may find these results on the seasonal variations in macroalgal DOC release relevant to current or future stewardship and harvesting practices.

In terms of biogeochemical knowledge gaps, the scope of this study addresses the scarcity of multi-year seasonal data on macroalgal DOC release (e.g., Abdullah and Fredriksen, 2004; Wada et al., 2007; Paine et al., 2023a) as well as conflicting experimental evidence.
regarding the links between macroalgal DOC release and active photosynthetic processes compared to passive leakage processes related to biological stress (e.g., Weigel and Pfister, 2021; Paine et al., 2021). Abdullah and Fredriksen (2004) commented that exudation was high during high production (March and June) although the difference was not significant. The seasonal trend in Paine et al. (2023a) was that DOC release was highest in spring with consecutively decreasing values in summer, fall, and winter. However, this trend was not attributable to biomass and instead was driven by nitrogen limitation (i.e., the ratio of carbon to nitrogen in the tissue as well as NO$_3^-$ availability in the seawater). The seasonal trend in Wada et al. (2007) was that DOC release was highest during growth (April and May), lower in fall (October) and winter (December), but lowest in late summer (August). The assumption given was that DOC release was associated with primary production, not biomass, although the significantly lower August values were not explained. Based on these limited data, contrasting trends, and differing drivers, there is a need for more long-term seasonal DOC release studies with sufficient sample sizes to gain insights into the underlying mechanisms driving variations.

While there is clear evidence that a primary driver of seasonal DOC release variability is nitrogen limitations (Paine et al., 2023a), previous and concurrent data analyzed by our laboratory indicates that *S. japonica* var. *religiosa* C:N ratios are typically between 8–11 (maximum of 13) at Oshoro Bay, without significant seasonal differences (Okazaki, unpublished; Nakanishi, unpublished; Togawa, unpublished). For context, a C:N ratio less than 10 generally indicates nitrogen sufficiency and above 20 indicates nitrogen limitation (Hurd et al., 2014). The global mean molar ratio of seaweed C:N is 20 based on 495 species (Sheppard et al., 2023). Given this, we instead test the hypothesis that, without nitrogen limitations, seasonal DOC release varies according to in situ growth and decay conditions. To do so, this study
assesses the impact of variable photosynthetically active radiation (PAR), correlations between DOC release and biomass, and tests for significant differences intra-annually between winter “early growth”, spring “late growth”, summer “early decay”, and autumn “late decay” life cycle-based seasonal stages.

Therefore, 16 sampling events and incubation experiments were conducted seasonally (4 to 6 times per year) over three years (2020–2022) to characterize DOC release from the kelp *Saccharina japonica* var. *religiosa* (class Phaeophyceae), collected from Oshoro Bay. Clarifying these mechanistic relationships may be particularly useful to Indigenous coastal stewards. For example, if macroalgal DOC release were strongly controlled by photosynthetic processes and primary production, Indigenous stewards could use biomass growth as an appropriate proxy. On the other hand, if DOC release were strongly influenced by passive leakage, signs of macroalgal biological stress or environmental change may be more relevant proxies.

While the precise cellular mechanisms controlling DOC release are outside the scope of this study, previous research has highlighted the importance of the “overflow hypothesis” where photosynthesis outpaces growth requirements (Nagata, 2000), as well as DOC release being enhanced by both nitrogen limitations (Mizuta et al., 1994; Weigel and Pfister, 2021; Paine et al., 2023a) and iron limitations (Paine et al., 2023b). Moreover, while the seasonality of macroalgal DOC release has been established for decades (Mann, 1973; Hatcher et al., 1977; Johnston et al., 1977), the lack of recent empirical data has resulted in synthesis papers making annualized generalizations based on few or no seasonal data (e.g., Barrón et al., 2014). Such generalizations are typically based on the disputed assumption that DOC release is proportional to primary production or biomass (Khailov and Burlakova, 1969; Sieburth, 1969). Estimates based on limited data therefore critically overlook essential seasonal, environmental, or biological
variations related to passive exudation.

With this in mind, this study aims to improve understanding of the variability of kelp DOC release, with a particular focus on the effects of PAR and other environmental parameters, in addition to seasonal variability related to growth stage. While the results of this study on macroalgal DOC release mechanisms will have the greatest place-based relevance to Oshoro Bay and Ainu Mosir, they may also provide useful insights to Indigenous Peoples globally. However, it must be emphasized that any insights discussed here cannot take precedence over place-based Indigenous Knowledge of seaweed cultivation, which has persisted for millennia and continues to evolve (Abbott and Williamson, 1974; Kobluk et al., 2021; Reid et al., 2022). Nevertheless, settler-colonialism has disrupted Indigenous Knowledge and systems of governance in many places (Whyte, 2018). Therefore, general insights from this study may be relevant to the processes of rebuilding more nuanced place-based stewardship practices globally.

Methods

Field site

Hokkaido University’s Oshoro Marine Biological Station was originally founded in 1908 as an affiliated facility of Tohoku Imperial University. Oshoro Bay is on the west coast of the Shakotan Peninsula, largely protected from wind and waves due to rocky cliffs, with an inlet facing northwest. Tidal level differences on the Sea of Japan coast are relatively small, creating good habitat for marine flora and fauna as well as facilitating collection and measurement activities. Reported flora and fauna on the shore include 208 species of marine algae, 389 species of invertebrates, 85 species of fishes, and 291 species of plankton (Motoda, 1971; Motoda et al., 1987; Yotsukura, 2021).

*S. japonica* var. *religiosa* is one of the dominant autotrophs within a shallow, sub-tidal
ecosystem along the southwestern portion of Oshoro Bay. The surveyed area is delimited by the temperature and salinity sampling points shown in Figure 1. *S. japonica* var. *religiosa* is an annual prostrate kelp with cyclical biomass at a minimum in winter, growth up to two meters in length from spring to summer, and declining biomass due to grazing, erosion, and natural senescence in autumn (Abe et al., 1985). This kelp forms dense beds in Oshoro Bay that are mainly monospecific, with some *Costaria costata* and *Undaria pinnatifida* interspersed, and cover approximately 500 m$^2$ along the northeastern shelf edge as well as 1000 m$^2$ in the southeastern portion of the surveyed area. Within this coastal shelf area, an additional 3000 m$^2$ features mixed macroalgal beds and other marine flora and fauna (Figure 1). Other smaller macroalgae commonly observed during this study include green (e.g., *Ulva sp.*), red (e.g., *Mazaella japonica* and *Neodilsea yendoana*), and brown (e.g., *Sargassum sp.* ) macroalgae. Several other species of macroalgae have been reported at Oshoro Bay in recent (Hoshikawa et al., 2018) and older literature (Matsuyama, 1983; Kawai, 1997).

**Field sampling**

Field surveys to collect whole kelp individuals for ex situ incubation were completed 16 times (January, early March, late March, June, August, and October 2020; January, March, April, July, October, and November 2021; and May, July, October, and November 2022). In situ sea surface temperature and salinity field probe measurements were taken at eight locations within the macroalgal bed area and one reference location within the bay (Figure 1). Sampling was seasonal to account for various growth stages and biological conditions. The kelp samples were detached from the underlying rocky substrate (typical depth of 30–50 cm) along the northeastern shelf edge (Figure 1) where tidal motion was active but moderate. Care was taken to minimize damage to the holdfast and kelp samples were transported in a polyethylene bag with
in situ seawater to our laboratory on the Hokkaido University campus in Sapporo within a few hours of collection (42 km drive). Up to six individual kelp were incubated in separate incubation tanks per sampling event.

Acid-washed 18-L polyethylene tanks were filled with pre-screened (100 µm mesh) reference seawater for the incubation tanks. This seawater was collected from a pier on the northeast side of the bay about 100 m from the kelp bed sampling location (Figure 1) for all sampling events except August 2020, October 2020, and July 2022, when seawater was collected in the bay by a small fishing boat (Figure 1).

**Incubation experiment**

After returning to our laboratory in Sapporo, kelp samples were gently rinsed with seawater from the reference point outside the macroalgal bed area. Rinsing was done to reduce the effect of DOC released during transportation and to reduce epibiota such as attached microalgae or grazers, while minimizing disruptions to the kelp microbiome. The kelp samples were then placed in clear polystyrene tanks filled with approximately three liters of the same reference seawater, fully submerging the kelp while leaving approximately two liters of headspace. The top of each tank was covered in a plastic wrap. Tank seawater volumes were confirmed by weight. Kelp and control tanks of reference seawater were incubated at the in situ sea surface temperature for 4–9 days ($t = 9$ days in January 2020, $t = 7$ days from early March 2020 through January 2021, $t = 5$ days from March 2021 through July 2021, and $t = 4$ days from October 2021 through November 2022).

The multi-day incubation period was chosen to assess the linearity of kelp DOC release rates. Non-linear kelp DOC release may be an indication of an adverse incubation environment, such as the development of anoxia or insufficient nutrients. Concerns regarding decreasing
nitrogen concentrations are particularly relevant given that previous studies indicate DOC release should increase with nitrogen limitations (Weigel and Pfister, 2021; Paine et al., 2023a).

Likewise, live photosynthesizing tissue and dead tissue are expected to release DOC at significantly different rates (Paine et al., 2021), and a sufficiently long incubation may be able to clearly differentiate between the two phases.

The potential effect of irradiance on DOC release rates was tested in each of the sampling events \((n = 16\) events) by treating half of the incubated kelp to a lower irradiance treatment and half to a higher irradiance treatment. The lower irradiance exposure was 200 µmol photons · m\(^{-2}\) · s\(^{-1}\) from artificial LED sources for all sampling events. The higher irradiance exposures varied and were 400 (incubation experiments from January 2020 to July 2021) or 1200 (incubation experiments from May 2022 through November 2022) µmol photons · m\(^{-2}\) · s\(^{-1}\) from artificial LED. The artificial cool white LED light sources were controlled by a light/dark timer set according to the actual daylight hours (photoperiod). When testing the effect of natural irradiance, the higher (natural) irradiance treatment levels were 2500 (October 2021) and 1500 (November 2021) µmol photons · m\(^{-2}\) · s\(^{-1}\).

Because the *S. japonica* var. *religiosa* bed was shallow (less than 50 cm depth), dense, and subject to tidal motion, in situ sub-surface irradiance was variable and certain in situ kelp would be periodically exposed to direct irradiance. Therefore, the 200 µmol photons · m\(^{-2}\) · s\(^{-1}\) irradiance was representative of kelp subject to dense community shading, 400 µmol photons · m\(^{-2}\) · s\(^{-1}\) was representative of submerged kelp not subject to community shading, and 1200 µmol photons m\(^{-2}\) s\(^{-1}\) was an upper limit representative of kelp periodically exposed by tidal motions to direct irradiance on a sunny day. Maximum above water irradiance on sunny summer days was approximately 2500 µmol photons · m\(^{-2}\) · s\(^{-1}\).
Two 3-L control tanks of filtered reference seawater were also incubated, one on each tier. These control samples assessed the potential for DOC contamination from the incubator environment or during the sampling process, as well as any detectable DOC trends due to plankton activity. The incubation experiments lasted between 4–9 days to assess potential impacts from artificial stress responses. Incubated control seawater DOC concentrations showed no evidence of accumulation, despite some fluctuations within the incubation periods (Figure 2e, Figure S1). The mean coefficients of variation (± SE) over the course of the incubation experiments were 5.2% ± 0.96% \((n = 16)\) for DOC concentrations in control tanks treated to lower irradiance, 4.3% ± 0.53% \((n = 15)\) for higher irradiance treatments, and 4.8% ± 0.56% \((n = 31)\) overall. These fluctuations were likely due to a combination of minor spatial heterogeneities within the incubated control seawater and phytoplankton and bacterial DOC dynamics. Mean control DOC concentrations were used as the baseline to determine kelp-derived DOC inventory accumulation.

Seawater samples from the incubation tanks were taken with a 25-mL syringe and immediately filtered through a pre-combusted \((450 °C \text{ for } 5 \text{ hours})\) 0.7 µm Whatman glass-fiber filter (GF/F). The syringes were rinsed with 4-mL seawater samples three times before taking a 50-mL sample. The decreasing volume in the tank was accounted for to normalize the increasing DOC concentrations throughout the incubation period, and the final seawater volume was confirmed by weight at the end of the incubation. The seawater samples were frozen at −30 °C in 60-mL amber borosilicate glass sample bottles until analysis. The bottles were cleaned before use, first in a detergent bath, followed by a 1.2 M HCl bath, then pre-combusted at 550 °C for 5 hours.

At the end of the incubation, kelp wet weight was measured by weighing the incubation
tank before and after removing the kelp sample, in addition to confirming the wet weight on
aluminum foil before drying. The removed kelp samples were then dried at 60 °C on aluminum
foil until a constant dry weight was achieved, typically after 4 to 5 days. DOC release results for
each kelp were normalized by their respective dry weight.

**DOC analyses**

All seawater samples were analyzed for DOC by a total organic carbon analyzer
(Shimadzu TOC-5000A or TOC-V) according to the high-temperature combustion method. DOC
samples were acidified by adding 100 µL of 2 M HCl to ensure the pH was lower than 3 and that
dissolved inorganic carbon (DIC) would be removed after sparging for 10 minutes. Each sample
was injected into the combustion column at least 3 times with a coefficient of variation within
2%.

Potassium hydrogen phthalate was used to make a standard stock solution of $8.3 \times 10^4$
µmol · L$^{-1}$ (1000 ppm) DOC. The standard stock solution was typically diluted to concentrations
between 42 and $2.7 \times 10^3$ µmol · L$^{-1}$ DOC (0.5–32 ppm) to calibrate the analyzer with a five or
six point linear regression model (100 µL analytical injections). For higher concentrations,
standard stocks up to $3.3 \times 10^4$ µmol · L$^{-1}$ DOC (400 ppm) were used to calibrate separate five
point linear regression models (13 µL analytical injections). In general, the analyzer was initially
conditioned with Milli-Q, standard potassium hydrogen phthalate samples, and seawater
standards. Subsequently, Milli-Q and standard samples were checked after analyzing five
seawater samples. Other methods of ensuring replicability included re-analyzing samples within
or between analysis runs. Coefficients of variation greater than 2% or significant deviations
between standard sub-samples triggered re-conditioning or maintenance before proceeding. To
assess spatial heterogeneity of DOC within the incubation tanks, duplicate ($n = 12$) DOC
samples were collected at the end of the November 2021 incubation experiments and triplicate \((n = 18)\) DOC samples were collected at the end of the May 2022, July 2022, October 2022, and November 2022 incubation experiments (Figure S2).

**Results**

**Biomass and environmental parameters**

Mean biomass (grams dry weight per individual) of the incubated kelp varied consistent with its annual life cycle (± SE between \(n\) kelp replicates) (Figure 2a). Inter-annually, while the highest average biomass was recorded in July 2021 (10.8 ± 2.0, \(n = 6\)), followed by June 2020 (7.93 ± 0.48, \(n = 6\)), then July 2022 (5.95 ± 1.2, \(n = 6\)), the differences were not significant (one-way ANOVA, \(p > 0.05\)) despite a four-fold range in individual magnitudes (3.97–17.9) (Figure S1). Average peak biomass can therefore be summarized over the three-year period as 8.2 ± 0.89 g DW (\(n = 18\)). Interannual differences in biomass were also not significant for other periods.

In situ sea surface temperature and salinity means represented eight sampling locations within the macroalgal bed area and one reference point within Oshoro Bay for each sampling event (± SE between sampling locations). Sea surface temperature varied from 5.1 ± 0.17 in January 2020 to 23.5 ± 0.17 °C in August 2020 (Figure 2c). Salinity varied from 28.9 ± 0.3 in late March 2020 to 33.71 ± 0.09 in July 2021 (Figure 2d).

Mean incubated in situ seawater DOC concentrations (± SE of \(n\) samples during the given incubation) varied from a low of 66 ± 3.8 µmol · L\(^{-1}\) (\(n = 8\)) in January 2020 to a high of 88 ± 0.78 µmol · L\(^{-1}\) (\(n = 12\)) in July 2022. There were no significant paired differences in DOC concentrations between control seawater incubated at lower or higher irradiances (Figure 2e).

**Individual kelp DOC release rate variability**

Mean kelp DOC release rates (mg C · gram dry weight\(^{-1}\) d\(^{-1}\)) (± SE between kelp
replicates) were calculated as least squares means from linear regressions of elapsed incubation
time and the corresponding kelp-derived DOC inventory (mg C · g DW$^{-1}$). These results and
supporting statistical parameters are summarized in Table S1. Kelp DOC release was
significantly ($p < 0.05$) linear for all kelp incubations except one kelp in January 2021 and the
three dead kelp in October 2021. All kelp samples enhanced the DOC inventory relative to the
initial inventory and to the concurrent control inventories (Figure 3, Figure 4, and Figure S1).
Mean DOC release rates varied from a low of 0.18 ± 0.035 mg C · g DW$^{-1}$ · d$^{-1}$ ($n = 6$) in
August 2020 to a high of 1.3 ± 0.26 mg C · g DW$^{-1}$ · d$^{-1}$ ($n = 6$) in November 2022 (Figure 3),
while the absolute minimum rate was 0.066 mg C · g DW$^{-1}$ · d$^{-1}$ for a kelp incubated in August
2020 and the absolute maximum rate was 2.4 mg C · g DW$^{-1}$ · d$^{-1}$ for a kelp incubated in
October 2020 (Table S1). Replicate DOC samples had coefficients of variation (representing
spatial heterogeneity of DOC within the incubation tanks) of 2.7% ± 1.0% (November 2021, $n =
6$), 3.8% ± 1.1% (May 2022, $n = 6$), 7.8% ± 3.1% (July 2022, $n = 6$), 2.0% ± 0.5% (October
2022, $n = 6$), and 3.4% ± 0.8% (November 2022, $n = 6$) (Figure S2).

Relationship of biomass and environmental parameters with DOC release

A linear regression of biomass (g DW · ind$^{-1}$) and DOC release rates per individual kelp
(mg C · ind$^{-1}$ · d$^{-1}$) (Figure 5a) was significant ($p = 1.2 \times 10^{-5}$) and indicates that biomass
explains 21% of the variation in the DOC release rates. However, individual linear regressions
between DOC release rates and biomass within each sampling event indicated that only five (late
March 2020, March 2021, July 2021, November 2021, and May 2022) of the 16 experiments
were significantly linear (Table S2).

A linear regression of photoperiod (hours) and biomass-normalized DOC release rates
(mg C · g DW$^{-1}$ · d$^{-1}$) was significant ($p = 0.011$), explaining 7.5% of the variation in DOC
release rates. The linear relationship was negative, indicating a tendency for kelp treated to longer photoperiods to have lower DOC release rates. There were no statistically significant linear relationships between salinity or sea surface temperature and DOC release rates (Figure 4c and 4d).

**Relationship of irradiance with DOC release**

Mean (± SE) DOC release rates (mg C · g DW⁻¹ · d⁻¹) for each of the four irradiance levels were 0.53 ± 0.084 (200 µmol photons · m⁻² · s⁻¹, n = 44), 0.43 ± 0.10 (400 µmol photons · m⁻² · s⁻¹, n = 26), 0.65 ± 0.12 (1200 µmol photons · m⁻² · s⁻¹, n = 12), and 0.62 ± 0.095 (1500 µmol photons · m⁻² · s⁻¹, n = 3) (Figure 6a). Therefore, there was a slight tendency for higher DOC release rates at higher irradiance exposures, but the differences between groups were not significant and there was no significant (p = 0.36) linear relationship between DOC release rate and PAR exposure (Figure 6a). In addition, the mean daily PAR exposure (mol photons m⁻² s⁻¹), which factors in the seasonally variable photoperiod, did not have a significant (p = 0.73) linear relationship with DOC release rate (Figure 6b).

Paired comparisons of the experimental effect of irradiance from artificial LED sources between qualitatively lower or higher irradiances (i.e., excluding the October and November 2021 results) were also made. Overall, the low irradiance treatment incubations were associated with slightly higher DOC release rates (0.52 ± 0.096, n = 38) compared to the DOC release rates (0.50 ± 0.080, n = 38) from the higher irradiance treatments. There were also no significant differences (p < 0.05, two-tail t-tests) within any of the sampling events comparing mean DOC release rates treated to low or high irradiance levels (Figure 6c).

The October 2021 experiment attempted to compare the effect of artificial and natural irradiance exposure (200 and 2000 µmol photons · m⁻² · s⁻¹), but the kelp (n = 3) treated to
natural irradiance died and DOC release was non-linear. Specifically, DOC release rates were 1.9 ± 0.43 mg C · g DW⁻¹ · d⁻¹ (n = 3) during the initial incubation period (t = 0–1 d), 88 ± 25 mg C · g DW⁻¹ · d⁻¹ (n = 3) in the following period (t = 1–2 d), and −4.7 ± 3.8 mg C · g DW⁻¹ · d⁻¹ (n = 3) in the final period (t = 2–4 d) (Figure 7, Table S1).

The effect of natural irradiance (1500 µmol photons · m⁻² · s⁻¹) was again investigated in the November 2021 experiment, with tissue death avoided (further details provided in the Discussion). There was no significant difference (p = 0.16, two-tail t-test) in DOC release rates between the artificial and natural treatments in that experiment (0.52 ± 0.060 [n = 3] and 0.62 ± 0.096 mg C · g DW⁻¹ · d⁻¹ [n = 3], respectively) (Figure 6c, Figure 7).

**Seasonal growth stage and DOC release**

To quantitatively test significant intra-annual differences, the aggregated data was separated according to the four boreal seasons (based on equinox dates). In terms of overall biomass trends and peak biomass being reached between June and July (Figure 2a), the four seasonal categories can also be ascribed qualitative “growth stage” labels, as follows: winter (early growth), spring (late growth), summer (early decay), and autumn (late decay). The data was also categorized into eight bi-seasonal periods for additional insights, as follows: early winter (n = 6), late winter (n = 4), early spring (n = 12), late spring (n = 12), early summer (n = 18), late summer (n = 6), early autumn (n = 15), and late autumn (n = 12) (Figure 8).

A two-way fixed factor analysis of variance (ANOVA) tested the effect of seasonal period and irradiance level (200, 400, 1200, or 1500 µmol photons · m⁻² · s⁻¹) on DOC release rates using Type-II sums of squares for an unbalanced design. The statistical results indicated that irradiance was not a significant factor (p = 0.83) while seasonal period was a significant factor (p = 0.0013). Tukey Honest Significant Differences pairwise tests then confirmed the
significant differences ($p < 0.05$) were between the autumn “late decay” (0.82 ± 0.12) seasonal period and the winter “early growth” (0.20 ± 0.028, $p = 0.0051$) and summer “early decay” (0.34 ± 0.066, $p = 0.0044$) seasonal periods (Figure 8, Figure S1, Appendix S1, Appendix S2).

**Discussion**

**Linearity of kelp DOC release rates**

Regressions of DOC accumulation over the corresponding incubation period for each individual kelp ($n = 85$) were significantly ($p < 0.05$) linear except for one individual experiment (“low light kelp 1”) in January 2021. This non-linearity was because the DOC concentrations of the third and fifth samples (76 and 77 µmol · L$^{-1}$) were significantly higher than the other samples in that incubation period (68 ± 0.49 µmol · L$^{-1}$, $n = 7$). However, they were not considered true outliers given their concentrations were within two standard deviations of the control tank DOC concentrations for the same period (72 ± 1.3 µmol · L$^{-1}$, $n = 5$, standard deviation = 2.8). In addition, the absolute low mean biomasses in January 2020 (0.45 g DW, $n = 2$) and January 2021 (0.45 g DW, $n = 4$), just 4% of the mean peak biomass in July 2021, resulted in highly sensitive fluctuations in the biomass-normalized kelp-derived DOC inventories (mg C · g DW$^{-1}$). This also explains why biomass-normalized DOC release rates were lower in August 2020 compared to January 2020 and January 2021, despite the actual enhancement of DOC concentration (µmol · L$^{-1}$) being less in the winter months.

While linear regressions between elapsed incubation time and kelp-derived DOC inventory were significant for all other incubated kelp, visual inspections of the individual curves indicate potential non-linearity due to saturation (Figure S1). For example, “low light kelp 1” in late March 2020 reached a peak of 3.6 mg C · g DW$^{-1}$ at $t = 1.7$ days, decreasing to 2.6 mg C · g DW$^{-1}$ by $t = 6.7$ days. Therefore, while the least squares mean DOC release rate was 0.63 mg C
\[ \text{g DW}^{-1} \cdot \text{d}^{-1} \ (R^2 = 0.68, p = 3 \times 10^{-5}), \] the initial phase of positive accumulation (at \( t = 1.7 \) days) might be better represented as 2.1 mg C \cdot g \text{DW}^{-1} \cdot \text{d}^{-1}, while the final DOC inventory (at \( t = 6.7 \) days) implies an overall rate of 0.39 mg C \cdot g \text{DW}^{-1} \cdot \text{d}^{-1}. Therefore, the use of least squares mean DOC release rates in this study is only for relative comparison and is not intended as a predictive tool or an assumption that actual kelp DOC release is linear.

However, it should be noted that concentrations above 600 µmol \cdot L^{-1} were routinely achieved without clear saturating effects, including a peak DOC concentration of 2000 µmol \cdot L^{-1} achieved by “high light kelp 3” in October 2020 (Figure S1k). Also, when including results from the dead kelp in October 2021, “high light kelp 2” exceeded 25000 µmol \cdot L^{-1} (Figure S1w). Therefore, it is unlikely that the “potentially non-linear” outliers can solely be explained by a saturating effect of high DOC concentrations. Instead, more nuanced studies of the complex interactions of ecophysiology are needed. While the results in this study are simplified to assume linear DOC release rates (based on the majority of results exhibiting statistically significant linear regressions), it is important to keep in mind the reality of complex and non-linear interactions when making any generalizations.

**Active exudation: relationship of biomass with DOC release**

To explain the variability in DOC release between biological replicates, the potential for biomass to drive DOC release was considered. Normalizing DOC release rates for biomass is standard practice given the expectation that they are strongly linked. In this study, there was a weak linear relationship indicating that biomass explained 21% of the variation in individual DOC release rates (Figure 5a). However, when assessing kelp biomass and DOC release rate results for each sampling event separately, only five of the 16 sampling event data sets demonstrated a significant linear relationship between biomass and DOC release rate (Table S2).
This indicates that biomass cannot generally be considered significantly linearly correlated with DOC release rates. Moreover, DOC release rates per individual kelp (mg C \cdot ind^{-1} \cdot d^{-1}) did not increase seasonally with increasing biomass before decreasing as biomass was lost due to grazing and erosion. Instead a second peak in DOC release rates was reached in the autumn (October–November) (Figure 8a and 8b) despite the lower biomass (Figure 2a). These multiple lines of evidence demonstrate that biomass was a weak factor in kelp DOC release.

**Active exudation: relationship of irradiance with DOC release**

Irradiance levels are considered an explanatory factor on DOC release rate variability. For example, Reed et al. (2015) indicated that sea surface irradiance was the most significant factor contributing to high temporal variation in *Macrocystis pyrifera* DOC release rates. In that study, irradiance accounted for 13% of the DOC release variation, when blade stage, sampling date, epiphyte load, blade C:N, and temperature were included as the other tested factors (Reed et al., 2015). However, there was no significant effect of irradiance in our results, even after extending the initial experimental design to compare irradiance levels of 200 and 1200 µmol photons \cdot m^{-2} \cdot s^{-1} in the 2022 incubation experiments. In addition, the mean daily PAR exposure (mol photons m^{-2} s^{-1}), which factors in the seasonally variable photoperiod, did not have a significant ($p = 0.73$) linear relationship with DOC release rate (Figure 6b). This indicates that the significant linear relationship between photoperiod and DOC release rate is not related to irradiance exposure but to another seasonal variable.

Because artificial irradiance is qualitatively different from natural light (e.g., ultraviolet spectrum), it is possible that natural irradiance would induce different DOC release responses. We tested this complication in the October 2021 and November 2021 experiments by comparing three kelp incubated at artificial LED irradiance levels of 200 µmol photons \cdot m^{-2} \cdot s^{-1} and three
kelp incubated at natural irradiance levels varying between 1000–2500 µmol photons · m$^{-2}$ · s$^{-1}$.

However, due to kelp death, the October 2021 results were not representative of the effect of natural irradiance. Therefore, only the November 2021 results are discussed in this section on natural irradiance and the October 2021 results will be discussed in the following section on the roles of death and decay.

The November 2021 incubation experiment averaged natural irradiance levels of 1500 µmol photons · m$^{-2}$ · s$^{-1}$ with no signs of tissue death induced. The difference between November 2021 kelp DOC release rates treated to artificial and natural irradiance was not significant (0.52 ± 0.060 [$n = 3$] and 0.62 ± 0.096 mg C · g DW$^{-1}$ · d$^{-1}$ [$n = 3$], respectively) (Figure 6c). Based on this, the primary use of artificial LED instead of natural irradiance in this study was determined to have not significantly skewed DOC release rates.

**Passive exudation: DOC release from decaying and dead kelp**

The October 2021 incubation experiment induced tissue death of the three kelp exposed to direct natural irradiance exceeding 2000 µmol photons · m$^{-2}$ · s$^{-1}$ (in contrast to the decaying but not dead kelp exposed to the lower artificial irradiance). This October 2021 (“late decay” period) result was also an indication that tissue death had not unknowingly occurred in samples from other sampling events. This was concluded based on the nearly two orders of magnitude in difference between DOC release from naturally decaying (but otherwise healthy) kelp and dead kelp in October 2021.

Specifically, DOC release rates were comparable to previous high results (1.9 ± 0.43 mg C · g DW$^{-1}$ · d$^{-1}$, $n = 3$) for the initial incubation period ($t = 0–1$ d), unprecedentedly high (88 ± 25 mg C · g DW$^{-1}$ · d$^{-1}$, $n = 3$) in the following period ($t = 1–2$ d), and negative or stable (−4.7 ± 3.8 mg C · g DW$^{-1}$ · d$^{-1}$, $n = 3$) in the final period ($t = 2–4$ d) (Figure 7, Table S1). The
extremely high DOC release upon death was more than an order of magnitude higher than the highest individual DOC release rate recorded from live kelp (2.4 mg C \cdot g^{-1} \cdot d^{-1}, October 2020) (Table S1). For further context, approximately 10 mg C \cdot g^{-1} \cdot d^{-1} could be considered a critical threshold indicating potential tissue death as it corresponds to 1% of the dry weight biomass being shed as DOC on a daily basis. This indicates that the kelp were likely still alive in the initial period and the extreme DOC release during the following period was a short-term response upon death. Post-death, some DOC may have continued to be passively exuded but microbial consumption of DOC appears to have been the dominant dynamic.

When also considering the lack of a significant difference in DOC release rates treated to natural irradiance in the November 2021 experiment, the high DOC release in the kelp exposed to natural irradiance in October 2021 was determined to be due to tissue death and not representative of the qualitatively different characteristics of natural light. The DOC release rates from the dead kelp (n = 3) in October 2021 are therefore presented separately from the primary dataset.

**Passive exudation: relationship of salinity and temperature changes with DOC release**

A previous study at Oshoro Bay associated the drop in seawater salinity observed from April to May 1999 and concurrent seawater temperature increase with severe lesions and thallus bleaching on *S. japonica* var. *religiosa* (Vairappan et al., 2001) and these seasonal fluctuations may also impact DOC release. Elsewhere, the effect of in situ experimental heatwaves on *Caulerpa prolifera* DOC release indicated no significant difference in DOC release rates between temperature treatments in the summer and a significantly lower (negative) DOC flux in the winter (Egea et al., 2023). At Oshoro Bay, sea surface temperatures have historically varied from 5–22 °C (Motoda, 1971; Motoda et al., 1987; Yotsukura, 2021). Therefore, the August
2020 temperature (23.5 °C) represents a historical exceedance, but the impact of historically high temperatures on DOC release is not clear from this study. Overall, however, linear regressions of temperature and salinity with DOC release rates in our study were not significant ($p = 0.53$ and $p = 0.57$, respectively).

**Comparability of macroalgal DOC release rates**

A wide range of DOC release rates have been reported for different macroalgae (Paine et al., 2021). Three recent studies with useful comparisons are highlighted here. From these comparisons, we can conclude that the results of our study show some comparability to other studies, are generally on the lower end of reported DOC release rates, and that overall, there are several environmental and biological factors that complicate generalized reviews of macroalgal DOC release rate data.

First, a seasonal study on the kelp *Ecklonia cava* in a subtropical bay on the main island of Japan reported mean (± SE) DOC release rates with the minimum value (0.12 ± 0.093 mg C · g DW$^{-1}$ · d$^{-1}$) in August 2003 and the maximum (5.8 ± 1.0 mg C · g DW$^{-1}$ · d$^{-1}$) in April 2004 (Wada et al., 2007). Even though the low August values of Wada et al. (2007) are similar to the August results of this study (0.14 ± 0.038 mg C · g DW$^{-1}$ · d$^{-1}$, $n = 6$; Figure 3 and Table S1), the maximum value was several times that of the highest mean values reported in this study (November 2022, 1.3 ± 0.26 mg C · g DW$^{-1}$ · d$^{-1}$, $n = 6$; Figure 3 and Table S1), and also higher than the overall maximum individual DOC release rate (October 2020, 2.4 mg C · g DW$^{-1}$ · d$^{-1}$; Figure 3 and Table S1). Another study in subtropical Japan by Watanabe et al. (2020), on the brown macroalgae *Sargassum horneri*, was limited to February and March 2020 during the period of peak primary productivity, and results were comparable (1.5 ± 0.62 in February and 1.8 ± 0.62 mg C · g DW$^{-1}$ · d$^{-1}$ in March) to the highest results of this study from the spring “late
growth” and autumn “late decay” periods (Figure 8a). Similar to the highest results from Wada et
al. (2007) and several times higher than the results for this study, cultured *S. japonica* in a
temperate bay in China was reported to release 6.2 (January) and 7.0 (April) mg C · g DW$^{-1}$ ·
d$^{-1}$, despite being the same species as the kelp in this study and of a similar size (0.74 m in
January, 1.3 m in April; Gao et al., 2021). Based on the high individual variability seen in these
studies, it is not clear if such variability between and within studies can also be attributed to
biological stress or are inherent to other local abiotic- and biotic- factors such as physiology,
climate, or in situ natural irradiance.

**Implications of passive DOC release for Blue Carbon estimates**

Quantifying DOC outwelling (i.e., lateral export) from macroalgal habitats remains an
important, yet unresolved component of marine carbon sequestration estimates, despite increased
interest (Santos et al., 2021). Based on the importance of the autumn “late decay” period (Figure
8) and the decoupling from PAR (Figure 6), these results indicate that passive DOC release is as
important or more important than active, photosynthetically driven, DOC release. This means
that extrapolating Blue Carbon estimates from data that does not account for seasonal variation
will be fundamentally flawed.

As a specific example, if only one sampling event had been conducted in this study, the
percent difference between that event’s mean DOC release rate and the weighted annualized
mean (0.45 mg C · g DW$^{-1}$ · d$^{-1}$) could be up to 186% ± 60% (November 2022, $n = 6$) and the
DOC release rate from a single kelp sample could be up to 441% higher (October 2020, “low
light kelp 1”) (Figure 9). Based on seasonal averages, mean DOC release rates from multiple
sampling events only conducted during a single seasonal period would have deviated from the
weighted annualized mean by −55% ± 6% in winter “early growth” ($n = 10$), +14% ± 25% in
spring “late growth” \((n = 24)\), \(-22\% \pm 15\%\) in summer “early decay” \((n = 24)\), or \(+85\% \pm 26\%\) in autumn “late decay” \((n = 27)\). Therefore, attempts to annualize kelp DOC release rates based on data from a few kelp individuals, a few sampling events, or a single season should be considered skeptically.

The kelp DOC release rate results in this study also add to the existing literature that challenges the assumption that DOC release is proportional to biomass or primary production. Instead, our results show that DOC release may be as significant or more significant during a period of senescence when there is effectively no primary production or biomass growth. Previous studies have acknowledged that estimating DOC release as a fraction of primary production is not ideal, but the decoupling shown here and elsewhere is an indication that this practice is highly misleading even as an approximation.

With respect to the growing interest in commercially farmed kelp as a Blue Carbon strategy, the explicit objective of minimizing stressed tissue and harvesting kelp in prime condition, before stress and decay conditions have advanced, could result in disproportionately reducing the actual DOC released in situ and ultimately sequestered. This effect could be further magnified due to the important role of microbially derived or transformed DOC, especially with regard to the microbial carbon pump and the generation of recalcitrant or refractory DOC (Brophy and Carlson, 1989; Ogawa et al., 2001; Jiao et al., 2010; Jiao et al., 2018). Likewise, while detrital export was not quantified in this study, this Blue Carbon pathway is likely also enhanced by stress and decay conditions (Newell et al., 1980; Krumhansl and Scheibling, 2012; Pedersen et al., 2020).

Yet, in light of the negative effects of industrial-scale Blue Carbon projects (Boyd et al., 2022; Ricart et al., 2022), it is critical to emphasize that any Blue Carbon credits should benefit
Indigenous Peoples and governance of balanced social-ecological systems. Indeed, projects centered on pursuing carbon credits for profit as the primary objective are already resulting in unintended consequences in addition to furthering Indigenous dispossession (Asiyanbi, 2016; Morrow et al., 2020).

Implications of passive macroalgal DOC release for Indigenous stewards

While this study was specific to the kelp *S. japonica* var. *religiosa* in Oshoro Bay, there are some qualitative implications that may at least provide insights for other locations and species. Therefore, Indigenous coastal stewards may find the following conclusions from this study relevant in confirming or supplementing existing knowledge from their experiences:

1) Kelp DOC release rates vary throughout the year, even when nutrients are sufficient, and should be closely and frequently monitored for their ecological impacts (Figure 3, 4, 6, and 8);

2) Kelp DOC release rates were highly variable between individual kelp regardless of biomass (especially in late March and October 2020 and 2021), and likely related to individual biological stress conditions (Table S1, Figure 3, 4, 6, and 7);

3) Kelp DOC release did not vary significantly with in situ temperature or salinity, but these are important parameters to monitor for other ecological impacts (Figure 5);

4) Kelp DOC release only required low levels of light, with no significant differences in DOC release rates between irradiance levels from 200 (representative of submerged dense self-shading) to 1500 (representative of periodic direct exposure to sunny weather) \( \mu \text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) or as a function of mean daily PAR (\( \text{mol photons} \cdot \text{m}^{-2} \cdot \text{d}^{-1} \)) (Figure 6);

5) The autumn “late decay” period may be commonly overlooked because of lower biomass
and no growth, yet even DOC release rates factoring in total biomass (mg C · ind\(^{-1}\) · d\(^{-1}\)) were seasonally higher in autumn compared to summer, and both early autumn and late autumn were comparable to the bi-seasonal peak in early summer (Figure 8b);

6) The significantly higher kelp DOC release rates during the autumn “late decay” period and during kelp death may fuel significant microbial activity and potential deoxygenation in more stagnant areas (Figure 7 and 8);

7) Monitoring kelp DOC release is primarily relevant for managing its ecological implications—ecosystem services such as Blue Carbon sequestration should be considered co-benefits of holistic Indigenous social-ecological stewardship that is fully-funded without the need for accounting.

**Conclusion**

This study showed statistically significant (\(p < 0.05\)) seasonal differences in kelp DOC release rates between the autumn “late decay” (0.82 ± 0.12 mg C · g DW\(^{-1}\) · d\(^{-1}\), \(n = 27\)) period and the winter “early growth” (0.20 ± 0.028 mg C · g DW\(^{-1}\) · d\(^{-1}\), \(n = 10\)) and summer “early decay” (0.34 ± 0.066 mg C · g DW\(^{-1}\) · d\(^{-1}\), \(n = 24\)) periods. These seasonal variations in kelp DOC release rates were not attributable to seasonal variations in salinity, sea surface temperature, or biomass. There was also no significant relationship between PAR and DOC release rates across three artificial irradiance levels of 200, 400, and 1200 \(\mu\)mol photons · m\(^{-2}\) · s\(^{-1}\) or when compared to natural irradiance levels of 1500 \(\mu\)mol photons · m\(^{-2}\) · s\(^{-1}\). In total, these three years of year-round data (based on 1091 DOC samples, 88 incubated kelp, and 16 sampling events) provide substantial evidence for the importance of passive exudation over active exudation as the primary driver stimulating seasonal variations in kelp DOC release, even when nitrogen is sufficient year-round. These conclusions indicate that annualizing non-seasonal data
(e.g., in recent Blue Carbon estimates) based on the assumed proportionality of DOC release and primary production is a fundamentally flawed approach.

Indeed, the complex variability of macroalgal DOC release shown here highlights the need for Indigenous stewardship of these ecosystems. Where this fundamental right is already being exercised, these results may guide monitoring and cultivation practices toward greater attention to seasonal variations linked to stress and decay conditions. By monitoring macroalgal ecosystems year-round, Indigenous stewards may be able to assess and anticipate periods of acute environmental changes as well as natural decay periods that could stimulate enhanced macroalgal DOC release. Indigenous stewardship of macroalgal health, including DOC release as an ecophysiological stress response, remains critical for broader social-ecological health.

**Authors’ contributions**

A. K. Carlson: Conceptualization (Lead), Formal analysis (Lead), Funding acquisition (Equal), Investigation (Lead), Methodology (Lead), Writing—original draft preparation (Lead), Writing—review and editing (Lead).

T. Yoshimura: Funding acquisition (Equal), Resources (Equal), Supervision (Supporting), Writing—review and editing (Equal).

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Conflicts of interests

The authors declare that they have no conflicts of interest.

Supplementary information

Supplementary materials are available in the online version of this article.

Data availability

All data analyzed for this publication are included in this article and its supplementary information files. The database in spreadsheet format is available upon request.

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**Figure captions**

Figure 1. July 2021 aerial images showing an overview of Oshoro Bay, the kelp sampling location, temperature and salinity measurement locations, and reference seawater sampling locations. An inset of the relative location of Oshoro Bay on the Shaktan peninsula in western Ainu Mosir (Hokkaido) is also provided (Google Earth 2021).

Figure 2. Annual cycles of biomass and environmental parameters. Data are (a) means (± SE between kelp, \( n = 2–6 \)) of individual kelp dry weight biomasses (g DW · ind\(^{-1} \)), (b) photoperiod hours used for the incubation experiments, (c) means (± SE between sampling locations, \( n = 9 \)) of in situ sea surface temperature (°C), (d) means (± SE between sampling locations, \( n = 9 \)) of salinity (parts per thousand), and (e) concentrations of DOC in the incubated control seawater tanks (± SE between samples within the respective incubation period). In situ sampling locations are shown in Figure 1. Low irradiance designations indicate exposure to 200 µmol photons · m\(^{-2} \)
· s\(^{-1}\) from artificial LED sources. High irradiance designations vary and indicate exposure to 400 (incubation experiments from January 2020 to July 2021) or 1200 (incubation experiments from May 2022 through November 2022) µmol photons · m\(^{-2}\) · s\(^{-1}\) from artificial LED or 1500 (November 2021) µmol photons · m\(^{-2}\) · s\(^{-1}\) from natural irradiance. Some error bars are smaller than the data symbol.

Figure 3. Mean individual kelp DOC release rates by sampling event. Black data points are means (± SE) of the individual kelp DOC release rates (mg C · g DW\(^{-1}\) · d\(^{-1}\)) within each sampling event. Some error bars are smaller than the data symbol. White data points represent the individual kelp DOC release rates calculated as least squares means from linear regressions of DOC concentrations (\(n = 5–17\) samples per incubation) at regular intervals during the incubation period (\(t = 4–9\) days). Each sampling event incubated \(n = 6\) kelp except January 2020 (\(n = 2\)), January 2021 (\(n = 4\)), early March 2020 (\(n = 4\)), and October 2021 (\(n = 3\)).

Figure 4. Mean kelp-derived DOC inventory accumulation. Data are mean (± SE) kelp-derived DOC inventory (mg C · g DW\(^{-1}\)) of all kelp (\(n = 6\), except January 2020 [\(n = 2\)], January 2021 [\(n = 4\)], early March 2020 [\(n = 4\)], and October 2021 [\(n = 3\)]) for a given sampling event as a function of elapsed incubation time (\(t = 4–9\) days). Data are categorized seasonally into (a) winter, (b) spring, (c) summer, and (d) autumn.

Figure 5. DOC release rates and environmental parameters. Relationships between (a) the individual kelp DOC release rates (mg C · ind\(^{-1}\) · d\(^{-1}\)) and corresponding individual kelp biomass (g DW · ind\(^{-1}\)), as well as the individual biomass normalized kelp DOC release rates (mg C · g DW\(^{-1}\) · d\(^{-1}\)) and (b) photoperiod hours, (c) mean in situ sea surface temperature (°C), and (d) mean in situ salinity. DOC release rates (\(n = 85\)) are least squares means for each individual kelp across the 16 sampling events. Linear regression equations and significance of the slope
coefficient shown. Significant linear trendline \( (p < 0.05) \) only applicable to (a) and (b).

Figure 6. Irradiance and DOC release rates. Data are (a) individual kelp DOC release rates (mg C \( \cdot g \text{ DW}^{-1} \cdot d^{-1} \)) as a function of their corresponding irradiance treatment, with mean (± SE) DOC release rates in black aggregating values within each of the four irradiance treatment levels (200, 400, 1200, and 1500 \( \mu \text{mol photons} \cdot m^{-2} \cdot s^{-1} \)), (b) mean (± SE) individual kelp DOC release rates (mg C \( \cdot g \text{ DW}^{-1} \cdot d^{-1} \)) as a function of their corresponding mean daily PAR exposure (mol photons \( \cdot m^{-2} \cdot d^{-1} \)), and (c) mean DOC release rates (± SE) for their corresponding qualitative (low or high) irradiance treatment within each sampling event \( (n = 16) \). Low irradiance designations indicate exposure to 200 \( \mu \text{mol photons} \cdot m^{-2} \cdot s^{-1} \) from artificial LED sources. High irradiance designations vary and indicate exposure to 400 (incubation experiments from January 2020 to July 2021) or 1200 (incubation experiments from May 2022 through November 2022) \( \mu \text{mol photons} \cdot m^{-2} \cdot s^{-1} \) from artificial LED or 1500 (November 2021) \( \mu \text{mol photons} \cdot m^{-2} \cdot s^{-1} \) from natural irradiance. There were no significant differences \( (p < 0.05, \text{two-tail } t\text{-tests}) \) within any of the sampling events comparing mean DOC release rates treated to low or high irradiance levels.

Figure 7. Mean DOC release rates from live senescent kelp and three stages of kelp death (pre-death, death phase 1, and death phase 2) in October 2021. November 2021 results comparing live kelp under artificial and natural irradiance are included to demonstrate extreme DOC release in October 2021 was related to kelp death and not the effect of natural irradiance.

Figure 8. Mean DOC release rates by season. Data are means (± SE) of kelp DOC release rates represented as a) biomass normalized rates (mg C \( \cdot g \text{ DW}^{-1} \cdot d^{-1} \)) and (b) raw rates per individual kelp (mg C \( \cdot \text{ind}^{-1} \cdot d^{-1} \)), with results aggregated according to eight bi-seasonal categories. Shading and border effects visually differentiate results by the four seasons.
Figure 9. Percent difference (%) of experimental DOC release rates from weighted annualized mean DOC release rate. Data are (a) means (± SE) of the percent differences (%) for each sampling event in black, with individual kelp results in white or (b) means (± SE) of the % differences for each sampling event aggregated according to bi-seasonal period. The annualized mean DOC release rate of 0.45 mg C · g DW\(^{-1}\) · d\(^{-1}\) was calculated from bi-seasonal weighted averages.

**Supplementary material captions**

Figure S1. Incubation time-series of volume-corrected DOC concentrations (µmol · L\(^{-1}\)) and biomass-normalized kelp-derived DOC inventories (mg C · g DW\(^{-1}\) · d\(^{-1}\)) for all incubated kelp (\(n = 88\)) and control seawater (\(n = 31\)). Low light designations indicate exposure to photosynthetically active radiation (PAR) of 200 µmol photons · m\(^{-2}\) · s\(^{-1}\) from artificial LED sources. High light designations vary and indicate exposure to 400 (incubation experiments from January 2020 to July 2021) or 1200 (incubation experiments from May 2022 through November 2022) µmol photons · m\(^{-2}\) · s\(^{-1}\) from artificial LED sources, or 1500 (November 2021) or 2000 (October 2021) µmol photons · m\(^{-2}\) · s\(^{-1}\) from natural irradiance. Each pair of graphs correspond to the 16 incubation experiments conducted in (a)–(b) January 2020, (c)–(d) early March 2020, (e)–(f) late March 2020, (g)–(h) June 2020, (i)–(j) August 2020, (k)–(l) October 2020, (m)–(n) January 2021, (o)–(p) March 2021, (q)–(r) April 2021, (s)–(t) July 2021, (u)–(v) October 2021 (live kelp), (w)–(x) October 2021 (dead kelp), (y)–(z) November 2021, (aa)–(ab) May 2022, (ac)–(ad) July 2022, (ae)–(af) October 2022, and (ag)–(ah) November 2022.

Figure S2. Coefficients of variation (%) for replicated DOC samples at the end of incubation experiments in November 2021, May 2022, July 2022, October 2022, and November 2022. Results represent the spatial heterogeneity of DOC within the incubation tanks.
Table S1. Linearity of kelp DOC release rates. DOC release rates (mg C · g DW\(^{-1} \cdot d^{-1}\)) are least squares means from linear regressions of elapsed incubation time and the corresponding kelp-derived DOC inventory (mg C · g DW\(^{-1}\)). Sample sizes (\(n = 5–17\)) represent the number of incubation seawater samples taken at regular intervals throughout each incubation period (\(t = 4–9\) days) and analyzed for DOC. Kelp DOC release was significantly (\(p < 0.05\)) linear for all kelp incubations except one kelp in January 2021 and the three dead kelp in October 2021. Shading and borders correspond to seasonal categories (Figure 8).

Table S2. Linear regression of kelp biomass and DOC release rate by sampling event. Kelp biomass (g DW · ind\(^{-1}\)) is individual dry weight and DOC release rates are least squares means (mg C · ind\(^{-1} \cdot d^{-1}\)) calculated from linear regressions of volume-corrected DOC concentrations (\(n = 5–17\) samples per incubation) throughout the incubation period (\(t = 4–9\) days). Sample size corresponds to the number of kelp incubated for a given sampling event (\(n = 6\), except for January 2020 [\(n = 2\)], early March 2020 [\(n = 4\)], January 2021 [\(n = 4\)], and October 2021 [\(n = 3\)].

Appendix S1. Effect of bi-seasonal period and PAR on DOC release: two-way ANOVA (Type II) and Tukey HSD test results with R code

Appendix S2. Effect of seasonal period and PAR on DOC release: two-way ANOVA (Type II) and Tukey HSD test results with R code
Kelp DOC release rate (mg C·g DW⁻¹·d⁻¹)

Sampling date

Jan  Feb  Mar  Apr  May  Jun  Jul  Aug  Sep  Oct  Nov  Dec

○ 2020  □ 2021  △ 2022
Kelp-derived DOC inventory (mg C g DW$^{-1}$)

Incubation time (days)

Winter 2020–2022 experiments

- January 2020
- January 2021
- Early March 2020

Spring 2020–2022 experiments

- Late March 2020
- March 2021
- April 2021
- May 2022

Summer 2020–2022 experiments

- June 2020
- July 2021
- July 2022
- August 2020

Autumn 2020–2022 experiments

- October 2020
- October 2021
- October 2022
- November 2021
- November 2022
y = 0.3526x + 0.6711
$R^2 = 0.2072$, $p = 1.2 \times 10^{-5}$

Kelp DOC release rate (mg C · ind$^{-1}$ · d$^{-1}$) vs Kelp biomass (g DW · ind$^{-1}$)

y = -0.0717x + 1.4359
$R^2 = 0.0809$, $p = 0.0083$

Kelp DOC release rate (mg C · g DW$^{-1}$ · d$^{-1}$) vs Photoperiod (hours)

y = -0.0067x + 0.6321
$R^2 = 0.0047$, $p = 0.53$

Kelp DOC release rate (mg C · g DW$^{-1}$ · d$^{-1}$) vs Sea surface temperature (°C)

y = -0.0253x + 1.3473
$R^2 = 0.004$, $p = 0.57$

Kelp DOC release rate (mg C · g DW$^{-1}$ · d$^{-1}$) vs Salinity
\[ y = 0.0012x + 0.5165 \]
\[ R^2 = 0.0014, \quad p = 0.73 \]

(a) Kelp DOC release rate (mg C · g DW \(^{-1}\) · d\(^{-1}\)) vs. PAR (µmol photons · m\(^{-2}\) · s\(^{-1}\)).

(b) Kelp DOC release rate (mg C · g DW \(^{-1}\) · d\(^{-1}\)) vs. Mean daily PAR (mol photons · m\(^{-2}\) · d\(^{-1}\)).

(c) Sampling date:

- 2020 High irradiance
- 2021 High irradiance
- 2022 High irradiance
- 2020 Low irradiance
- 2021 Low irradiance
- 2022 Low irradiance

Kelp DOC release rate (mg C · g DW \(^{-1}\) · d\(^{-1}\))
(a) Percent difference (%) from annualized mean DOC release rate

(b) Percent difference (%) from annualized mean DOC release rate

Sampling date

Season

Early winter Late winter Early spring Late spring Early summer Late summer Early autumn Late autumn
(ae) DOC concentration (µmol · L$^{-1}$) over time for the period 19–23 October 2022.

(af) Kelp-derived DOC inventory (mg C · g DW$^{-1}$) over time for the period 19–23 October 2022.

(ag) DOC concentration (µmol · L$^{-1}$) over time for the period 29 November–4 December 2022.

(ah) Kelp-derived DOC inventory (mg C · g DW$^{-1}$) over time for the period 29 November–4 December 2022.

- **Low Light Kelp 1**
- **Low Light Kelp 2**
- **Low Light Kelp 3**
- **Average**
- **High Light Kelp 1**
- **High Light Kelp 2**
- **High Light Kelp 3**
- **Average (dead kelp)**
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<td>January 2020–November 2022</td>
<td>0.35</td>
<td>0.22</td>
<td>$7 \times 10^{-6}$</td>
<td>85</td>
<td>Yes</td>
<td></td>
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<tr>
<td>January 2020</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>2</td>
<td>NA</td>
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<tr>
<td>early March 2020</td>
<td>0.10</td>
<td>0.066</td>
<td>0.74</td>
<td>4</td>
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<tr>
<td>late March 2020</td>
<td>1.2</td>
<td>0.84</td>
<td>0.010</td>
<td>6</td>
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<tr>
<td>June 2020</td>
<td>$-0.92$</td>
<td>0.12</td>
<td>0.50</td>
<td>6</td>
<td>No</td>
<td></td>
<td></td>
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<tr>
<td>August 2020</td>
<td>0.31</td>
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<td>0.27</td>
<td>6</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>October 2020</td>
<td>1.1</td>
<td>0.13</td>
<td>0.48</td>
<td>6</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>January 2021</td>
<td>0.14</td>
<td>0.49</td>
<td>0.30</td>
<td>4</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 2021</td>
<td>2.5</td>
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<td>0.035</td>
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<tr>
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<td></td>
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<tr>
<td>July 2021</td>
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<tr>
<td>October 2021</td>
<td>$-0.64$</td>
<td>0.51</td>
<td>0.50</td>
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<tr>
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<td>0.32</td>
<td>0.24</td>
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<tr>
<td>May 2022</td>
<td>0.99</td>
<td>0.70</td>
<td>0.037</td>
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<tr>
<td>July 2022</td>
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<tr>
<td>October 2022</td>
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<td>0.15</td>
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<td></td>
</tr>
<tr>
<td>November 2022</td>
<td>0.27</td>
<td>0.038</td>
<td>0.71</td>
<td>6</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
> Bi_SeasonalDOC.lm <- lm(normDOC ~ Bi_Season + PAR, data = DOC_release_data)
> Bi_SeasonalDOC.II.aov <- car::Anova(Bi_SeasonalDOC.lm, type = 2)
> Bi_SeasonalDOC.II.aov
Anova Table (Type II tests)

Response: normDOC

| Sum Sq Df F value  Pr(>F) |
|------------------------|------------------------|
| Bi_Season  4.7036 7 2.8776 0.01014 * |
| PAR  0.0415 1 0.1778 0.67445 |
| Residuals 17.7465 76 |

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> glht.Tukey <- glht(Bi_SeasonalDOC.lm,linfct=mcp(Bi_Season="Tukey"))
> summary(glht.Tukey)

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: lm(formula = normDOC ~ Bi_Season + PAR, data = DOC_release_data)

Linear Hypotheses:

| Estimate Std. Error t value Pr(>|t|) |
|-------------------------------|------------------------|
| early_spring - early_autumn == 0 -0.2027678  0.1702270  -1.191  0.9273 |
| early_summer - early_autumn == 0 -0.3224207  0.1689397  -1.908  0.5343 |
| early_winter - early_autumn == 0 -0.5456850  0.2343551  -2.328  0.2795 |
| late_autumn - early_autumn == 0  0.2019817  0.1937306   1.043  0.9636 |
| late_summer - early_autumn == 0  0.3330988  0.2366305   1.408  0.8423 |
| late_winter - early_autumn == 0  0.6099900  0.2343551   2.603  0.1629 |
| late_spring - early_autumn == 0  0.5455741  0.2727291   2.000  0.4735 |
| late_summer - early_spring == 0 -0.1196530  0.1623023  -0.737  0.9951 |
| late_summer - late_spring == 0 -0.1303311  0.2355048  -0.553  0.9992 |
| late_summer - late_winter == 0 -0.4047494  0.1935688   2.091  0.4153 |
| late_summer - early_winter == 0 -0.3429172  0.2277942  -1.505  0.6158 |
| late_winter - early_summer == 0 -0.4033311  0.2355048  -1.788  0.6158 |
| late_summer - late_winter == 0 -0.3428064  0.2671124  -1.283  0.8959 |
| late_summer - early_summer == 0 -0.2232643  0.2286638  -0.976  0.9746 |
| late_summer - early_winter == 0  0.5244024  0.1871837   2.802  0.1038 |
| late_summer - late_winter == 0 -0.0106781  0.2312529  -0.046  1.0000 |
| late_summer - late_summer == 0 -0.2875693  0.2286638  -1.258  0.9054 |
| late_summer - late_summer == 0 -0.2231534  0.2678544  -0.833  0.9898 |
| late_summer - late_winter == 0  0.7476667  0.2518213   2.969  0.0690 |
| late_summer - early_winter == 0  0.2125861  0.2853202   0.745  0.9948 |
| late_summer - early_winter == 0 -0.0643050  0.2789898  -0.230  1.0000 |
| late_summer - early_winter == 0  0.0001108  0.3119201  0.000  1.0000 |
late_spring - late_autumn == 0  -0.5350805  0.2418720  -2.212  0.3425
late_summer - late_autumn == 0  -0.8119717  0.2518213  -3.224  0.0348 *
late_winter - late_autumn == 0  -0.7475558  0.2878764  -2.597  0.1646
late_summer - late_spring == 0  -0.2768911  0.2853202  -0.970  0.9754
late_winter - late_spring == 0  -0.2124753  0.3175948  -0.669  0.9973
late_winter - late_summer == 0  0.0644158  0.3119201   0.207  1.0000

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
> SeasonalDOC.lm <- lm(normDOC ~ Season + PAR, data = DOC_release_data)
> SeasonalDOC.II.aov <- car::Anova(SeasonalDOC.lm, type = 2)
> SeasonalDOC.II.aov

Anova Table (Type II tests)

Response: normDOC

<table>
<thead>
<tr>
<th>Sum Sq</th>
<th>Df</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>3.9956</td>
<td>3</td>
<td>5.7737</td>
</tr>
<tr>
<td>PAR</td>
<td>0.0112</td>
<td>1</td>
<td>0.0488</td>
</tr>
<tr>
<td>Residuals</td>
<td>18.4544</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

---

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> glht.Tukey <- glht(SeasonalDOC.lm, linfct = mcp(Season = "Tukey"))
> summary(glht.Tukey)

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: lm(formula = normDOC ~ Season + PAR, data = DOC_release_data)

Linear Hypotheses:

| Estimate | Std. Error | t value | Pr(>|t|) |
|----------|------------|---------|---------|
| Spring - Autumn == 0 | 0 -0.3190 | 0.1373 | -2.324 0.09923 . |
| Summer - Autumn == 0 | 0 -0.4780 | 0.1373 | -3.482 0.00442 ** |
| Winter - Autumn == 0 | 0 -0.6260 | 0.1823 | -3.435 0.00506 ** |
| Summer - Spring == 0 | 0 -0.1590 | 0.1386 | -1.147 0.65817 |
| Winter - Spring == 0 | 0 -0.3070 | 0.1813 | -1.694 0.32967 |
| Winter - Summer == 0 | 0 -0.1481 | 0.1813 | -0.817 0.84418 |

---

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Adjusted p values reported -- single-step method)