Vol. 11: 671–684, 2019
https://doi.org/10.3354/aei00340

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published December 19

Using macroalgal bioindicators to map nutrient plumes from fish farms and other sources at a bay-wide scale

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ABSTRACT: Human activities can elevate coastal levels of dissolved inorganic nitrogen (DIN). As macroalgae readily absorb and accumulate DIN, the elemental (total N and C:N ratio) composition of their tissues is less affected by temporal fluctuations compared to more direct measures of DIN concentration. Additionally, their isotopic ($\delta^{15}N$) composition can reflect that of the source, which could potentially be used to identify between multiple effluent sources. To investigate whether macroalgal 'bioindicators' could map and distinguish between multiple effluents, 2 species of macroalgae (*Chondrus crispus* and *Palmaria palmata*) were deployed in a bay containing a salmon farm and sewage treatment facility. Both species exhibited high total N and low C:N ratio near the salmon farm and sewage facility. However, the elemental composition of *C. crispus* was influenced over a greater distance than that of *P. palmata*. Differences were also observed between their isotopic composition, as *C. crispus* indicated that the salmon farm and sewage facility had distinct $\delta^{15}N$ signatures, whereas values of $\delta^{15}N$ in *P. palmata* had not changed after 10 d incubation in the field. Interestingly, the distinct isotopic signals observed in *C. crispus* were likely a result of higher DIN concentrations at the salmon farm, which likely caused macroalgae to fractionate and form biomass lighter in $\delta^{15}N$. Overall, this study suggests that macroalgal bioindicators can monitor and identify between multiple effluent sources, which could provide a useful tool for coastal management. However, some species of macroalgae may make more effective bioindicators than others, and the mechanisms underlying their fractionation require further investigation.

KEY WORDS: Aquaculture · $\delta^{15}N$ · Isotope · Macroalgae · Nitrogen · Salmon · Sewage · Wastewater

1. INTRODUCTION

Human activities can elevate nutrient levels in coastal environments (Nixon 1995, Smith 2003, Eddy 2005). Nitrogen (N) loading is of particular concern, as it can lead to algal blooms, oxygen depletion and biodiversity loss (Nixon 1995, Howarth et al. 2011). Given that net-pen aquaculture releases N and other wastes into the surrounding water, monitoring its effluents has developed into a very active area of research (e.g. Cloern 2001, Callier et al. 2013, Jansen et al. 2018). However, most water bodies also receive effluents from other sources, such as agriculture, urbanisation, industrialisation and wastewater treatment facilities (Leonard et al. 1997, Costanzo et al. 2005, Alquezar et al. 2013). Hence, there are calls for a change in the way aquaculture is monitored and managed, from the current approach where the localised effects of single farms are regarded, towards an ecosystem approach to aquaculture, whereby the

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effects of multiple farms and human activities are simultaneously considered at a wider scale (FAO 2007, 2010).

Fish farms release nutrients into the environment as either particulate or dissolved wastes. Particulate wastes derive from faeces and uneaten feed, and represent between 13 and 32% of all the N released from fish farms (Islam 2005, Holmer et al. 2007, Sanderson et al. 2008, Callier et al. 2013). These particulate wastes quickly settle onto the seafloor and rarely disperse more than a few hundred metres (Brager et al. 2015, Price et al. 2015, Bannister et al. 2016, Filgueira et al. 2017). Over the last 3 decades, the amount of particulate wastes produced by fish farms has been significantly reduced due to the development of more efficient feeds and feeding systems (Islam 2005, Sørensen 2012, Sprague et al. 2016). In contrast, dissolved wastes are excreted by fish directly into the water column and represent between 68 and 87% of N (Wang et al. 2012). Although this dissolved fraction makes up the majority of fish farm wastes, less is known about its dispersal and persistence within the marine environment (Price et al. 2015, Jansen et al. 2018).

Fish farm effluents are enriched in dissolved inorganic N (DIN), a term which includes nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_3) and ammonium (NH_4^+). Up to 90% of all the N excreted by marine fish occurs as NH_3, which quickly converts to NH_4^+ at the pH of seawater (reviewed by Leung et al. 1999). Consequently, several studies have reported elevated NH_4^+ concentrations close to fish farms (Navarro et al. 2008, Sanderson et al. 2008, Jansen et al. 2018). However, a recent and comprehensive review showed that most studies to date have found no evidence of fish farms increasing DIN concentrations (Price et al. 2015). These varying and conflicting results can be attributed to several factors. First, background nutrient concentrations, and the release of nutrients from fish farms, both exhibit strong daily pulses and seasonal fluctuations (Karakassis et al. 2001). Second, fish farms are often purposefully located in areas of high water exchange, quickly dissolving and dispersing any dissolved wastes (Dalsgaard & Krause-Jensen 2006). Third, any inputs of DIN are rapidly assimilated by marine organisms and lost to the atmosphere through volatilization (Dailer et al. 2010). Hence, any increase in DIN is likely to be small, short-lived and difficult to detect. For these reasons, monitoring dissolved nutrient concentrations is prone to large margins of error, making it time and cost expensive due to the need for continual and repeated sampling (Dalsgaard & Krause-Jensen 2006).

Using macroalgae as biological indicators (or 'bioindicators') is an alternative method of assessing dissolved nutrient levels (García-Seoane et al. 2018). Macroalgae readily absorb and accumulate N, meaning the N content of their tissues is less affected by short-term fluctuations compared to more direct measures of DIN concentration (Chopin et al. 1995, García-Sanz et al. 2010, Carballeira et al. 2013). Also, macroalgal bioindicators only absorb the fraction of nutrients that are bioavailable (i.e. the fraction responsible for eutrophication) and can be logistically simpler and more time effective (Costanzo et al. 2001, Dailer et al. 2010). Lastly, and of growing interest, is the potential for their N isotope composition to identify and distinguish between multiple effluent sources (Heaton 1986, Costanzo et al. 2001, Lemesle et al. 2016).

The N isotope composition of a sample is generally measured as the ratio of ^{15}N to ^{14}N relative to air, and expressed on a delta scale ($\delta^{15}N$) in units of ‰ (Peterson & Fry 1987). Although isotopes undergo the same biochemical pathways, the cells of most organisms will preferentially take up the lighter ^{14}N isotope due to faster reaction times and metabolic processes (Mariotti et al. 1982, Dailer et al. 2010, Newton 2010). This process of 'fractionation' generally occurs throughout the trophic web, which is why $\delta^{15}N$ usually increases with trophic level (Mill et al. 2007). Interestingly, fractionation can cause different effluent sources to exhibit distinct isotopic signatures. For instance, sewage tends to be high in $\delta^{15}N$ (8~30‰), as it is derived from the wastes of humans and animals at high trophic levels (Dailer et al. 2010, García-Sanz et al. 2010). Also, bacteria involved in sewage treatment fractionate the available pool of N, further enriching the effluent in ^{15}N (Middlebrooks & Pano 1983, Bannon & Roman 2008). In contrast, fish farm effluents tend to have moderate $\delta^{15}N$ values (8~11‰) due to the use of feeds derived from both animal (^{15}N-enriched) and plant (^{15}N-depleted) ingredients (García-Sanz et al. 2010, Wang et al. 2014). Pulp mill effluents tend to have low $\delta^{15}N$ values due to their use of terrestrially derived wood chips (Wayland & Hobson 2001, Oakes et al. 2010). Lastly, agricultural effluents tend to have $\delta^{15}N$ values close to 0 because of the application of fertilizers enriched in NH_4^+ or NO_3^- fixed from atmospheric N (Costanzo et al. 2001, Dailer et al. 2010). As macroalgae are widely believed to take up ^{15}N in proportion to its availability, the $\delta^{15}N$ of their tissues can reflect the signature of the dominant source of N (Gartner et al. 2002, García-Sanz et al. 2010, Lemesle et al. 2015).

Macroalgal bioindicators have been used to monitor effluents in coastal waters since the late 1970s

(reviewed by García-Seoane et al. 2018). The majority of these studies have relied on collecting native species occurring naturally within an area. However, a growing number have transplanted live individuals from one location to another. The advantages of this approach are that macroalgae can be: (1) deployed in areas where do they not naturally occur (e.g. in highly impacted areas or in deep water); (2) conditioned to be isotopically similar prior to their deployment; (3) starved of N so they absorb it more readily; and (4) exposed to effluents for controlled periods of time (Costanzo et al. 2001, Alquezar et al. 2013, García-Seoane et al. 2018). For example, Costanzo et al. (2001) used transplanted macroalgae to map wastewater effluents in Australia and were able to track improvements made to wastewater management practices within the region. Transplanted macroalgae have also been used to map effluents emanating from fish farms in Japan (Yokoyama & Ishihi 2010). However, to date, few attempts have been made to map aquaculture effluents in relation to other sources.

The aim of this study was to investigate whether macroalgal bioindicators could map the footprint of multiple effluents, and to gain a greater insight into the dispersal of dissolved aquaculture wastes. Two macroalgae species were deployed across a bay containing a salmon farm and a sewage treatment facility to test the following hypotheses: (1) macroalgae deployed near the salmon farm and sewage facility will display elevated $\delta^{15}N$ values, reflecting the composition of the dominant N source; and (2) the total N content of macroalgae will be higher near anthropogenic sources of N. If macroalgal bioindicators prove capable of mapping the footprint of multiple effluents, they could provide a useful tool in helping coastal management transition towards an ecosystem approach to aquaculture.

2. MATERIALS AND METHODS

2.1. Study region

Liverpool Bay is located along the southern shore of Nova Scotia in eastern Canada (Fig. 1). The bay measures approximately 6 km long and 2 km wide, and has a maximum depth of 40 m, a tidal range of 2 m and a flushing time of 65 h (Gregory et al. 1993,

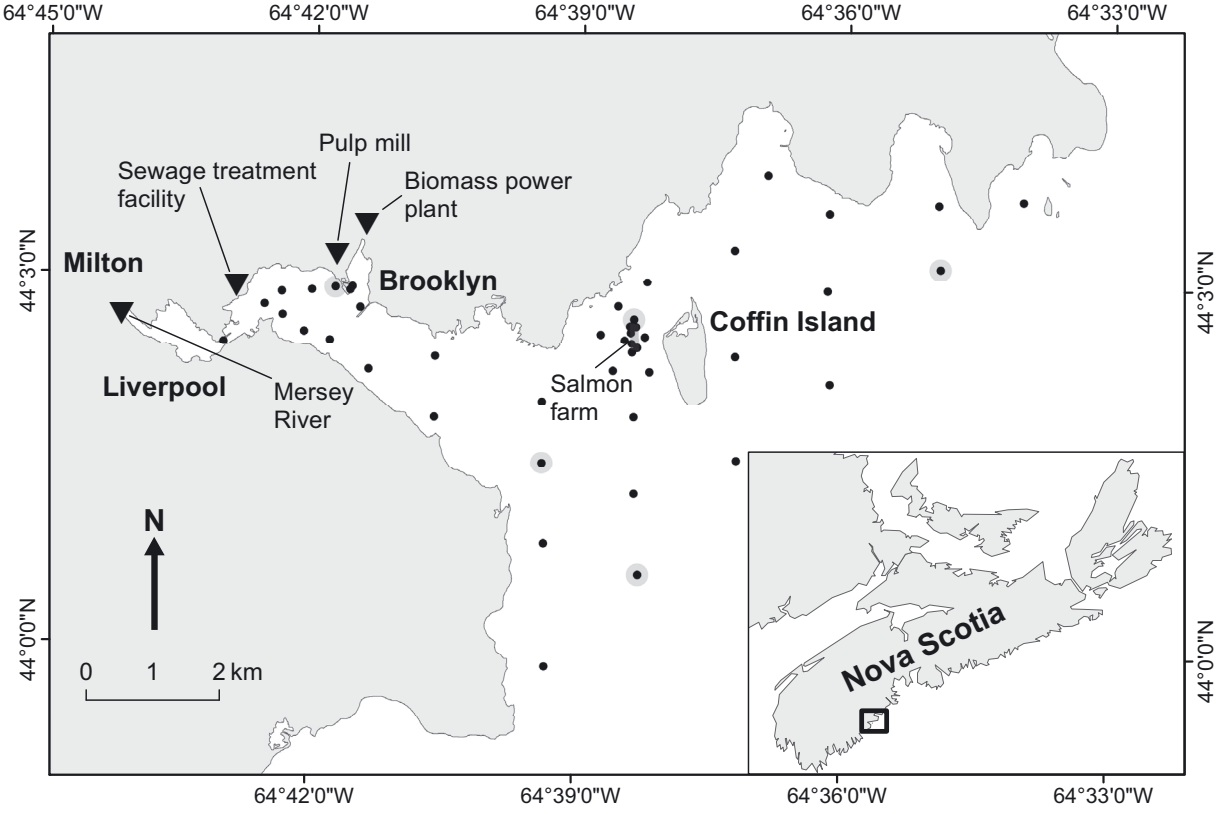

Fig. 1. Liverpool Bay, Nova Scotia, Canada, indicating towns, key geographical features and probable point sources. Points indicate the locations of the deployments of the macroalgae *Chondrus crispus* and *Palmaria palmata*. Grey circles indicate stations where surface water samples were taken

Stewart & White 2001). The Mersey River discharges into the northwest of the bay and has the largest outflow and watershed of all Nova Scotian rivers (Davis & Browne 1998). Between 2014 and 2018, the Mersey River exhibited the highest daily mean (±SE) discharge rate of 190 ± 21.4 $m^3 s^{-1}$ in December, and the lowest daily discharge of 18 ± 8.6 $m^3 s^{-1}$ in August (www.wateroffice.ec.gc.ca). The discharge of the Mersey River was <4 $m^3 s^{-1}$ at the time of this study.

The drainage catchment of Liverpool Bay encompasses the towns of Brooklyn, Liverpool and Milton, which have a combined population of 4460 (Statistics Canada 2016). Industrial and urban developments in the area include a biomass power plant (in part-time operation), a scallop processing plant, commercial docking wharfs (>50 registered fishing vessels), a shipyard and a marina (Stewart & White 2001). A pulp mill, which has been decommissioned since June 2012, is believed to have created large deposits of sawdust on the surrounding seafloor (V. Fisher pers. comm.). There is also a municipal wastewater treatment facility consisting of 3 aerated lagoons capable of ultraviolet disinfection that release treated sewage from the surrounding towns into Liverpool Harbour (A. Grant pers. comm.). Lastly, a salmon (Atlantic salmon *Salmo salar*) farm was built to the west of Coffin Island in 2010 and consists of 14 pens, 32 m in diameter, situated at depths of 12–16 m. Salmon production is approximately a 2 yr cycle which starts with the stocking of smolts (body mass ~110 g) in the spring. These are later harvested as full-size adults (body mass ~6 kg) during the winter of the following year, and the farm is left fallow until the next spring.

2.2. Field sampling

Fieldwork was conducted in Liverpool Bay in August 2018. Forty stations (Fig. 1) were designated to cover as wide an area as logistically possible but were spatially biased to provide greater sampling in areas close to probable point sources (i.e. the salmon farm and sewage treatment facility). At each station, approximately 20 g of the macroalgae *Chondrus crispus* and *Palmaria palmata* were placed in separate transparent, perforated chambers and suspended in the water column at a depth of 3 m using a combination of buoys, leaded ropes, weights and anchors (see Costanzo et al. 2001, 2005). An additional 5 chambers were hung directly from the outer rim of the salmon pens using just ropes and weights. All chambers were then left to incubate for 10 d based on immersion times trialled by Lemesle et al. (2016). In addition, 1 l surface water samples (n = 10) were collected using sterilised containers, half of which were collected during sample deployment and the other half during sample retrieval. After retrieval, all macroalgae and water samples were immediately stored in the dark at 5°C for 12 h before being relocated to a −20°C freezer.

This experiment took place 3 mo before the salmon harvest, when salmon biomass was near its peak. This likely corresponded with high rates of feeding in order to finish the salmon at maximum harvestable size. In addition, field sampling took place during the summer when sea surface temperatures are highest and background NH_4^+ and NO_3^- concentrations are at their lowest (Gregory et al. 1993, Keizer et al. 1996). Hence, this experiment represents an extreme case scenario of low ambient nutrient concentrations and high fish production.

2.3. NO_3^- and NH_4^+ concentration analysis

Water samples were defrosted and filtered using a 0.45 µm sterile syringe filter. NO_3^- was measured by hot vanadium reduction of NO_3^- to nitric oxide using an Analytical Sciences NOx 5100 Thermalox detector (Braman & Hendrix 1989). For this, 2 ml of each water sample (in duplicate) were injected into the vanadium (III) solution and were run with bracketing NO_3^- standards of between 0 and 10 µM. Samples were also measured for NO_2^- concentration using the sulfanilamide and napthal-ethylenediamine colorimetric method (Pai et al. 1990). However, NO_2^- was absent, indicating the samples measured on the NOx detector were NO_3^- only. NH_4^+ concentrations were analysed using the phenol blue method and were measured on a Thermo Scientific Evolution 260 Bio UV-Visible Spectrophotometer (Solórzano 1969). For this, 2 ml per sample (in duplicate) were reacted with bracketing standards of ammonium chloride between 0 and 10 µM.

2.4. Source of macroalgae

Macroalgae can exhibit large spatial and temporal variations in isotope composition which could confound their ability as bioindicators (Raimonet et al. 2013, Lemesle et al. 2016). Consequently, the *C. crispus* and *P. palmata* used in this study were reared at a land-based hatchery (www.acadianseaplants.com) and were collected 24 h before deployment and stored in the dark at 5°C. Unlike wild-collected specimens, these

macroalgae were grown from the same brood stock in identical environmental conditions, resulting in similar physical condition and isotopic composition prior to deployment. However, the 2 species were grown in separate tanks from one another and supplied with different sources of inorganic N. Hence, we expected isotope composition to differ between species.

2.5. Isotope analysis

Macroalgae samples were defrosted in filtered seawater and cleaned of any epibionts before being washed in deionised water and dried at 60°C for at least 48 h. This process was carried out on just the tips (outermost 10 mm) of fronds for *C. crispus*, whereas whole fronds were processed for *P. palmata*. The latter species exhibits uniform growth, meaning new tissues can form all across its fronds (Nunes et al. 2016). In contrast, *C. crispus* exhibits apical growth, meaning new tissues grow only at the tips (Chopin et al. 1990). Hence, the tips of *C. crispus* fronds should represent the newest tissues and be more representative of nutrient conditions in Liverpool Bay. Although somatic and reproductive tissues can have markedly different isotopic compositions (Fredriksen 2003), our samples did not contain any reproductive structures.

All dried samples were homogenised using an agate pestle and mortar and weighed to 3.25 ± 0.25 mg in tin capsules in triplicate. Analysis was performed with an elemental analyser coupled to a DeltaPlus XP – Conflo III continuous flow-isotope ratio mass spectrometer. This created estimates of total N, C:N ratio, $\delta^{13}C$ and $\delta^{15}N$ with an analytical precision of 0.02‰. $\delta^{15}N$ and $\delta^{13}C$ were calculated using the equations described by Peterson & Fry (1987).

2.6. Pigment extraction

Many of the *C. crispus* samples were noticeably paler in colour after the field incubation period. Hence, to investigate whether macroalgal pigment concentrations exhibited spatial trends, we measured the chlorophyll *a* (chl *a*) concentration in 1 *C. crispus* sample from each of the stations using the methanol extraction protocol outlined by Torres et al. (2014).

2.7. Spatial analysis

Mean values of $\delta^{15}N$, total N, chl *a* and C:N ratio were interpolated using the 'kernel interpolation with barriers' tool within ArcMap 10.5. This method uses the shortest distance between points without intersecting a barrier (i.e. the coastline), such that points on either side of a barrier have less influence on one another, allowing for contours to change abruptly along barrier edges (Gribov & Krivoruchko 2011). Bandwidth selection for the kernel interpolation was carried out using the visual inspection approach outlined by Wand & Jones (1995). This involved creating a series of exponential models from large (3000 m) to small (1750 m) bandwidths in increments of 250 m (Figs. S1–S7 in the Supplement at www.int-res.com/articles/suppl/q011p671_supp.pdf). The most efficient kernel function (e.g. polynomial, Gaussian and Epanechnikov) was then identified by selecting the function which generated the lowest root-mean-square error (RMSE) and mean prediction error (ME) closest to 0 (Table S1) as detailed by Lessio et al. (2014). Through this process, an exponential function with a 2500 m bandwidth was deemed the most appropriate. Lastly, to assess the accuracy of interpolated data, the coefficient of variation (CV) was calculated using the equation described by Costanzo et al. (2001).

A hotspot analysis was performed using the Getis-Ord Gi* statistic (Getis & Ord 1992). This tested whether high or low values clustered together more than if spatial patterns were generated by chance alone (Getis 2010). The resulting *z*-scores and p-values identified whether stations were 'hot spots' (high values surrounded high values) or 'cold spots' (low values surrounded low values) at the 95% significance level. A fixed distance band of 2500 m was used, and a false discovery rate correction was applied to account for multiple testing and spatial dependency.

3. RESULTS

3.1. Dissolved nutrient concentrations

NH_4^+ and NO_3^- concentrations were low across the study area (Fig. 2), with many samples containing concentrations below detection limit. NH_4^+ ranged from detection limit (these cases were assigned a value of 0) to 1.29 µM (±0.05) and NO_3^- ranged from 0 to 0.32 (±0.01). There were noticeable differences between the 2 sampling dates as the station closest to the pulp mill and sewage treatment facility had an NH_4^+ concentration of 0 µM on 18 August, which increased to 1.3 µM just 11 d later, the highest concentration of NH_4^+ recorded in this study. The other stations also displayed marginally higher NH_4^+ concentrations for this second sampling date. NO_3^- con-

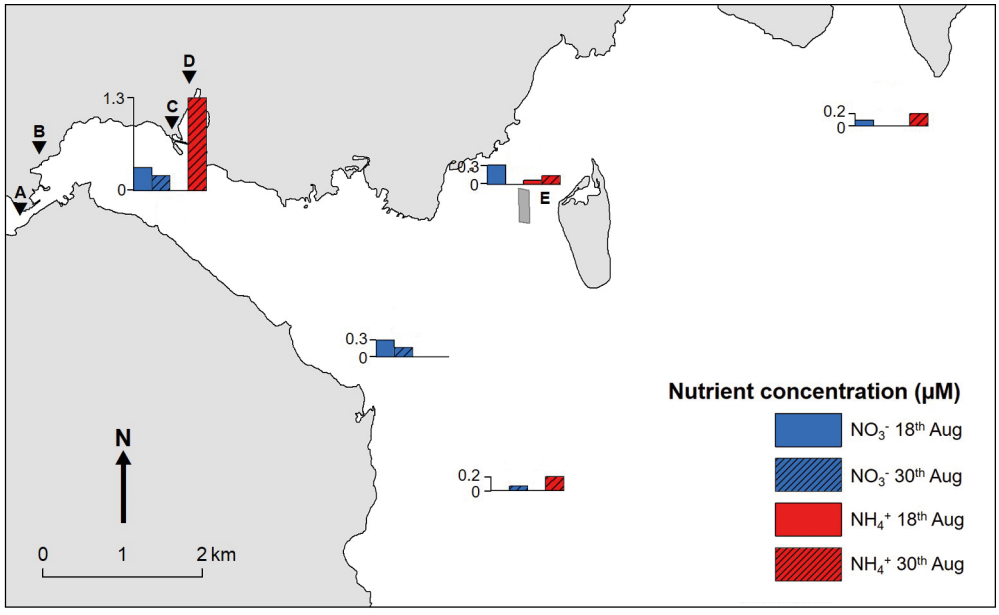

Fig. 2. NO_3^- and NH_4^+ concentrations of surface water samples collected at 2 different time periods. Chart position indicates sampling stations, and triangles indicate probable point sources (A: river mouth, B: sewage treatment facility, C: decommissioned pulp mill, D: biomass power plant). The grey box labelled E denotes the boundaries of the salmon farm

centrations were comparatively much lower than NH_4^+ and displayed no clear spatial or temporal trends.

3.2. End-member elemental and isotopic composition

Prior to their incubation in the field, the $\delta^{15}N$ and C:N ratio of *Chondrus crispus* were higher than those of *Palmaria palmata* (Table 1). In contrast, the total N content and $\delta^{13}C$ was lower in *C. crispus*. The $\delta^{15}N$ of the fish feed used at the salmon farm was slightly greater than that of *C. crispus.*

3.3. Elemental and isotopic composition of incubated macroalgae

Values of $\delta^{15}N$ in *C. crispus* (Fig. 3a) were lowest within the boundaries of the salmon farm (mean ± SE: 1.69 ± 0.06‰) and highest near the sewage treatment facility (4.43 ± 0.01‰). The $\delta^{15}N$ of all *C. crispus* samples deployed within the vicinity of the salmon farm had decreased by approximately 1‰ compared to initial values following the 10 d incubation period. The hot-spot analysis (Fig. 4a) indicated that $\delta^{15}N$ values were significantly low within the salmon farm ($p < 0.05$) and that these low values reached 2 km to the northeast and 2.4 km to the southwest of the farm. In contrast, the hot-spot analysis indicated that the inner bay area had significantly high $\delta^{15}N$ values ($p < 0.05$) which reached outwards over a distance of 3.5 km. Interpolations also suggested elevated $\delta^{15}N$ values 4 km northeast of the salmon farm, an area not known to have any obvious anthropogenic inputs of N. However, the hot-spot analysis indicated that these were not significant ($p > 0.05$). Unlike *C. crispus*, all *P. palmata* $\delta^{15}N$ values were negative and displayed a much narrower range of −2.32 to −1.14‰. These $\delta^{15}N$ values did not display any clear spatial patterns (Fig. 3b), and all samples were deemed statistically indistinguishable (Fig. 4b) by the hot-spot analysis ($p > 0.05$).

N content of the incubated macroalgae (Fig. 3c,d) was highest within the boundaries of the salmon farm for both species (*C. crispus* = 3.48 ± 0.01%; *P. palmata* = 4.05 ± 0.03%) and lowest at the northern outermost edge of the bay (*C. crispus* = 1.28 ± 0.02%; *P. palmata* = 2.17 ± 0.03%). The hot-spot analysis

Table 1. Isotope composition of hatchery-reared macroalgae *Chondrus crispus* and *Palmaria palmata* prior to incubation in the field, and of the fish feed used at the salmon farm. Error is ± 1 SE

Source	$\delta^{15}N$ (‰)	N (%)	$\delta^{13}C$	C:N ratio
C. crispus	3.36 ± 0.04	3.26 ± 0.01	−31.36 ± 0.01	10.21 ± 0.03
P. palmata	−1.72 ± 0.04	4.38 ± 0.02	−20.18 ± 0.1	7.8 ± 0.09
Fish feed	3.87 ± 0.09	7.1 ± 0.08	−20.38 ± 0.08	7.8 ± 0.06

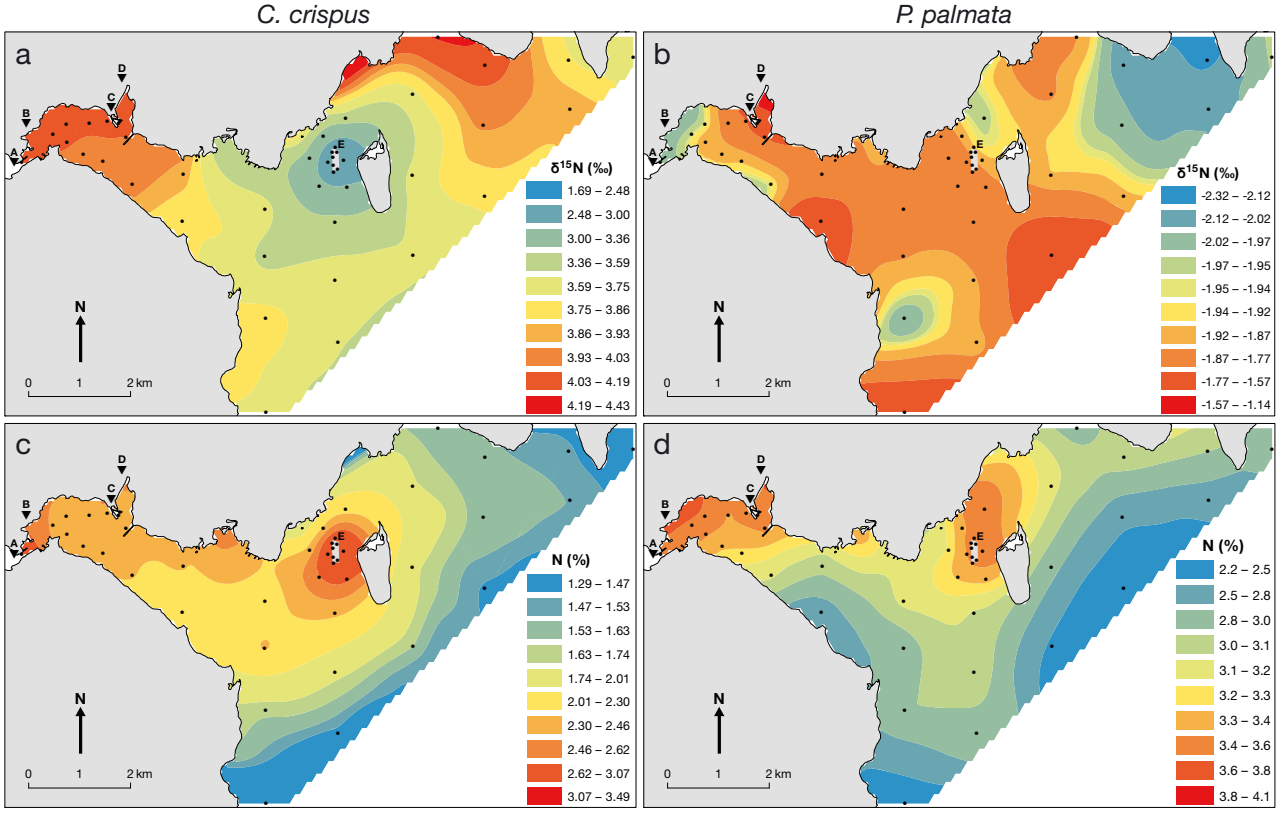

Fig. 3. (a,b) $\delta^{15}N$ and (c,d) total N content of the macroalgae *Chondrus crispus* (a,c) and *Palmaria palmata* (b,d). Points represent deployment stations. Triangles indicate probable point sources (labelled as in Fig. 2)

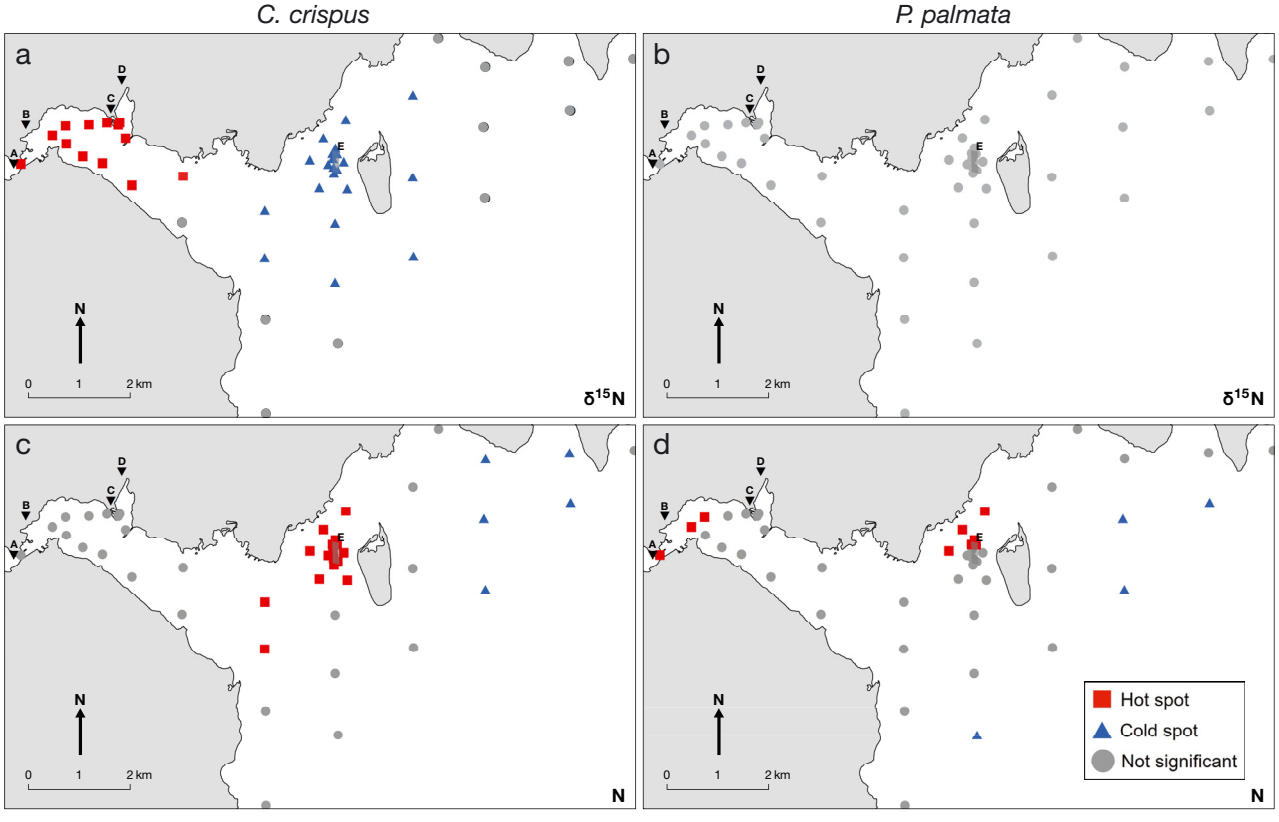

Fig. 4. Hot-spot analysis of (a,b) $\delta^{15}N$ and (c,d) N content of the macroalgae *Chondrus crispus* (a,c) and *Palmaria palmata* (b,d). Triangles indicate probable point sources (labelled as in Fig. 2)

(Fig. 4c,d) indicated that total N was significantly high ($p < 0.05$) within the boundaries of the salmon farm for both species. For *C. crispus*, these high N values reached 0.8 km to the north of the farm and 2.4 km to the southwest. In contrast, the high N values observed in *P. palmata* spread out from the farm in a predominantly northerly direction for 0.8 km. Only *P. palmata* exhibited significantly high values of total N ($p < 0.05$) within the inner bay area. Both species displayed significantly low total N values ($p < 0.05$) at the northern outermost edge of the bay.

Ratios of C:N (Fig. 5a) were lowest within the boundaries of the salmon farm for *C. crispus* (9.25 ± 0.01) and inner bay area nearest to the river (11.69 ± 0.02), and highest at the northern outermost edge of the bay (23.51 ± 0.06). *P. palmata* (Fig. 5b) was slightly different in that C:N ratios were lowest at the salmon farm (9.7 ± 0.04) but highest at the southern outermost edge of the bay (17.83 ± 0.22). The hot-spot analysis (Fig. 6a,b) confirmed that C:N ratios were significantly low ($p < 0.05$) within the boundaries of the salmon farm for both species. For *C. crispus*, these low C:N values reached 0.8 km to the north of the farm and 2.4 km to the southwest. In contrast, the low C:N values observed in *P. palmata* spread out from the farm in a northwest direction for just 0.3 km. The hot-spot analysis confirmed that the inner bay area also had significantly low C:N values ($p < 0.05$) which reached outwards over a distance of 2.8 km for *C. crispus* and 1.2 km for *P. palmata*. In contrast, C:N ratios were significantly high ($p < 0.05$) at the northern outermost edge of the bay for *C. crispus* and at the southern outermost edge of the bay for *P. palmata*.

Lastly, the chl *a* content of *C. crispus* (Fig. 5c) exhibited similar spatial trends to total N in that values were highest close to the salmon farm and inner bay area. However, all stations were deemed statistically indistinguishable in terms of chl *a* content (Fig. 6c). Overall, the interpolated data were between 80 and 90% accurate (Figs. S8 & S9).

4. DISCUSSION

The aim of this study was to investigate whether macroalgal bioindicators could map and identify between multiple effluent sources, and in doing so, provide a useful tool for helping management transition towards an ecosystem approach to aquaculture. Two species of macroalgae were incubated in a bay con-

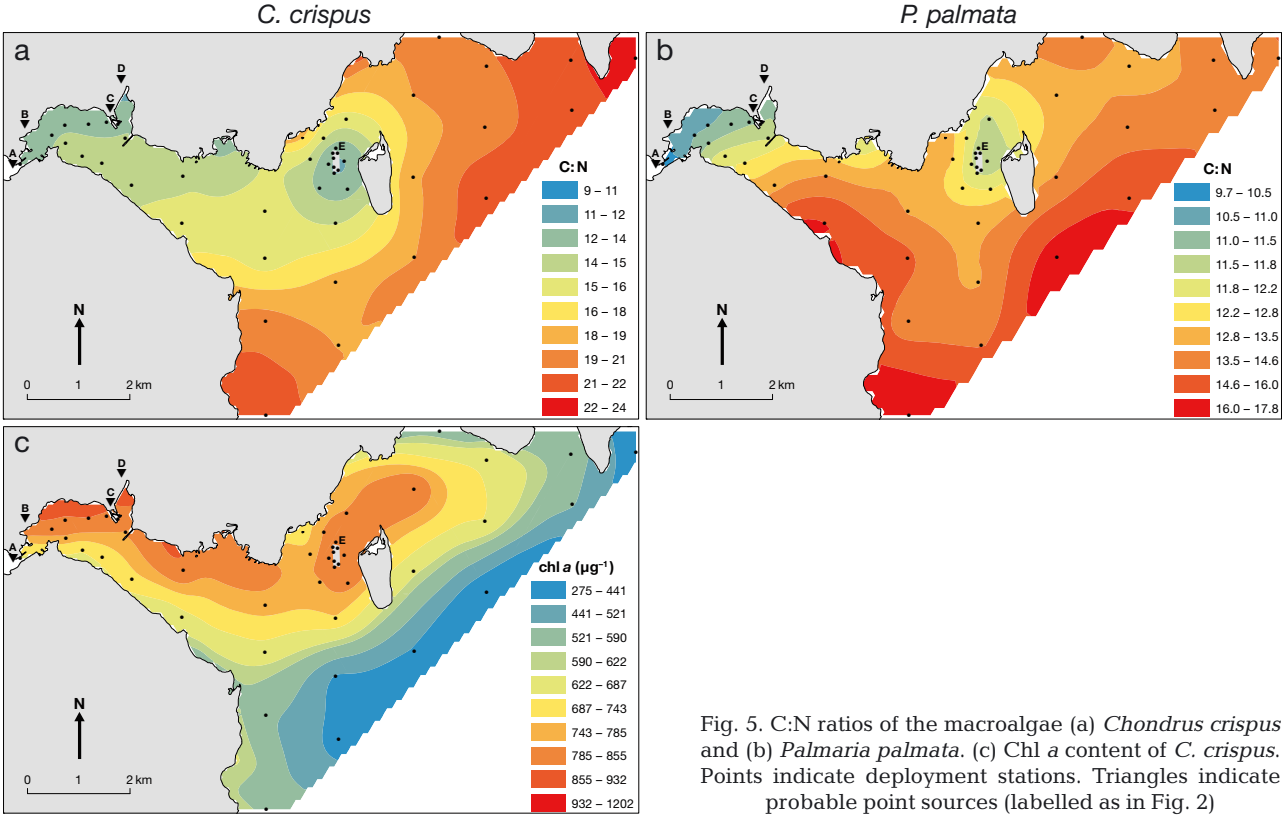

Fig. 5. C:N ratios of the macroalgae (a) *Chondrus crispus* and (b) *Palmaria palmata*. (c) Chl *a* content of *C. crispus*. Points indicate deployment stations. Triangles indicate probable point sources (labelled as in Fig. 2)

taining a salmon farm and sewage treatment facility, after which their elemental and isotopic composition was analysed.

4.1. Dissolved nutrient concentrations and the footprint of aquaculture wastes

The ambient nutrient concentrations of coastal waters, and the release of wastes from salmon farms and sewage treatment facilities, exhibit strong daily and seasonal fluctuations (Munksgaard & Young 1980, Karakassis et al. 2001). Such variation may explain why concentrations of NH_4^+ and NO_3^- differed greatly between the 2 sampling periods in our study. For example, NH_4^+ was undetectable in water samples taken near the sewage facility during the first sampling event, but this had increased to 1.3 ± 0.04 µM (mean ± SE) for the second sampling event. Similarly, the salmon farm had an initial NO_3^- concentration of 0.3 ± 0.01 µM but was undetectable during the second sampling event. Although DIN concentrations are lowest in Nova Scotia during the summer (Keizer et al. 1996), it is highly unlikely that NO_3^- and NH_4^+ concentrations were 0. Rather, these low and variable DIN concentrations were probably an artefact of experimental design, as the spatial and temporal coverage of water sampling was low.

4.2. Total N and C:N ratios of macroalgal bioindicators

Macroalgae take up N primarily in the form of NH_4^+ and NO_3^- (Hurd et al. 2014). When the supply of these nutrients exceeds what is needed for growth, macroalgae can accumulate N within their tissues for use during periods of low availability (Fong et al. 2004). Hence, the total N content of macroalgae tends to be higher after exposure to high DIN concentrations (Duarte 1992, Yokoyama & Ishihi 2010). In reverse, low C:N ratios tend to correspond with high DIN concentrations, as well as greater rates of photosynthesis and growth (Ahn et al. 1998, Umezawa 2002, Royer et al. 2013). As macroalgae deployed at the outer edges of the bay exhibited lower values of total N and chl *a*, and higher values of C:N, it suggests that they had grown under more N-limited conditions. In contrast, macroalgae near the salmon farm and sewage treatment facility exhibited higher values of total N and chl *a*, and lower C:N ratios, suggesting that the DIN emanating from these

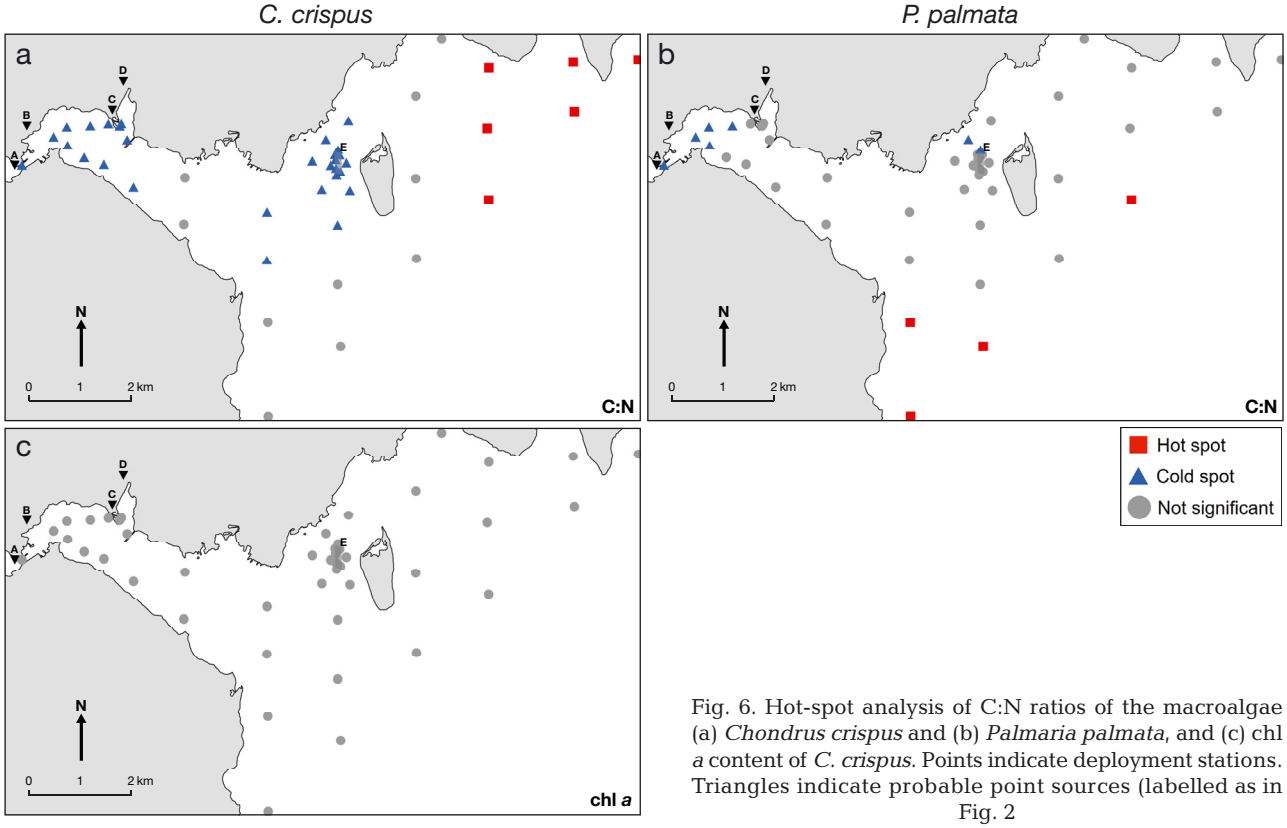

Fig. 6. Hot-spot analysis of C:N ratios of the macroalgae (a) *Chondrus crispus* and (b) *Palmaria palmata*, and (c) chl *a* content of *C. crispus*. Points indicate deployment stations. Triangles indicate probable point sources (labelled as in Fig. 2

effluent sources had promoted higher rates of growth, photosynthesis and N storage (Ahn et al. 1998, Umezawa 2002). Hence, our hypothesis that total N content would be higher near sources of anthropogenic N was supported.

One of the aims of this study was to use macroalgal bioindicators to provide greater insight into the dispersal of dissolved aquaculture wastes. The hot-spot analysis suggested that the tissues of *Chondrus crispus* exhibited significantly high total N values, and significantly low C:N ratios, up to 0.8 km northeast of the salmon farm and 2.4 km to the southwest. As both variables suggest that a transfer of N had occurred from the salmon farm to nearby macroalgae, these distances could indicate the extent of DIN emanating from the salmon farm. However, it must be noted that our results cannot be directly interpreted as a nutrient 'plume', but rather as a 'zone of influence' on organisms (García-Sanz et al. 2010, Carballeira et al. 2013).

Overall, our results suggest that the DIN released from the salmon farm and sewage treatment facility had increased the growth of macroalgal bioindicators. Determining to what degree these DIN inputs affect food webs and the wider ecosystem was beyond the scope of the present study. However, it is likely that other marine organisms (e.g. bacteria, phytoplankton, seagrass and other species of macroalgae) are also absorbing this N and experiencing faster rates of growth and reproduction (reviewed by Price et al. 2015). For example, Lapointe et al. (2005) attributed an increase in macroalgal blooms and invasions on coral reefs in Florida (USA) to increasing DIN inputs from sewage outfalls and septic tanks. Similarly, Robinson et al. (2005) reported that the development of salmon farming in the Bay of Fundy coincided with the growth of extensive algal mats along the surrounding shoreline. Hence, monitoring and reducing coastal N will undoubtedly continue to be an important aspect of coastal management as coastal human populations, wastewater treatment facilities and aquaculture, agricultural and industrial developments increase around the world (Yang et al. 2015, Clements & Chopin 2017).

4.3. Isotopic composition of macroalgal bioindicators

Several studies have reported elevated $\delta^{15}N$ levels in macroalgae exposed to effluents from aquaculture (Vizzini et al. 2005, Carballeira et al. 2013, Wang et al. 2014) and sewage treatment facilities (Gartner et al. 2002, Costanzo et al. 2005). Correspondingly, some of the highest $\delta^{15}N$ values observed in this study (4.32 ± 0.03‰) were from *C. crispus* samples deployed near the sewage treatment facility. However, the lowest $\delta^{15}N$ values (1.7 ± 0.06‰) were observed within the boundaries of the salmon farm. Hence, our hypothesis that macroalgae would display elevated $\delta^{15}N$ values close to the salmon farm and sewage facility was only partially supported.

There are several possible explanations why *C. crispus* near the salmon farm had such low $\delta^{15}N$ values. First, the effluents from the salmon farm may simply have been depleted in $\delta^{15}N$. However, salmon excretion is typically enriched by ~1.2‰ relative to their feed (Wang et al. 2014). As the salmon feed had a $\delta^{15}N$ of 3.87 ± 0.09‰, the effluent from the salmon farm was likely to be ~5.1‰. Alternatively, it may be that the salmon farm was producing very little in the way of dissolved wastes. However, this is unlikely, as the farm was at peak biomass at the time of sampling. Instead, effluents from the salmon farm could have negatively affected the physiology and growth of *C. crispus*, and consequently, its isotopic composition. However, total N, C:N ratio and chl *a* content suggested that *C. crispus* near the farm was growing faster in response to elevated DIN concentrations. Hence, the most probable explanation for these low $\delta^{15}N$ values is that *C. crispus* was fractionating near the salmon farm.

Many authors have claimed that fractionation within macroalgae is minimal to non-existent, meaning they should take up ^{14}N and ^{15}N in direct or close proportion to the supply (Gartner et al. 2002, Cohen & Fong 2005, Deutsch & Voss 2006, García-Sanz et al. 2010, Lemesle et al. 2015). However, several species of macroalgae have now been shown to fractionate when exposed to high DIN concentrations (Cohen & Fong 2005, Swart et al. 2014, Wang et al. 2014). For example, Swart et al. (2014) found that *Ulva lactuca* fractionated NH_4^+ at concentrations of ≥10 µM. Hence, the salmon farm could have increased DIN concentrations above the threshold required for fractionation, causing macroalgae to grow new biomass more isotopically negative than the source. This would explain why the $\delta^{15}N$ of *C. crispus* deployed within a 400 m radius of the salmon farm had decreased by an average of 1‰ compared to their initial nitrogen composition. This study therefore joins a growing number which suggest that the $\delta^{15}N$ of macroalgae is not simply a function of the source, but also of the rate of fractionation when levels of DIN are in excess (Chopin et al. 1995, Wang et al. 2014). However, these results should not be inter-

preted as a direct measure of farm-induced eutrophication since the DIN threshold required for *C. crispus* to fractionate remains unknown.

If elevated DIN concentrations caused *C. crispus* to fractionate within the salmon farm, why was the same response not observed near the sewage treatment facility? This difference may be due to differences in DIN composition between the 2 sources. While NO_3^- and NH_3 account for the majority of DIN discharged by sewage facilities, NH_4^+ is the principal form of N released by fish farms (Leung et al. 1999, Pehlivanoglu & Sedlak 2004). As macroalgae preferentially take up NH_4^+ because it is energetically 'cheaper' than NO_3^-, and because they fractionate NH_4^+ at a faster rate, this could have resulted in stronger fractionation rates near the salmon farm (Cohen & Fong 2005, Hurd et al. 2014). Additionally, DIN concentrations near the sewage treatment facility may have been below what was required to promote fractionation. In support of this, total N and C:N ratios in *C. crispus* suggested that DIN levels were higher at the salmon farm. However, the only way to truly understand whether *C. crispus* fractionated at the salmon farm would be to conduct a controlled lab-based study investigating the mechanisms (e.g. NO_3^- and NH_4^+ concentrations) underlying its fractionation dynamics.

4.4. Comparisons between species

This study used 2 different species of macroalgae to test and compare their suitability as potential bioindicators. To our knowledge, this is the first study to trial *Palmaria palmata* as a bioindicator, and only the second to use *C. crispus* (Lemesle et al. 2016). Prior to its deployment in the field, *P. palmata* was substantially lighter in $\delta^{15}N$ than *C. crispus.* As macroalgae depleted in ^{15}N typically respond faster to elevated $\delta^{15}N$ signals, this could have given *P. palmata* an advantage over *C. crispus* as a bioindicator (Cohen & Fong 2006, García-Seoane et al. 2018). However, the $\delta^{15}N$ values of *P. palmata* exhibited very little change after the 10 d incubation period and displayed no response to the effluents from the salmon farm and sewage facility. In addition, values of N and C:N ratios in *P. palmata* exhibited a much narrower range than in *C. crispus* and were less responsive to anthropogenic effluents. These results therefore suggest that $\delta^{15}N$ in *P. palmata* is not an effective bioindicator, and that N and C:N ratios in *P. palmata* are less effective bioindicators than they are in *C. crispus*.

The differences observed between these 2 macroalgae species might be because *P. palmata* has thicker and flatter fronds, and a different cell wall composition, and therefore different rates of diffusion and nutrient uptake (Chopin et al. 1999, Gartner et al. 2002, Dailer et al. 2010, Hurd et al. 2014). Alternatively, it may be due to differences in their growth characteristics and how tissue samples were prepared in the lab. In this study, only the tips of *C. crispus* fronds were processed and analysed, as this species is known to exhibit apical growth (Chopin et al. 1990). Hence, it was assumed that these tissues should be the newest, and therefore, provide a better reflection of recent nutrient concentrations and isotope composition in Liverpool Bay. In contrast, *P. palmata* is known to exhibit uniform growth, meaning fronds should have contained a combination of new and old tissues (Nunes et al. 2016). Consequently, whole fronds were processed and analysed, which may have diluted any signals.

4.5. Future research

Macroalgal bioindicators have become a popular tool for monitoring anthropogenic effluents in coastal areas. However, this approach is limited by the lack of a standardized methodology, with many studies employing different incubation times, depths, distances, pre-exposure conditioning procedures and tissue selection processes (reviewed by García-Seoane et al. 2018). Also, while bioindicator studies have been conducted at many different latitudes, they have been restricted to species that can be locally sourced or can survive being transplanted to the area of interest. Consequently, over 40 different macroalgae species have been used as bioindicators to date. This is problematic, as rates of nutrient uptake vary between species due to differences in morphology, tissue composition and growth rates (Gartner et al. 2002, Deutsch & Voss 2006). Hence, there is a need to test how results vary between species, and between tissues, in macroalgae transplanted to the same location. While this study and several others have tried to address this research gap, further research is required before they can be widely adopted as a biomonitoring tool.

4.6. Conclusions

This study investigated whether macroalgal bioindicators could be used to map and identify between

multiple effluent sources. Maps of total N and C:N ratios suggested that both macroalgae species had absorbed N from the salmon farm and sewage treatment facility, but that the salmon farm had influenced the elemental composition of *C. crispus* over a greater distance than that of *P. palmata*. Differences were also observed between their isotopic composition, as *C. crispus* indicated that the fish farm and sewage treatment facility had distinctly different $\delta^{15}N$ signatures, whereas values of $\delta^{15}N$ in *P. palmata* remained largely unchanged after the incubation period. This evidence suggests that *P. palmata* is a poor bioindicator compared to *C. crispus*. There was also evidence that *C. crispus* was fractionating N in response to elevated DIN concentrations, which could complicate its use as a bioindicator. Overall, this study suggests that macroalgal bioindicators have the potential to monitor and identify between multiple effluent sources, which could provide a useful tool for helping the transition towards an ecosystem approach to the management of aquaculture.

Acknowledgements. This study was funded by an NSERC Collaborative Research and Development Grant (NSERC-Cooke Aquaculture CRD 511841-17). We thank Anne McKee, Claire Normendeau, Duncan Bates, Mark Merrimen and all staff at the fish farm in Liverpool Bay.

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Editorial responsibility: Adam Hughes, Oban, UK

Submitted: August 7, 2019; Accepted: November 4, 2019
Proofs received from author(s): December 4, 2019