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Geographical variation in the carbon, nitrogen, and phosphorus content of blue mussels, *Mytilus edulis*.

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Highlights

- Rope cultured mussels captured significantly more N and P than bottom cultured mussels.
- The shell of bottom cultured mussels removed significantly more C than rope cultured mussels
- Higher seawater temperatures significantly reduced the levels of phosphorus in mussel tissue.

Abstract

Shellfish farming contributes to nutrient removal in coastal and estuarine systems, as bivalves incorporate nutrients into their tissues and shells, which is removed from the marine system on harvest. Fourteen locations around the UK were surveyed to explore geographic variation in carbon, nitrogen and phosphorus content of tissue and shell in blue mussels. Phosphorus in

tissue had a significant negative relationship with mean annual seawater temperature for both rope and bottom cultured sites. Per tonne of live mussel, rope culture removed significantly more nitrogen (8.50±0.59 kg) and phosphorus (0.95±0.07 kg) than bottom cultured (5.00±0.013 kg nitrogen and 0.43±0.01 kg phosphorus). Bottom culture, however, provides significantly more C removal in shell (60.15±0.77 kg) than in rope cultured (46.12±1.69 kg). Further studies are required to examine the effect of growth rate, on the nitrogen and phosphorus remediation, and carbon stored in shell, of rope culture and bottom cultured mussel aquaculture.

Keywords: Nutrient remediation, ecosystem services, regulating services, shellfish, bivalves, bivalve aquaculture

1 Introduction

Human activities have substantially increased the inputs of nutrients to coastal and estuarine waters (Boyer and Howarth, 2008) through increased use of chemical fertilisers and organic waste in intensive agriculture and human sewage in the form of nutrient waste from expanding cities (Petersen *et al*., 2019). Biogeochemical cycling of nutrients plays a significant role in nearshore coastal and estuarine systems in controlling densities of micro- and macro-algae (Clements and Comeau, 2019; Gobler *et al*., 2016; Rose *et al*., 2014). Excess nutrients can lead to biogeochemical imbalance and substantial perturbation to coastal systems, and has led to an increased occurrence of eutrophic estuaries around the world (Rose *et al*., 2014).

In recent years mussel farms have been discussed as a mechanism for reducing the impact of terrestrial nutrient inputs to estuaries, through their ability to filter phytoplankton and incorporate nitrogen (N), phosphorus (P) and carbon (C) into their shells and tissue (Buer et al., 2020; Clements and Comeau, 2019; Hedberg et al., 2018; Kotta et al., 2020; Petersen et al., 2019; Rose et al., 2015). N is considered the primary limiting factor in coastal environments, although P also encourages the growth of phytoplankton (Petersen *et al*., 2019; Rose *et al*., 2015). To estimate the nutrient remediation potential of bivalve aquaculture, it is important to understand how different environmental conditions may influence the amount of C, N and P that is incorporated in shellfish tissue and shell. A range of environmental factors may influence mussel biology and potentially vary the capacity for incorporation of N and P into tissue and shell. Temperature is a key factor which has long been understood to influence the metabolism of the blue mussel *Mytilus edulis* (Widdows, 1973), including filtration rates, absorption and the utilisation of available food (Zippay & Helmuth, 2012). *M. edulis* are more

likely to grow faster and to a larger size at warmer sites (Lesser *et al*. 2010). Meanwhile, low salinity has been shown to reduce filtration and growth rates (Seed and Suchanek, 1992). Cultivation methods have also been shown to affect the growth rates of mussels, with faster growth in rope culture than bottom culture (Kamermans and Capelle, 2019). Food availability may also influence growth rates, and this is often represented by chlorophyll-*a* concentration (Rosland *et al*., 2009; Thomas *et al*., 2011). Suspended particulate material (SPM) is sometimes used as a proxy for food supply (Smaal and Haas, 1997), whilst tidal range is used as a proxy for tidal velocity and hence water flow and food supply (Coen and Luckenbach, 2000).

Nutrient loading of the environment may influence the N and P uptake by bivalves. Increased catchment nutrient concentrations increases phytoplankton production in estuarine and coastal waters (Bricker *et al*., 1999). Mussels have the potential to optimize their nutrient balance by regulating food uptake quantitatively as well as qualitatively, and are able to store nutrients (Jansen *et al*., 2012). This would imply that with increased food supply, N and P concentration in flesh would increase, although studies have shown that mussels will reach an algal saturation level, after which filtration, and by association, growth is reduced (Dolmer, 2000; H. U. Riisgård et al., 2013; Hans Ulrik Riisgård et al., 2013).

As well as its role in nutrient remediation, bivalve aquaculture is also gaining widespread attention because of its potential role in the C cycle (Filgueira *et al*., 2015; Hickey, 2009; Tang *et al*., 2011; Waldbusser *et al*., 2013), due to the growing drive to mitigate climate change. Carbon is stored in shells for long periods of time, with some authors arguing that the C stored in shell represents a long-term sink. Others, however, argue that due to biogeochemical transformations during the calcification process, particularly the CO_2 released into the water column, incorporation of C into shell material by bivalves should be considered to be a source of atmospheric CO_2 (Filgueira *et al*., 2015; Fodrie *et al*., 2017). Whether carbonate formation in bivalve shell is considered a net sink or a net source, the amount of C stored in shell, and therefore removed from the marine system at harvest is one way to estimate the magnitude of this process carried out by cultured mussels.

Previous estimates of nutrient remediation by shellfish have used a single look-up value for tissue nutrient composition applied to data from many different environmental settings (Hedberg et al., 2018; Kotta et al., 2020; van der Schatte Olivier et al., 2018). Typically, variation in tissue nutrient content due to regional or environmental factors has not been taken into account, although some work has been carried out on oysters in eastern Canada (Clements

and Comeau, 2019), and on mussels in the Baltic (Buer et al. 2020). The role of environmental factors on nutrient composition in mussels remains poorly understood. Several studies have highlighted mussels as a potential mechanism for removing excess nutrients from eutrophic estuaries (Timmermann et al., 2019; van der Schatte Olivier et al., 2018). Some studies focus on net storage of nutrients within the mussel shell and tissue (Petersen et al., 2016), and their removal during harvest, while others focus on their role in in-situ biogeochemical processes such as denitrification in the sediment beneath mussel beds (Carlsson et al., 2012). The removal of whole animals at harvest presents a clear, calculable quantity of nutrient removal. However, there is a need to better understand geographical variation in nutrient uptake. Mcleod and Mcleod (2019) highlight the need for site specific assessments to be carried out in order to gain site specific values for C, N and P, but the role of environmental conditions in driving variation in tissue and shell nutrient content remains unclear.

The UK is an ideal case study for investigating the potential for shellfish farming in coastal nutrient remediation, due to the wide ranges in environmental and physical conditions represented, including nutrient concentrations in catchments, sea surface temperatures, hydrodynamics, and coastal morphology. Therefore, this study aimed to investigate regional variation in C, N and P content in tissue and shells of *M. edulis* in fourteen estuaries around the UK. The study examined potential relationships with a range of environmental predictors, including estuarine nutrient concentrations, salinity, sea surface temperature and shellfish culture method.

2 Methods

2.1 Site selection

Fourteen sites were selected around the UK (Figure 1), chosen to include a range of annual water temperature and a range of high and low catchment nutrient supply.

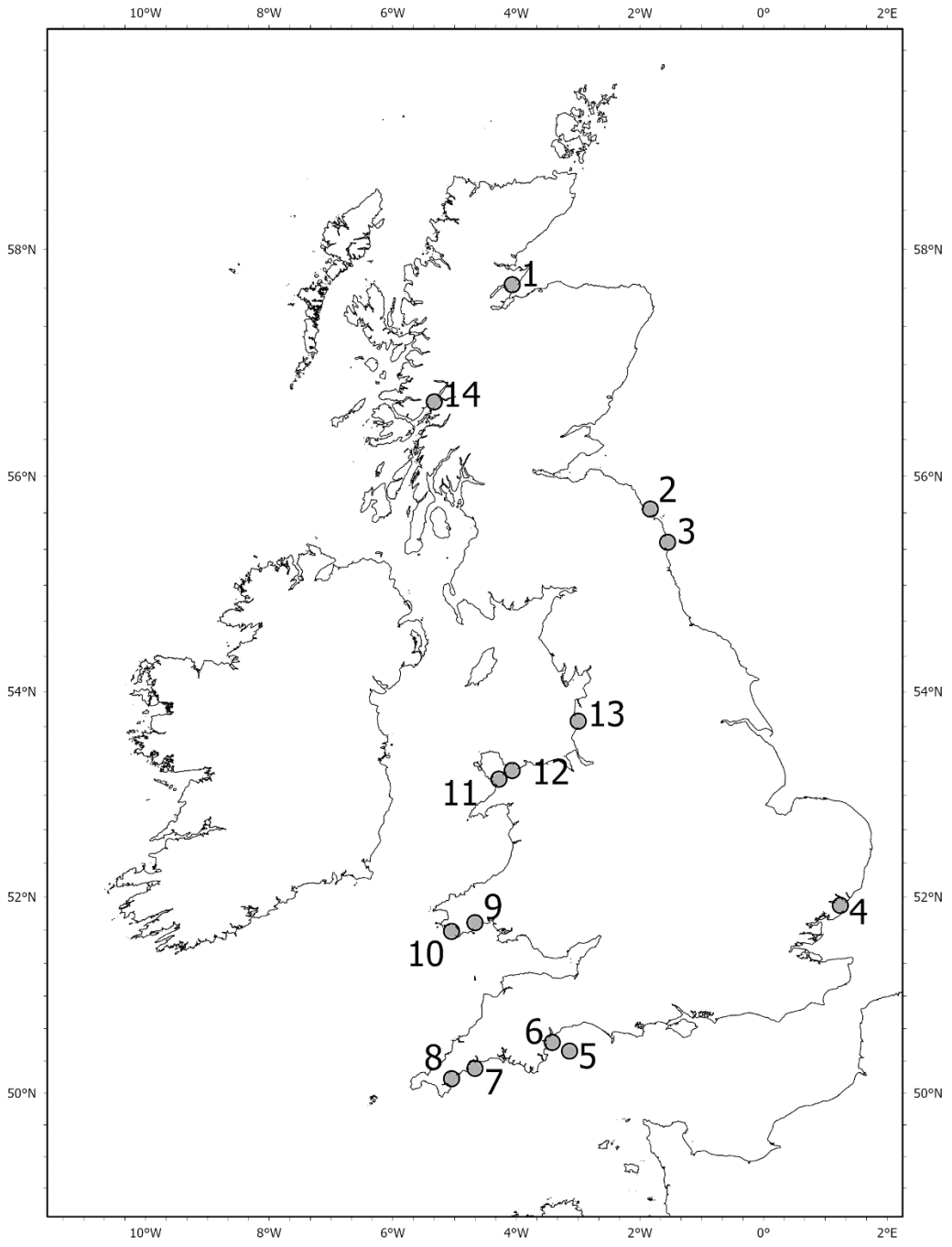

Figure 1 Map of sample sites around the United Kingdom. 1- Cromarty Firth, 2- Lindisfarne, 3- River Coquet, 4- Deben Estuary, 5- Lyme Bay, 6- River Teign, 7- River Fowey, 8- River Fal, 9- Swansea docks, 10- Milford Haven, 11- River Braint, 12- Menai Strait, 13- River Ribble, 14- Loch Leven

Suitable sites were chosen based on shellfish production area reports (CEFAS, available from https://www.cefas.co.uk/data-and-publications/sanitary-surveys/scotland/ and

https://www.cefas.co.uk/data-and-publications/sanitary-surveys/england-and-wales/reports/) selecting estuaries where blue mussels (*M. edulis*) were present. For each site, the following ancillary data was collected. Sea surface temperature was obtained from satellite imagery using the Copernicus Marine Environment Monitoring Service (CMEMS, available from http://marine.copernicus.eu/). Nitrate concentrations in the input rivers to each estuary were collated from data in the harmonised river monitoring scheme (HRMS, available from https://data.gov.uk/dataset/bda4e065-41e5-4b78-b405-41c1d3606225/historic-uk-water-quality-sampling-harmonised-monitoring-scheme-summary-data). Suspended particulate material (SPM) data were obtained from satellite images available at http://data.cefas.co.uk/#/View/18133 (Silva *et al*., 2016). Tidal range was obtained from the Enhanced UK Estuaries Database (Manning and Whitehouse, 2012). Site characteristics are summarised in Table 1.

Table 1 Site characteristics of 14 selected sampling locations (Figure 1). All data except tidal range are annual means. * indicates a wild bed.

ID	Site name	Seawater temperature (°C)	River nitrate concentration (mg l^{-1})	Chlorophyll-*a* (mg m^{-3})	Tidal range (m)	salinity (ppt)	Suspended particulate matter (mg l^{-1})	Culture method
1	Cromarty Firth	8.2	0.11	2.20	2.62	32.9	1.78	Bottom
2	Lindisfarne*	9.1	3.68	1.49	3.16	33.5	7.55	Bottom
3	River Coquet*	9.0	0.25	1.62	3.04	33.6	5.20	Bottom
4	River Deben*	11.5	11.50	3.70	2.24	33.7	58.68	Bottom
5	Lyme Bay	12.9	4.35	0.84	2.60	35.1	3.14	Rope
6	The Teign	12.6	2.03	3.26	2.64	34.0	2.43	Bottom
7	River Fowey	12.4	1.82	1.05	3.40	34.6	2.12	Rope
8	River Fal*	12.6	3.43	1.25	3.42	34.4	1.70	Bottom
9	Swansea Docks	11.8	0.73	1.91	6.34	29.5	7.42	Rope
10	Milford Haven*	11.9	3.25	0.95	4.46	33.4	6.55	Bottom
11	Afon Braint*	11.0	2.5	2.13	3.02	32.9	8.09	Bottom
12	Menai Strait	10.8	0.60	4.50	5.14	30.3	6.48	Bottom
13	River Ribble*	10.6	4.73	2.17	6.16	27.8	23.46	Bottom
14	Loch Leven	9.6	4.73	1.61	3.70	28.0	0.96	Rope

2.2 Sample collection

Preliminary analysis was conducted at two of the survey sites: Menai Strait and Afon Braint, in January 2018, to assess between-individual and between-location variation, in order to develop the sampling strategy for the multi-site survey. Results showed that five random locations around each site and six mussels were needed from each location to adequately account for within-site variation. Mussels were chosen that were between 40-90mm in length to represent mussel size at harvest. Mussels were collected from around the UK during September and October of 2018, cleaned and frozen before being returned to laboratory for storage/processing.

2.3 Sample preparation

After collection, mussel shells were scraped clean of encrusting barnacles, patted dry using paper towels and the live weight was recorded. Shell length was measured using digital Vernier callipers. The mussels were placed in labelled zip lock bags before being frozen at -20ºC prior to analysis. In the laboratory, the mussels were defrosted, and the tissue completely removed from the shell. The wet weight of tissue was determined after gently rolling samples in paper towel to remove excess external water. Tissue samples were placed into tin dishes and frozen to -20°C before being placed in a freeze dryer for 120 h at a vacuumed temperature of -40°C. Once completely dry, the dry weight was taken. The mussels were hand-ground with a pestle and mortar and then placed into Precellys tubes with stainless steel ball bearings and ground to a fine powder. The tissue of the six mussels from each location were pooled and homogenised, creating five replicate samples to be analysed from each site.

Shells from the pooled samples of six mussels were patted dry using paper towels and wet weight taken, then oven dried at 60°C for 120 h and dry weight taken. The dried shells were crushed with a hammer into small pieces, then placed in a hammer mill and ground to 1mm particles, and finally ground to a fine powder in a ball mill.

2.4 Elemental analysis

N and C content was measured in subsamples of dried tissue and shell, using a Flash elemental analyser, with flash combustion (950°C) and measurement of gaseous products by gas

chromatography. The dry weights of samples analysed were between 1 to 1.5 mg for tissue, and 9.5 to 10.5 mg for shell. Optimum sample weights were determined prior to analysis. All samples were run with carrier gas blanks, sample blanks (empty tin capsules), a duplicate sample, then acetanilide standards following every ten samples run. Each day Certified Reference Material was also analysed (Apple leaves NIST 1515 and NIES mussel tissue) to ensure the machine-maintained accuracy. There was no observed drift in the calibration with time, and so elemental composition was calculated based on a mean for all blanks and acetanilide standards.

2.4.1 Phosphorus analysis

Phosphorus analysis was based on the methods of Solórzano and Sharp (1980). Mussel shell and tissue samples were placed in a muffle furnace for 3 hours at 450°C. Once cooled, samples were acidified with 3.5 M HCl. These were placed on an orbital shaker for 16 hours, before centrifugation until all the supernatant was clear. These then had a colour developing reagent added and were allowed to stand for 15 minutes until the colour had developed and were analysed using a spectrophotometer (Evolution 201) within one hour.

2.5 Statistical analysis

2.5.1 Percentage carbon, nitrogen, and phosphorus analysis

Linear models (S1, Supplementary material) were used to explore which factors explain variation in the mussel C, N and P content. The Shapiro–Wilk test was used to test for normality and the Levene's test for homogeneity of variance in the response data. Percentage P in tissue was log transformed in order to meet the assumptions of normality. Explanatory variables were tested for collinearity using Pearson correlation. SPM was highly correlated with annual nitrate (greater than 0.80 (Garson, 2012) and therefore SPM was removed from the model.

A linear model was then prepared accounting for all the environmental variables still included following the collinearity checks, all were continuous variables with the exception of culture type which was a categorical variable. Interactions were not included due to the coarse nature of the environmental data. The best model was selected using the dredge function from the Package MuMIn (version 1.43.6). This function selects the lowest Akaike information criterion (AIC) score and a delta AIC score below 2 (Burnham and Anderson, 2004). Following the linear model an ANOVA F-test was carried out to test the significance of each

variable. Post model validation was carried out using QQ plots and residuals vs fitted graphs (Zuur *et al*., 2010).

2.5.1.1 Upscaled values of kg per tonne of harvested live weight

The percentage C, N, and P can give different results to the kg of C, N, and P tonne^{-1} of live mussels, due to variation in the proportion of tissue and shell in mussels around the UK. To find the mass of C, N and P tonne^{-1} of live mussels, the percentage contents were upscaled using conversion factors derived from the live weight, wet weight and dry weight of each component (shell and tissue) measured during processing, applying the following formulae:

$$\text{Dry Weight per tonne}^{-1}\text{ of mussels (kg)} = \frac{\text{Average dry weight of animal (g)}}{\text{Average Live weight of animal (g)}} \times 1000\text{ kg}$$

$$\text{Dry Shell (kg) per tonne}^{-1}\text{ of mussels (kg)} = \frac{\text{Average Shell dry weight (g)}}{\text{Average dry weight of animal (g)}} \times \text{Average dry weight (kg)}$$

$$\text{Dry Tissue (kg) per tonne}^{-1}\text{ of mussels (kg)} = \text{Average dry weight of animal (kg)} - \text{Average Dry shell weight (kg)}$$

This was calculated for the five locations and then averaged for each site. The data were then analysed using linear models, with model selection carried out as above and significance of each variable tested ($p \leq 0.05$), using the ANOVA F-test.

All statistical analyses were conducted using R 3.5.2 (R Foundation for Statistical Computing 2011).

3 Results

3.1 Percentage content nitrogen, and phosphorus analysis

Percentage C, N, and P content in mussel tissue was much greater than that of shell (Table 2). Tissue content of all elements was also more variable between sites than that of shell for all the response variables. The greatest range across sites was recorded for tissue percentage C content (38.77% to 45.69%), followed by tissue percentage N content (7.86% to 10.22%), while tissue percentage P content had the lowest range (0.87% to 1.41%). By contrast, the variability in shell nutrient content was much lower. Shell percentage C content varied from 12.48% to

13.54%, shell percentage N content (0.28% to 0.78%), while tissue percentage P content had the lowest range (0.009% to 0.018%).

Impact of culture method on nutrient content

There was significantly greater percentage C and P content in the tissue of rope cultured mussels (Figure 2A and 2C) compared with bottom cultured mussels (43.50 ± 0.48 %C, 1.30 ± 0.07 %P and 40.97 ± 0.20 %C, 1.03 ± 0.03 %P, respectively). There was no significant difference in percentage N content between the tissue of rope cultured mussels and bottom cultured mussels (Figure 4B). There was significantly greater percentage content of C and N in shell of rope cultured mussels (Figure 2D and 2E) compared with bottom cultured mussels (13.10 ± 0.08 %C, 0.59 ± 0.03 %N and 12.70 ± 0.04 %C, 0.42 ± 0.02 %N, respectively). However, there was no difference in the percentage P content in shells of rope and bottom cultured mussels (Figure 2F).

Impact of mean environmental factors on nutrient content

There was a significant negative relationship between percentage P content in tissue and mean annual seawater temperature, for both rope and bottom cultured mussels separately (Figure 3C). There was also a significant negative relationship between percentage P content in shell and mean annual salinity, when data from both rope and bottom cultured sites were pooled (Figure 4F). There was no significant effect of seawater temperature or salinity on the percentage C and N content in either tissue or shell.

The average annual nitrate concentration of the survey sites varied from 0 to 6 mg l^{-1}, apart from the Deben estuary having an exceptionally high nitrate concentration of 11.5 mg l^{-1}. However, there was no significant relationship between mean annual nutrient concentration and the percentage C, N, and P content in mussel tissue or shell (Figure 7).

Table 2 Percentage (%) composition of carbon, nitrogen and phosphorus in sampled mussel shell and tissue from the 14 sampled sites and overall mean. Cell values are mean ± SE of five sampling locations per site. * indicates rope cultured mussels.

ID	Site name	%C in tissue	%N in tissue	%P in tissue	%C in shell	%N in shell	%P in shell
1	Cromarty Firth	43.01±0.17	9.65±0.09	1.18±0.07	12.54±0.06	0.38±0.026	0.010±0.001
2	Lindisfarne	38.77±0.27	8.25±0.07	1.24±0.09	12.35±0.07	0.28±0.03	0.012±0.001
3	River Coquet	40.72±0.23	8.42±0.06	1.15±0.10	12.88±0.10	0.53±0.04	0.012±0.000
4	Deben Estuary	40.18±0.19	8.25±0.07	0.95±0.07	12.71±0.12	0.46±0.06	0.013±0.001
5	Lyme Bay *	44.78±0.22	8.89±0.09	1.00±0.03	12.92±0.05	0.55±0.02	0.012±0.001
6	River Teign	40.37±0.13	8.16±0.04	0.89±0.08	12.78±0.18	0.46±0.06	0.012±0.001
7	River Fowey *	42.98±0.46	10.22±0.14	1.61±0.09	12.74±0.10	0.45±0.04	0.009±0.000
8	River Fal	41.38±0.42	8.67±0.10	1.05±0.14	12.93±0.06	0.54±0.03	0.015±0.002
9	Swansea Docks *	40.53±0.44	8.46±0.17	1.16±0.15	13.19±0.07	0.56±0.07	0.013±0.001
10	Milford Haven	40.06±0.29	8.63±0.13	0.89±0.03	12.48±0.06	0.31±0.03	0.013±0.001
11	Afon Braint	41.67±0.29	8.27±0.09	1.09±0.05	12.53±0.05	0.31±0.02	0.015±0.002
12	Menai Strait	40.72±0.59	7.86±0.12	0.97±0.01	12.96±0.06	0.51±0.01	0.015±0.001
13	River Ribble	43.07±0.24	9.22±0.09	0.87±0.06	12.69±0.21	0.43±0.09	0.012±0.001
14	Loch Leven *	45.69±0.32	8.57±0.07	1.41±0.14	13.54±0.06	0.78±0.27	0.018±0.002
	Overall Mean ±SE	41.71±0.24	8.68±0.08	1.10±0.033	12.80±0.04	0.47±0.02	0.013±0.001

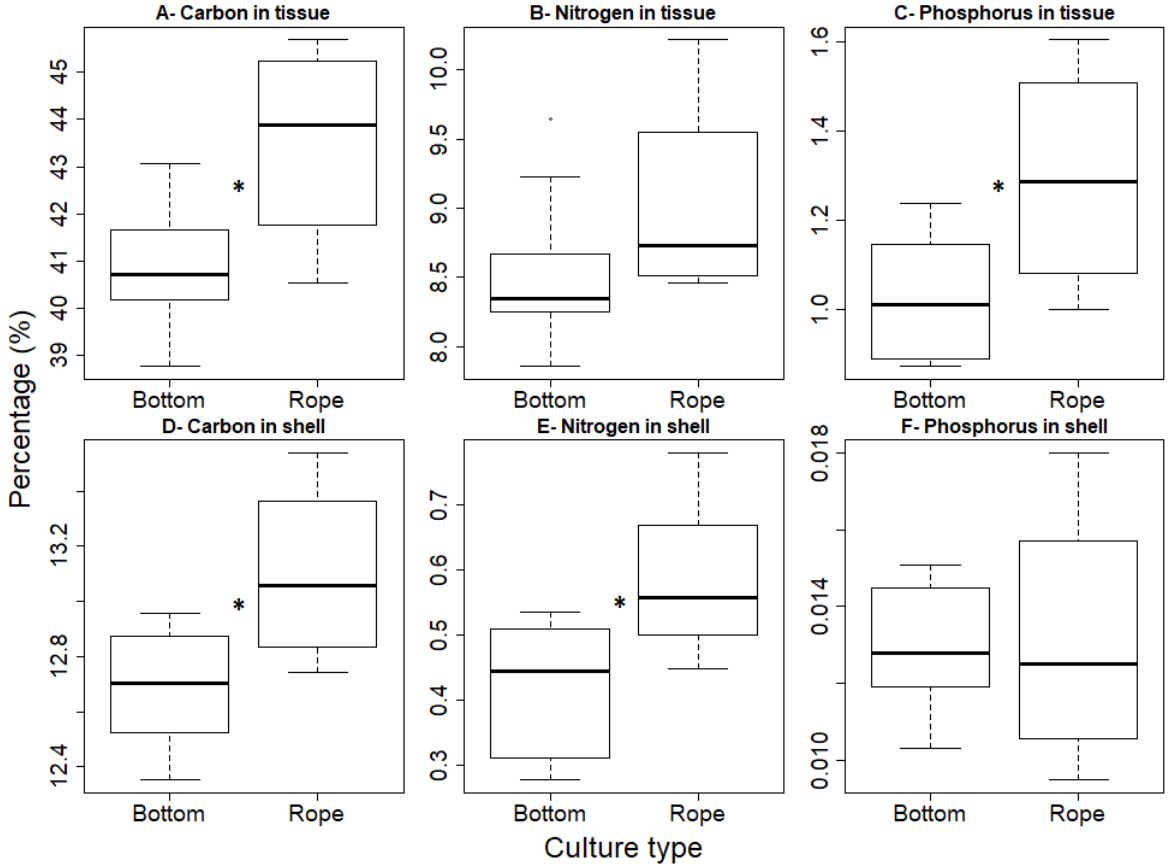

Figure 2 A comparison of the percentage (%) of (A,D) carbon, (B,E) nitrogen, and (C,F) phosphorus in mussel tissue (top row) and shell (bottom row). The boxes indicate the 25th and 75th percentiles, median (thick line), error bars indicating the 1.5 times inter-quartile range. The asterisks indicate a significant difference between culture methods. *GLM $p<0.05$.

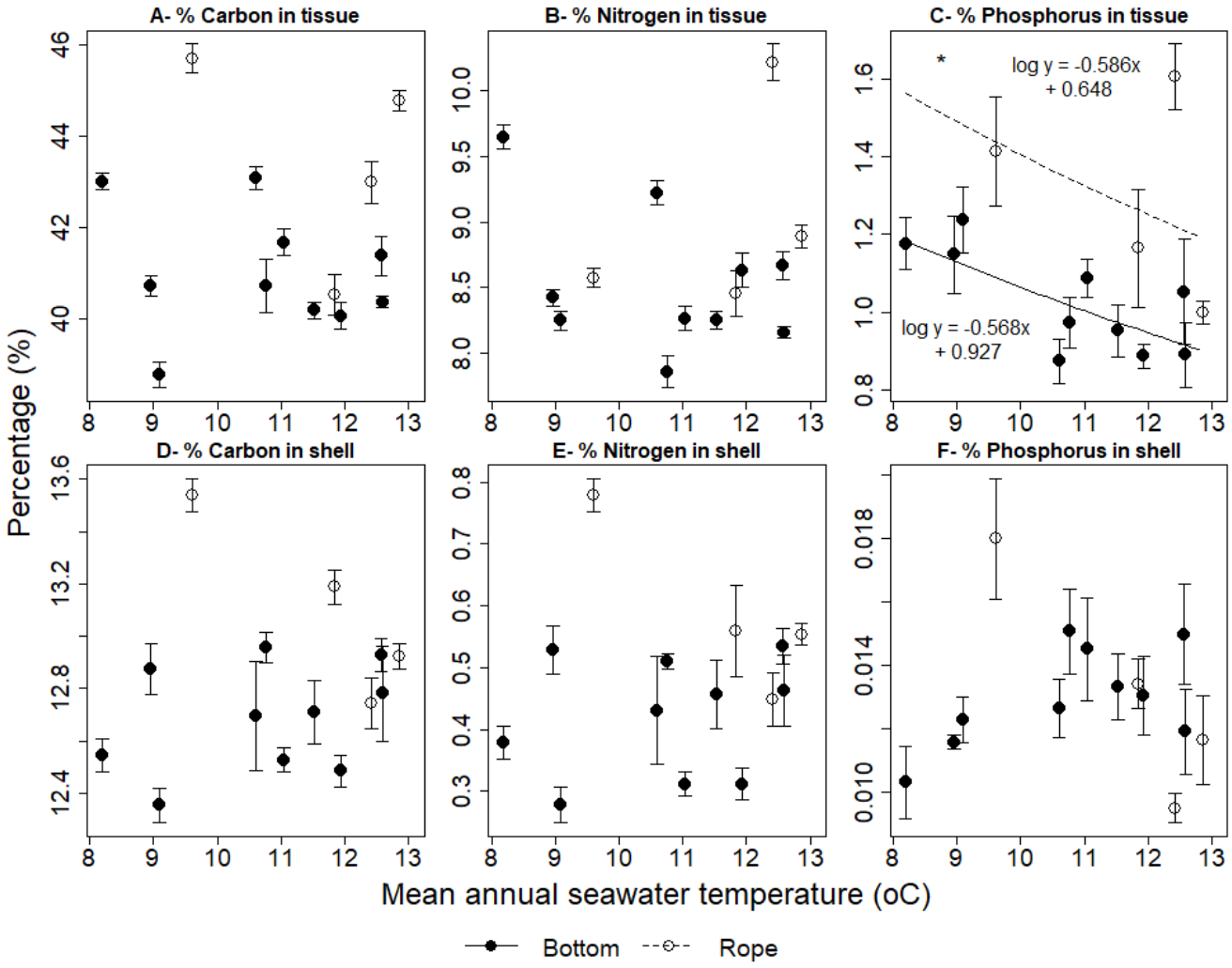

Figure 3 The percentage (%) of (A, D) carbon, (B, E) nitrogen, and (C, F) phosphorus (with SE bars) in mussel shell against the mean annual temperature (°C). Lines on graph indicate significant linear relationships. *GLM $p<0.05$. Filled circles and solid lines represent bottom cultured mussel sites and empty circles and dashed lines represent rope cultured.

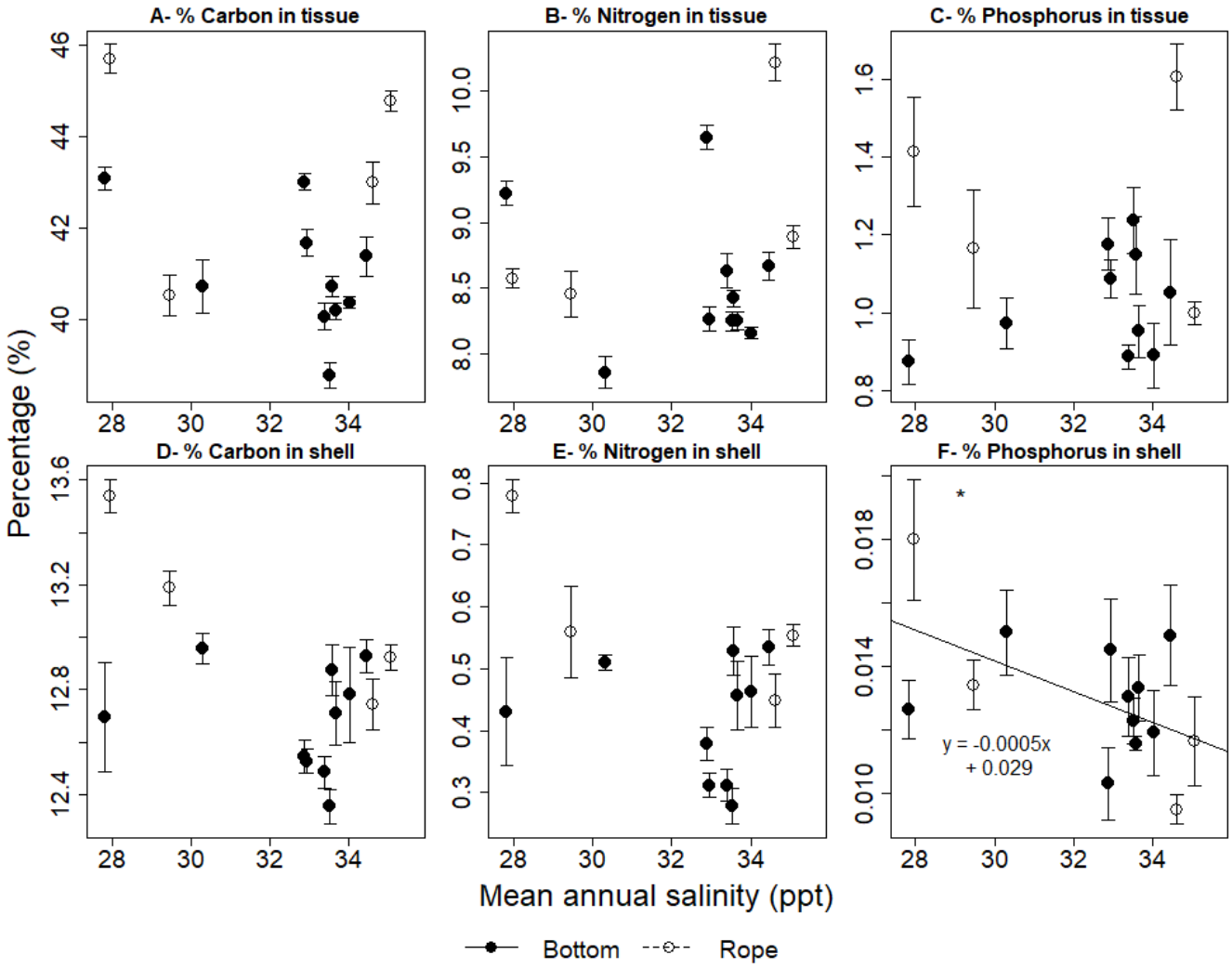

Figure 4 The percentage (%) of (A, D) carbon, (B, E) nitrogen, and (C, F) phosphorus (with SE bars) in mussel shell against the mean annual salinity (ppt). *GLM $p<0.05$. Filled circles represent bottom cultured mussel sites and empty circles represent rope cultured sites. Line on graph indicate significant linear relationship for pooled bottom and rope culture (3F).

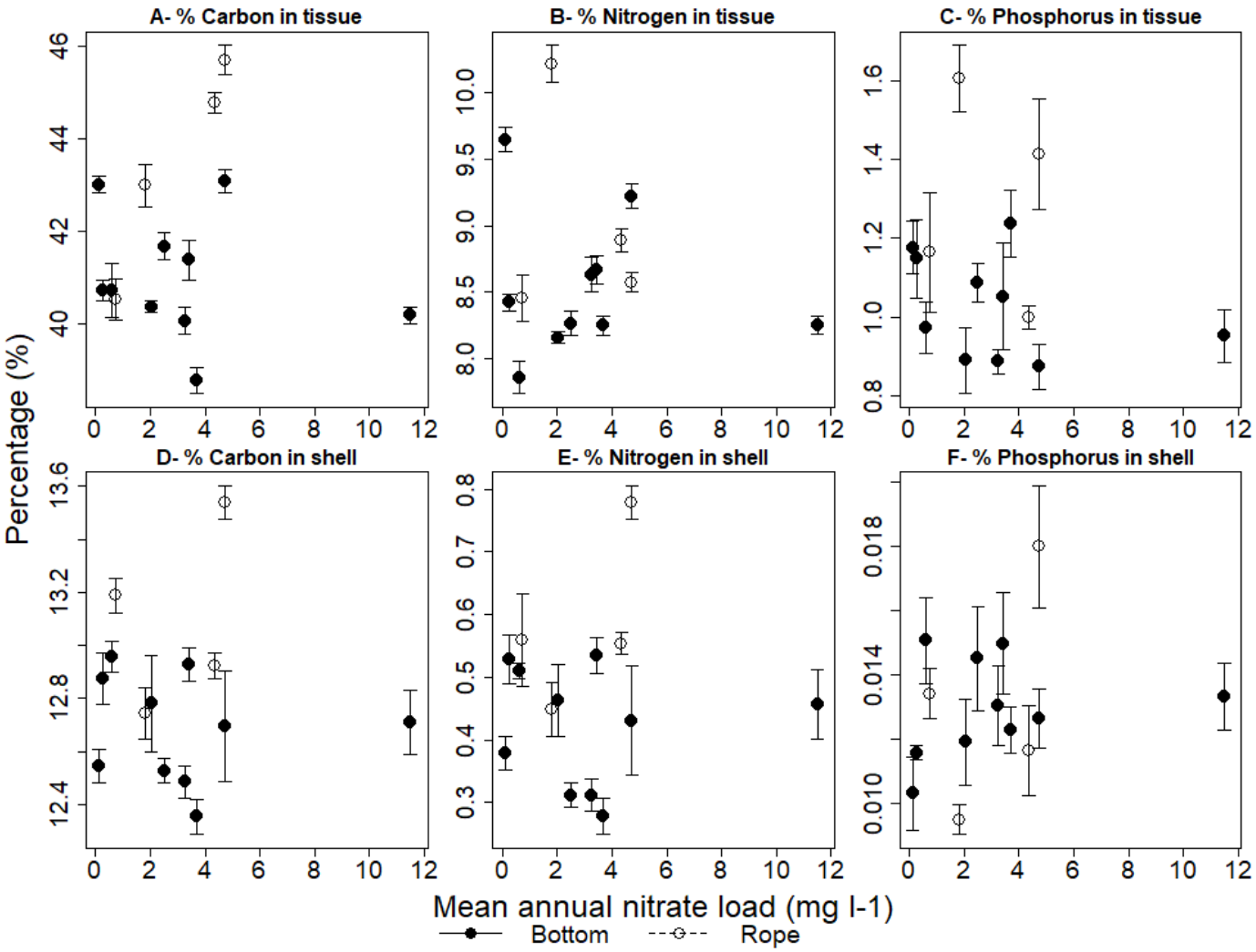

Figure 5 The percentage (%) content of (A, D) carbon, (B, E) nitrogen, and (C, F) phosphorus (with SE bars) in mussel shell against the average catchment annual nitrate concentration (mg l^{-1}) from the 14 sampled sites.

3.2 Kg of nutrient per tonne of live mussel

Impact of culture method on nutrient content

When expressed as a proportion of total weight of live mussel (kg $tonne^{-1}$), culture method had a significant effect on the kg of C, N, and P in tissue and shell (Figure 6). The carbon content in tissue (kg C $tonne^{-1}$) of rope cultured mussels (31.40 ± 2.88 kg) was double that in bottom cultured (14.59 ± 0.55 kg, Figure 6A). Conversely, the kg C $tonne^{-1}$ in shell of bottom cultured mussels (60.15 ± 0.77 kg) was significantly higher than in rope cultured (46.12 ± 1.69 kg,

Figure 6D). However, overall, there was no significant difference in the C kg tonne^{-1} of rope cultured (77.52 ± 3.65 kg) and bottom cultured mussels (74.74 ± 0.68 kg, Figure 6G).

The kg N tonne^{-1} in tissue of rope cultured mussels (6.48 ± 0.58) was also double that of bottom cultured (14.59 ± 0.55, Figure 6B). There was no significant difference in the N content of shell between bottom and rope cultured mussels (Figure 6E). Overall, there was significantly more N (kg N tonne^{-1}) in rope cultured (8.50 ± 0.59) than bottom cultured (5.00 ± 0.013, Figure 6H).

The kg P tonne^{-1} in tissue of rope cultured mussels (0.90 ± 0.07) was significantly higher than that of bottom cultured (0.69 ± 0.01, Figure 6C). In shell, bottom cultured mussels (0.06 ± 0.001) had significantly more kg P tonne^{-1} than that of rope cultured (0.04 ± 0.001, Figure 6F). Overall, there was significantly more kg P tonne^{-1} in rope cultured (0.95 ± 0.07) than bottom cultured (0.43 ± 0.01, Figure 6I).

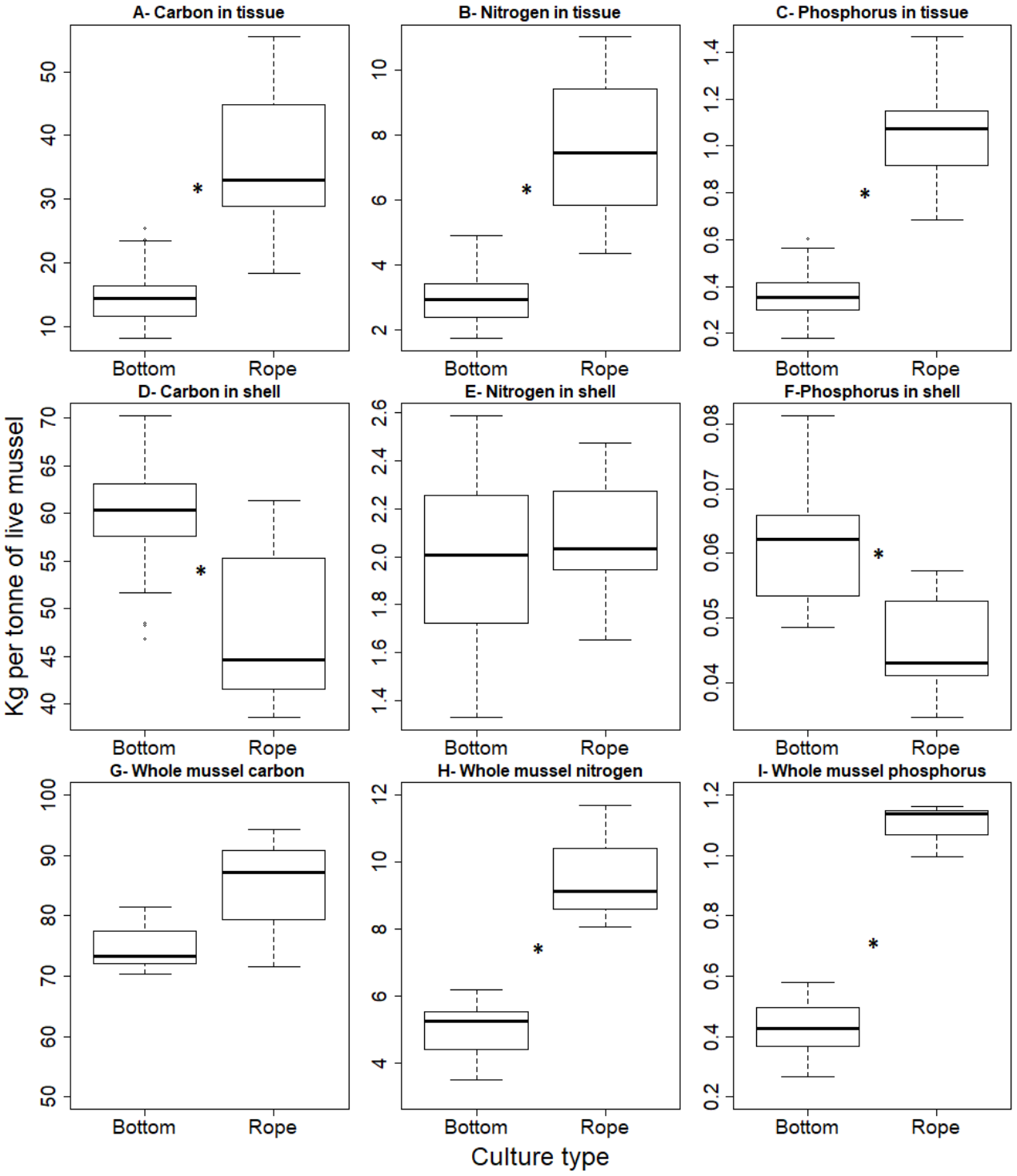

Figure 6 A comparison of kilograms (Kg) of carbon (A, D, G), nitrogen (B, E, H), and (C, F, I) phosphorus per tonne of live mussel. The boxes indicate the 25th and 75th percentiles, median (thick line), error bars indicating the 1.5 times inter-quartile range. The asterisks indicate a significant difference between culture methods. * $p<0.05$.

4 Discussion

This study showed that average annual seawater temperature and salinity did influence the P content of mussel tissue and shell, respectively. Average annual nutrient concentration, annual mean chlorophyll-*a*, tidal range and mean annual salinity had no influence on N, P or C content. This study has also shown that a crucial factor to consider when assessing the potential of C and nutrient removal services is the culture method used. There was significantly more C and P found in the tissue content of rope cultured mussels and C and N content in the shell of rope cultured mussels. When this was upscaled to predict the kg of C, N, and P tonne^{-1} of live mussels there was significantly more C, N and P in the tissue of rope grown mussels and significantly more C and P in the shell of bottom cultured mussels. When looking at the mussel overall, there was no significant difference in the C between rope and bottom cultured, however, significantly more N and P in the rope cultured mussels. Comparing these findings to a similar study in the Baltic (Buer et al., 2020) they found that the nutrient content within mussel tissue was not strongly related to the environmental parameters: salinity, average chlorophyll-*a* levels, or maximum water temperatures. They too found that mussels cultivated on suspended substrate in the upper water column exhibited considerably higher nutrient contents than mussels on the seabed. Tissue content of N and P in our study was similar to, but slightly higher than values reported for the Baltic by Buer et al. (2020), particularly for N in tissue of benthic mussels, around 30% higher, while that of rope-grown mussels were more similar.

The significant negative relationship between P in tissue and sea water temperature may relate to temperature influences on metabolism. Previous work by Widdows (1973) found that as water temperatures increased over a range from 5ºC to 20ºC, there was an increase in metabolism and oxygen uptake. Smaal and Vonck (1997) showed that respiration rates follow a seasonal pattern, with high values in early spring and summer, and relatively low values in autumn and winter. Their study also found that in the summer and autumn, there was relatively low P content, but this increased over winter and into spring, potentially due to a temperature effect on metabolism. Globally, average surface temperatures have increased by 0.7°C and are projected to rise by up to 4°C by the end of the century potentially causing large metabolic changes in intertidal *M. edulis* of which the body temperature of aerially exposed mussels can fluctuate by 20°C within 12 h (Mangan et al., 2019). The findings of our study could

demonstrate that in the future with a warming ocean, the increased water temperatures will reduce the potential of *M. edulis* to remediate phosphorus.

A number of studies have shown that temperature affects growth rates, with warmer waters facilitating faster growth (Riisgård et al., 2012; Mackenzie L Zippay and Helmuth, 2012). This would be an important factor to consider when using mussels as a tool for dealing with nutrient remediation, as this leads to a shorter grow out time from seed to harvest on mussel farms, and therefore more N would be removed. Whilst our findings show that warmer temperatures will have a negative relationship on P content in tissue, this could potentially be offset by the higher rate of nutrients removal due to the faster production cycle. This would require further modelling of nutrient uptake and retention with growth and the reproductive cycle to confirm.

The significant negative relationship between P in shell and salinity suggests that the ability to remove P through shell production will be more efficient in systems with lower salinity. Mussel shells are primarily composed of $CaCO_3$ (95–99% of CaCO3 as aragonite) but can also contain phosphate (P_2O_5) in the periostracum layer (Miculescu *et al*., 2018). The periostracum is a thin organic coating which is the outermost layer of the shell (Taylor and Kennedy, 1969). Mussels from less saline environments have thinner and weaker shells (Nagarajan et al., 2006). Kautsky et al. (1990) showed that the calcium carbonate content of mussel shells was higher in the highly saline environment of the North Sea (salinity 28 ppt) than in the Baltic Sea (salinity 7 ppt). Therefore, in mussels with thicker shells, the greater shell mass compared with the periostracum layer may lead to lower P content in shell samples. Studies have found reduced growth with decreasing salinity, and mussels in lower salinity environments will generally be smaller (Maar et al., 2015; Riisgård et al., 2012). Smaller shells would therefore have a greater surface area: volume ratio, leading to great P concentrations and potentially removal. This may have positive implications for using shellfish as a tool for nutrient removal in regions where coastal waters are cooler and less saline (Carlsson *et al*., 2012; Petersen *et al*., 2014). Mussels at lower salinities (<10 psu), may be stressed due to the extra maintenance costs from osmoregulation (Buer et al., 2020; Landes et al., 2015; Hans Ulrik Riisgård et al., 2013) and only reached a size of 30–45 mm after 18 months. However, it is important to note that the amount of nutrients remediated within shell is negligible towards the total nutrient content.

Whilst recognising the ongoing debate on the use of shellfish as a method of C sequestration

(Filgueira *et al*., 2015), it is still useful to estimate the tonnage of C being taken out of the system at harvest. This study found that despite having a similar weight of C removed in both bottom and rope cultured mussels, bottom cultured sites removed significantly more C in shell, similarly to previous work (Beadman *et al*., 2003). C incorporated in shell can be considered a long-term C store (Mangerud and Gulliksen, 1975), whilst C in tissue is consumed and respired. This indicates that if mussels were to be used as a method to capture C, it would be best achieved through bottom culture.

The most influential factor on potential for nutrient remediation was the culture method used. Globally bottom culture only accounts for approximately 15% of overall mussel production, with suspended and off-bottom culture accounting for 85% (McKindsey *et al*., 2011). The UK does not follow this trend, with more bottom culture than rope. In rope cultured mussels, due to lower predation pressure (Kamermans and Capelle, 2019), there is increased production of meat, with less energy put into producing thicker shells as a defence mechanism against predation. Bottom cultured mussels are also exposed to different environmental conditions such as tripton advection over mussels and the movement or reorganisation of mussels due to sedimentation (Hutchison et al., 2016) and in less well mixed waters can suffer from reduced oxygen events (Dolmer and Frandsen, 2002). All these factors can influence the growth of bottom cultured mussels.

As the tissue contains the highest proportion of nutrients, this led to significantly higher N and P content (kg tonne^{-1}) in rope cultured mussels. Taylor et al. (2019) found through modelling, that in Limfjorden using high-density spat collector configurations could produce yields in excess of 1600 t per model farm (~85 t ha^{-1}) within a 6-month growth cycle, were able to exceed 2500 t (~133 t ha^{-1}). They also estimated that it would be possible to extract 0.6-1.4 t N ha^{-1} within one growth season from July till March, similar to other studies in the region (Nielsen et al., 2016; Petersen et al., 2014). Taylor et al. (2019) also found in their study that through optimising culture methods the potential production of mussels and associated nutrient mitigation could be improved. Modelling the potential nutrient remediation potential of rope farms in the Baltic Sea has been estimated to remove between 17- 65 t N farm^{-1} year^{-1}, depending on the scale of farms being used and the density at which mussels are produced (Holbach et al., 2020). While it should be noted that the Baltic Sea presents a very different environment from around the UK, with lower salinity and lower tidal ranges, and generally

greater nutrient concentrations, these are important factors to consider when selecting sites for mussel farms to be used purely for nutrient remediation.

Bottom culture is typically regarded as the least efficient method for culturing mussels, due to the high density dependent losses (Cubillo *et al*., 2012) and predation pressure (Capelle *et al*., 2017). Therefore, overall production levels are greater on rope than for bottom culture. Kamermans and Capelle (2019) summarised mussel production across several studies. While there was a large variation in the areal density of mussels produced on ropes, the average density was 69.6 kg m^2, whilst bottom aquaculture typically having an average 6.4 kg m^2. Combined with the higher nutrient content of rope-grown mussels, this would indicate that future developments for nutrient remediation would look to utilise suspended rope mussel aquaculture due to the potential higher nutrient yield per area. In addition, there is limited potential to expand current intertidal sites or expand to new intertidal sites. As such, moving mussel farming offshore would seem the logical next step, with large offshore sites already being developed off the south coast of England (Sheehan *et al*., 2020). Buck et al. (2010) look at the potential for utilising offshore wind farms as sites to co-locate mussel aquaculture, suggesting that it could provide a suitable environment for seed mussel collection in the German Bight. Further work by Brenner et al. (2012) highlights the importance of ensuring that such sites are suitably offshore where the influence of estuarine run-off is reduced. Offshore rope cultivation therefore seems to be the location with the greatest potential for increasing production but may not be the best location to maximise nutrient removal.

1 Conclusions

The findings of this study suggest that when planning a large-scale policy approach to using mussels as nutrient remediators, the most important factor to consider is the method of culture. Rope cultured mussels removed double the amount of N and P per tonne of live mussel compared with bottom cultured mussels. Increasing average sea surface temperature had a negative effect on P concentration in tissue, however, with faster growth more kg of N would be removed, and potentially the increased growth could offset the decrease in P concentration caused by the warmer water. Similarly, while more kgs of C is incorporated in the shell of bottom cultured mussels, this too could be offset by the greater growth rates found in rope-

cultured mussels. In this study, the effect of growth was not calculated. We suggest that further work should explore how much additional potential for nutrient remediation is possible when variation in growth rates is accounted for.

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Supplementary material

S1 Supplementary Material Best linear model for percentage content of C, N and P and kg of C, N and P in shell and tissue. Asterisks indicate parameter significance. * GLM, p≤0.05, ** p≤0.01, *** p≤0.001.

Model Number	Response Variable	Best Linear Model	Adjusted R^2 value	Model Significance
1	%C in tissue	culture type*	0.3087	$P \leq 0.001$
2	%P in tissue	culture type* + mean annual seawater temperature*	0.4855	$P \leq 0.05$
3	%C in shell	culture type* + salinity	0.4841	$P \leq 0.001$
4	%N in shell	culture type*	0.3031	$P \leq 0.001$
5	%P in shell	Salinity*	0.2367	$P \leq 0.01$
6	Kg C in tissue	culture type*	0.5272	$P \leq 0.001$
7	Kg N in tissue	culture type**	0.5464	$P \leq 0.001$
8	Kg P in tissue	culture type***	0.6309	$P \leq 0.001$
9	Kg C in shell	culture type**	0.5587	$P \leq 0.001$
10	Kg P in shell	culture type**	0.4728	$P \leq 0.001$