



# European lobsters utilise Atlantic salmon wastes in coastal integrated multi-trophic aquaculture systems

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**ABSTRACT:** In this study, we investigated if juvenile European lobsters *Homarus gammarus* would eat waste from Atlantic salmon *Salmo salar* cages in a coastal integrated multi-trophic aquaculture (IMTA) setup and if there were any impacts on growth. Trophic interactions between salmon and lobsters were assessed using  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  stable isotope analysis and fatty acid profiling from fish feed as indicators of nutrient flow. Analysis revealed that lobsters directly utilised particulate waste from salmon production, as levels of indicator fatty acids from salmon feed were significantly higher in lobster tissues near the fish cages compared to the control site. Route of uptake may have been direct consumption of waste feed or faecal material or indirectly through fouling organisms. Stable isotope analysis did not indicate nutrient transfer to lobsters, suggesting that the duration of the study and/or the amount of waste consumed was not sufficient for stable isotope analysis. Lobsters grew significantly over the trial period at both sites, but there was no significant difference in lobster growth between the sites. Our results show a trophic relationship between salmon and lobsters within this IMTA system, with no apparent advantage or disadvantage to growth.

**KEY WORDS:** Integrated multi-trophic aquaculture · IMTA · Lobster · Salmon · Fatty acids · Stable isotopes · Ecosystem services

## 1. INTRODUCTION

The European lobster *Homarus gammarus* (Linnaeus, 1758) is an important economic decapod crustacean, with a natural distribution ranging from Morocco to northern Norway (Wilson 2008). Global demand for lobster is increasing, but over the past few decades wild stocks have been decreasing (Drengstig & Bergheim 2013, Ellis et al. 2015, Nillos Kleiven et al. 2019), prompting research into commercial hatchery production of larvae for restocking purposes (Addison et al. 1994, Schmalenbach et al. 2011). However, lobsters grown in land-based hatcheries and then directly released into the environment are known to be vulnerable to immediate predation as they have limited exposure to environmental stimuli

(Agnalt et al. 2017). To improve survivability, sea-based containers have been used successfully to acclimatise juvenile lobsters to environmental conditions before final release (Beal et al. 2002, Perez Benavente et al. 2010, Beal & Protopopescu 2012, Daniels et al. 2015, Halswell et al. 2016). At the release stage, juvenile lobsters form small burrows in sediment and feed on zooplankton and organic matter particles suspended in the water column, using currents created by their pleopods (swimmerets). The particle sizes consumed at this stage are normally between 60 and 100  $\mu\text{m}$  (Lavalli & Barshaw 1989), suggesting that they are able to consume fine particulate wastes from fish farms.

Atlantic salmon *Salmo salar* cage farms are generally sited in coastal environments and release nutrient-

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rich particulate and dissolved wastes such organic carbon and nitrogen from uneaten food and faecal wastes and excreted soluble nitrogen into the surrounding environment (Wang et al. 2012). The concept of integrated multi-trophic aquaculture (IMTA) endeavours to utilise these wastes, thereby increasing the resource efficiency of trophically linked co-cultured species to improve nutrient utilisation for environmental mitigation while producing additional organisms (Chopin et al. 2012). Placing lobsters in sea-based containers next to fish cages may provide a regular food supply, together with other potentially beneficial conditions (e.g. shelter, co-location for efficiency improvements), for improved growth and management. This may also function as an ecosystem service from salmon aquaculture and provide positive societal benefits; for example, if there is improvement of growth and better survival when released, it could aid enhancement of local lobster populations and any resulting fishery.

Performance of IMTA can be measured empirically by comparing growth or nutrient uptake by the consumer species between the culture site and a reference location. This approach has been used to assess shellfish growth (Sarà et al. 2009, Lander et al. 2013) and nutrient uptake and growth of seaweeds (Blouin et al. 2007, Abreu et al. 2009). Direct nutrient transfer between species is commonly investigated using biochemical tracers, such as fatty acids (FAs) and stable isotopes (Redmond et al. 2010, Colombo et al. 2016, White et al. 2019, Sardenne et al. 2020).

FAs have been used to assess the dispersal of particulate-derived waste, both spatially and through food webs, with studies assessing the level of influence aquaculture has on wild fish and benthic communities (e.g. Fernandez-Jover et al. 2009, 2011, White et al. 2017, 2019, Woodcock et al. 2018). However, assessing the performance of an IMTA system with FAs is dependent on the target species, as there have been variable results using blue mussels *Mytilus edulis* (Redmond et al. 2010, Irisarri et al. 2015), although more consistency has been found for zooplankton (Fernandez-Jover et al. 2009), shrimp (Olsen et al. 2012) and sea urchins (George & Parrish 2015).

Stable isotopes of carbon and nitrogen have been used widely to assess the flow of nutrients through food webs, and more recently, to trace waste products from aquaculture (Marín Leal et al. 2008, Deudero et al. 2012). Stable isotopes assimilate within the tissues of consumers, with heavier isotopes,  $^{13}\text{C}$  and  $^{15}\text{N}$ , remaining longer in animal tissues than lighter ones,  $^{12}\text{C}$  and  $^{14}\text{N}$ , which are rapidly utilised during metabolism (Marín Leal et al. 2008). Consequently, the tissues of

organisms tend to adopt the same stable isotopic signature as their food source (Paulet et al. 2006).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios have been applied for nutrient tracking within IMTA systems with fish and mussels in the Bay of Fundy, Canada (Irisarri et al. 2015), fish with oysters and mussels (Navarrete-Mier et al. 2010) and in multiple farms with fish and mussels in the Western Mediterranean (Sanz-Lazaro & Sanchez-Jerez 2017).

The aim of this study was to investigate if juvenile European lobsters would consume wastes from salmon cages by assessing the nutrient transfer between salmon waste and lobsters within a pilot-scale coastal site and whether there were any positive or negative effects on growth. FA analysis and stable isotopes were used as tracers of nutrient transfer, and growth was determined as an increase in carapace length (CL).

## 2. MATERIALS AND METHODS

### 2.1. Experimental site and data collection

The study was conducted at the Lehanagh Pool marine research site, a small-scale experimental IMTA site in Bertraghboy Bay, Connemara, County Galway, Ireland (Fig. 1), between January 2018 and March 2019. The site comprised 2 polar circle cages (50 m circumference and 8 m deep) at a water depth of 21 m. These were stocked in April 2018 with 7660 Atlantic salmon post-smolts (5360 and 2300 in the 2 cages), averaging 90 to 100 g. In addition, 400 lumpfish *Cyclopetrus lumpus*, averaging 40–50 g, were also stocked into each cage as a preventative method for controlling sea lice as per commercial standards. The fish were hand-fed a maintenance diet according to the manufacturer's feeding tables. The site was managed according to organic farm standards, with no prescription medicines or antifoulants used. A control site for the study was set up approximately 300 m west of the cages. European lobsters (Stage IV juveniles,  $n = 204$ ) were deployed in May 2018 (108 at the cage site, 96 at the control site) within sea-based container culture (SBCC) structures (see Daniels et al. 2015). Lobsters ( $n = 36$ ) were housed individually and labelled in each stack suspended from the cages at 2 m depth and from a longline at the control site at the same depth.

Particulate organic matter loading from the salmon cages was modelled with a spreadsheet-based particulate dispersion model (Telfer et al. 2006) to assess the organic load dispersal and deposition around the cages and ensure that the SBCC structures were situated in locations where the lobsters would be exposed to salmon waste and that the control site was far enough

away not to be influenced by particulate wastes. The model was run using data from fish production and measured current speeds and directions collected using a MIDAS ECM self-recording electromagnetic current meter (Valeport) deployed at 2 depths (9 and 14 m) within 50 m of the cages between 1 and 15 July 2018.

During this time, 3 sediment traps were positioned to assess the numerical and distributional accuracy of the model. Two sediment traps were placed at a depth of 19 m, one 0 m and one 5 m from the south-west cage; the latter was between the 2 cages. The third sediment trap was deployed at the control site at 5 m depth. Each sediment trap had 4 cylinders with a height:diameter ratio 7.5:1 (60:8 cm) secured vertically on a gimballed stainless steel frame. A clear container (plastic fixed pot) was screwed onto the bottom of every cylinder. The design of the sediment traps was based on specifications in Blomqvist & Hakanson (1981). Total sedimentation rate per m<sup>2</sup> over 15 d was calculated by dry weight (DW). Model outputs were compared to amount of waste material collected in deployed sediment traps next to the cages to assess model accuracy.

## 2.2. Lobster growth

Lobsters were deployed in SBCCs at the cage site at the beginning of April 2018, and growth measurements commenced after a period of 1 mo. Growth of lobsters was assessed by measuring CL (mm) on 9 occasions over a 319 d period, between May 2018 and March 2019. Due to variable weather conditions, between 20 and 60 lobsters were randomly subsampled from the cage and control sites, at each sampling event. Each lobster was removed from the SBCC unit and placed on gridded paper. A digital image was taken at 90 degrees to each specimen. The lobster was then returned to its container and to the water. CL was measured from the images using Digimizer image analysis software (MedCalc Software).

CL gain (CLG) was used as an indicator of growth at the cage and control sites and was calculated as:

$$\text{CLG} = \text{CL}_t - \text{CL}_0 \quad (1)$$

where CL<sub>t</sub> is final CL and CL<sub>0</sub> is initial CL.

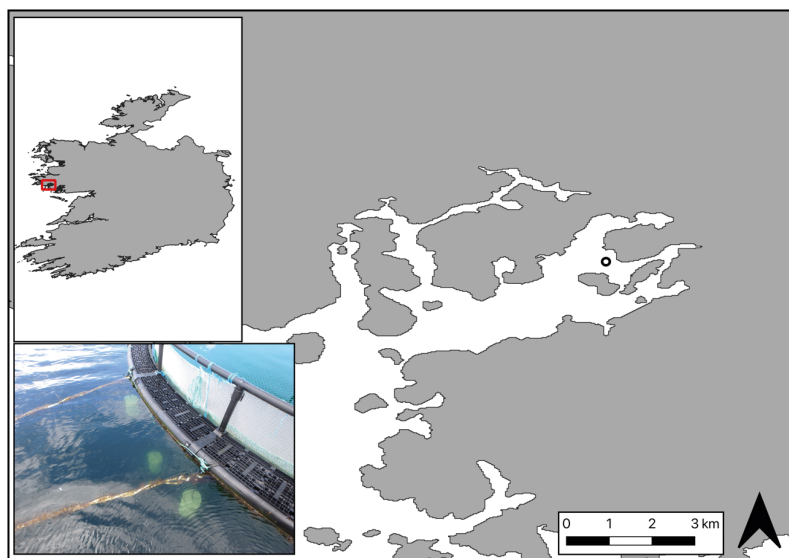


Fig. 1. Bertraghboy Bay, Ireland, and the location of the marine research site. The photograph shows the cage site and the sea-based container culture (SBCC) structures. The black circle indicates the location of the experimental site in relation to the Irish West Coast

A von Bertalanffy growth curve (von Bertalanffy 1938) was fitted to the mean CL measurements during the trial:

$$L(t) = L_{\infty} \{1 - \exp[-K(t - t_0)]\} \quad (2)$$

where  $L(t)$  is CL (mm) as a function of time ( $t$ ),  $L_{\infty}$  is the largest lobster CL, and  $K$  is the growth coefficient ( $\text{yr}^{-1}$ ).

## 2.3. Lipid and FA analysis

At the end of the trial (March 2019), 20 juvenile lobsters were sampled and stored in a freezer ( $-20^{\circ}\text{C}$ ) overnight. The next day, samples were shipped on ice to the University of Stirling for analysis. Ten individual juvenile lobsters from the control and 10 from the cage location, and a sample of the salmon aqua-feed used at the cage site were individually homogenised and subjected to lipid extraction.

Total lipids were extracted from 0.5–1.0 g of feed and lobster tissue in ice-cold chloroform:methanol solution (ratio 2:1, v/v). Extraction of lobster tissue was achieved using 20 ml of solution and feed using 36 ml of solution. The samples were homogenised in an Ultra-Turrax tissue disruptor (Fisher Scientific). Lipid content was determined gravimetrically (Folch et al. 1957).

FA methyl esters (FAMES) were separated from total lipids by acid-catalysed transmethylation at  $50^{\circ}\text{C}$

for 16 h using 2 ml of 1 % (v/v) sulphuric acid (95 %, Aristar®, BDH Chemicals) in methanol and 1 ml toluene (Christie 1993). FAMES (6 ml) were extracted and purified by adsorption chromatography using 500 mg sorbent acid washed solid-phase extraction cartridges (Clean-up® silica extraction columns; UCT). Cartridges were pre-conditioned with 5 ml of isohexane before the sample was added and the FAMES eluted with 10 ml isohexane:diethyl ether (95:5, v/v) and separated and quantified by gas-liquid chromatography using a Fisons GC-8160 (Thermo Scientific) equipped with a 30 m × 0.32 mm i.d. × 0.25 µm ZB-wax column (Phenomenex). Hydrogen was used as the carrier gas with an initial oven gradient of 50 to 150°C at 40°C min<sup>-1</sup> to a final temperature of 230°C at 2°C min<sup>-1</sup>. Individual FAMES were identified by comparison to standards (Supelco™ 37- FAME mix; Sigma-Aldrich). All data were collected and processed using Chrom-Card™ for Windows (Version 1.19; Thermoquest Italia) software. 17:0 heptadecanoic acid was used as internal standard to calculate FA content per g of tissue.

Predominant FAs measured within the salmon feed were used as tracers. A total of 5 FAs were selected. Three FAs, oleic acid (OA, 18:1n-9), linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3), were based on the increased 'terrestrial' FAs derived from an increasing inclusion level of vegetable oil used within salmon feed (Sprague et al. 2016). Cetoleic acid (22:1n-11) and eicosenoic acid (20:1n-9) were also chosen, as they tend to be typically found in higher quantities within salmon feed and have consequently been observed in fish farm waste (Henderson et al. 1997).

#### 2.4. Stable isotope analysis

Five samples of lobster leg muscle from each site (n = 5 control site, n = 5 cage site) and 2 samples of salmon feed were subjected to stable isotope analysis. Each sample was frozen at -20°C prior to lyophilisation in a Christ Alpha 1-4 LSC freeze-drier (Martin Christ Gefriertrocknungsanlagen). For the analysis, 0.7 mg of lobster leg muscle and 1.5 mg of feed were weighted into 3 × 5 mm tin capsules and loaded onto an Elementar Procure analyser, which converted organic N and C in the samples to N<sub>2</sub> and CO<sub>2</sub> for measurement of δ<sup>15</sup>N and δ<sup>13</sup>C, respectively, on a Thermo-Fisher-Scientific Delta XP Plus isotope ratio mass spectrometer.

Units of isotope ratios were expressed in δ<sup>15</sup>N and δ<sup>13</sup>C:

$$\delta X = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \quad (3)$$

where  $X$  is <sup>13</sup>C or <sup>15</sup>N, and  $R$  is either the <sup>13</sup>C:<sup>12</sup>C ratio or the <sup>15</sup>N:<sup>14</sup>N ratio. In-house reference materials used were: gelatine solution, alanine-gelatine solution spiked with <sup>13</sup>C-alanine, and glycine-gelatine solution, each dried for 2 h at 70°C. Four USGS 40 glutamic acid standards (Qi et al. 2003, Coplen et al. 2006) were used as independent checks of accuracy.

#### 2.5. Statistical analyses

FA percentages for each sample were arcsine transformed prior to statistical analysis to correct for the binomial distribution of proportional data (Sokal & Rohlf 1995). FAs occupying more than 0.1 % of the feed FA profile were compared between the cage and the control site, using a 2-sample Student's *t*-test. Statistical tests were performed using Minitab™ statistical software. Principal component analysis (PCA) was used to classify and discriminate between the FA profiles of the lobster samples at different locations. PCA creates 2 orthogonal values (principal components, PCs) which are representative of the original variables. The higher the PC value is, the more representative of the data set it is. PCA was performed using MVSP statistical software (KCS). A non-parametric multivariate ANOSIM was performed with PRIMER 5 (Clark & Gorley 2001) to detect significant differences between *a priori* sources of variation for the cage site and control site results (as defined factors) using a Bray-Curtis similarity matrix.

Stable isotopes (δ<sup>13</sup>C and δ<sup>15</sup>N) for lobster leg muscle were compared between the 2 locations using a 2-sample Student's *t*-test, using Minitab software.

Growth was compared between the 2 locations over time with a repeated-measures ANOVA, using SPSS Ver 26 software (IBM). As sphericity is an important assumption of a repeated-measures ANOVA (where the variances of the differences between all possible pairs of within-subject conditions are equal), the growth data were tested using Mauchly's test of sphericity.

### 3. RESULTS

#### 3.1. Waste dispersion and current flow at the cage site

The hydrographic data collected by the current meter showed that the cage site was characterised by moderate to slow average current speeds of 0.040 m s<sup>-1</sup> in mid-water and 0.028 m s<sup>-1</sup> near the seabed. The

residual current flow showed a very slow movement in a southerly direction over time. Distribution of particulate waste from the cages over the trial period was very local to the fish cages, with only low amounts of particulate waste travelling beyond 20 m from the cage edge (Fig. 2).

Model predictions of sedimentation for suspended solids near the SBCC deployment locations ( $100\text{--}200\text{ g m}^{-2}\text{ 15 d}^{-1}$ ) showed broad agreement with average sediment trap-collected material ( $252\text{ g m}^{-2}\text{ 15 d}^{-1}$ ). However, the model underestimated the distribution of solid waste between the cages, with modelled values of 50 and  $100\text{ g m}^{-2}\text{ 15 d}^{-1}$  vs. an average measured value of  $368\text{ g m}^{-2}\text{ 15 d}^{-1}$ . Both model and sediment traps illustrated that the SBCCs near the cages were placed in areas of high particulate waste distribution of between 100 and  $200\text{ g m}^{-2}\text{ 15 d}^{-1}$ . The models also showed little possibility that much particulate waste from the cages would reach the control lobster site approximately 300 m to the west.

### 3.2. FAs

The FA profile of lobster tissues varied with location, as the amount of FA tracers (given as % of total lipid) showed significant differences ( $p < 0.05$ ) between the cages and control stations (Table 1). Lobsters located near the cages had a significantly higher total lipid content ( $1.22 \pm 0.34\text{ mg g}^{-1}\text{ DW}$ ; mean  $\pm$  SD) than those at the control site ( $0.93 \pm 0.19\text{ mg g}^{-1}\text{ DW}$ ) ( $p < 0.05$ ). The FA profile of the supplemented sal-

mon feed was largely characterised by OA (18:1n-9; 24.4 %), eicosenoic acid (20:1n-9; 7.2 %), cetoleic acid (22:1n-11; 12.8 %), LA (18:2n-6; 11.6 %) and ALA (18:3n-3; 4 %), confirming that these FAs are indicative and appropriate for use as tracers. The relative percentages of these FAs were significantly higher ( $p < 0.05$ ) in lobsters located near the cage site (Fig. 3) than in those at the control site. Additionally, n-6 polyunsaturated FA (PUFA) levels were found to be significantly higher at the cage site ( $12.24 \pm 0.77\%$ ) compared to the control ( $10.27 \pm 0.65\%$ ) ( $p < 0.05$ ), with LA (18:2n-6) being higher in the cage ( $6.3 \pm 1.4\%$ )

Table 1. Fatty acid (FA) profile (% FA of total lipid) of lobster muscle. Values are means ( $\pm$ SD). Last column indicates level of significance between the 2 locations (\*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ ). PUFA: polyunsaturated FA; DMA: dimethyl acetal. Total saturated FAs also include 15:0, iso17:0, anteiso17:0, iso18:0, anteiso18:0, 22:0 and 24:0; total monoenes also include 16:1n-9, 17:1, 20:1n-11, 20:1n-7 and 22:1n-9; total n-6 PUFAs also include 18:3n-6, 20:3n-6, 22:4n-6 and 22:5n-6; total n-3 PUFAs also include 21:5n-3; total PUFAs also include 16:2, 16:3 and 16:4; total DMA includes 16:0 DMA, 18:0 DMA, 18:1 DMA and 20:0 DMA. NA: not applicable

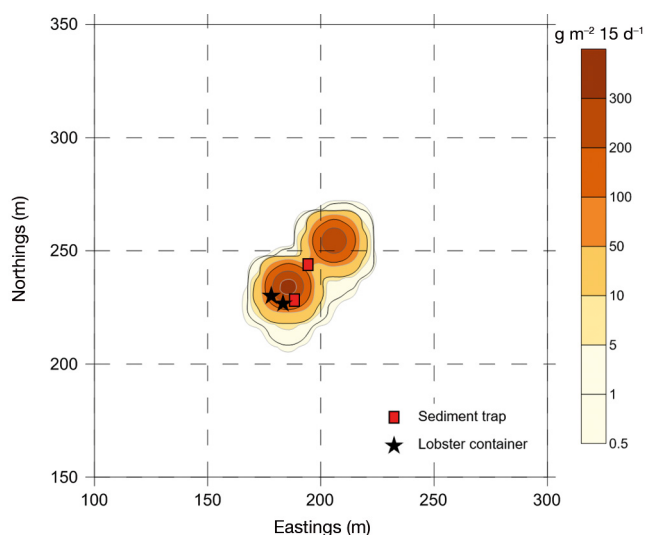


Fig. 2. Contour plot of suspended solids settlement ( $\text{g m}^{-2}\text{ 15 d}^{-1}$ ) around cages 1 and 2, presented as output from a spreadsheet-based dispersion model

	Feed	— Lobsters — Cages	Control	Signifi- cance
<b>Total lipid (%)</b>	<b>24.16</b>	<b><math>1.22 \pm 0.34</math></b>	<b><math>0.93 \pm 0.18</math></b>	
Fatty acids				
14:0	4.6	$1.2 \pm 0.3$	$0.5 \pm 0.1$	***
16:0	10.6	$12.1 \pm 0.5$	$11.8 \pm 0.5$	NA
18:0	1.5	$4.6 \pm 0.6$	$6.8 \pm 0.4$	***
20:0	0.3	$0.4 \pm 0.0$	$0.5 \pm 0.1$	***
<b>Total saturated</b>	<b>17.4</b>	<b><math>19.3 \pm 0.8</math></b>	<b><math>20.8 \pm 0.5</math></b>	***
16:1n-7	3.4	$3.1 \pm 0.5$	$2.7 \pm 0.4$	*
18:1n-9	24.0	$14.6 \pm 1.6$	$8.4 \pm 0.8$	***
18:1n-7	2.0	$5.2 \pm 0.4$	$6.3 \pm 0.6$	***
20:1n-9	7.2	$3.4 \pm 0.5$	$1.5 \pm 0.2$	***
22:1n-11	12.7	$3.9 \pm 1.2$	$0.1 \pm 0.1$	***
24:1n-9	0.7	$0.4 \pm 0.1$	$0.1 \pm 0.1$	***
<b>Total monoenes</b>	<b>51.9</b>	<b><math>33.8 \pm 3.6</math></b>	<b><math>21.6 \pm 1.3</math></b>	***
18:2n-6	11.5	$6.4 \pm 1.4$	$0.9 \pm 0.1$	***
20:2n-6	0.2	$1.2 \pm 0.2$	$1.4 \pm 0.1$	*
20:4n-6	0.3	$3.6 \pm 0.7$	$6.6 \pm 0.9$	***
<b>Total n-6 PUFAs</b>	<b>12.0</b>	<b><math>12.2 \pm 0.8</math></b>	<b><math>10.3 \pm 0.7</math></b>	***
18:3n-3	4.0	$0.8 \pm 0.1$	$0.5 \pm 0.1$	***
18:4n-3	2.0	$0.6 \pm 0.2$	$0.4 \pm 0.1$	**
20:3n-3	0.1	$0.4 \pm 0.0$	$0.5 \pm 0.0$	***
20:4n-3	0.3	$0.5 \pm 0.1$	$0.5 \pm 0.1$	NA
20:5n-3	5.0	$14.9 \pm 2.0$	$22.8 \pm 1.0$	***
22:5n-3	0.6	$1.2 \pm 0.2$	$1.4 \pm 0.8$	NA
22:6n-3	5.5	$13.8 \pm 1.5$	$17.3 \pm 1.5$	***
<b>Total n-3 PUFAs</b>	<b>17.8</b>	<b><math>32.3 \pm 3.0</math></b>	<b><math>43.3 \pm 1.4</math></b>	***
<b>Total PUFAs</b>	<b>30.7</b>	<b><math>44.7 \pm 2.6</math></b>	<b><math>54.1 \pm 1.4</math></b>	***
<b>Total DMA</b>	<b>0.0</b>	<b><math>2.2 \pm 0.5</math></b>	<b><math>3.4 \pm 0.5</math></b>	***



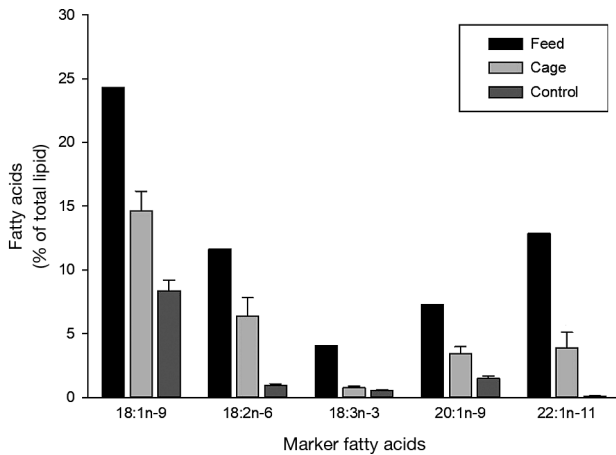


Fig. 3. Contribution of selected fatty acids (% of total lipid, mean  $\pm$  SD) to the total fatty acid content in the lobster tissue at the end of the experiment (March 2019) at the control and cage sites. Feed levels are included for reference

compared to the control ( $0.9 \pm 0.1\%$ ) ( $p < 0.05$ ) (see Table 1). Overall, tracer FAs accounted for 29.08% of the total lipid content of the cage station, which was significantly higher when compared to the control station (11.36%). Conversely, total n-3 PUFA levels were significantly higher ( $p < 0.05$ ) at the control site ( $43.27 \pm 1.38\%$ ) compared to the cage site ( $32.3 \pm 3\%$ ). FAs contributing to the difference between the control and the cage site were eicosapentaenoic acid (EPA,  $22.8 \pm 1\%$  and  $14.9 \pm 0.9\%$ ) and docosa-

hexaenoic acid (DHA,  $17.2 \pm 1.46\%$  and  $13.8 \pm 1.4\%$ ), respectively.

The PCA plot (Fig. 4) indicated clear differences between FA profiles of lobster leg muscle from the cage site and from the control site along PC-1, which accounted for 92.8% of the total variance in the data. Post hoc ANOSIM confirmed the groups from control and cage sites were significantly different ( $p < 0.05$ ,  $R = 0.994$ ). The FAs primarily driving this difference at the cage site were OA (18:1n-9) and LA (18:2n-6) of terrestrial origin, along with eicosenoic acid (20:1n-9) and cetoleic acid (22:1n-11). These are marine oil-based, and both were incorporated at high levels within the salmon feed. On the other hand, EPA and DHA together with arachidonic acid (20:4n-6) were more dominant in defining the FA profile at the control site, making it likely that the diet of the lobsters was primarily influenced by naturally derived marine oils, accounting for 40.4% of total FAs compared to 28.7% for lobsters at the cages (Table 1).

### 3.3. Stable isotopes

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures for lobster tissue at the control and cage sites were similar, as shown by their similar positions in the plot (Fig. 5). However, the signature for salmon feed differed from those found for lobsters (Fig. 5). A 2-sample Student's *t*-test

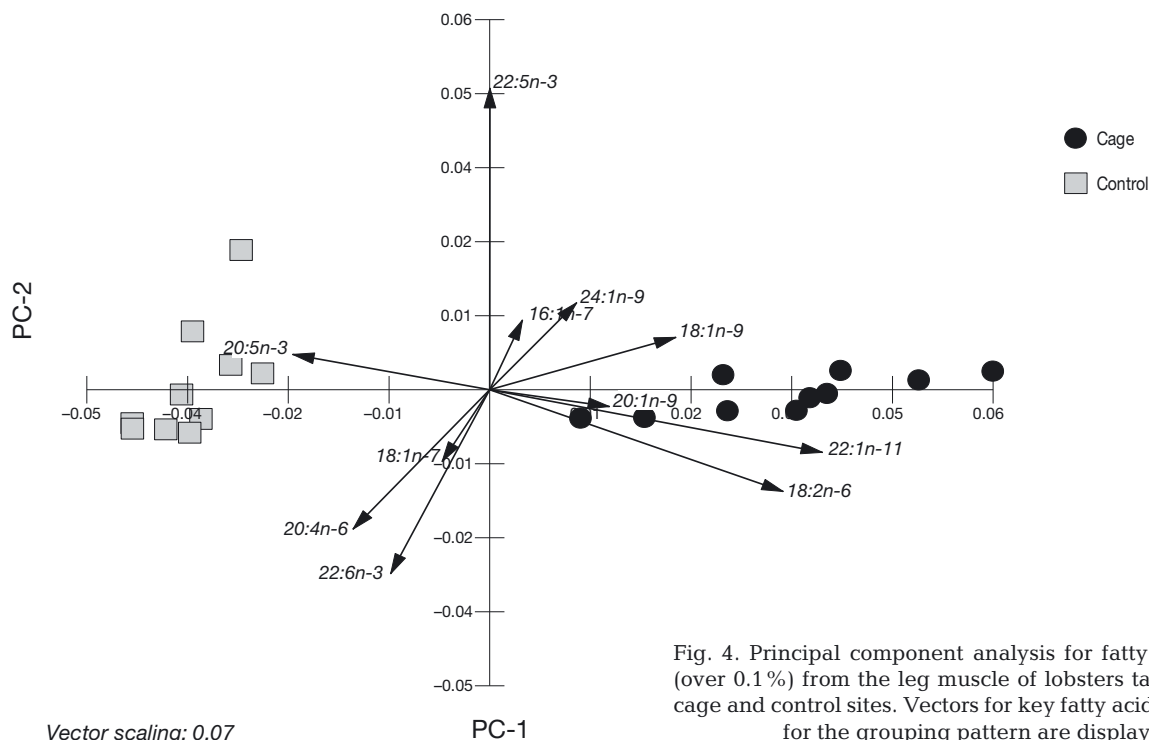


Fig. 4. Principal component analysis for fatty acid profiles (over 0.1%) from the leg muscle of lobsters taken from the cage and control sites. Vectors for key fatty acids responsible for the grouping pattern are displayed

indicated no significant differences for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  between cage and control sites for lobsters ( $t = 1.33$ ,  $\text{df} = 5$ ,  $p = 0.240$ ), and neither showed any similarity to the signatures for the salmon feed.

### 3.4. Growth of lobsters

An increase in CL was observed over the trial period at both cages and control sites. These were fitted to von Bertalanffy growth curves (Fig. 6) using calculated values of  $K = 0.0048$ ,  $t_0 = -32.08$  for the cage site and  $K = 0.0044$ ,  $t_0 = -0.4318$  for the control site. After Mauchly's test showing that the data conformed to sphericity ( $W = 0.0224$ ,  $p = 0.150$ ), the repeated-measures ANOVA indicated significant growth in lobsters over the trial period ( $F = 77.5$ ,  $p < 0.001$ ) at both sites, although there was no significant difference in lobster growth between the sites ( $F = 20.9$ ,  $p = 0.149$ ).

## 4. DISCUSSION

The aim of this study was to determine if juvenile European lobsters would feed on waste from salmon cages in a coastal IMTA setup and assess if there was a subsequent impact on growth of the lobsters. Other studies have shown that the flow of wastes from coastal finfish aquaculture could potentially be used for aquaculture-based production of extractive species such as kelp (Fossberg et al. 2018) and bivalves (Lander et al. 2012). In this study, we evaluated a novel combination of co-cultured species wherein the juvenile lobsters would be used for restocking purposes rather than as an additional economic crop.

The results from the FA analysis showed that the content of OA (18:1n-9), LA (18:2n-6), ALA (18:3n-3), cetoleic acid (22:1n-11) and eicosenoic acid (20:1n-9), which were characteristic of the salmon feed used, were each significantly higher in the tissues of the lobsters located near the cages than in those at the control location. The PCA further confirmed these results, as the lobsters at the cage and control sites showed distinctly different FA profiles, which suggests different food sources. The FA profile of lobsters at the cage site was clearly influenced by the salmon feed, whereas at the control site, lobster nutrition was dominated by natural marine oils.

In contrast, stable isotope analysis of lobster leg muscle taken at the cage and control sites showed little difference in the  $\delta^{15}\text{N}$  ratio between locations, nor

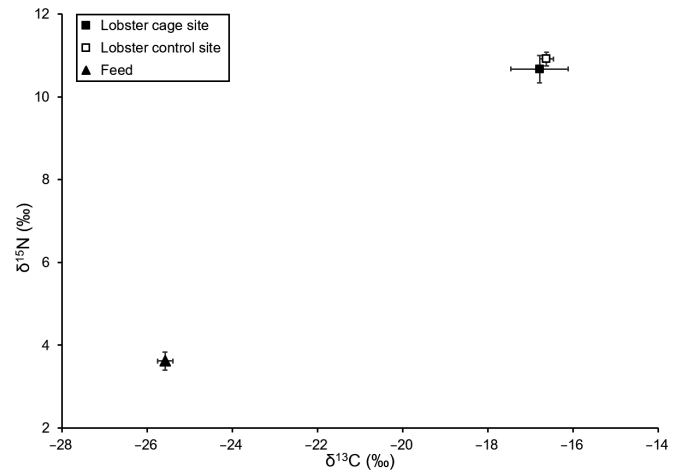


Fig. 5. Biplot of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (mean  $\pm$  SD) stable isotopes of lobster leg muscle and supplemented feed between cage and control stations at the end of the trial in March 2019

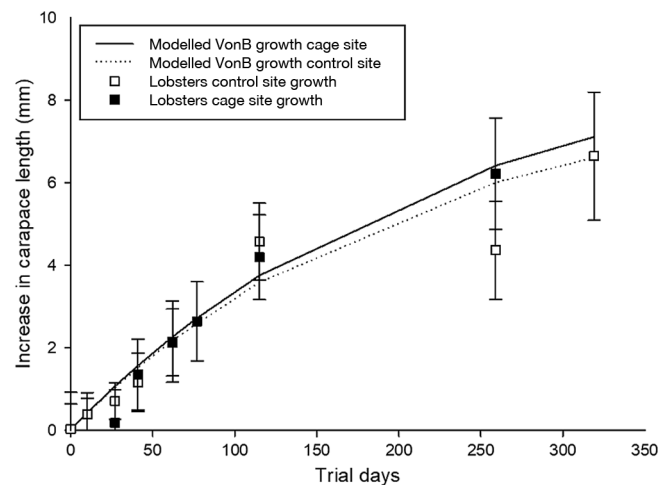


Fig. 6. Growth curves of lobsters between control and cage stations, based on measured values (mean  $\pm$  SD). Von Bertalanffy growth functions plotted by trial day and increases in carapace length for 319 d of the trial. Parameters estimated for the cage site were  $L_{\infty} = 9.43$ ,  $K = 0.0044$ ,  $t_0 = -0.4318$ , and control site  $L_{\infty} = 8.44$ ,  $K = 0.0048$ ,  $t_0 = -0.3208$

was there a similarity between the signature ratio for lobster tissue and salmon feed. There may be several reasons for the differences between the FA tracers and the stable isotope analysis. Bethoney et al. (2011) demonstrated that  $\delta^{15}\text{N}$  values in lobster tissue reflect their long-term diet, so the time-period of our study may not have been long enough. Another consideration is that there may have been an insufficient amount of waste consumed to establish an isotopic signal, as lobsters are slow and periodic feeders (Bordner & Conklin 1981).

Based on their FA profiles, juvenile lobsters at the cage site consumed fish farm nutrient waste, although it was not clear if this was direct or indirect. The sizes of waste feed and faecal particles in the water column from the salmon cages were not measured in this experiment, but other studies have shown that suspended particulate organic matter originating from cages is often between 1 and 10  $\mu\text{m}$  (Lander et al. 2013) and up to 300  $\mu\text{m}$  (Law et al. 2014). As the internal mesh size of the SBCC structures was  $2.5 \times 2.5 \text{ mm}$  (Daniels et al. 2015), particles of waste this size could have entered the SBCC and been within the size range eaten directly by juvenile lobsters (Lavalli & Barshaw 1989). Studies have demonstrated direct uptake of waste particles by consumer IMTA species (Handå et al. 2012, Bergvik et al. 2019). However, in the marine environment there will be a wider dietary choice and a complex food web. An *in situ* study showed that mussels did not directly assimilate wastes and had a selective diet, preferring other sources of food (Sanz-Lazaro & Sanchez-Jerez 2017). Even within the SBCC system, there would be a dietary choice (Daniels et al. 2015), and the uptake of wastes by lobsters could have been indirect via fouling organisms.

Despite the differences in the FA profiles of the juvenile lobsters at the cage site and the reference site, there was no significant difference in growth. Information on growth rates of wild juvenile European lobsters is scarce (Mercer et al. 2001, Wahle et al. 2013), which prevents comparison to natural conditions. Stage IV is the first postlarval stage of the life cycle, and juvenile lobsters are transitioning to benthic organisms (Charmantier et al. 1991). Therefore, the position of the SBCC may not have been deep enough in the water column for their feeding behaviour and could have influenced the results, as other studies have shown that depth can be an important factor in IMTA systems (Sanz-Lazaro et al. 2018). There will always be multiple factors to consider in designing the optimal setup and there will often be trade-offs (Halswell et al. 2018). In this case, the scale of the experimental site meant there were limited options to position the SBCC within the waste stream of the fish cage. This study focussed on 1 cage site and 1 control site, but the dispersal of wastes around fish cages varies depending on site characteristics, and other locations may have had different results. Furthermore, this research used a pilot-scale experimental site, but the results may be different if the research was repeated at a full-scale commercial farm, as there would be higher volumes of waste.

Previous studies have demonstrated the advantage of acclimatising juvenile lobsters using *in situ* containers as a way of improving survivability (Beal et al. 2002, Perez Benavente et al. 2010, Beal & Protopopescu 2012, Daniels et al. 2015, Halswell et al. 2016), and locating an SBCC next to salmon cages might offer additional shelter from storm events. However, it is also important to note that fish medication can be harmful to juvenile lobsters, and is often used to treat fish diseases (Burridge et al. 2014, Cresci et al. 2018). The effect of routine operations, such as disease treatment, on the different components of an IMTA system is not only an issue for lobsters and salmon, but is an essential consideration for any combination of species in an IMTA system. For example, lobsters could only be deployed at sites which use non-medicinal treatments.

The interaction between salmon cages and wild lobsters is an important area of research, especially in Canada where studies have explored the conflict over use of space between salmon farms and lobster fisheries (Walters 2007, Grant et al. 2019). In Canada and Norway, research has also focussed on the potential impact of medicinal treatments used for salmon aquaculture on lobsters (Burridge et al. 2014, Cresci et al. 2018). This present study focussed on potential benefits of having lobsters in an IMTA system alongside salmon cages prior to release into the wild for restocking purposes. The results clearly demonstrated utilisation of wastes from the salmon cages, but this had no obvious impact on growth. Other potential implications for lobster physiology, metabolic processes and behaviour were not explored. Sardenne et al. (2020) suggested that a diet shift toward waste fish feed may have an influence on reproductive success in some wild crustaceans. Therefore, although a trophic connection has been established within this system, further research and trials will be required to determine if a coastal salmon–lobster IMTA setup would be appropriate as a stage in lobster restocking programmes.

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