




Stable isotopes demonstrate seasonally stable benthic-pelagic coupling as newly fixed nutrients are rapidly transferred through food chains in an estuarine fish community

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Abstract

Seasonal differences in the availability of resources potentially result in the food web architecture also varying through time. Stable isotope analyses are a logistically simple but powerful tool for inferring trophic interactions and food web structure, but relatively few studies quantify seasonal variations in the food web structure or nutrient flux across multiple trophic levels. We determined the temporal dynamics in stable isotope compositions (carbon, nitrogen and sulphur) of a fish community from a highly seasonal, temperate estuary sampled monthly over a full annual cycle. Sulphur isotope values in fish tissues discriminated among consumers exploiting pelagic and benthic resources but showed no seasonal variation. This implied limited change in the relative consumption of pelagic and benthic resources by the fish community over the study period despite major seasonal changes in phytoplankton biomass. Conversely, carbon and nitrogen isotope values exhibited seasonality marked by the commencement of the spring phytoplankton bloom and peak chlorophyll concentration, with $\delta^{13}\text{C}$ values following expected trends in phytoplankton growth physiology and variation in $\delta^{15}\text{N}$ values coinciding with changes in major nitrogen sources to plankton between nitrate and ammonium. Isotope shifts in fish muscle were detected within 2 weeks of the peak spring phytoplankton bloom, suggesting a rapid trophic transfer of carbon and nitrogen along food chains within the estuarine food web during periods of high production. Therefore we caution against the assumption that temporal averaging effectively dampens isotopic variability in tissues of higher trophic-level animals in highly dynamic ecosystems, such as temperate estuaries. This work highlights how stable isotope analyses can be combined with environmental data to gain a broader understanding of ecosystem functioning, while emphasising the need for temporally appropriate sampling in stable isotope-based studies.

KEYWORDS

food web dynamics, isotope incorporation, nutrient pathways, SIA, sulphur, trophic transfer

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1 | INTRODUCTION

Seasonal variations in the abundance, quality and availability of resources have the potential to alter consumer behaviour, affecting the temporal structure and flux of nutrients through food webs. Despite this, many representations of food web structure or consumer-resource relationships are based on limited static snapshots of the ecosystem. In estuarine, lake and coastal systems, changes in light, temperature and nutrient availability commonly trigger seasonal phytoplankton blooms from spring to summer (Cloern & Dufford, 2005; Cloern *et al.*, 2014). Life cycles of zooplankton are adapted to match phytoplankton blooms, resulting in pulses of autochthonous and secondary production across an annual cycle (Kohlbach *et al.*, 2021; Maps *et al.*, 2014). Tertiary and higher consumers may accommodate large, predictable temporal variations in production by moving between ecosystems (*e.g.*, Hutchison *et al.*, 2020), seasonal resource switching (*e.g.*, Young & Ferguson, 2013) or concentrating feeding activity and somatic or reproductive tissue growth into narrow temporal windows (*e.g.*, Feeney *et al.*, 2012). Traits expressed by consumers within a food web in a seasonally varying setting will therefore determine the temporal variability of the food web structure and patterns of nutrient flux (McMeans *et al.*, 2015).

Using stable isotope compositions as naturally occurring tracers of nutrient flux and trophic interactions in food webs (Grey, 2016; Jennings *et al.* 2008a, Layman *et al.*, 2012; Trueman *et al.*, 2014), some studies have successfully identified seasonal shifts in food web structure, particularly in relation to the utilisation of different production sources. For example, Bergamino and Richoux (2015) demonstrated increased assimilation of marsh grass in primary consumers during autumn and winter in the Kowie estuary, South Africa. In the Baltic Sea region, stable isotopes of carbon and nitrogen have been used to demonstrate that production from blooms of nitrogen-fixing cyanobacteria following the spring diatom bloom is rapidly incorporated into benthic invertebrates (Karlson *et al.*, 2014; Lesutiene *et al.*, 2014). Although stable isotope analyses provide an indirect view of trophic interactions, they are logistically easier to perform than observational sampling such as stomach content work. Consequently, the use of stable isotope tracers facilitates a potential increase in the spatial or temporal focus and resolution of studies in food web research (Boecklen *et al.*, 2011).

Despite these advantages, few stable isotope-based studies in aquatic systems readily quantify seasonal variations in nutrient flux or food web structure that include higher trophic-level compartments of food webs, particularly beyond single time-point sampling (but see Claudino *et al.*, 2013). This may be in part due to earlier work suggesting an apparent lack of seasonality in the stable isotope composition of fish tissues. Jennings *et al.* (2008b) did not detect temporal variability in the $\delta^{15}\text{N}$ of North Sea fishes despite observing fluctuations in zooplankton, whereas Perga & Gerdeaux (2005) argued that the seasonal fluctuations they observed within a lake whitefish species' $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were a result of internal remobilisation of nutrients for gonadal growth rather than the seasonally varying

isotopic compositions of their zooplankton prey. More recent anecdotal evidence suggests, however, that seasonal variations in primary production can be expressed in the stable isotope compositions of higher trophic-level organisms. Lesutiene *et al.* (2014) showed that secondary consumer fish had isotope values more consistent with the assimilation of newly fixed nitrogen, implying incorporation of cyanobacterial production across two trophic levels in their study of a Baltic Sea lagoon. In the tropical eastern Pacific, the $\delta^{13}\text{C}$ values of a zooplanktivorous fish species have been shown to be negatively correlated with sea surface temperatures, the main driver of phytoplankton $\delta^{13}\text{C}$ in the region (MacKenzie *et al.*, 2019). To date, there are few studies quantifying temporal variability in fluxes of seasonally pulsed production within multiple higher trophic level compartments of a food web. Understanding seasonal dynamics in the food web structure and nutrient flux is particularly important for estuarine systems given that they function as key nursery grounds for species of economic and cultural importance (Cobain *et al.*, 2019; Sheaves *et al.*, 2015). Commonly, estuaries are sites with high levels of human impacts, but are also accessible in terms of developing scientifically informed management strategies to limit negative impacts on biodiversity and ecosystem service provision.

In this study, we characterised the stable isotope composition of the fish community in a highly seasonal, marine-dominated temperate estuary on a monthly basis spanning an annual cycle. We exploit expected temporal variations in isotopic compositions of production and relative availability of pelagic and benthic nutrient sources to assess: (a) flux dynamics of phytoplankton production into higher trophic-level fishes; and (b) seasonal variations in the relative use of benthic and pelagic resources in consumer functional guilds. To track seasonal variations in assimilation of potential production sources through the food web, we measured $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values in white dorsal muscle tissues. Different primary production sources are typically separated by $\delta^{13}\text{C}$ values due to differing fixation pathways and, in aquatic systems, benthic and pelagic production sources typically display differing $\delta^{13}\text{C}$ values (Doi *et al.*, 2010; Duffill Telsnig *et al.*, 2019). $\delta^{15}\text{N}$ values in primary production reflect the isotopic composition of nitrogen sources used during protein synthesis and may therefore vary if nitrogen sources switch between (for instance) nitrate, ammonia or atmospheric N_2 fixation (Lesutiene *et al.*, 2014; Radice *et al.*, 2019; Ryabenko, 2013). In estuarine environments, $\delta^{34}\text{S}$ values are particularly useful in providing spatial separation as not only are freshwater/terrestrial end-members distinct compared to marine sources, but the isotopic fractionation associated with anoxic microbial production in the sediment subsurface labels benthic production allowing benthic nutrient pathways to be differentiated from those fuelled from overlying pelagic primary production (Connolly *et al.*, 2004; Fry *et al.*, 1982). Further, it is well documented that the dense phytoplankton blooms and associated nutrient cycles that characterise temperate estuaries have associated temporally varying isotopic expressions (*e.g.*, Cifuentes *et al.*, 1988; Goering *et al.*, 1990; Kukert & Riebesell, 1998).

We fit linear mixed effects models to carbon, nitrogen and sulphur isotope ratios in three functional groups that comprise the fish

community (benthic, pelagic and benthopelagic species) to explicitly test the hypothesis that mean community stable isotope ratios change with calendar month. This approach accounts for potential differences in physiology and diet associated with species and individual body size, the composition of which can vary over the seasonal cycle. Comparing observed temporal isotopic trends, if any, with chlorophyll-*a* and nutrient data, we explore whether isotope dynamics in the fish community coincided with the dynamic environmental context, specifically the timing of the spring phytoplankton bloom. In doing so, we determine fish community trophic structuring, and the extent and rate at which inferred temporal isotopic variability at the base of the food web is propagated into higher trophic levels in a highly dynamic estuary.

2 | MATERIALS AND METHODS

2.1 | Site description

The Southampton Water, Test and Itchen estuarine system (50° 52' N and 1° 22' W, hereafter Southampton Water) is a well-studied estuarine system on the southern coast of England, Figure 1. The main water body is approximately 10 km long and 2 km wide, and fed by three rivers, the Itchen, Test and Hamble, with a total watershed area of 1716 km² (Nedwell *et al.*, 2002). The central channel of the estuary is maintained at a depth of at least 12 m by periodic dredging, and much of the shoreline has been extensively modified for industrial activity. A marine-dominated system, tidal ranges vary between 1.5 and 4.5 m, with an atypical tidal regime consisting of a “young flood stand” and a double high water (Levasseur *et al.*, 2007). Both surface

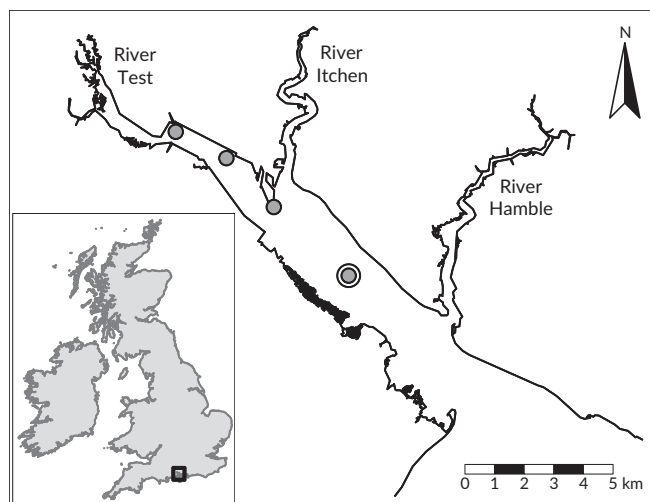


FIGURE 1 Map of Southampton Water and its location within the British Isles (inset). Grey circles indicate locations of water sampling for nutrient analyses, and approximately demarcate the stretch of estuary from which fish were sampled. The double-bound grey circle marks the approximate location of Southampton Water Hound Buoy where chlorophyll, temperature and turbidity data were recorded

and bottom water currents readily reach approximately 0.5 to 1.0 ms⁻¹ during neap and spring tidal cycles, respectively, resulting in well- to partially mixed vertical water profiles across the entire estuary with surface and bottom salinities rarely below 20 and 30 psu, respectively (Levasseur *et al.*, 2007; Townend, 2008). Littoral and sublittoral areas consist mainly of mud to sandy sediment habitats, with saltmarshes constituting less than 20% of the 1.1 × 10⁷ m² intertidal area and limited to fringing southern shores and the lower reaches of the river Test (Townend, 2008). Southampton Water is a hypereutrophic environment (Torres-Valdés & Purdie, 2006), with large effluent inputs dominating ammonium and phosphate levels within the estuary (90 and 75% of nutrient loads, respectively, Nedwell *et al.*, 2002). High nutrient concentrations are coupled with low turbidity: suspended particulates average from 40 mg⁻¹ at the estuary mouth down to 5–10 mg⁻¹ at the upper reaches, making the estuary a highly productive environment (Townend, 2008).

2.2 | Sample collection, processing and data sources

Physical sampling of the fish community was conducted opportunistically towards the end of each calendar month, from October 2015 through to January 2017 inclusive as part of an ongoing, licenced, environmental monitoring programme of Southampton Water linked to the industrial activity within the estuary. Briefly, monitoring was conducted as follows (see Supporting Information Figure S1 for schematic diagram). Water from the estuary is continuously drawn through a large intake pipe and passes through a series of filtrations: very large items are removed *via* a coarse grid that is periodically cleaned by a grab system; rotating drum screens are then employed that separate out smaller debris, including fish and invertebrates, which are returned directly back to the estuary *via* a fish-return system. The fish-return system is netted (10 mm mesh-size) for a 24 h period (separated into one 18 h overnight collection and 6 hourly collections), and the grab system monitored. Catches were sorted, identified to species level, counted and length-frequency measurements taken (highly abundant species are subsampled). Samples representative of the long-term time series records of observed species and their size distributions were retained and supplemented from quarterly trawls (part of the same monitoring survey). All trawls fell within 3 km of the intake pipe within Southampton Water, using a 10 m bottom otter trawl with a 10 mm cod end, towed for 10 min with up to 10 trawls per quarter. Samples were frozen post-collection until further analysis.

Sub-samples of fish were selected for isotope analyses (approximately *n* = 25 per month when available) based on monthly size-frequency abundance averages for all species calculated from 7 years of survey data. Fish were defrosted, standard lengths and wet weight recorded with small plugs of white muscle (*ca.* 0.5 cm³) excised from below the dorsal fin and stored in Eppendorf tubes. Whole fillets were taken with skin, bone and spines removed for small fish. Samples were then freeze-dried at –55°C for 24 h (Heto Power dry LL3000) and stored in sealed containers at room temperature. Dehydrated muscle

tissues were homogenised and samples of 1.9 ± 0.1 mg weighed out using a Sartorius microbalance with a precision of 0.001 mg and sealed in tin capsules. All stable isotope analyses were conducted at the NERC Life Science Mass Spectrometry Facility (now NEIF) based at SUERC, East Kilbride, using an Elementar vario Pyrocube (Hanau, Germany) coupled to an IsoPrime (now Elementar) VisiON mass spectrometer (Cheadle Hulme, UK). Isotope values are reported relative to their respective international standards: Pee Dee Belemnite for carbon, atmospheric air for nitrogen and Cañon Diablo Troilite for sulphur, expressed in delta (δ) per mil (‰) notation:

$$\delta X = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 10^3$$

where X is either ^{13}C , ^{15}N or ^{34}S and R is the ratio of $^{13}\text{C}:^{12}\text{C}$, $^{15}\text{N}:^{14}\text{N}$ or $^{34}\text{S}:^{32}\text{S}$. Laboratory reference materials were run between every 10 samples to ensure accuracy, to correct for linearity effects and to correct for drift over time (MSAG2, methanesulfonamide and gelatine; M2, methionine and gelatine; and SAAG2, sulphanilamide and gelatine). Further international standards of IAEA-S-1, S-2 and S-3 silver sulphides were also run to ensure the full range of $\delta^{34}\text{S}$ values was captured by reference materials. Analytical measurement standard deviations were up to 0.1, 0.2 and 0.6‰ for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$, respectively.

Nutrient data for nitrate, ammonium and silicate, along with oxygen saturation measurements, were sourced from the UK Environment Agency water quality data archive (public sector information licenced under the Open Government Licence v3.0, accessed 13 February 2018), with the four sampling locations shown in Figure 1. Temperature, chlorophyll-*a* and turbidity time series data were obtained from the Xylem data buoy located approximately at the Southampton Water Hound Buoy location, Figure 1 (data accessed 13 February 2018). The buoy was fitted with a YSI EX02 multi-parameter probe (maintained at a water depth of 1–2 m) mounted with antifouling guards and cleaning rotary wiper brushes, providing near-continuous environmental monitoring (data recorded every 15 min). Data are limited from 12 November 2015 to 31 January 2017 as the buoy was out for maintenance prior to this period, with values reported as 3 day running means to average over full tidal and diurnal cycles.

In addition to fish, and to try and quantify potential isotopic variability in pelagic estuarine production, we sampled suspended particulate matter (SPM) from the cooling water intake system, collected each month by pumping pre-screened water through a 100 μm plankton net (submerged in a tank to reduce flow-through pressure) and preserved with Lugol's iodine and stored until further analysis. The composition of SPM samples was determined by visual analysis of sub-samples (5 μl) under a dissecting microscope with the remaining sample filtered onto pre-combusted GF/F filters (Whatman1825-047, 0.7 μm effective mesh size), frozen and later freeze-dried. Small semi-circular plugs (12 mm diameter) were taken and sealed in tin capsules (plug area determined beforehand through a small pilot study) for isotopic analysis. During the month of July 2016, additional samples of various macroalgal species and terrestrial leaf litter were taken to

provide context in the diversity of isotopic composition in differing production sources within the estuary. Samples were cleaned of epiphytes, freeze-dried, homogenised and analysed for stable isotope composition.

2.3 | Ethical statement

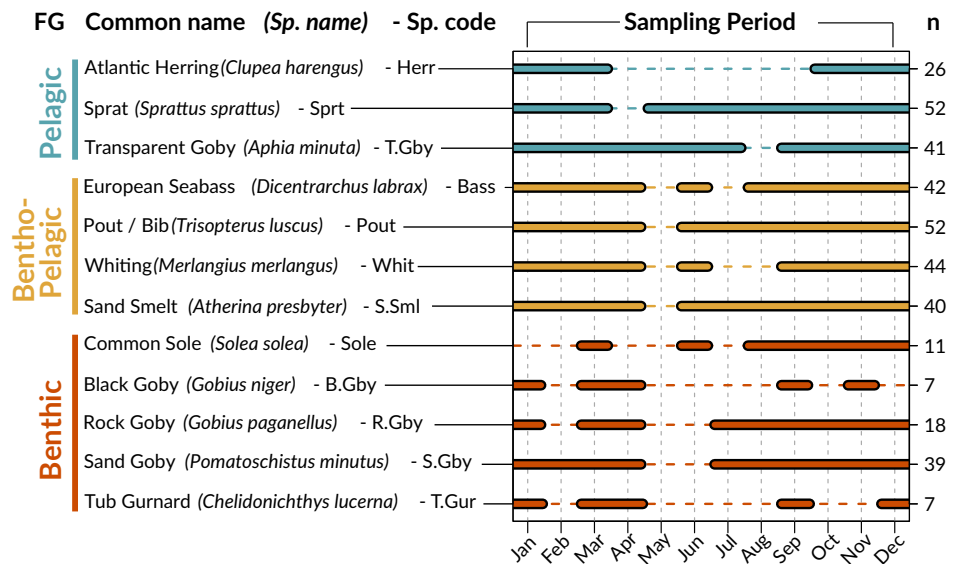
As part of the survey's consent and exemption from the MMO (Marine Management Organisation) for scientific surveys, coupled with the cooperation of the Southampton harbour authority, all fish were treated as humanely as possible and only individuals suffering damage within the intake system and nets and deemed to stand little to no chance of survival upon return to the estuary were retained. The majority of fish were sampled during the overnight collection (ca. 90%) where survival rates were limited.

2.4 | Statistical analyses

The initial data set of fish muscle isotope composition consisted of 453 individual measurements from a total 32 species. For statistical analyses, data were limited to those species with a minimum number of seven individuals sampled over the whole period. Flounder, *Platichthys flesus*, were excluded as the majority of individuals were young-of-the-year (standard length <80 mm, $n = 7$ of 10) sampled in March and April with juveniles being known to inhabit freshwater-dominated reaches of estuaries (Kerstan, 1991). Thornback rays, *Raja clavata*, were also excluded ($n = 9$) due to their urea-based osmotic regulation (Kim & Koch, 2012). The final data set consisted of 379 individual isotopic measurements spanning 12 species (Figure 2) that comprise most of the biomass of the fish community in Southampton Water (80% of total fish biomass surveyed over the whole period). The 12 species were assigned to one of three functional groups: benthic (B), pelagic (P) or benthopelagic (BP) based on trophic behaviours derived from the literature (see Appendix S1 for brief dietary and life-history descriptions of each species). Sampling during May 2016 coincided with a large jellyfish bloom (*Aurelia aurita*), resulting in net rupture and the loss of the 18 h overnight sampling; therefore fish could only be collected from the six hourly daytime samples. Sampled individuals for this month were limited to pelagic species and flounder only, likely due to avoidance of shallow water areas during daytime hours in the warm summer period (pers. obs.) and increased survival rates in hourly catches. Although mostly within expected values (mean = 3.32), the range of C:N ratios exceeded the recommended value of 3.5 (3.05 to 4.52, Skinner *et al.*, 2016); therefore all $\delta^{13}\text{C}$ values were mathematically corrected for lipid content following Kiljunen *et al.* (2006).

Mixed-effects models were employed to estimate the influence of key factors and random species effects on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values separately. Model selection was conducted following Zuur *et al.* (2009): random effects (random species intercepts and/or slopes) were selected by conditional AIC (cAIC, Säfken *et al.*, 2021) on models with the full suite of fixed effects estimated by REML; fixed effects

FIGURE 2 Overview of the 12 species analysed in this study, including functional group assignment (FG), whether they were sampled (solid bar) or absent (dashed line) during each calendar month of the sampling period, and the total number of individuals analysed for stable isotopes (n)



were then tested using likelihood ratio tests on nested models fitted with maximum likelihood (at the $\alpha = 0.05$ level); and final parameter estimates were derived for the optimal model using a Bayesian framework. The initial full model fitted was:

$$\delta X \sim \text{Mass} + \text{FG} + \text{Month} + \text{FG} : \text{Mass} + (1|\text{Species}) + (0 + \text{Mass}|\text{Species})$$

where “Mass” is individual wet mass, logarithmically transformed with base 10 (continuous), “FG” is functional group (categorical), “Month” is calendar month (categorical), “FG:Mass” is the interaction of FG on Mass, (1|Species) is random species intercepts, and (0+Mass|Species) is random species slopes with Mass. During model selection, random species intercepts and slopes were fitted independently (*i.e.*, assumed covariance of zero, due to singular fits); however, Bayesian model random effects were fitted in an unstructured manner and therefore could co-vary. Bayesian posterior estimates were drawn from 2×10^6 iterations with a burn-in of 1.5×10^6 and a thinning of 250. Model error distributions were fitted as Gaussian, and graphical checking during model selection indicated that variances were homogenous and residual errors were well described by the normal distribution (although slightly over-dispersed). All statistical analyses were conducted in R 3.5.0 (R Core Team, 2018), with mixed-effects models conducted using the packages “lme4” (Bates *et al.*, 2015) and “MCMCglmm” (Hadfield, 2010), and conditional AIC values estimated using the package “caic4” (Säfken *et al.*, 2021).

3 | RESULTS

3.1 | Environmental data

Temperature and chlorophyll-a data indicate that the period covered was environmentally typical (Figure 3a,b, turbidity data provided in Supporting Information Figure S3). Water temperature seasonally

varied, with minima in the late winter of approximately 7°C rising to a maximum of about 20°C in the late summer. Chlorophyll-a showed a strong peak in late spring (mid-May) of approximately $500 \mu\text{g l}^{-1}$, and a prolonged but smaller secondary peak in later summer (mid-August) of up to $12 \mu\text{g l}^{-1}$ after which chlorophyll levels slowly decline to a winter minimum of approximately $0.5 \mu\text{g l}^{-1}$.

All measured nutrients exhibited concentration minima in June 2016, of 26, 3.2 and $5.6 \mu\text{mol l}^{-1}$ for nitrate, ammonium and silicate, respectively (Figure 3), although data were not available for all months. A peak nitrate concentration of $110 \mu\text{mol l}^{-1}$ occurred in May with high concentrations throughout the preceding winter (Figure 3c). Summer nitrate concentrations remained low but slowly increased from the June minimum until reaching a concentration of $79 \mu\text{mol l}^{-1}$ by December 2016. Ammonium concentrations fluctuated throughout the year, with the minimum in June but similarly low concentrations of 3.6 and $3.7 \mu\text{mol l}^{-1}$ also observed in December 2015 and January 2017 (Figure 3d). Peak concentration occurred in May 2016 at $14.6 \mu\text{mol l}^{-1}$, but the large standard deviation of $8.5 \mu\text{mol l}^{-1}$ indicated considerable differences between the four sample sites. Silicate concentrations also varied seasonally, with late winter maxima of 39.3 and $40.2 \mu\text{mol l}^{-1}$ in January 2016 and February 2017, respectively (Figure 3e). Temporal trends in dissolved oxygen saturation levels consisted of supersaturation during the early summer (May and June), with a peak of 112% in May 2016 (Figure 3f). For the rest of the year, oxygen remained subsaturated within Southampton Water typically at around 90%, although levels dropped to 83% in September 2016.

SPM samples showed high variability between phytoplankton, zooplankton and detritus concentrations (Supporting Information Figures S4 and S5), including sediment entrainment. Nevertheless, the sample at the end of May was wholly diatoms and their phytodetritus, confirming the spring phytoplankton bloom. Stable isotope ratios of SPM varied through time for all three elements (Supporting Information Figure S6); nevertheless, differing samples compositions (including inorganic content) coupled with no sample replication

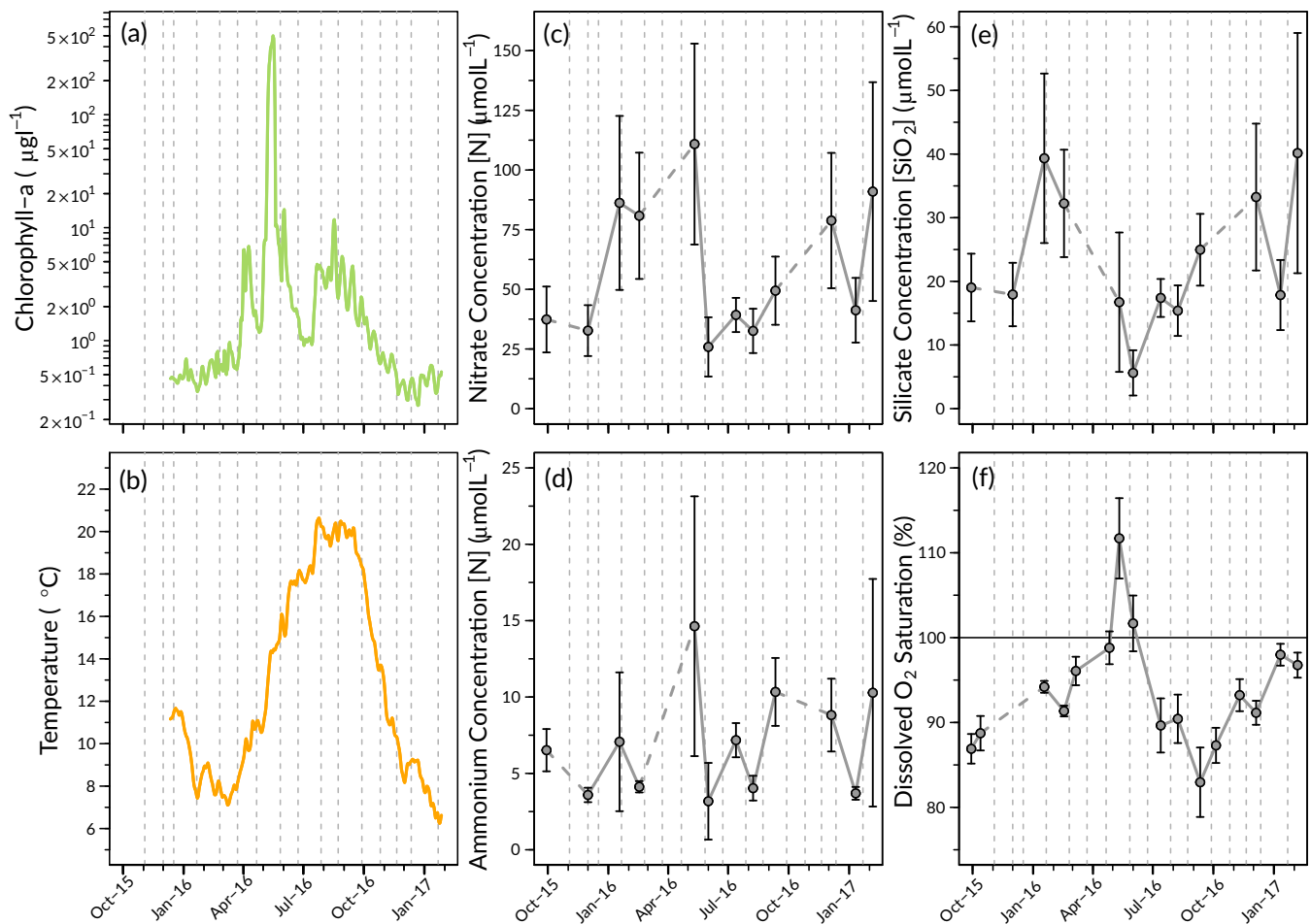


FIGURE 3 Plots of environmental data from Southampton Water during the sampling period. Chlorophyll-a (a) and temperature (b) time series plotted as 3-day running means. Mean nutrient concentrations plus standard deviations of nitrate (c), ammonium (d), and silicate (e), plus oxygen saturation (f). Vertical dashed grey lines indicate dates when the fish community was sampled. The solid horizontal line in (f) indicates 100% saturation level. Sampling locations shown in Figure 1

means true seasonal baseline effects could not be separated out. Although the diatom-dominated May sample had low $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, proportionally high concentrations of detritus in Jan-16 and Nov-16 (Supporting Information Figure S4) also coincided with considerable reductions in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Supporting Information - Figure S6). C:N ratios were very low (<1.5) across all SPM samples, suggesting air intrusion due to filter packing, reducing the confidence that $\delta^{15}\text{N}$ values accurately reflect true SPM values. Isotope biplots of limited basal resource sampling from July are provided in Supporting Information Figure S7, and demonstrate ranges of approximately 22, 10 and 12‰ across basal sources for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$, respectively, within the estuary.

3.2 | Fish community data

Individual stable isotope data for the Southampton Water fish community exhibited large variation in all three elements, with ranges from -21.3 to -13.9 , 10.4 to 18.7 , and 9.8 to 19.5 ‰ for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$

and $\delta^{34}\text{S}$, respectively (see Supporting Information Figure S2 for biplots). Variance was highest for sulphur (2.69%) and lowest for carbon (1.35%), with carbon and nitrogen values positively co-varying, Table 1. The optimal mixed effects models explaining variation in isotope ratios in the fish community data were:

$$\delta^{13}\text{C} \sim \text{Mass} + \text{Month} + (1|\text{Species}) + (0 + \text{Mass}|\text{Species})$$

$$\delta^{15}\text{N} \sim \text{Mass} + \text{FG} + \text{Month} + (1|\text{Species}) + (0 + \text{Mass}|\text{Species})$$

$$\delta^{34}\text{S} \sim \text{Mass} + \text{FG} + \text{FG} : \text{Mass} + (1|\text{Species})$$

For $\delta^{34}\text{S}$, inclusion of both random intercepts and slopes led to singular fits. A non-singular model fitted with only random intercepts and slopes (no fixed effects) partitioned a much larger proportion of variation to random intercepts than slopes (variances of 1.24 and 0.34% , respectively) and therefore only random intercepts were selected. Bayesian parameter estimates are provided in Table 1 (model selection results and species random effects given in Supporting Information Tables S1 and S2, respectively). The explanatory power of the

TABLE 1 Optimal mixed effects model parameter estimates for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$, and variance structure of the data

Predictors (n = 379)	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$	
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Intercept	-18.33	-18.93 to -17.79	13.20	12.12 to 14.19	14.79	13.65 to 15.92
$\log_{10}\text{Mass}$	0.48	0.10 to 0.86	0.47	0.07 to 0.94	-0.70	-1.33 to -0.07
FG-BP	-	-	1.46	0.09 to 2.88	1.42	-0.21 to 3.10
FG-P	-	-	0.47	-1.03 to 2.14	1.80	0.05 to 3.63
Feb	0.07	-0.39 to 0.52	0.24	-0.13 to 0.64	-	-
Mar	0.15	-0.30 to 0.58	0.23	-0.11 to 0.59	-	-
Apr	-0.03	-0.49 to 0.47	-0.13	-0.53 to 0.27	-	-
May	2.60	1.89 to 3.33	-1.02	-1.68 to -0.37	-	-
Jun	0.59	0.03 to 1.10	-1.31	-1.74 to -0.86	-	-
Jul	0.51	0.00 to 1.05	-1.19	-1.63 to -0.72	-	-
Aug	0.57	0.05 to 1.11	-0.88	-1.34 to -0.44	-	-
Sep	-0.33	-0.82 to 0.20	-0.75	-1.16 to -0.34	-	-
Oct	0.23	-0.17 to 0.60	-0.45	-0.78 to -0.13	-	-
Nov	0.23	-0.12 to 0.65	-0.14	-0.48 to 0.16	-	-
Dec	0.18	-0.15 to 0.56	-0.26	-0.55 to 0.05	-	-
$\log_{10}\text{Mass:BP}$	-	-	-	-	-0.00	-0.69 to 0.67
$\log_{10}\text{Mass:P}$	-	-	-	-	0.81	0.05 to 1.59
Random effects						
Intercept variance	0.57	0.12 to 1.36	1.17	0.18 to 2.76	1.25	0.23 to 1.78
Slope variance	0.26	0.02 to 0.67	0.44	0.00 to 1.15	-	-
Intercept-slope covariance	-0.23	-0.67 to 0.03	-0.12	-0.71 to 0.38	-	-
Residual variance	0.89	0.77 to 1.04	0.59	0.51 to 0.69	1.55	1.32 to 1.78
Marginal r^2	0.21	0.10 to 0.36	0.42	0.15 to 0.64	0.27	0.08 to 0.46
Conditional r^2	0.43	0.28 to 0.64	0.80	0.66 to 0.92	0.57	0.39 to 0.79
Variance-covariance structure of data						
$\delta^{13}\text{C}$	1.35		-		-	
$\delta^{15}\text{N}$	0.81		2.00		-	
$\delta^{34}\text{S}$	0.10		-0.57		2.69	

Note: Intercept values represent the average isotope value for a benthic (reference functional group (FG) denoted as B, BP: benthic-pelagic and P: pelagic) fish with a mass of 1 g, captured in the reference month of January. Estimates given are the mean plus the 95% credible intervals (CI) of posterior distributions. Predictors whose credible intervals do not encompass zero are highlighted in bold.

mixed effects models was generally good: fixed effects alone explained 21%, 42% and 27% of the variation in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$, respectively (mean marginal r^2), increasing up to 80% of variation when random effects are included in the case of $\delta^{15}\text{N}$ (conditional r^2). Mean residual variance ranged from 0.59‰² for $\delta^{15}\text{N}$ to 1.55‰² for $\delta^{34}\text{S}$.

The intercept of -18.3‰ for $\delta^{13}\text{C}$ did not differ by functional group, therefore representing the average $\delta^{13}\text{C}$ value for a fish with a mass of 1 g, captured in January. For $\delta^{15}\text{N}$, the estimated intercept value was 13.2‰ for a 1 g benthic fish in January, increasing by 1.5‰ for benthic-pelagic fishes. Credible intervals for pelagic fishes span zero, and therefore any difference between benthic and pelagic $\delta^{15}\text{N}$ values is not statistically clear. The intercept of 14.8‰ for $\delta^{34}\text{S}$ values represents the annual average value for a 1 g benthic fish, with

benthic-pelagic and pelagic fishes being 1.4 and 1.8‰ higher, respectively.

Calendar month was a key factor in explaining variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values: see Figure 4. For carbon, a large increase from stable winter values of -18.3 to -15.7‰ was observed from April to May, although only the pelagic functional group was represented in the latter. Fish caught in the months of June, July and August also showed slightly elevated $\delta^{13}\text{C}$ values with values of -17.7, -17.8 and -17.7‰, respectively. $\delta^{13}\text{C}$ values for all other months did not differ from those of January. For $\delta^{15}\text{N}$, the months of November through to April inclusive were all statistically similar, whereas May through to October was a prolonged period of lower isotope values (Figure 4). A reduction from 13.1 to 12.2‰ was observed between April and May, reaching a minimum $\delta^{15}\text{N}$ value of 11.9‰ in June, after which values

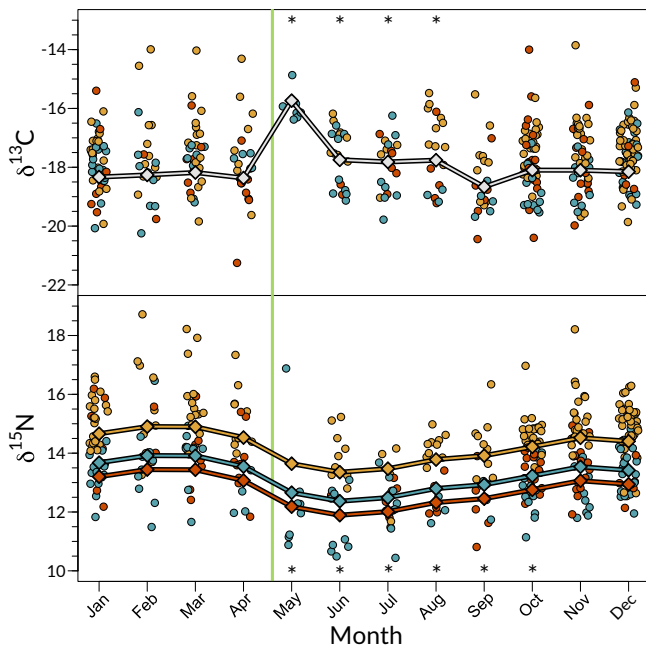


FIGURE 4 Plots of $\delta^{13}\text{C}$ (top) and $\delta^{15}\text{N}$ (bottom) against calendar month of sampling with mixed effects model monthly intercepts plotted as diamonds and solid bars (values in Table 1). Colours as in Figure 2, with grey indicating a global estimate across functional groups for $\delta^{13}\text{C}$. Months whose credible intervals excluded zero (i.e., statistically distinguishable from January values) are denoted by an asterisk at the top of the plot for positive intervals and at the bottom of the plot for negative intervals. The solid vertical green line demarks the peak phytoplankton bloom. Data are jittered along the x-axis for clarity

slowly increased again through to November. For $\delta^{34}\text{S}$, the optimal model excluded calendar month, and therefore evidence suggests that average $\delta^{34}\text{S}$ values do not vary throughout the year within the fish community.

Mass was an important predictor in explaining variation in all three isotope ratios, Figure 5. The influence of wet weight on $\delta^{13}\text{C}$ was estimated to be an increase of 0.48‰ per order of magnitude increase in mass, similar to the estimated trend with $\delta^{15}\text{N}$ values with a 0.47‰ difference per order of magnitude of mass. The relationship of $\delta^{34}\text{S}$ with mass shows a negative trend; nevertheless, the slope is dependent on the functional group, Figure 5. For benthic and benthopelagic fishes, an order of magnitude increase in mass resulted in a decrease of 0.7‰ in $\delta^{34}\text{S}$ (statistically indistinguishable between the two functional groups), but for pelagic fishes, $\delta^{34}\text{S}$ increased with mass at a rate of 0.11‰ but with credible intervals bounding zero.

4 | DISCUSSION

Food web architecture is ultimately dependent on basal resource inputs. In many ecosystems, rates of these inputs vary in time due to seasonal changes in nutrient dynamics, and fluxes of primary and secondary production sources. Food web structure is therefore expected to also vary seasonally due to differing consumer responses such as switches in resource reliance or diapause (McMeans *et al.*, 2015). We sought to evaluate the dynamics in whole food web structure as changes in basal resource dependency (benthic vs. pelagic reliance) detailed at a monthly time scale over

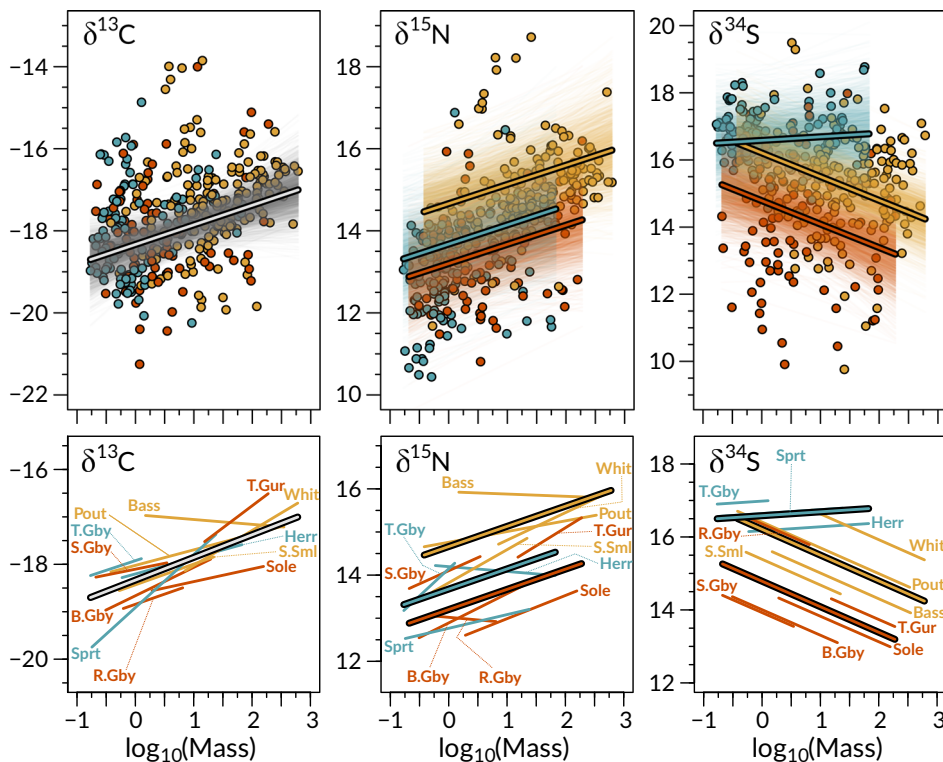


FIGURE 5 Dual plots of $\delta^{13}\text{C}$ (left), $\delta^{15}\text{N}$ (middle) and $\delta^{34}\text{S}$ (right) against individual body size, with mean mixed effects model intercept and slopes by functional group estimates plotted as solid bars (values as in Table 1 and colours as in Figure 2, grey indicating a common intercept and slope among functional groups for $\delta^{13}\text{C}$). Top plots show individual isotope data and uncertainty in functional group posterior estimates as individual opaque lines for each posterior draw ($n = 2000$). Bottom plots show random species intercepts and slopes (values in Supporting Information Table S2) labelled with species labelled with sp. codes as in Figure 2. Sprt random species intercept for $\delta^{34}\text{S}$ is overlapped by the pelagic functional group bar

an entire seasonal cycle for an estuarine fish community through the use of stable isotope analyses.

4.1 | Interpreting model outputs: temporal trends

The observed temporal trends in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition of the fish community mapped well with the observed environmental variables that describe phytoplankton dynamics within Southampton Water, notably the initiation of the spring bloom (cf. Figures 3 and 4). This alignment implies that phytoplankton production, its associated physiology and isotopic signatures propagate through the estuarine food web and are detectable in fish tissues, and therefore phytoplankton contribute a major production source that underpins the fish community biomass. Indeed, the average $\delta^{13}\text{C}$ intercept value of -18.3‰ , which was representative of the fish community across most of the year, is consistent with a dominant phytoplankton source (France, 1995). Previous work has demonstrated that within Southampton Water almost all phytoplankton production is consumed by ciliates and mesozooplankton within the estuary (Muxagata, 2005) and therefore directly enters the food web.

Temporal trends in $\delta^{13}\text{C}$ consisted of a stable winter value of -18.3‰ until approximately 2 weeks prior to peak chlorophyll-*a* concentrations in April when it showed a sudden increase reaching -15.7‰ in May, sampled 2 weeks post the observed chlorophyll maximum of *ca.* $500\ \mu\text{g l}^{-1}$. This increase was short-lived as $\delta^{13}\text{C}$ values then reduced again in June to -17.7‰ , although they remained marginally elevated compared to winter values until August. Elevations in $\delta^{13}\text{C}$ values associated with phytoplankton blooms are consistent with reduced isotopic fractionation during photosynthetic carbon fixation in phytoplankton, as the diffusion rate of $\text{CO}_{2(\text{aq})}$ becomes a limiting factor for phytoplankton growth (Cifuentes *et al.*, 1988; Fogel *et al.*, 1992; Goering *et al.*, 1990; Kukert & Riebesell, 1998; Savoye *et al.*, 2003). Additionally, $\delta^{13}\text{C}$ values of dissolved inorganic carbon increase during intense blooms if the rates of $\text{CO}_{2(\text{aq})}$ uptake into cells exceed those of diffusion of atmospheric CO_2 into surface waters, resulting in further elevated $\delta^{13}\text{C}$ values of newly fixed production (Fogel *et al.*, 1992). This is evidenced in Southampton Water by the supersaturation of oxygen in May (Figure 2f). As the bloom progresses, processes other than $\text{CO}_{2(\text{aq})}$ diffusion subsequently limit growth of phytoplankton biomass. Specifically, within Southampton Water, both direct grazing by zooplankton (Muxagata, 2005) and phosphate levels (Xiong, 2000) are known to limit phytoplankton production during bloom progression. Consequently, $\delta^{13}\text{C}$ values in new phytoplankton production return to “baseline” values, matching the observed temporal trends in both fish muscle $\delta^{13}\text{C}$ and dissolved oxygen concentrations.

The seasonal depletion observed in $\delta^{15}\text{N}$ values spanning from May until October is more protracted than the temporal dynamics of $\delta^{13}\text{C}$, but the commencement with the spring bloom still suggests an association with phytoplankton dynamics. Photosynthetic production is strongly linked to biogeochemical regimes in marine ecosystems (Cloern, 1996). Within Southampton Water, ammonium is the

dominant nitrogen source utilised within the water column ($>60\%$ total nitrogen uptake annually), with significant nitrate uptake only occurring when chlorophyll-*a* levels are elevated in the spring–summer period ($>5\ \mu\text{g l}^{-1}$, cf. Figure 3, Torres-Valdés & Purdie, 2006). Most (90%) of the ammonium inputs into Southampton Water are sourced from treated effluent discharge (Nedwell *et al.*, 2002), and such ammonium sources are enriched in ^{15}N compared to nitrate (Savage, 2005; Thorner *et al.*, 2008). The observed temporal trends in $\delta^{15}\text{N}$ in the fish community are therefore consistent with a switch from ammonium-dominated production and recycling in the winter to nitrate-based production in the summer when diatoms dominate the microalgal community (Ali, 2003; Dransfield, 2000). This is corroborated by the large reductions in nitrate and dissolved silicate observed over the period (Figure 3). Mesocosm experiments demonstrate a nitrate uptake preference in diatoms (Glibert & Berg, 2009), and the slow return to winter $\delta^{15}\text{N}$ values from June could be indicative of phytoplankton community succession from predominantly diatoms through to dinoflagellates over the summer period, and the associated changes in nitrate to ammonium preferences (Ali, 2003; Glibert *et al.*, 2016).

The seasonal reduction in $\delta^{15}\text{N}$ values may also be explained by an overall reduction in food chain length within the fish community during the summer period. Due to the increase in primary production, the abundance of small-bodied prey increases, resulting in an increase in realised predator–prey mass ratios and therefore a lower at-size trophic position of predators (Reum & Hunsicker, 2012). This would result in a reduction in fish $\delta^{15}\text{N}$ values across the community (assuming a temporally stable $\delta^{15}\text{N}$ value in basal production) due to the trophic discrimination of *ca.* 3‰ in nitrogen during the assimilation process (Perkins *et al.*, 2014; Sweeting *et al.*, 2007a). The maximum reduction in $\delta^{15}\text{N}$ values of 1.3‰ in June would therefore imply an average reduction of almost half a trophic level throughout the fish community during this period. Nevertheless, $\delta^{13}\text{C}$ values also display trophic discrimination in fishes of approximately $1\text{--}2\text{‰}$ (Duffill Telsnig *et al.*, 2019; Sweeting *et al.*, 2007b), and therefore a reduction in $\delta^{13}\text{C}$ values of $0.5\text{--}1\text{‰}$ would be expected over the same period. This is not evident from the study data as values remained elevated by approximately 0.6‰ during June, July and August and stabilised to winter values thereafter (Table 1; Figure 4). Therefore, the seasonality in $\delta^{15}\text{N}$ values is likely driven by the propagation of phytoplankton–nutrient dynamics and associated isotopic signatures rather than large-scale reductions in trophic positions within the fish community.

Although attempts were made to quantify the isotopic seasonality within the pelagic primary production pathway, logistical constraints limited efforts to a single monthly sample of suspended particulate matter ($>100\ \mu\text{m}$) from the cooling water intake system. Visual analysis of samples revealed abundances of diatoms and zooplankton that aligned with expected seasonal trends (Supporting Information - Figure S5, Ali, 2003; Muxagata, 2005) but also variable amounts of both organic matter and inorganic sediment (combined as detritus, Supporting Information Figure S4). Varying fluctuations of detrital content will mask any underlying temporal trends in phytoplankton (and zooplankton) isotope composition, as can be seen from the low $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values when detritus dominated the mixture (cf.

Supporting Information Figures S3 and S5). Bulk SPM samples are therefore unlikely to be a good isotopic representation of biologically available pelagic resources due to potentially high proportions of refractory material (Cloern *et al.*, 2002). Physical separation of samples post collection could allow for more precise fractions to be quantified, including phytoplankton and zooplankton, but represents a considerable increase in logistical effort (Gearing *et al.*, 1984). Previous studies have also highlighted the considerable isotopic variability within estuarine particulate organic matter, both spatially at the local scale (e.g., Cloern *et al.*, 2002; Gearing *et al.*, 1984) and temporally, including isotopic shifts within the same tidal cycle (Bouillon *et al.*, 2000). It is therefore unlikely that individual samples collected at a single locality, as was the case here, will accurately reflect seasonal temporal trends in the isotope values of the basal resource within the estuary.

4.2 | Interpreting model outputs: functional group differences

Dividing the fish community into three broad functional groups based on traditional trophic guilds facilitates determining the strength of couplings between the pelagic and benthic pathways. From an isotopic perspective, strong couplings should lead to a convergence of isotopic signatures between groups as basal resources from the typically isotopically distinct pathways are integrated into tissues across the community. Importantly, this does not necessarily require changes in fish feeding behaviours if production exchanges occur lower down in the food web. Benthic-pelagic coupling (incorporation of benthic-derived resources into the pelagic food web) can occur *via* resuspension of sedimentary material into the water column, including microphytobenthos (Hernández Fariñas *et al.*, 2017), which is subsequently grazed by zooplankton. Conversely, pelagic-benthic coupling (incorporation of pelagic material into the benthos) can result from phytoplankton production settling out of the water column or being directly intercepted by the benthic community (Gaudron *et al.*, 2016; Norén *et al.*, 1999). Such couplings are influenced by environmental settings, increasing in areas that are eutrophic (Gaudron *et al.*, 2016) and vertically well mixed (Hernández Fariñas *et al.*, 2017). Shallow water depths further elevate coupling strength due to increased proximity between the two pathways and the absence of hydrographic boundaries such as thermoclines (Giraldo *et al.*, 2017). Southampton Water is a marine-dominated, tidally well-mixed, and relatively shallow estuary (Levasseur *et al.*, 2007; Townend, 2008); therefore the expectation is for strong benthic-pelagic couplings to occur within the ecosystem.

Fish functional groups were not statistically distinguishable in their $\delta^{13}\text{C}$ values (Figure 5 and Table 1). This is despite $\delta^{13}\text{C}$ being frequently utilised to discriminate between benthic and pelagic pathways in fish communities (Duffill Telsnig *et al.*, 2019; Giraldo *et al.*, 2017) with microphytobenthos typically enriched by around 5‰ in ^{13}C compared to phytoplankton (Doi *et al.*, 2010; France, 1995). The lack of separation therefore suggests strong benthic-pelagic couplings based on carbon flow between the two food web compartments. $\delta^{34}\text{S}$ values can also be used as an independent marker differentiating between

pelagic and benthic pathways due to bacterial reduction of sulphates within sediments resulting in ^{34}S depletion (Duffill Telsnig *et al.*, 2019; Fry & Chumchal, 2011; Fry *et al.*, 1982). Functional groups within the Southampton Water fish community were statistically separated by $\delta^{34}\text{S}$ values (Figure 5 and Table 1). Pelagic fishes were enriched by 1.8‰ in ^{34}S compared to benthic fishes with benthic-pelagic fishes occupying intermediate values, implying less incorporation of secondary sedimentary production in pelagic fishes compared to other functional groups. Nevertheless, $\delta^{34}\text{S}$ values in pelagic fishes averaged approximately 17‰, below the expected marine end member value (marine water sulfates, $\delta^{34}\text{S}$ ca. 21‰, Connolly *et al.*, 2004; Fry *et al.*, 1982). Given that trophic discrimination for $\delta^{34}\text{S}$ is approximately zero (McCutchan *et al.*, 2003), pelagic fishes appear to be incorporating some biomass derived from the benthic pathway. Freshwater sulphates also exhibit low $\delta^{34}\text{S}$ values (−5 to 5‰); nevertheless marine sulphate signatures dominate even at very low salinities within estuaries (approximately 1 psu) due to the greater sulphate concentrations in sea water (Fry & Chumchal, 2011). Freshwater sulphate sources are therefore unlikely to influence results here where salinities measured 31 psu on average.

The incorporation of benthic resources into pelagic fish diets has been inferred from $\delta^{34}\text{S}$ values in other estuaries (e.g., Fry & Chumchal, 2011) and, as inferred from convergent $\delta^{13}\text{C}$ values, supports the premise of significant two-way benthic-pelagic couplings within the estuary. Nevertheless, $\delta^{34}\text{S}$ appears to be more sensitive at distinguishing between functional groups despite its higher measurement error compared to carbon and nitrogen (0.6‰ s.d.). This is potentially due to the much greater separation expected between benthic and pelagic end-member values in $\delta^{34}\text{S}$ compared to carbon (up to ca. 40‰ compared to only ca. 5‰, France, 1995; Fry *et al.*, 1982). Given its sensitivity, it is interesting that no statistical support was found for temporal trends in $\delta^{34}\text{S}$ values across the fish community (Supporting Information Table S1). This implies that the seasonal changes in production sources within Southampton Water do not correspond to large-scale restructuring of the food web in terms of benthic-pelagic reliance as a mechanism for temporal stability (McMeans *et al.*, 2015).

Within consumers, $\delta^{15}\text{N}$ is indicative of differences in trophic level due to relatively high trophic discrimination (McCutchan *et al.*, 2003; Sweeting *et al.*, 2007a). The differences observed in $\delta^{15}\text{N}$ values between fish functional groups here, with benthic-pelagic species having values of 1.5‰ compared to benthic or pelagic species, reflect the more predatory nature of this trophic group which contains larger predatory species such as whiting and European seabass (Heessen *et al.*, 2015 and Appendix S1).

4.3 | Interpreting model outputs: individual size and species effects

Given that fish communities are notably size structured (Jennings *et al.*, 2002) and that both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fractionate during trophic assimilation in fishes (Sweeting *et al.*, 2007a, 2007b), it is not surprising that positive relationships were found between these isotopes

and individual body size at the community level. Assuming a predator–prey mass ratio of 1000, similar to other coastal fish communities (e.g., Trebilco *et al.*, 2016), the slope of 0.48 with logarithmic body mass determined here for $\delta^{13}\text{C}$ would equate to an approximate trophic discrimination factor (TDF) of 1.44‰, within the 1–2‰ range estimated for marine fishes (Sweeting *et al.*, 2007b). For $\delta^{15}\text{N}$, the estimated slope of 0.47 suggests a TDF of 1.41‰; nevertheless as the functional group also affects $\delta^{15}\text{N}$ and the groups are not distributed evenly across body sizes, this value is not representative of the whole community (see $\delta^{15}\text{N}$ against mass in Figure 5). Simple linear regression between body mass and $\delta^{15}\text{N}$ values across the whole fish community yields an estimated slope of 0.92 and therefore implies a community TDF of 2.76‰, similar to previous values of 3.15‰ + 1.28‰ s.d. determined for fishes (Sweeting *et al.*, 2007a).

At the individual level, changes in the relative contribution of benthic vs. pelagic production can also be related to body size in fishes (Duffill Telsnig *et al.*, 2019). As $\delta^{34}\text{S}$ does not fractionate during trophic assimilation (McCutchan *et al.*, 2003), slopes of the relationships between $\delta^{34}\text{S}$ values and body size indicate ontogenetic changes in benthic vs. pelagic reliance. Benthic and benthopelagic fishes exhibited negative slopes (−0.70 for both functional groups), suggesting an increasing reliance on benthic resources with increasing body size for fishes within these guilds. This is in contrast with pelagic fishes that displayed near-zero slopes (0.11 but not statistically distinguishable from 0). This suggests that the contributions of benthic-derived sulphur are independent of body size in pelagic fishes (Figure 5), implying a consistent relative reliance on pelagic and benthic pathways throughout ontogeny within the estuary.

One of the strengths of the mixed-effects modelling approach is that it not only facilitates partitioning of variance among complex data structures but also provides inference within those structures (Zuur *et al.*, 2009). Species-level differences in average dietary behaviours, as well as life histories and physiologies (see Appendix S1), are expected to result in differences in isotope compositions, which are accounted for in the random effects structure (shown in Figure 5). This structure potentially provides insight into ecological processes that facilitate the coexistence of similar species and the functioning of the food web. For example, the congeneric rock and black gobies (*Gobius paganellus* and *Gobius niger*, respectively) exhibit similar morphologies, reproductive cycles and trophic behaviours. Isotopically however, rock gobies have elevated $\delta^{34}\text{S}$ values compared to the black gobies sampled in Southampton Water (mean difference of 2.4‰, see Figure 5 and Supporting Information Table S2) implying greater incorporation of pelagic resources in rock gobies and a potential mechanism for reducing competition between these two species. Conversely, the third benthic goby, the sand goby (*Pomatoschistus minutus*), exhibits a similar $\delta^{34}\text{S}$ with size trend to that of the black goby yet displays elevated $\delta^{15}\text{N}$ values across their overlapping size ranges. This suggests that sand gobies occupy a higher trophic position at size compared to black gobies (Figure 5), another potential mechanism for reducing interspecific competition (note that rock and black gobies occupy similar $\delta^{15}\text{N}$ at size values).

Where species random effects on slopes with body size were estimated ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), further insight can be garnered with respect to ontogeny. For example, most fish species examined here exhibited positive trends of $\delta^{15}\text{N}$ with size (Figure 5); nevertheless, it is notable that herring are one of the few species that do not. As is common among clupeids, herring can acquire prey both *via* selective particle feeding or filter feeding, the latter causing increased omnivory in the diet (Möllmann *et al.*, 2004). It has been suggested that this can result in a reduction in trophic position with size in herring when filter feeding becomes more prevalent (Duffill Telsnig *et al.*, 2019) and has been demonstrated isotopically in *Sardina pilchardus*, an ecologically similar, warmer water clupeid species (Bode *et al.*, 2007). This contrasts with sprat, which are one of the few clupeid species that are obligate selective particle feeders (Möllmann *et al.*, 2004), and show a positive $\delta^{15}\text{N}$ trend with body size here (Figure 5).

4.4 | Implications for stable isotope ecology

Pronounced changes in fish community $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values coinciding with, and inferred to reflect, phytoplankton physiology during the peak spring bloom suggests that isotopic changes were rapidly transferred through food chains during the period of high production, discernible across at least two trophic steps in approximately 2 weeks to a month. This contrasts with earlier studies that have suggested seasonal variations in the isotopic composition in lower portions the food web are unlikely to be reflected in muscle tissues of higher trophic levels such as fishes due to slow turnover and trophic dampening (e.g., Jennings *et al.*, 2008b; Perga & Gerdeaux, 2005). Unfortunately, as we lack direct isotopic observations of pure phytoplankton, they cannot assess whether the amplitude of temporal fluctuations in plankton isotopic compositions was attenuated during trophic transfer to fish tissues. Nevertheless, experimentally derived isotopic half-lives of species sampled were 24 and 50 days in sand gobies (Guelinckx *et al.*, 2007) and juvenile European sea bass, respectively (Sweeting *et al.*, 2005), which themselves only account for a single trophic step, implying slower turnover rates than those observed. Meta-analyses of (mostly) experimentally derived isotopic half-lives highlight the effects of body size and temperature linked to metabolic processes (Thomas & Crowther, 2015; Vander Zanden *et al.*, 2015), yet applying these derived equations yields turnover rates of 16–30 days when taking the average fish body size and water temperature from May, the month when rapid changes were observed. Among the small number of field-based studies in fishes however, isotope half-lives as low as 8–18 days have been estimated (McIntyre & Flecker, 2006; Weidel *et al.*, 2011). We suggest that these apparent discrepancies are potentially due to (a) mismatches between metabolic conditions in laboratory set-ups vs. those experienced in the field; and (b) the kinetics associated with isotope incorporation.

Isotope incorporation experiments typically restrict fish to voluntary swimming only (e.g., Ankjær *et al.*, 2012; Sweeting *et al.*, 2005). Yet aquaculture studies have long shown that both growth rates and food conversion efficiencies (i.e., mass gain per unit food intake)

increase when fish are maintained in flow-through currents and forced to swim (Davison, 1997). Such effects are induced at low but sustained swim speeds and do not result in increased food intake (Brown *et al.*, 2011; Christiansen *et al.*, 1989; Houlihan & Laurent, 1987). Increased protein turnover rates within muscle tissue also result from sustained swimming activity (Felip *et al.*, 2013; Houlihan & Laurent, 1987), resulting in higher retention of newly derived dietary proteins. We are unaware of any study directly testing for the effect of exercise on determined isotope incorporation rates in fishes, but such an effect has recently been demonstrated in bird lipid reserves (Carter *et al.*, 2018). Laboratory experiments also typically operate under conditions of uniform (and high) food availability and diet quality, whereas in field conditions growth is commonly maximised over relatively short timescales, as evidenced in the seasonally varying growth increment widths observed in otoliths, the foundation of fish age and growth analysis. As isotopic incorporation represents the sum of isotopic assimilation into newly grown tissue and tissue turnover (Fry & Arnold, 1982), variations in the relative proportion of newly assimilated tissue compared to maintenance turnover probably significantly influence estimates of incorporation rate. We argue therefore that experimental fish that are forced to actively swim, similar to the natural behaviours such as foraging and predator evasion, with seasonal food fluxes are therefore likely to reveal faster but more realistic isotopic incorporation rates due to concurrent physiological increases in growth rate, food conversion efficiency and protein turnover as experienced in the field.

Isotope incorporation is well described by first-order kinetics (Fry & Arnold, 1982; Martínez del Río *et al.*, 2009) and therefore the most rapid changes in tissue isotopic composition will always occur immediately following the isotopic change in assimilated material. It is therefore important to recognise that statistical differences may be observed prior to equilibration or even the time taken to reach an isotopic signature that reflects 50% of the initial composition and 50% of the incorporated material (the isotopic half-life, Martínez del Río *et al.*, 2009). This is especially the case where isotopic shifts in assimilated resources are sudden and stark (Haas *et al.*, 2009).

To accommodate temporal variability in baselines, Jardine *et al.* (2014) suggested using slow-growing primary consumers such as mussels to provide an average isotopic baseline for data interpretation. Nevertheless, such an approach could potentially lead to erroneous conclusions, such as interpreting changes in $\delta^{15}\text{N}$ as changes in the trophic level rather than nutrient regimes, particularly if the baseline organism is only a minor component of the overall food web. Being in isotopic equilibrium with diet is an explicit assumption of many applications of stable isotopes in ecology such as the use of mixing models to infer dietary contributions or the use of isoscapes to infer location (Phillips *et al.*, 2014). Accounting for temporal variation in prey types, prey isotopic compositions and tissue growth rates, if present, significantly complicates the underlying mathematics associated with mixing models or with comparisons to isoscapes, but may be critical to avoid erroneous assumptions, especially in seasonally dynamic environments. This could be particularly problematic if study sample designs result in individuals being sampled over time periods

of many weeks such as opportunistic sampling over an entire growth season. Extensions to isotopic mixing models may allow consideration of temporal variability (Woodland *et al.*, 2012), but currently there is a lack of data or theory to effectively predict temporal isotope dynamics throughout multi-trophic-level food webs. We therefore caution against blindly applying assumptions that seasonalities in baseline isotope compositions are not expressed in tissues of higher trophic-level animals without careful consideration, especially in marine settings where primary producers have extremely rapid life cycles.

4.5 | Conclusions

The research presented here adds to the growing evidence of isotopic seasonality in higher-trophic level consumers (*e.g.*, Lesutiene *et al.*, 2014; MacKenzie *et al.*, 2019). Uniquely, we verified isotopic seasonality across a fish community encompassing differing species with various physiologies and life-history traits (see Appendix S1). This excludes other species-specific hypotheses for isotopic seasonality such as the internal nutrient shuttling for gonad development related to specific reproductive cycles (Perga & Gerdeaux, 2005). By measuring the stable isotope composition of a fish community on a monthly basis over a full seasonal cycle, coupled with environmental measurements of the estuary, we were able to infer of the Southampton Water food web:

1. The fish community is supported by both pelagic and benthic production with:
 - a. Pelagic fishes showing consistently lower incorporation of benthic-derived sulphur compared to benthic and benthopelagic functional groups
 - b. Benthic and benthopelagic fishes incorporating relatively more benthic-derived production with increasing body size
2. Temporal variations in fish muscle reflect:
 - a. Physiological differences in isotopic fractionation in phytoplankton production from the $\delta^{13}\text{C}$ values
 - b. Differential nutrient (nitrate vs. ammonium) utilisation in $\delta^{15}\text{N}$ values
3. The relative use of benthic resources does not exhibit seasonal variation despite dramatic shifts in the availability of pelagic production.

We qualitatively demonstrated through stable isotopes the biogeochemical functioning of an estuarine ecosystem, highlighting the dependency of fish biomass on seasonally varying phytoplankton production and nutrient pathways. Ecological applications of stable isotopes are at their most powerful when stable isotope data can be interrogated alongside other data sources that provide the biogeochemical context to the system(s) of interest.

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AUTHOR CONTRIBUTIONS

M.R.D.C. and C.N.T. conceptualised the study, M.R.D.C. conducted field sampling, R.A.R.M. and M.R.D.C. analysed samples, M.R.D.C. conducted statistical analyses, M.R.D.C. wrote first draft of manuscript and all authors reviewed and edited the manuscript. The authors declare no conflicts of interest.

DATA AVAILABILITY

Stable isotope and Xylem buoy data available on Figshare (10.6084/m9.figshare.16761415), a sub-set of which has already been published in Cobain *et al.* (2019). Nutrient data available as UK government public data: <https://environment.data.gov.uk/water-quality/view/landing>

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