SUPPLEMENTARY INFORMATION:

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

Matthew R. D. Cobain[1,2]*, Rona A. R. McGill[3], Clive N. Trueman[1]

1. Ocean and Earth Science, University of Southampton, Southampton, SO14 3ZH, UK

2. School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

3. National Environmental Isotope Facility (formerly NERC LSMSF), Scottish Universities Environmental Research Centre, East Kilbride, Glasgow, G75 0QF, UK

* Corresponding Author (mrdcobain@gmail.com)

This supplementary information contains additional information providing environmental and ecological context to the Southampton Water estuarine system. It includes fish species descriptions; isotope biplots and figures relating to sampling and environment not reported in the results of the main article; and tables of model selection outputs and fish species random effects for the mixed effect models.

Species Descriptions

Below we provide brief descriptions of the twelve species (common name followed by species name with authority), separated by functional group, included in the stable isotope-based fish community analysis in Southampton Water. Specific information includes their reproductive behaviour, their occurrence within shallow reaches of Southampton Water throughout the year based on survey data (water intake only) and the typical diet of each species.

Pelagic (P) Trophic Functional Group

Atlantic Herring - Clupea harengus (Linnaeus, 1758)

A commercially important, cold-water forage fish, Atlantic herring constitute distinct populations of reproductively synchronised individuals of single batch spawners, but with different populations spawning at various times of the year across all seasons throughout their range (Heessen et al., 2015). Herring spawn demersally on gravel substrates, with spawning around the southern coast of England restricted to winter-spring (Haegele & Schweigert, 1985; Bréchon et al., 2013) - herring larvae are almost absent within zooplankton ichthyofauna by June (Grioche & Koubbi, 1997). Juveniles >3cm are first observed within Southampton Water in June with the 0-group remaining abundant within the estuary until February and absent post April. Larger individuals (up to 22cm) appear to over winter within the estuary.

The diet of herring is well studied, comprising predominantly of zooplankton, particularly calanoid copepods throughout their range (Heessen et al., 2015). Larval fish are also a common occurrence in herring diets (Segers et al., 2007). Herring utilise both selective particle feeding and filter feeding depending on size and prey density (Möllmann et al., 2004).

European Sprat - Sprattus sprattus (Linnaeus, 1758)

Sprat are a small bodied clupeid, tolerant of varying salinities, widely distributed in coastal, shelf and estuarine areas from the Black and Mediterranean Seas in the South, through to the North and Baltic seas (Peck et al., 2012). They are an indeterminate batch spawner, releasing pelagic eggs over a prolong period, varying with temperature but concentrated in the summer

(Peck et al., 2012). In Southampton Water, sprat are highly abundant all year round although segregate by size. Young juveniles dominate abundances in the summer months from May through to October with larger individuals (>6cm) almost completely absent during this period, however adults of up to 20cm over winter in the estuary.

Unlike other clupeids that may switch between particle and filter feeding, sprat are obligate particle feeders (Peck et al., 2012). Their diet is similar to that of herring, being dominated by late stage calanoid copepods (Möllmann et al., 2004), although other larger planktonic organisms may also be taken with diel selection patterns observed (Peck et al., 2012).

Transparent Goby - Aphia minuta (Risso, 1810)

A very small and short-lived species, much of the research focus on the transparent goby has focused on the Mediterranean and Black Sea populations where they are commercially exploited. A separate, larger-bodied sub-species occurs in the NE Atlantic from Portugal to southern Norway (La Mesa et al., 2005). They are considered semelparous, where fish die shortly after benthic spawning, however females may lay two batches of eggs (Caputo et al., 2000), and due to the short life cycle, potentially three cohorts spawning per year (Heessen et al., 2015), although concentrated in the summer months (La Mesa et al., 2005). In Southampton Water, transparent gobies are found all year round, often in high densities, with smaller individuals (<4cm) dominating abundances in the late autumn and winter, and larger individuals (4-6cm) from early spring to August.

Stomach content analyses suggest that transparent gobies feed almost exclusively on zooplankton, with the diet dominated by calanoid copepods but decapod and polychaete larvae also making significant contributions (La Mesa et al., 2008).

Bentho-Pelagic (BP) Trophic Functional Group

European Seabass - Dicentrachus labrax (Linnaeus, 1758)

A coastal water species with economic importance, bass typically migrate offshore to spawn from February to May (Jennings & Pawson, 1991). The southern and western British Isles are the northern limit of their geographic range and they are a late maturing fish, taking up to seven years of growth to reach maturity (Heessen et al., 2015). In Southampton Water, 0-group individuals are first observed in August, with the cohort easily tracked through to the following spring. Frequency of occurrence appears to decrease with size for other cohorts over the same period (individuals up to 26cm are recorded). During June and July, bass appear almost absent, either moving out to more open waters or taking refuge in deeper parts of the estuary.

The diet of bass is broad and varies with size. Young of the year consume calanoid copepods and a variety of benthic invertebrates such as polychaetes, bivalves and amphipods whereas larger individuals also take crabs and other fish species (Cobain et al., 2019).

Pout / Bib - Trisopterus luscus (Linnaeus, 1758)

Pout are a medium sized gadoid found from the Skagerrak and North Sea down to the Moroccan coast and into the western Mediterranean (Heessen et al., 2015). Pout are batch spawners with a protracted spawning period from early spring to summer (Merayo, 1996; Heessen et al., 2015). In Southampton water, 0-group fish are observed from April/May and throughout the autumn, often in high densities. Larger adults (up to 25cm) are observed almost continuously throughout the year.

The smallest of pout consume almost exclusively calanoid copepods, however with increasing size the diet is quickly expanded to include larger crustaceans, notably shrimps, amphipods and crabs, with small fishes, such as gobies and sprats, also taken by larger individuals (Hamerlynck & Hostens, 1993; Reubens et al., 2011; Heessen et al., 2015).

Whiting - Merlangius merlangus (Linnaeus, 1758)

A gadoid found across the north-east Atlantic, whiting have a prolonged spawning period stretching from January through to July in the English channel and North Sea (Heessen et al., 2015; González-Irusta & Wright, 2017). Spawning occurs in deeper waters (>100m depth) but post metamorphosis, juveniles are found mainly in shallow waters including estuaries (Heessen et al., 2015). In the Southampton Water surveys, small whiting (<10cm) can be observed from July, although more typically from August or September. Otherwise observations of whiting occur throughout the year but more sporadic compared to pout. There is a notable reduction in abundance in the survey during late spring to early summer. Individuals of up to 31cm have been recorded within the estuary.

Whiting are typically described as a piscivorous predator, with fish including clupeids, sand eels and Norway pout found in the stomachs of large adults (Hislop et al., 1991, Heessen et al., 2015). However, particularly at smaller body sizes, crustaceans such as crangon, euphausids and amphipods also make major contributions to individual diets (Hislop et al., 1991).

Sand Smelt - Atherina presbyter Cuvier, 1829

Sand smelt are a small fish living for only a few years, with most individuals maturing after their first year (Turnpenny et al., 1981; Pajuelo & Lorenzo, 2000). They are a typical estuarine fish with a range from northern Europe to the western Mediterranean. Spawning occurs during the summer, a period which can be prolonged with potential repeat spawning (Henderson et al., 1984, Turnpenny et al., 1981; Pajuelo & Lorenzo, 2000). In Southampton Water, 0-group individuals (~4cm) are typically first observed from August / September, with all age groups relatively abundant throughout the year except for mid-summer (June / July).

Although often considered a surface particle feeder, both pelagic zooplankton and benthic macrofauna are readily observed in the diet of sand smelt (Turnpenny et al., 1981; Pombo et al., 2005). Isotopic evidence of the co-occurring, morphologically similar but now considered separate species *A. boyeri* suggests considerable incorporation of benthic derived sources and trophic plasticity (Vizzini & Mazzola, 2002).

Benthic (B) Trophic Functional Group

Common Sole - Solea solea (Linnaeus, 1758)

One of several flatfish species found in southern England, sole are shallow-water batch spawners, the timing of which shifts from February at the southern reaches of their range to as late as June in the North Sea (Heessen et al., 2015). In the eastern English Channel, spawning is concentrated in April (Eastwood et al., 2001), with juveniles almost exclusively restricted to shallow coastal areas (<20m depth, Symonds and Rogers, 1995). In Southampton Water, individuals are typically <25cm in size and occur sporadically throughout the year in surveys, although notably reduced in occurrence from January to March possibly due to the cold-water temperatures.

The diet consists of benthic invertebrates, notably polychaetes, decapods and molluscs, being nocturnal feeders that rely on olfactory senses to locate prey (Molinero & Flos, 1992; Heessen et al., 2015).

Black Goby - Gobius niger Linnaeus, 1758

One of the largest species of goby found in the British Isles, the black goby does not mature until approximately 5cm in size, typically in the second year (Vesey & Langford, 1985). The main spawning period is April-May on the southern English coast, although gamete retention indicates possible batch spawning from between March and November (Vesey & Langford, 1985; Heessen et al., 2015). Larger males build and protect egg clutches within nests, although as with other *Gobius spp.* smaller 'sneaker' male forms occur (Heessen et al., 2015). In Southampton Water, black gobies are observed throughout the year, although peak abundances coincide with the peak spawning period in April and May.

The diet of black gobies is dominated by polychaetes and amphipods, and larger individuals may feed upon smaller gobies (Vesey & Langford, 1985; Heessen et al., 2015). There appears to be seasonal dynamics in diet, whereby more errant species are consumed in warmer months versus sedentary species in the winter, likely due to temperature effects on fish motility (Vesey & Langford, 1985).

Rock Goby - Gobius paganellus Linnaeus, 1758

Similar in size to the black goby, however the rock goby's life history includes a more protracted spawning period, concentrated in the late spring to early summer but potentially spanning from December to July, with likely multiple broods (Miller, 1961; Dunne, 1978; Heessen et al., 2015). In Southampton Water, rock gobies are observed more frequently than black gobies throughout the year, with notable higher abundances over the autumn and winter period. The smallest individuals (<3cm) almost exclusively occur during the autumn (August to November).

Dietary analyses reveal that, like other gobies, rock gobies rely heavily on benthic invertebrate prey, notably amphipods, other crustaceans such as ostracods, and polychaetes (Dunne, 1978).

Sand Goby - Pomatoschistus minutus (Pallas, 1770)

The sand goby is a small but common goby found in shallow waters from Norway to Spain (Heessen et al., 2015). Females lay clutches of eggs on hard substrate with males building and protecting clutches in nests, with peak spawning between April and June in waters as shallow as 1m (Hesthagen, 1977). In Southampton Water, sand gobies are observed all year round. Small individuals (3cm) are commonly observed from August with sand gobies often dominating fish abundances through the autumn. Adults of up to 7cm occur year-round although abundances are markedly reduced from April until June.

The diet consists predominantly of benthic invertebrates, including meiofauna, and mainly consists of polychaetes, molluscs and amphipods (Leitão et al., 2006).

Tub Gurnard (Chelidonichthys lucerna Linnaeus, 1758)

The largest gurnard in the north east Atlantic, tub gurnards are found from Norway to the west African coast and in the Mediterranean (Heessen et al., 2015). Spawning season varies depending on the region, but in the western English Channel is reportedly between April and September (Baron, 1985). Observations within Southampton Water are less frequent compared to other species listed here but occur throughout the year with individuals ranging from 9cm to 30cm.

Tub gurnards appear to focus on epi- and suprabenthic prey, including benthic fishes in larger individuals, with the diet consisting of a majority of crabs and shrimps (López-López et al., 2011; Stagioni et al., 2012).

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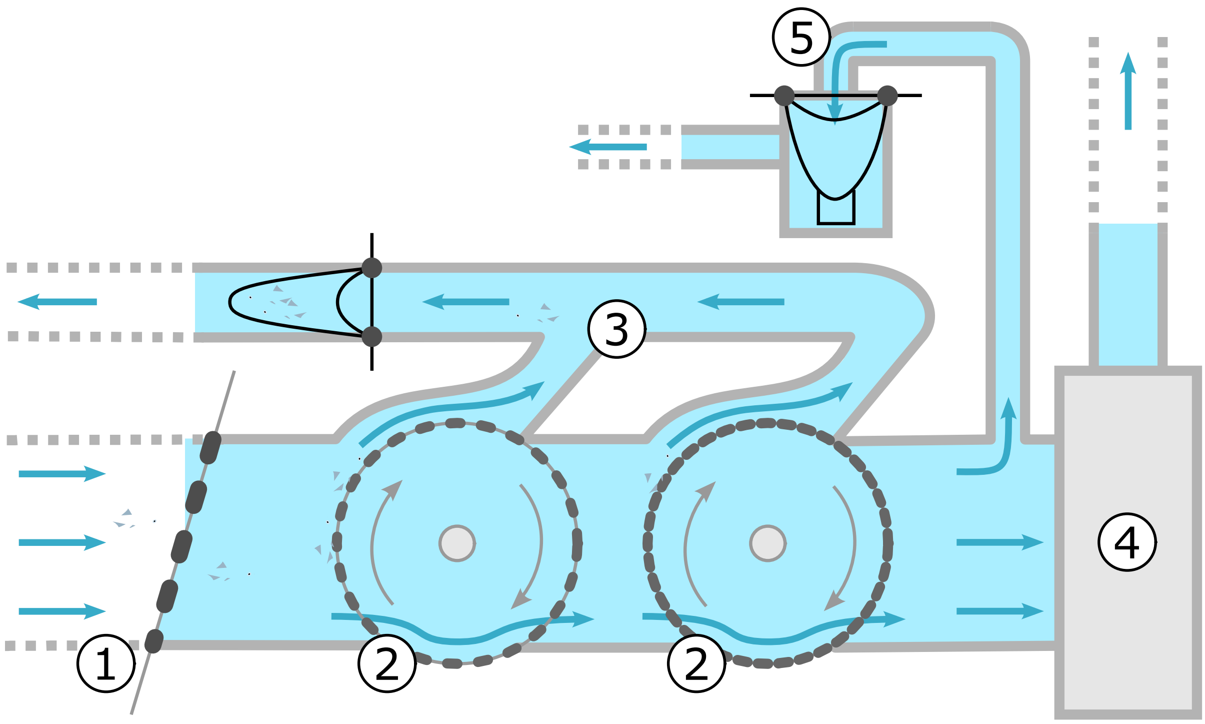

Figure S1: Conceptual diagram of the industrial water intake, screening and fish-return system that was used for fish and SPM sampling in Southampton water. 1) Water is drawn from the estuary (water flow direction indicated by blue arrows) with entrained fish and passes through a static coarse grid. The grid separates out large debris items such as branches or kelp and is periodically cleaned by a grab system (not shown). 2) Rotating drum screens of decreasing mesh size then filter out smaller debris, including macroscopic fish and invertebrates, by impingement as water flows through them. As the drums rotate, impinged material is continually redirected away from the main water flow. 3) The fish-return system returns material directly back to the estuary, with fish sampling conducted by periodic netting of the fish-return system waterway. 4) Screened water is then taken up and used for industrial activity before being returned to the estuary elsewhere. 5) Screened water can additionally be pumped to a separate tank with known flow rates, which is used for SPM sampling.

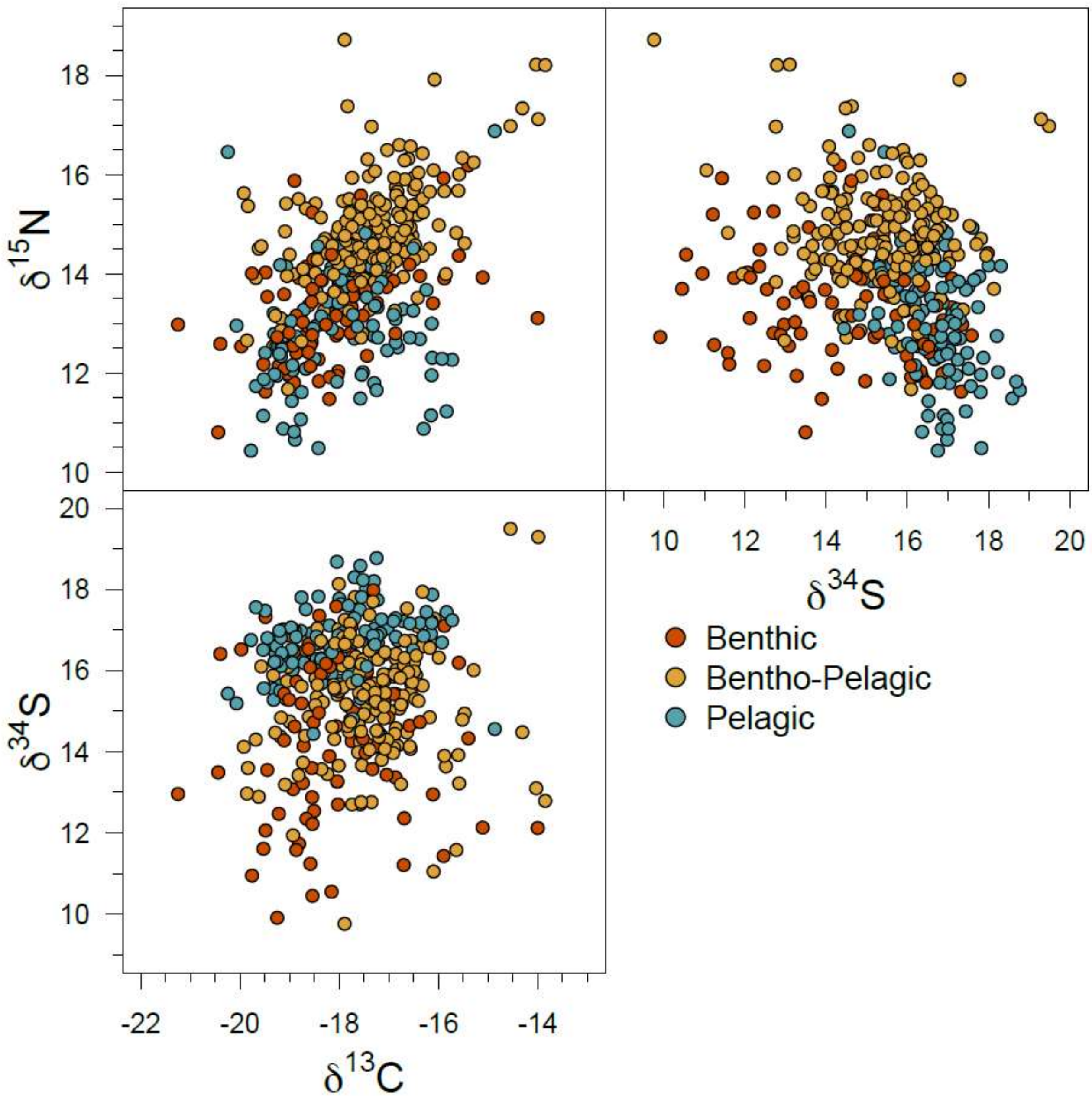

Figure S2: Isotope biplots of CN (top left), CS (bottom left) and NS (top right) of fish muscle data used in statistical analyses with points coloured by functional group.

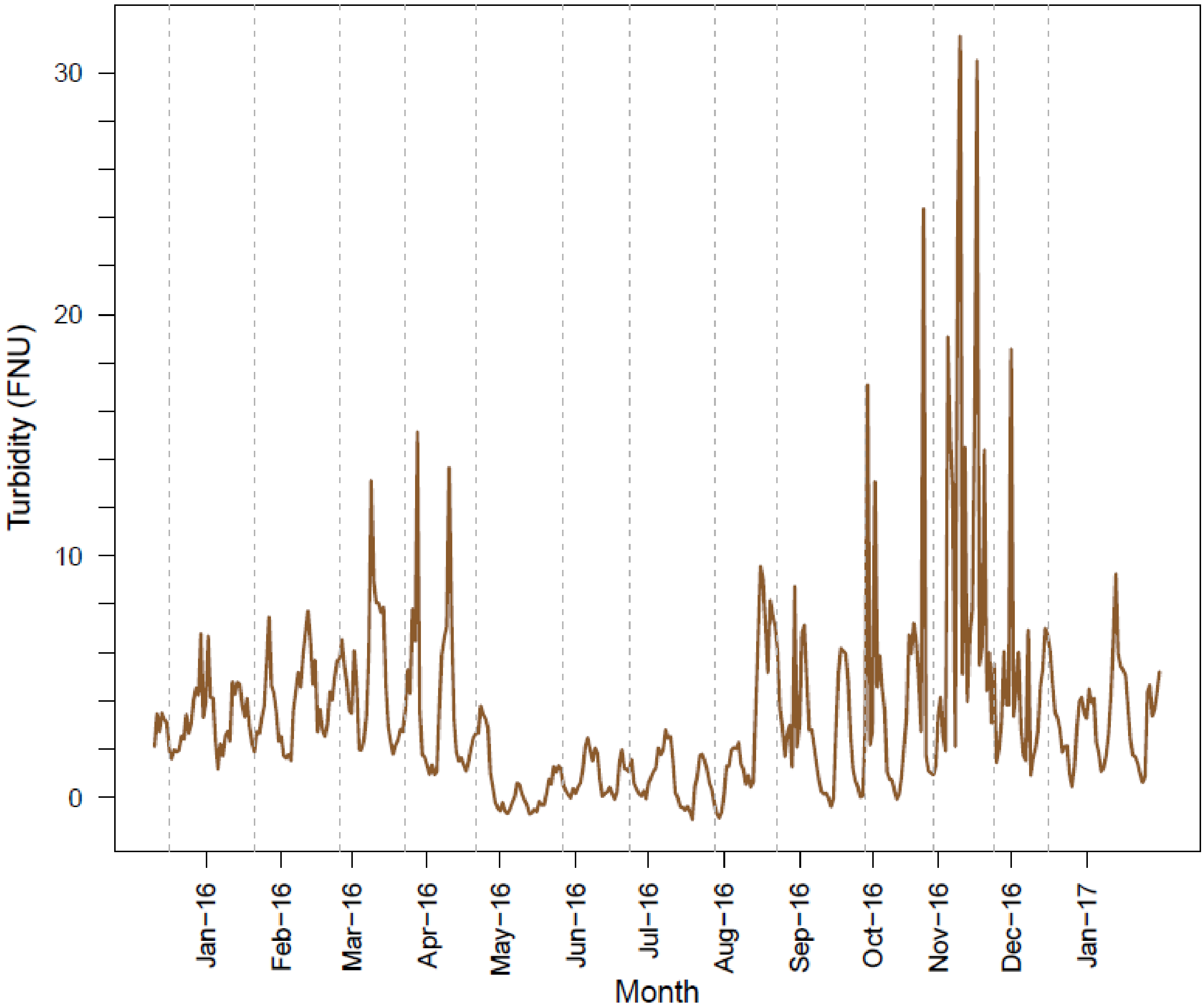

Figure S3: Time series of average daily water turbidity, given in Formazin Nephelometric Units (FNU), estimated from the degree of scattering of emitted infrared light. Ticks on the x-axis show the commencement of individual months. Dashed grey lines indicate dates when SPM samples were collected. For clarity, three high data points have been removed: 14^{th}, 15^{th} and 18^{th} of August 2016 with values of 73.5, 66.9 and 88.7FNU respectively.

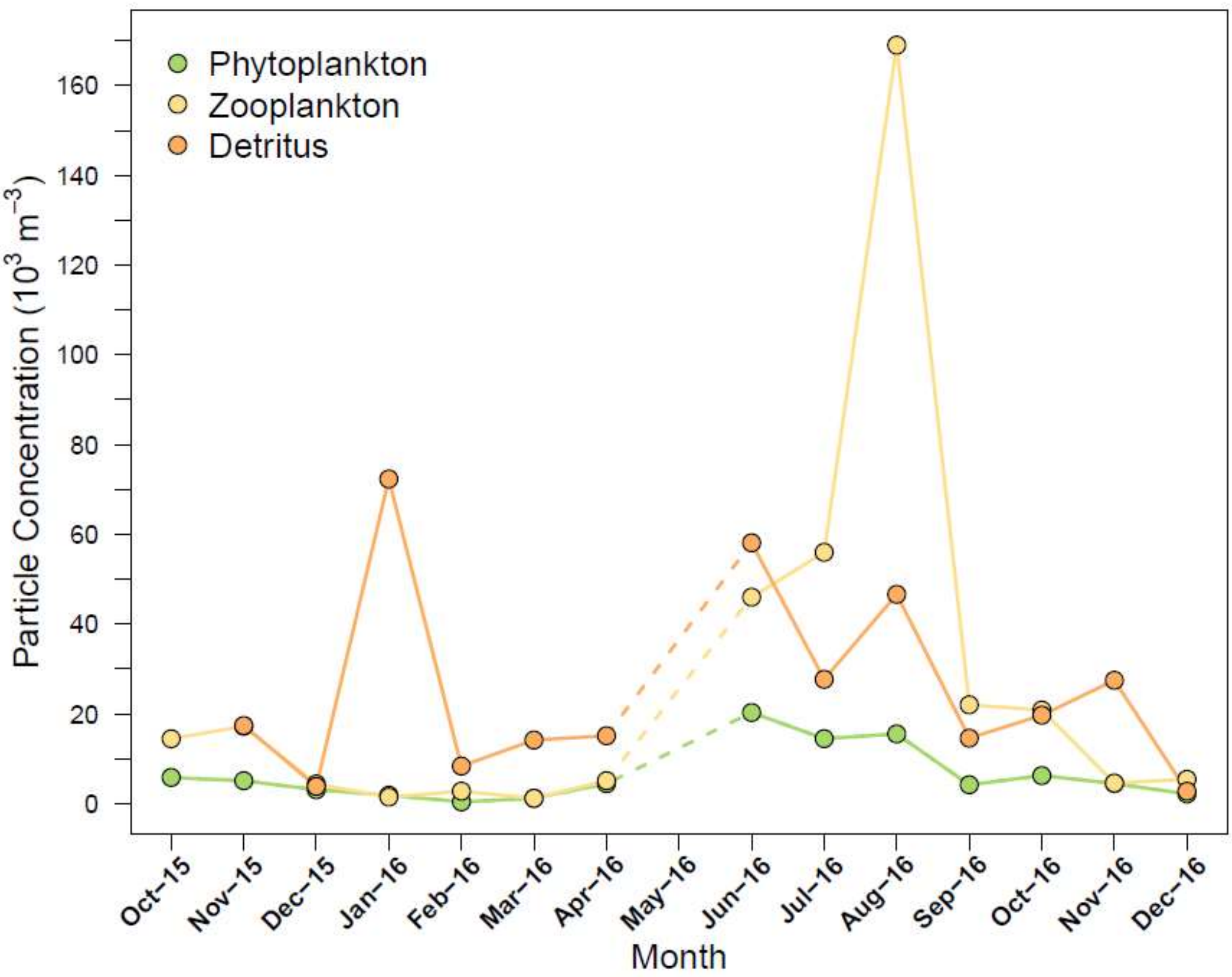

Fig S4: Time series of phytoplankton, zooplankton and detritus abundance estimates from October 2015 through to December 2016. No estimates are available for May 2016 due to high densities of diatoms and their associated phyto-detritus causing obscuration even under large subsample dilutions. No detritus estimate is available for October 2015.

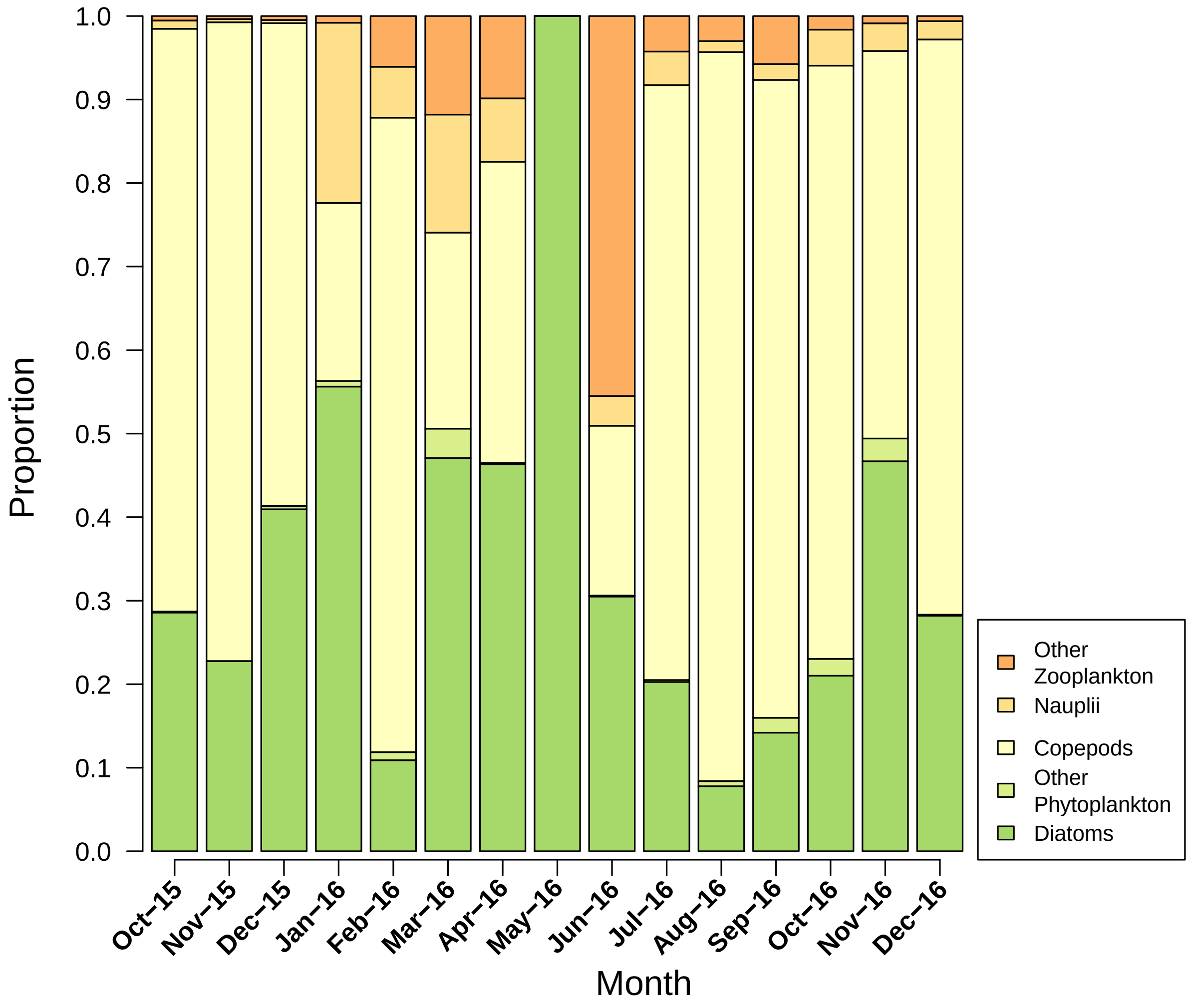

Figure S5: Overview of the relative plankton community composition, excluding detritus, from October 2015 through to December 2016.

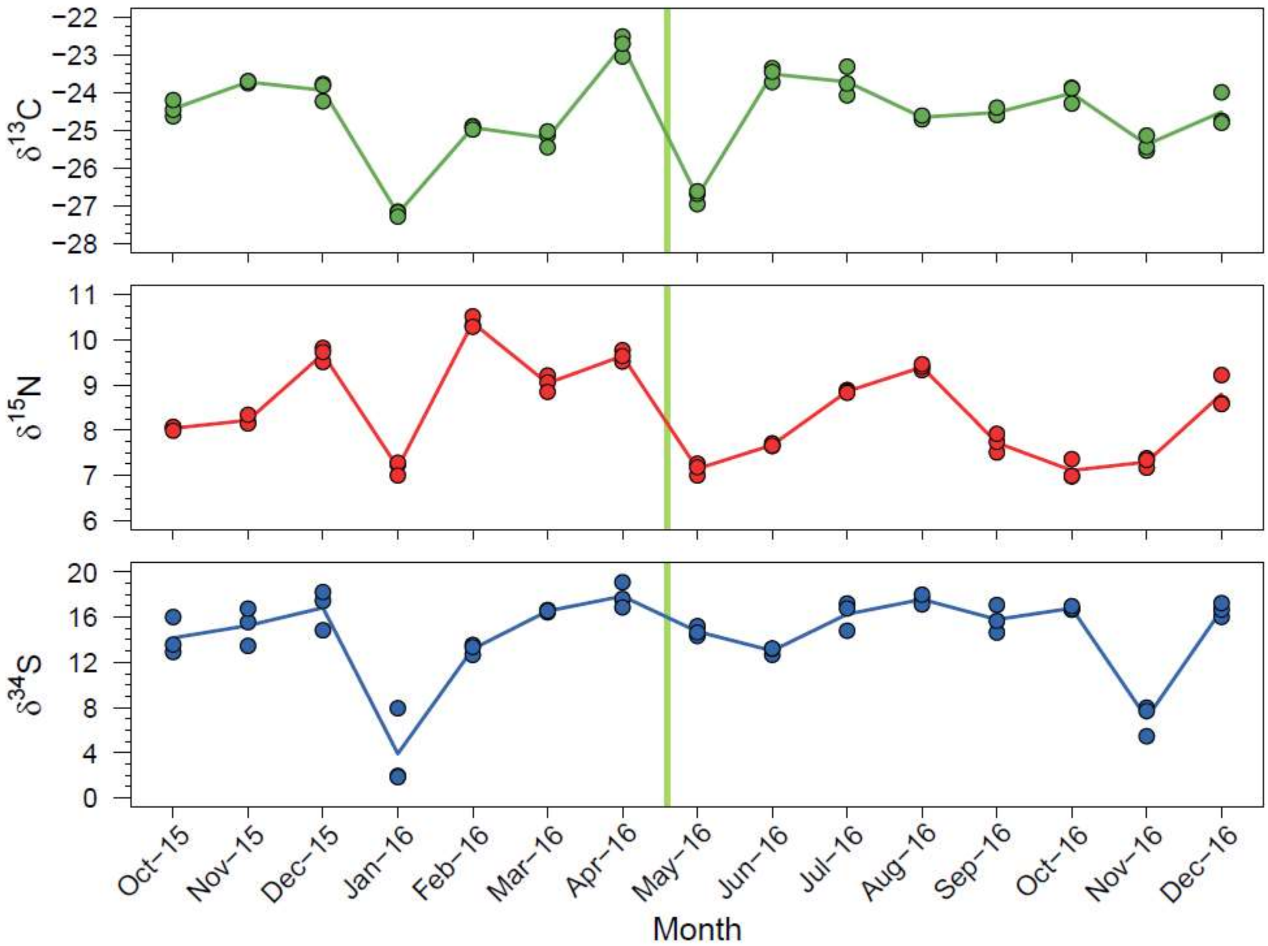

Figure S6: Time series of the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ isotopic composition of suspended particulate matter filtrate sampled from Southampton Water. Solid lines connect monthly means of 3 replicate measurements. Green vertical line demarcates the approximate position of the peak phytoplankton bloom determined by chlorophyll-a concentration.

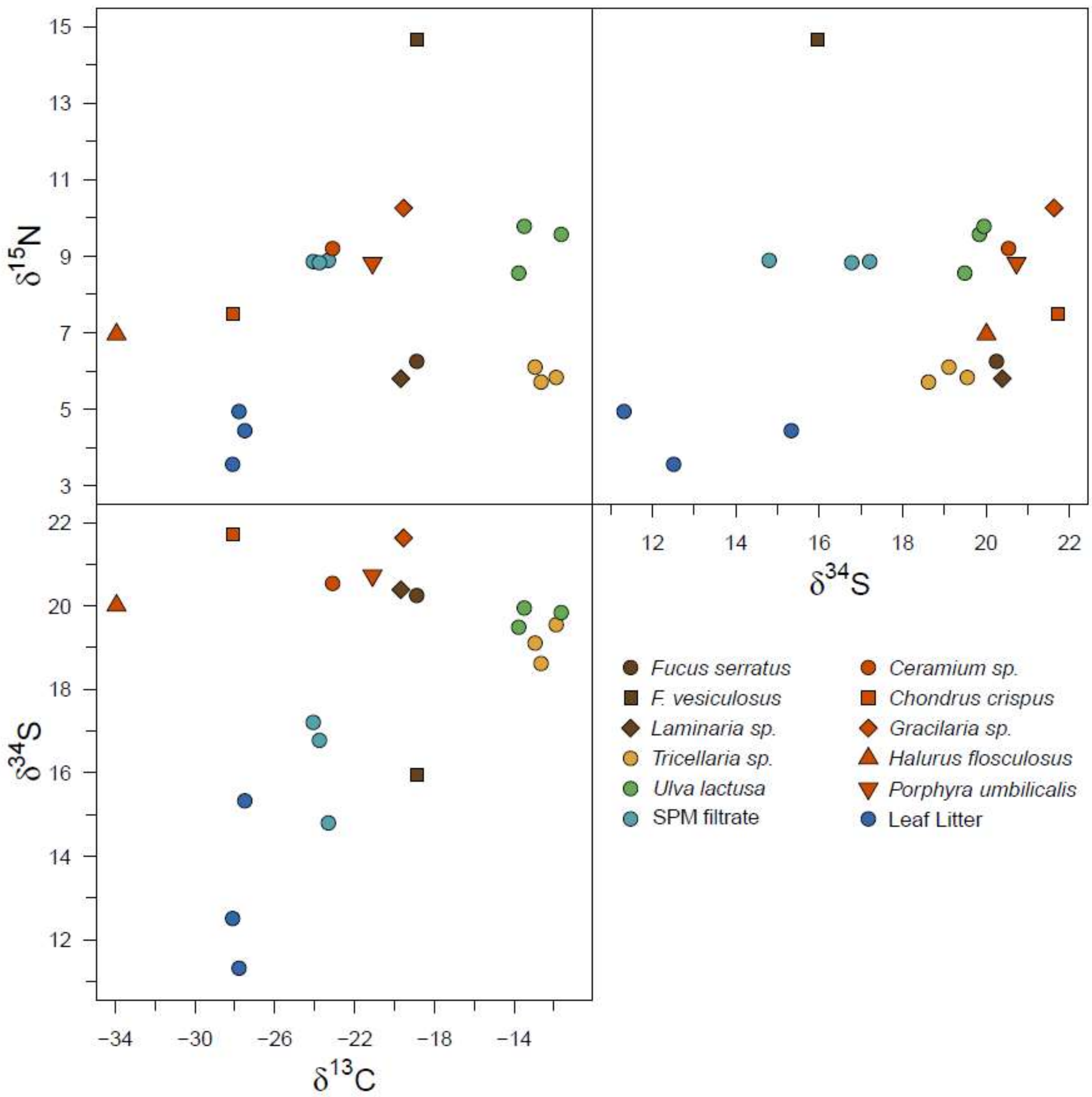

Figure S7: Isotope biplots of CN (top left), CS (bottom left) and NS (top right) of various macroalgae, leaf litter, a bryozoan species and suspended particulate matter (SPM) sampled in July 2016. Colours indicative of broad taxonomic clade (red = red algae, green = green algae, brown = brown algae, yellow = bryozoa, dark blue = leaf litter and light blue = plankton filtrate). Unique symbols within brown and red algae denote separate species. Samples of Ulva lactuca and Tricellaria sp. were taken from the same macroalgae cluster and colony respectively.

δ^{13}C Models	Restricted ML	cAIC	cLog-Likelihood	cDF	AIC	Log-Likelihood	DF	LRT	p-value
Mass + Month + FG + Mass:FG + (1|Species) + (0 + Mass|Species)	Y	1060.0	-500.0	29.98					
Mass + Month + FG + Mass:FG + (1|Species)	Y	1071.1	-510.0	25.57					
Mass + Month + FG + Mass:FG + (0 + Mass|Species)	Y	1095.0	-521.7	25.76					
Mass + Month + FG + Mass:FG + (1|Species) + (0 + Mass|Species)	N				1089.4	-524.7	20		
Mass + Month + FG + (1|Species) + (0 + Mass|Species)	N				1088.8	-526.4	18	3.48 (2)	0.176
Month + FG + (1|Species) + (0 + Mass|Species)	N				1093.1	-529.6	17	6.29 (1)	0.012
Mass + FG + (1|Species) + (0 + Mass|Species)	N				1123.9	-555.0	7	57.11 (11)	<0.001
Mass + Month + (1|Species) + (0 + Mass|Species)	N				1087.1	-527.6	16	2.27 (2)	0.322
Month + (1|Species) + (0 + Mass|Species)	N				1091.4	-530.7	15	6.32 (1)	0.012
Mass + (1|Species) + (0 + Mass|Species)	N				1122.4	-556.2	5	57.32 (11)	<0.001
Optimum δ^{13}C Model									
Mass + Month + (1|Species) + (0 + Mass|Species)	Y	1059.3	-500.6	29.06					
δ^{15}N Models									
Mass + Month + FG + Mass:FG + (1|Species) + (0 + Mass|Species)	Y	908.15	-420.7	33.4					
Mass + Month + FG + Mass:FG + (1|Species)	Y	931.8	-439.7	26.24					
Mass + Month + FG + Mass:FG + (0 + Mass|Species)	Y	1018.90	-483.6	25.9					
Mass + Month + FG + Mass:FG + (1|Species) + (0 + Mass|Species)	N				956.93	-458.46	20		
Mass + Month + FG + (1|Species) + (0 + Mass|Species)	N				953.11	-458.56	18	0.18 (2)	0.912
Month + FG + (1|Species) + (0 + Mass|Species)	N				957.33	-461.67	17	6.22 (1)	0.012
Mass + FG + (1|Species) + (0 + Mass|Species)	N				1017.18	-501.59	7	86.07 (11)	<0.001
Mass + Month + (1|Species) + (0 + Mass|Species)	N				955.52	-461.76	16	6.41 (2)	0.041
Optimum δ^{15}N Model									
Mass + Month + FG + (1|Species) + (0 + Mass|Species)	Y	909.32	-422.31	32.35					
δ^{34}S Models									
Mass + Month + FG + Mass:FG + (1|Species)	Y	1263.31	-605.58	26.08					
Mass + Month + FG + Mass:FG + (0 + Mass|Species)	Y	1296.87	-622.3	26.13					
Mass + Month + FG + Mass:FG + (1|Species)	N				1288.4	-625.22	19		
Mass + Month + FG + (1|Species)	N				1291.4	-628.65	17	6.86 (2)	0.032
Mass + FG + Mass:FG + (1|Species)	N				1282	-632.98	8	15.52 (11)	0.16
Mass + Month + Mass:FG + (1|Species)	N				1292.2	-629.08	17	7.72 (2)	0.021
Mass + Mass:FG + (1|Species)	N				1285.2	-636.57	6	7.19 (2)	0.027
Optimum δ^{34}S Model									
Mass + FG + Mass:FG + (1|Species)	Y	1256.61	-613.2	15.1					

Table S1: Model selection for mixed effects models. ML – maximum likelihood; AIC –Akaike's Information Criterion; DF –degrees of freedom; LRT – likelihood ratio test statistic. Prefix c indicates effective sample size corrected statistic.

	δ13C corrected			δ15N			δ34S		
Intercept	**Estimate (mean)**	**CI lower (95%)**	**CI upper (95%)**	**Estimate (mean)**	**CI lower (95%)**	**CI upper (95%)**	**Estimate (mean)**	**CI lower (95%)**	**CI upper (95%)**
Bass	1.38	0.66	2.25	1.27	0.17	2.39	-0.49	-1.66	0.65
Black Goby	-0.33	-1.21	0.47	-0.31	-1.45	0.76	-0.78	-2.01	0.4
Herring	0.13	-0.65	0.95	0.52	-0.74	1.81	-0.41	-1.78	0.94
Pout	0.31	-0.38	1.05	0.1	-1.04	1.25	0.2	-0.95	1.31
Rock Goby	-0.51	-1.24	0.18	-0.18	-1.27	0.93	1.57	0.47	2.74
Sand Goby	0.23	-0.34	0.85	0.9	-0.12	1.96	-0.86	-2.11	0.25
Sand Smelt	-0.1	-0.74	0.59	-0.84	-1.97	0.25	-0.82	-2.02	0.34
Sole	-0.3	-1.31	0.72	-0.75	-2.08	0.45	-0.26	-1.44	0.98
Sprat	-0.58	-1.12	0	-0.91	-2.2	0.3	0.06	-1.25	1.44
Transparent Goby	0.41	-0.2	1.05	0.47	-0.78	1.73	0.4	-0.9	1.75
Tub Gurnard	-0.29	-1.79	0.9	0.22	-1.33	1.82	0.35	-0.91	1.71
Whiting	-0.43	-1.42	0.48	-0.49	-1.73	0.71	1.13	-0.03	2.34
Slope with log_{10}(Mass)									
Bass	-0.58	-1.15	-0.08	-0.52	-1.08	-0.02			
Black Goby	0.12	-0.66	0.92	0.21	-0.59	1.07			
Herring	-0.14	-0.77	0.48	-0.58	-1.3	0.05			
Pout	-0.15	-0.69	0.34	-0.22	-0.76	0.27			
Rock Goby	-0.05	-0.93	0.71	-0.59	-1.71	0.24			
Sand Goby	-0.23	-0.94	0.37	0.14	-0.6	0.95			
Sand Smelt	-0.04	-0.65	0.5	0.3	-0.27	0.91			
Sole	-0.21	-0.92	0.41	0.07	-0.59	0.75			
Sprat	0.64	0.11	1.26	-0.15	-0.8	0.36			
Transparent Goby	-0.07	-0.66	0.63	0.79	-0.11	1.98			
Tub Gurnard	0.45	-0.28	1.42	0.37	-0.4	1.29			
Whiting	0.26	-0.26	0.79	0.17	-0.37	0.74			

Table S2: Random species intercepts and slopes with mass for mixed effects models. CI – credible interval