Supporting information for:

Reproduction explains seasonal eDNA variation in a temperate marine fish community

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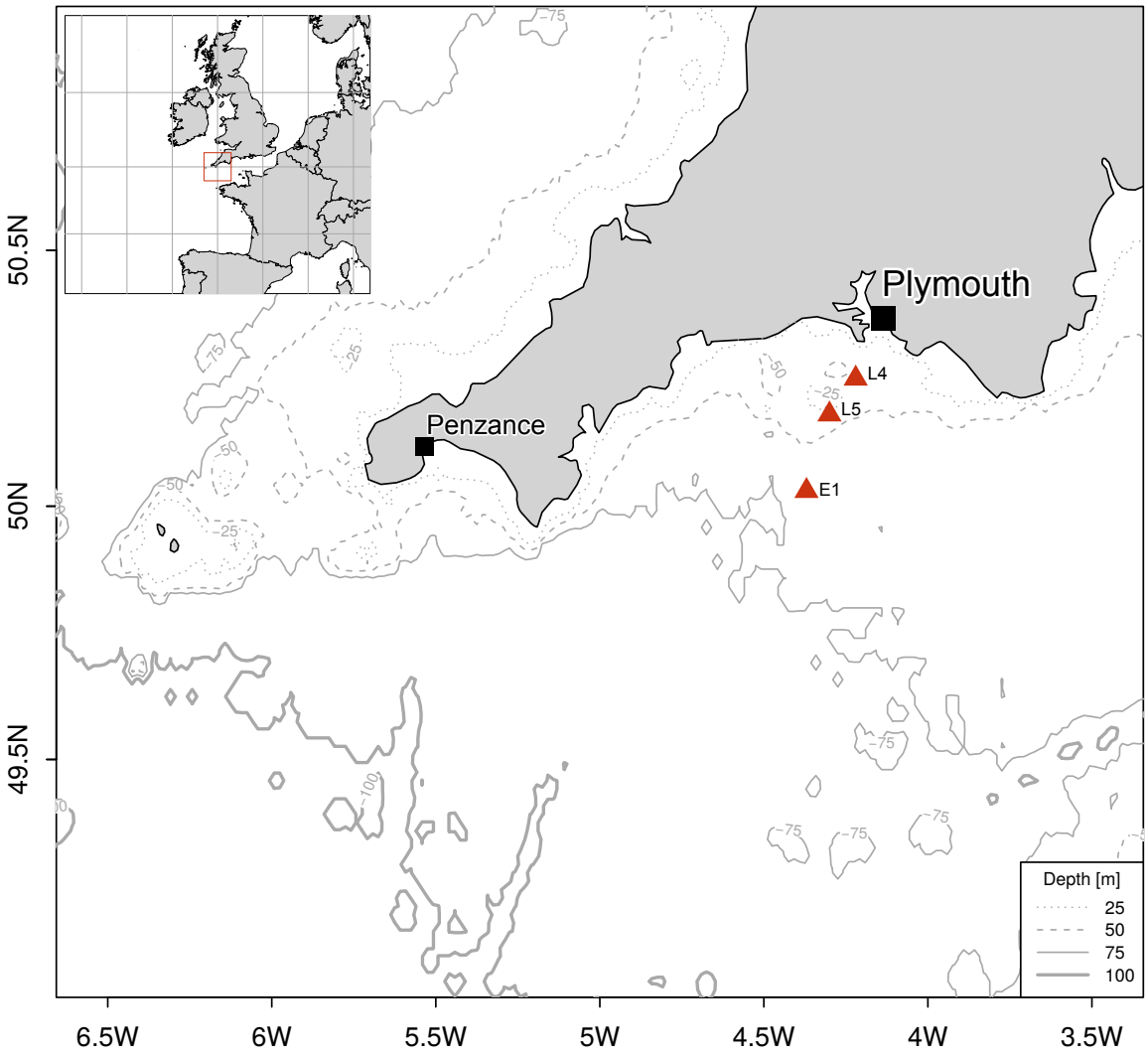
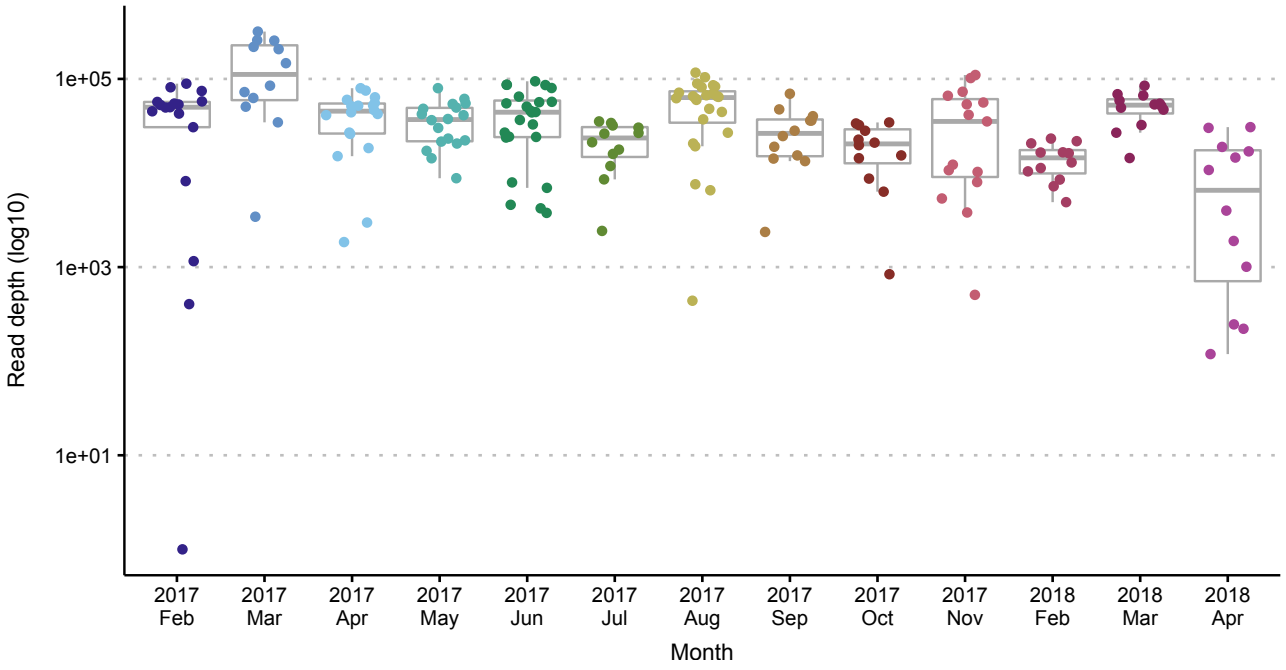
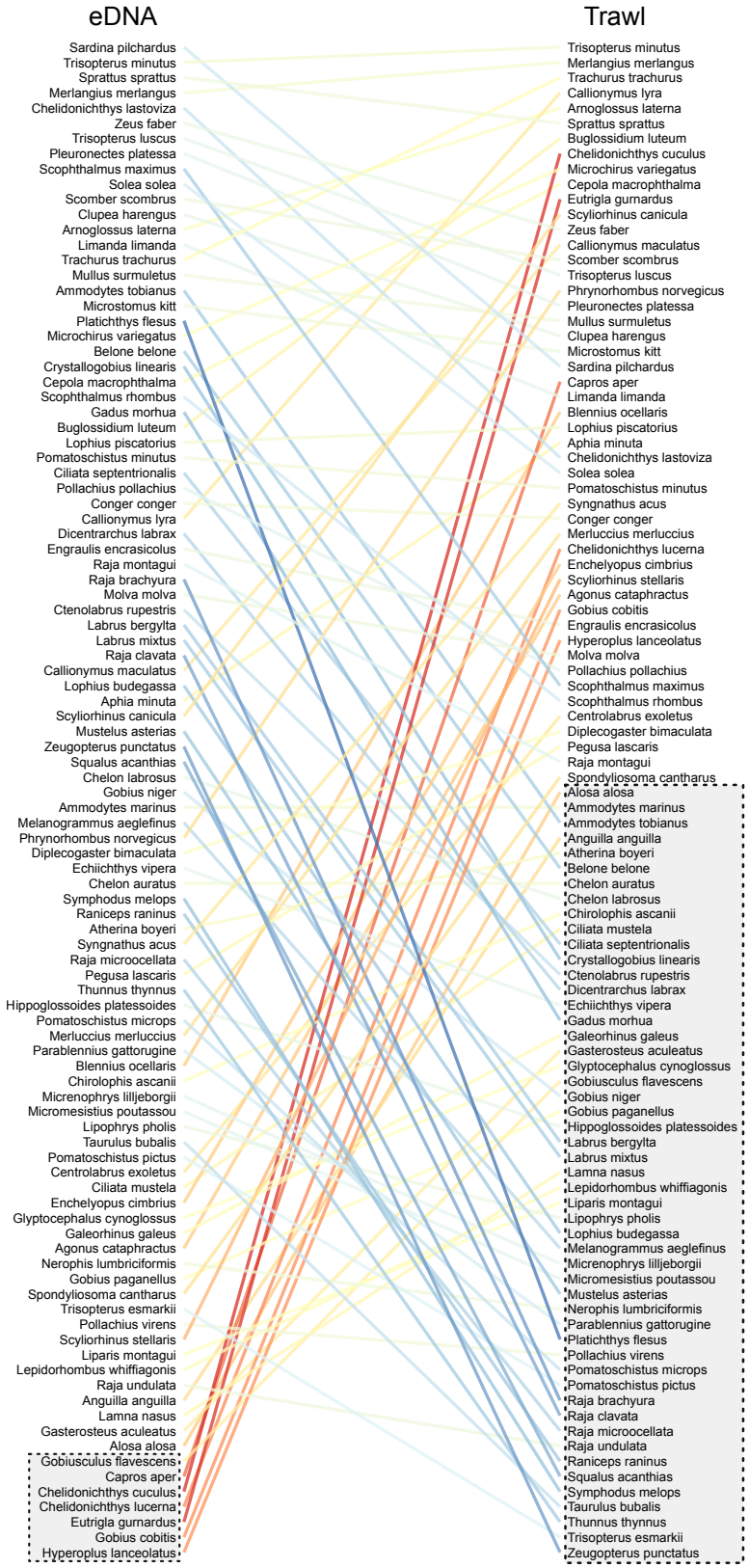


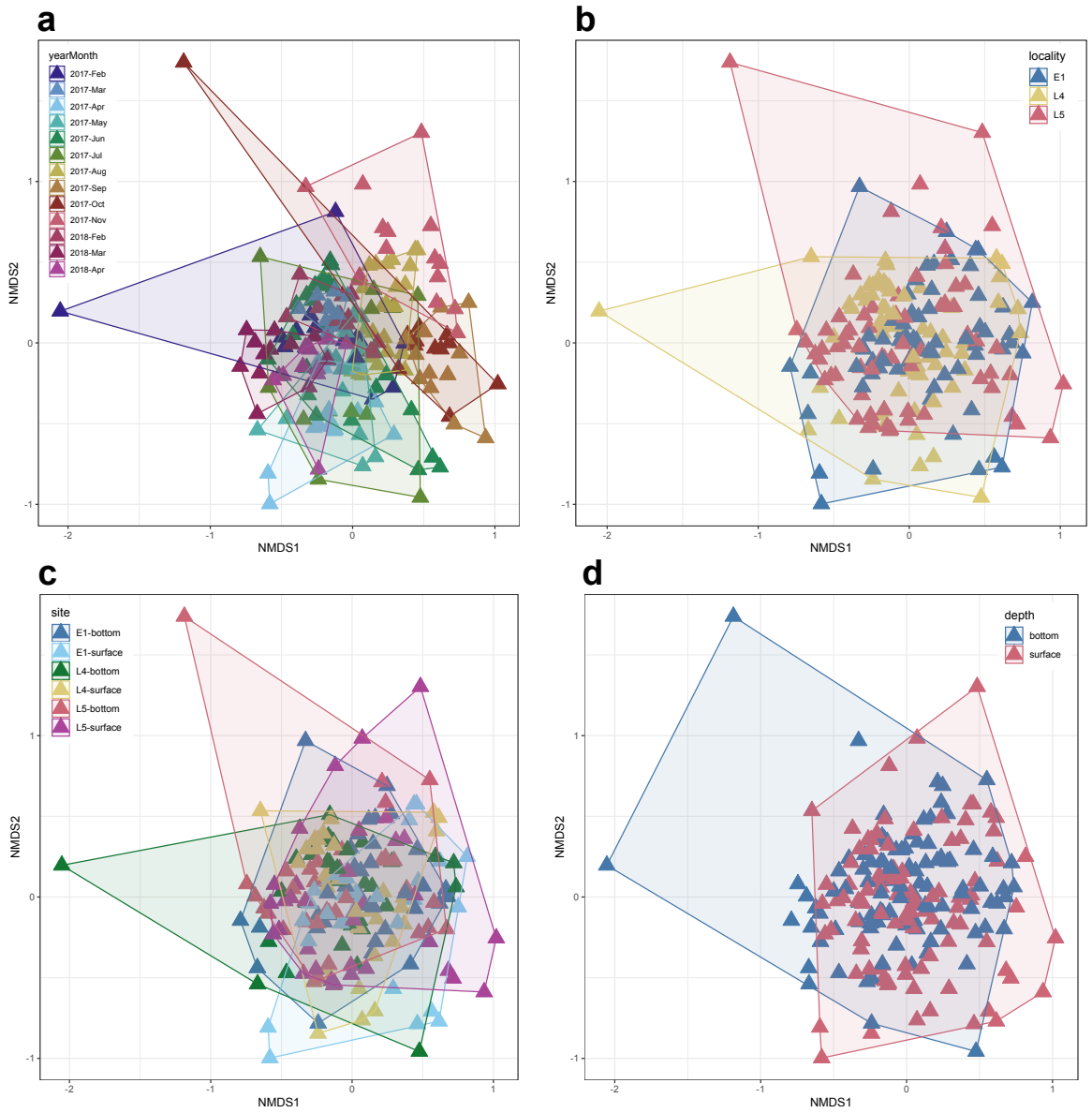
Supporting Figure S1: Map of sampling locations in relation to the Western English Channel. Inset panel shows position of study area in reference to the UK and western Europe. Sampling locations L4, L5 and E1 are marked with red triangles.



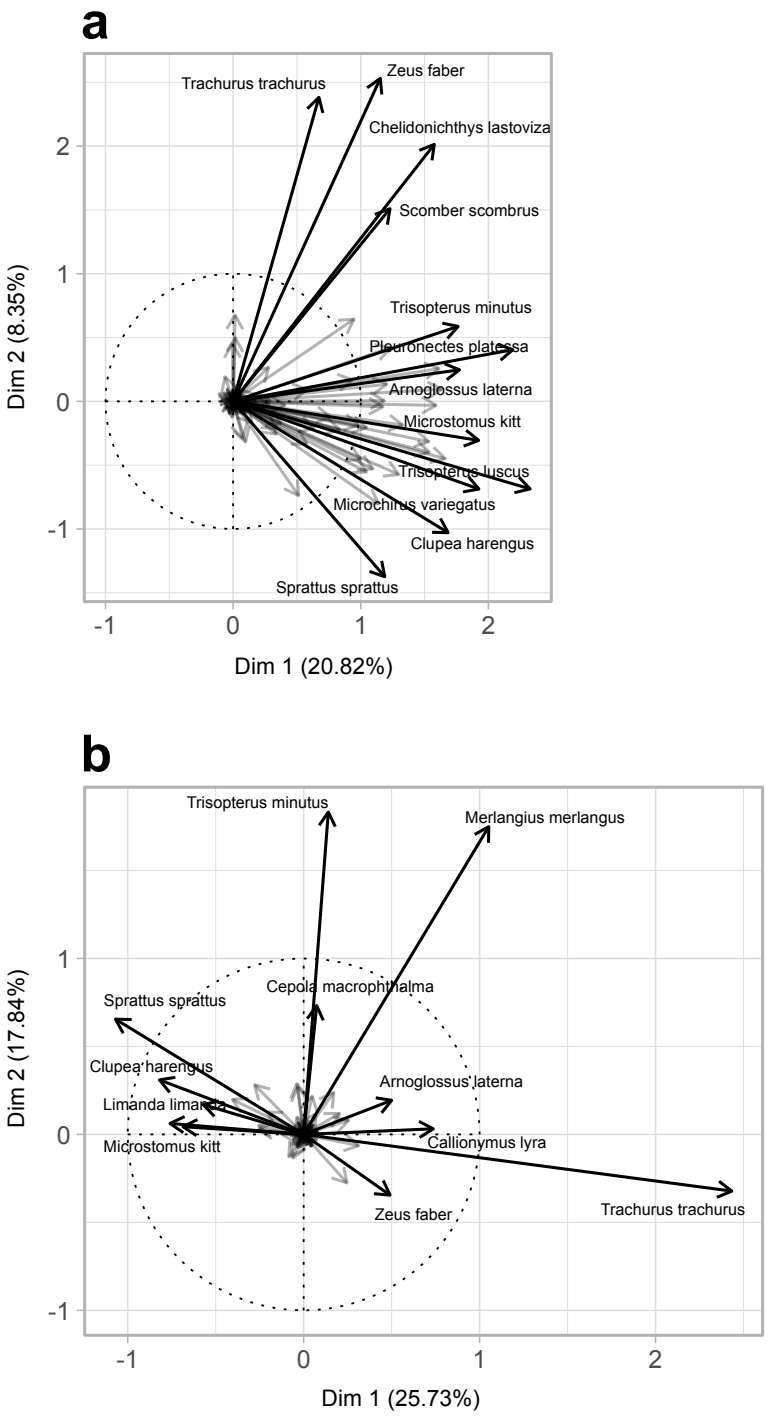
Supporting Figure S2: Sequencing read depth per sample by month after bioinformatic processing and taxonomic assignment. All locations; samples $n = 200$; reads $n = 8,633,038$.



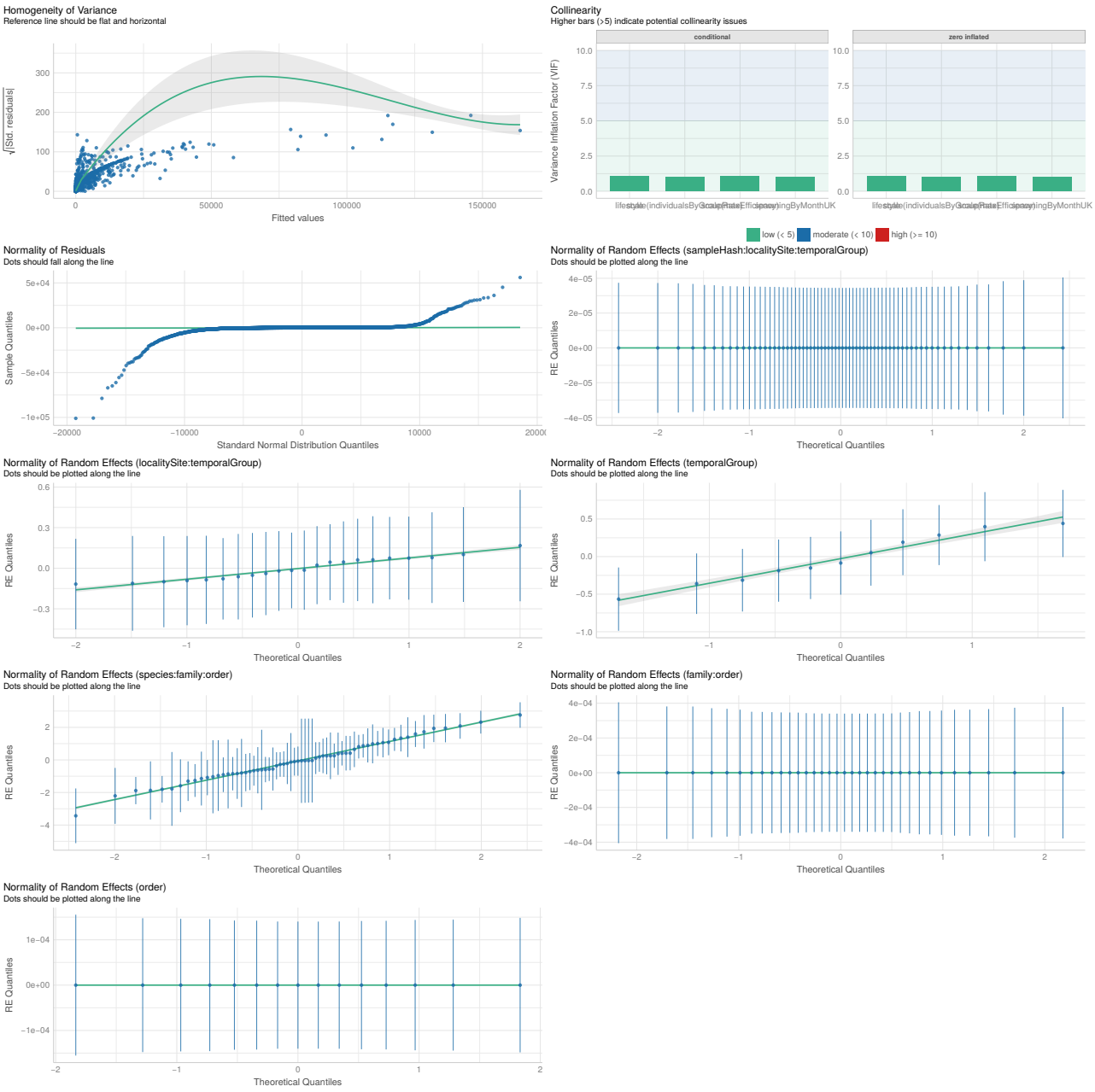
Supporting Figure S3: Ranked species abundances for eDNA (all locations; species $n = 94$; reads $n = 8,633,038$) and demersal trawl (site L4; species $n = 49$; individuals $n = 99,172$) over survey period February 2017 to April 2018. Blue indicates eDNA rank higher than demersal trawl rank; red indicates demersal trawl rank higher than eDNA rank. Colour intensity reflects degree of difference in rank. Species in grey boxes are those with zero abundance.



Supporting Figure S4: Patterns in eDNA variation. Non-metric multidimensional scaling (NMDS) ordinations of fish community eDNA by (a) month; (b) location; (c) location and water depth; and (d) water depth. Period Feb 2017 to Apr 2018; species $n = 94$; samples $n = 200$; reads $n = 8,633,038$. Stress $= 0.21$.



Supporting Figure S5: Species contributions to principal coordinates (PCoA) for (a) eDNA at all sites (period Feb 2017 to Apr 2018; samples $n = 200$; species $n = 94$; reads $n = 8,633,038$); and (b) demersal trawl at location L4 (period Jan 2016 to Nov 2017; samples $n = 62$; species $n = 70$; individuals $n = 180,884$). Only the top twelve species with the greatest contributions are labelled. These plots show a seasonal signal in different axes; in the demersal trawl data (b), this information is contained in the first PCoA axis (Dim 1), while in the eDNA data (a), this signal is contained in the second PCoA axis (Dim 2), with many of the same seasonal species represented. This is the same analysis as used for the seasonal GAM plots in main Figure 1a,b.



Supporting Figure S6: Model performance and diagnostics for the zero-inflated negative-binomial generalised-linear mixed-model fitted in the *glmmTMB* v1.1.2.3 package. Simplified model formula: reads ~ offset(sampleTotalReads) + trawlCPUE + PCRefficiency + reproductionMonth + lifestyle + (1|event/location/sample) + (1|order/family/species). Diagnostics were estimated with the *performance* v0.8.0 package.

Supporting Table S1: Abundances by combined species ($n = 100$) for eDNA (all locations; species $n = 94$; reads $n = 8,633,038$) and demersal trawl (site L4; species $n = 49$; individuals $n = 99,172$) over the survey period (Feb 2017 to Apr 2018). Species merged reflect those that are not resolved in eDNA (*) or trawl survey (†) to species level.

Family	Species	Merged taxa as	Total eDNA reads	Total trawl abundance
Anguillidae	*Anguilla anguilla*		41	0
Congridae	*Conger conger*		21,425	6
Atherinidae	*Atherina boyeri*		969	0
Belonidae	*Belone belone*		88,575	0
Clupeidae	*Alosa alosa*		1	0
Clupeidae	*Clupea harengus*		161,642	90
Clupeidae	*Sardina pilchardus*		1,735,705	60
Clupeidae	*Sprattus sprattus*		921,237	1,739
Engraulidae	*Engraulis encrasicolus*		14,978	2
Gadidae	*Gadus morhua*		43,992	0
Gadidae	*Melanogrammus aeglefinus*	*Merlangius/Melanogrammus**	2,124	0
Gadidae	*Merlangius merlangus*	*Merlangius/Melanogrammus**	631,654	35,885
Gadidae	*Micromesistius poutassou*		518	0
Gadidae	*Pollachius pollachius*		22,327	2
Gadidae	*Pollachius virens*		72	0
Gadidae	*Raniceps raninus*		979	0
Gadidae	*Trisopterus esmarkii*		83	0
Gadidae	*Trisopterus luscus*		382,281	152
Gadidae	*Trisopterus minutus*		1,409,597	45,427
Lotidae	*Ciliata mustela*		376	0
Lotidae	*Ciliata septentrionalis*		23,853	0
Lotidae	*Enchelyopus cimbrius*		268	4
Lotidae	*Molva molva*		10,462	2
Merlucciidae	*Merluccius merluccius*		770	6
Gasterosteidae	*Gasterosteus aculeatus*		2	0
Gobiesocidae	*Diplecogaster bimaculata*		2,014	1
Lophiidae	*Lophius budegassa*		4,450	0
Lophiidae	*Lophius piscatorius*		39,061	31
Mugilidae	*Chelon auratus*		1,602	0
Mugilidae	*Chelon labrosus*	*Chelon labrosus/ramada**	2,727	0
Ammodytidae	*Ammodytes marinus*	Ammodytidae*	2,310	0
Ammodytidae	*Ammodytes tobianus*	Ammodytidae*	138,619	0
Ammodytidae	*Hyperoplus lanceolatus*	Ammodytidae*	0	2
Blenniidae	*Blennius ocellaris*		704	33
Blenniidae	*Lipophrys pholis*		503	0
Blenniidae	*Parablennius gattorugine*		731	0
Callionymidae	*Callionymus lyra*		20,676	3,698
Callionymidae	*Callionymus maculatus*		5,641	231
Caproidae	*Capros aper*		0	44
Carangidae	*Trachurus trachurus*		140,328	5,044
Cepolidae	*Cepola macrophthalma*		73,381	534
Gobiidae	*Aphia minuta*	*Aphia/Crystallogobius*†	4,353	24
Gobiidae	*Crystallogobius linearis*	*Aphia/Crystallogobius*†	76,700	0
Gobiidae	*Gobius cobitis*	*Gobius*†	0	3
Gobiidae	*Gobius niger*	*Gobius*†	2,583	0
Gobiidae	*Gobius paganellus*	*Gobius*†	126	0
Gobiidae	*Gobiusculus flavescens*		1	0
Gobiidae	*Pomatoschistus microps*	*Pomatoschistus*†	837	0
Gobiidae	*Pomatoschistus minutus*	*Pomatoschistus*†	24,275	11
Gobiidae	*Pomatoschistus pictus*	*Pomatoschistus*†	423	0
Labridae	*Centrolabrus exoletus*		382	1
Labridae	*Ctenolabrus rupestris*		9,961	0
Labridae	*Labrus bergylta*		8,426	0
Labridae	*Labrus mixtus*		8,101	0
Labridae	*Symphodus melops*		1,412	0
Moronidae	*Dicentrarchus labrax*		17,359	0
Mullidae	*Mullus surmuletus*		139,673	95
Scombridae	*Scomber scombrus*		188,730	182
Scombridae	*Thunnus thynnus*		852	0
Sparidae	*Spondyliosoma cantharus*		85	1
Stichaeidae	*Chirolophis ascanii*		637	0
Trachinidae	*Echiichthys vipera*		1,714	0
Bothidae	*Arnoglossus laterna*		155,940	1,791
Pleuronectidae	*Glyptocephalus cynoglossus*		208	0
Pleuronectidae	*Hippoglossoides platessoides*		845	0
Pleuronectidae	*Limanda limanda*		152,373	41
Pleuronectidae	*Microstomus kitt*		95,864	77
Pleuronectidae	*Platichthys flesus*		93,687	0
Pleuronectidae	*Pleuronectes platessa*		299,641	97
Scophthalmidae	*Lepidorhombus whiffiagonis*		49	0
Scophthalmidae	*Phrynorhombus norvegicus*		2,119	116
Scophthalmidae	*Scophthalmus maximus*		201,003	2
Scophthalmidae	*Scophthalmus rhombus*		62,668	2
Scophthalmidae	*Zeugopterus punctatus*		3,761	0
Soleidae	*Buglossidium luteum*		41,142	891
Soleidae	*Microchirus variegatus*		92,396	864
Soleidae	*Pegusa lascaris*		890	1
Soleidae	*Solea solea*		189,264	14
Agonidae	*Agonus cataphractus*		192	3
Cottidae	*Micrenophrys lilljeborgii*		557	0
Cottidae	*Taurulus bubalis*		426	0
Liparidae	*Liparis montagui*		59	0
Triglidae	*Chelidonichthys cuculus*	Triglidae*	0	891
Triglidae	*Chelidonichthys lastoviza*	Triglidae*	410,382	15
Triglidae	*Chelidonichthys lucerna*	Triglidae*	0	4
Triglidae	*Eutrigla gurnardus*	Triglidae*	0	494
Syngnathidae	*Nerophis lumbriciformis*		186	0
Syngnathidae	*Syngnathus acus*		920	9
Zeidae	*Zeus faber*		394,102	249
Scyliorhinidae	*Scyliorhinus canicula*		4,106	296
Scyliorhinidae	*Scyliorhinus stellaris*		62	4
Triakidae	*Galeorhinus galeus*		201	0
Triakidae	*Mustelus asterias*		4,074	0
Lamnidae	*Lamna nasus*		4	0
Rajidae	*Raja brachyura*		11,037	0
Rajidae	*Raja clavata*		5,658	0
Rajidae	*Raja microocellata*		910	0
Rajidae	*Raja montagui*		12,137	1
Rajidae	*Raja undulata*		48	0
Squalidae	*Squalus acanthias*		2,819	0

Supporting Table S2: Bioinformatic steps. Number reads remaining at each bioinformatic step for each library. Taxonomy assigned reads are reads assigned to species level using the curated British Isles fishes reference library (https://doi.org/10.5281/zenodo.5235648), and after exclusion of contaminant reads. Libraries 1 and 2 additionally include reads from other projects. Total study reads $n = 8,633,038$.

Filtering step	Library1	Library2	Library3	Library4
Total passing filter	11,870,442	8,452,438	5,121,196	5,521,444
Detect primers	9,117,734	6,267,911	4,164,044	4,379,211
Demultiplex	6,208,992	4,191,401	3,162,978	3,540,113
Trim primers	6,184,538	4,183,246	3,048,387	3,501,779
Quality filter	6,148,772	4,157,127	3,046,156	3,492,755
Merge	5,172,732	3,920,681	2,980,238	3,064,476
Remove chimaeras	5,039,142	3,808,741	2,928,314	3,038,129
Homology search	4,962,411	3,776,154	2,881,205	2,983,483
Taxonomy assigned	3,137,378	2,743,855	2,076,159	2,481,765

Supporting Table S3: Model output for the zero-inflated negative-binomial generalised-linear mixed-model fitted in the *glmmTMB* v1.1.2.3 package for R. Simplified model formula: reads ~ offset(sampleTotalReads) + trawlCPUE + PCRefficiency + reproductionMonth + lifestyle + (1|event/location/sample) + (1|order/family/species).

Effect	Component	Grouping	Term	Estimate	Std. error	Statistic	*p* value
fixed	cond		(Intercept)	-5.54	0.29	-19.40	7.96e-84
fixed	cond		scale(individualsByGroupRate)	0.13	0.04	3.33	8.54e-04
fixed	cond		scale(maxEfficiency)	0.77	0.18	4.27	1.93e-05
fixed	cond		spawningByMonthUKTRUE	0.85	0.13	6.66	2.83e-11
fixed	cond		lifestylebenthopelagic	0.51	0.42	1.20	2.29e-01
fixed	cond		lifestylepelagic	1.37	0.57	2.41	1.60e-02
fixed	zi		(Intercept)	1.12	0.57	1.98	4.80e-02
fixed	zi		scale(individualsByGroupRate)	-4.56	1.89	-2.41	1.60e-02
fixed	zi		scale(maxEfficiency)	0.15	0.34	0.45	6.52e-01
fixed	zi		spawningByMonthUKTRUE	-0.20	0.12	-1.65	9.91e-02
fixed	zi		lifestylebenthopelagic	-0.05	0.72	-0.06	9.49e-01
fixed	zi		lifestylepelagic	-1.27	0.96	-1.31	1.89e-01
ranpars	cond	sampleHash:localitySite:temporalGroup	sd(Intercept)	0.00			
ranpars	cond	localitySite:temporalGroup	sd(Intercept)	0.17			
ranpars	cond	temporalGroup	sd(Intercept)	0.37			
ranpars	cond	species:family:order	sd(Intercept)	1.31			
ranpars	cond	family:order	sd(Intercept)	0.00			
ranpars	cond	order	sd(Intercept)	0.00			
ranpars	zi	sampleHash:localitySite:temporalGroup	sd(Intercept)	0.25			
ranpars	zi	localitySite:temporalGroup	sd(Intercept)	0.30			
ranpars	zi	temporalGroup	sd(Intercept)	0.89			
ranpars	zi	species:family:order	sd(Intercept)	1.53			
ranpars	zi	family:order	sd(Intercept)	1.44			
ranpars	zi	order	sd(Intercept)	0.00			

Supporting Table S4: Average number reads by library after bioinformatic processing and taxonomic assignment, including mean and standard deviation (sd). Samples total $n = 200$; reads $n = 8,633,038$.

Library	Samples (n)	Reads (mean)	Reads (sd)
lib1	36	45,783	30,450
lib2	53	46,019	24,579
lib3	72	28,743	23,628
lib4	39	63,579	80,702

Supporting Table S5: Summary of control samples by library ($n = 93$).

Library	Control type	Sample ID	Number reads
lib1	Extraction blank	aa00a01558fc	115
lib1	Extraction blank	fc7e08b091ba	43
lib1	Field blank	0047ab44449d	62
lib1	Field blank	fb41386052ce	0
lib1	PCR blank	e41eef009613	83
lib2	Extraction blank	2723335dd699	75
lib2	Extraction blank	1d6a9604b69a	35
lib2	Extraction blank	18e13d408025	1
lib2	Extraction blank	e56ed77c409c	0
lib2	Field blank	60574c8b9eba	7,076
lib2	PCR blank	a802ceaed424	23
lib2	Tag blank	fd9979c924ea	44
lib2	Tag blank	837dffd2089f	31
lib2	Tag blank	ee8208a32209	26
lib2	Tag blank	6e39d4ff8cc0	22
lib2	Tag blank	e421fcbbf3d9	19
lib2	Tag blank	66dcca9289f0	7
lib2	Tag blank	98be275d78bd	7
lib2	Tag blank	273b42d31b7d	5
lib2	Tag blank	c92254f55b76	3
lib2	Tag blank	6385960f439b	2
lib3	Extraction blank	ab51842343ee	0
lib3	Extraction blank	ad48ae2c8844	0
lib3	Field blank	5b418dd9a365	6,695
lib3	Field blank	6ef9609590d0	0
lib3	PCR blank	45e5b060e9a6	0
lib3	PCR blank	5c16cb6d6673	0
lib3	PCR blank	aae911df243d	0
lib3	PCR blank	fa599638a519	0
lib3	Tag blank	0befac7a352d	0
lib3	Tag blank	2958706ff8f2	0
lib3	Tag blank	2d27a3ee1f02	0
lib3	Tag blank	3de083adb338	0
lib3	Tag blank	5907f3ea2903	0
lib3	Tag blank	5dad9ae4982b	0
lib3	Tag blank	785f96048ec4	0
lib3	Tag blank	855cbc8af0d0	0
lib3	Tag blank	8f2cf8de203f	0
lib3	Tag blank	a1875aaaf2da	0
lib3	Tag blank	a1face388953	0
lib3	Tag blank	b999389d8ce7	0
lib3	Tag blank	bc171adbdb4a	0
lib3	Tag blank	be6d9f88dc9f	0
lib3	Tag blank	defe144e2282	0
lib3	Tag blank	f5c0f9c31109	0
lib4	Extraction blank	6c91ad5ee0a6	0
lib4	Extraction blank	8d7d1a314a0d	0
lib4	Field blank	a448daac3e5e	1,105
lib4	Field blank	c420024be825	1,096
lib4	Tag blank	01a25be35e22	0
lib4	Tag blank	0cc22a1a1497	0
lib4	Tag blank	140141bf6315	0
lib4	Tag blank	15f304f0eb64	0
lib4	Tag blank	1f5538f9345c	0
lib4	Tag blank	2d0519ce967f	0
lib4	Tag blank	2ebbe5f3151e	0
lib4	Tag blank	3c2964eefd5c	0
lib4	Tag blank	4226a7249142	0
lib4	Tag blank	442363d7f0ea	0
lib4	Tag blank	4486f8f53fab	0
lib4	Tag blank	4a3198fc1bf4	0
lib4	Tag blank	4a99e71fc03b	0
lib4	Tag blank	4e617b435cd9	0
lib4	Tag blank	5005696b37f8	0
lib4	Tag blank	64a858406351	0
lib4	Tag blank	69c12c4a2362	0
lib4	Tag blank	74ae607effb5	0
lib4	Tag blank	81ac963d71f3	0
lib4	Tag blank	88ec6aebca28	0
lib4	Tag blank	8a18852f4533	0
lib4	Tag blank	92b29f0ec50c	0
lib4	Tag blank	9304480254b6	0
lib4	Tag blank	950236cfaa59	0
lib4	Tag blank	9b9ca0e27633	0
lib4	Tag blank	9ca2fc590464	0
lib4	Tag blank	aebd388759f1	0
lib4	Tag blank	b0cec63878a3	0
lib4	Tag blank	b72a233792db	0
lib4	Tag blank	ba4c134f870f	0
lib4	Tag blank	bed1533422e4	0
lib4	Tag blank	c64b68fa8a1b	0
lib4	Tag blank	c8bf098fc98b	0
lib4	Tag blank	cb4acdc15c90	0
lib4	Tag blank	cce48ca850ac	0
lib4	Tag blank	cceb0b3241d2	0
lib4	Tag blank	d1f5b2446f09	0
lib4	Tag blank	d62693c7cfb0	0
lib4	Tag blank	dc22e1b82e37	0
lib4	Tag blank	e3edbae02aff	0
lib4	Tag blank	e422478bcee9	0
lib4	Tag blank	f136beeadf8b	0
lib4	Tag blank	f1d036b2dc7c	0
lib4	Tag blank	fb00d702c29b	0