eDNA summary report

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Cronfa Treftadaeth Heritage Fund

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Executive Summary

Environmental DNA (eDNA) methodologies allow us to assess biodiversity by taking samples from the environment (e.g water, sediment or air) and extracting DNA from the samples to identify the presence of species. During Project SIARC, we designed an eDNA survey to gather information on elasmobranch (shark, skate and ray) presence in the Carmarthen Bay and Estuaries (CBAE) Special Area of Conservation (SAC). Sampling sites were informed using hydrodynamic and tracer modelling based on residual patterns, led by Bangor University. Water samples were taken from 10 sites across CBAE SAC each month, for 12 months.

These data were brought together with samples taken in 2020/2021 by Angel Shark Project: Wales, which covered 10 sites across 12 months in the Pen Llyn A'r Sarnau (PLAS) SAC, to provide a vital assessment of the presence and diversity of elasmobranchs off the Welsh coast. A total of eleven elasmobranch species were detected across the two SACs. Tope (*Galeorhinus galeus*) was the only Project SIARC focal species detected in samples from both SACs. The species was detected across three sites in the PLAS SAC, in the months of February, March, July and November; and at just one site in the CBAE SAC, in June. The angelshark (*Squatina squatina*) was only detected in the PLAS SAC in March and May, at two different sites and common stingray (*Dasyatis pastinaca*) was detected in the PLAS SAC at one site in July. Spurdog (*Squalus acanthias*), was not detected in the eDNA samples from either SAC but was present in the BRUVS eDNA samples.

The other elasmobranch species detected were smallspotted catshark (*Scyliorhinus canicula*), nursehound (*Scyliorhinus stellaris*), starry smoothhound (*Mustelus asterias*), thornback ray (*Raja clavata*), blonde ray (*Raja brachyura*), small-eyed ray (*Raja microocellata*), spotted ray (*Raja montagui*) and undulate ray (*Raja undulata*).

In CBAE SAC, elasmobranch species were detected most often in April, May and June and there were no elasmobranch detections in October. In the PLAS SAC, elasmobranch species were detected most often in March and there were no elasmobranch detections in September and October.

Key Findings

- 240 monthly water samples analysed across PLAS SAC and CBAE SAC with a further 36 samples analysed taken concurrently with BRUVS
- Eleven elasmobranch species were detected, including all four Project SIARC focal species
- Angelshark was detected at one site in the PLAS SAC in March and at an estuarine site in May
- Tope was detected in both SACs and was the highest detected species in the BRUVS samples
- Common stingray was detected at one site in the PLAS SAC in July
- Spurdog was detected in the BRUVS samples
- No significant seasonal signals for any species but detection of smallspotted catshark was significantly impacted by water temperature

Crynodeb Gweithredol

Mae methodolegau DNA amgylcheddol (eDNA) yn ein galluogi i asesu bioamrywiaeth trwy gymryd samplau o'r amgylchedd (e.e. dŵr, gwaddod neu aer) a thynnu DNA o'r samplau i nodi presenoldeb rhywogaethau. Yn ystod Prosiect SIARC, gwnaethom gynllunio arolwg eDNA i gasglu gwybodaeth am bresenoldeb elasmobranciaid (morgwn a morgathod) yn Ardal Cadwraeth Arbennig Bae ac Aberoedd Caerfyrddin. Llywiwyd lleoliadau samplu gan ddefnyddio modelu hydrodynamig ac olrhain yn seiliedig ar batrymau gweddilliol, dan arweiniad Prifysgol Bangor. Cymerwyd samplau dŵr o ddeg safle ar draws Ardal Cadwraeth Arbennig Bae ac Aberoedd Caerfyrddin bob mis am 12 mis.

Daethpwyd â'r data hyn ynghyd â samplau a gymerwyd yn 2020/2021 gan Brosiect Maelgwn: Cymru, a oedd yn cwmpasu deg safle dros 12 mis yn Ardal Cadwraeth Arbennig Pen Llŷn a'r Sarnau, a hynny i ddarparu asesiad hanfodol o bresenoldeb ac amrywiaeth elasmobranciaid oddi ar arfordir Cymru. Canfuwyd cyfanswm o un-ar-ddeg rhywogaeth o elasmobranciaid ar draws y ddwy ACA. Y ci glas (*Galeorhinus galeus*) oedd yr unig rywogaeth ffocal SIARC a ganfuwyd yn y ddau leoliad: yn ystod misoedd Chwefror, Mawrth, Gorffennaf a Thachwedd mewn tri lleoliad yn Ardal Cadwraeth Arbennig Pen Llŷn a'r Sarnau ac yn ystod mis Mehefin mewn un lleoliad yn Ardal Cadwraeth Arbennig Bae ac Aberoedd Caerfyrddin. Canfuwyd y maelgi (*Squatina squatina*) yn Ardal Cadwraeth Arbennig Pen Llŷn a'r Sarnau yn unig, ym mis Mawrth a mis Mai mewn dau leoliad, a chanfuwyd y forgath ddu (*Dasyatis pastinaca*) yn Ardal Cadwraeth Arbennig Bae ac Aberoedd Caerfyrddin mewn un lleoliad ym mis Gorffennaf. Ni chafodd y ci pigog (*Squalus acanthias*) ei ganfod yn y samplau eDNA o'r naill Ardal Cadwraeth Arbennig na'r llall, ond roedd yn bresennol yn y samplau BRUVS.

Y rhywogaethau elasmobranciaid eraill a ganfuwyd oedd y morgi lleiaf (*Scyliorhinus canicula*), y morgi brych (*Scyliorhinus stellaris*), y morgi llyfn (*Mustelus asterias*), y forgath stŷds (*Raja clavata*), y forgath felen gwta (*Raja brachyura*), y forgath lygaid-bach (*Raja microocellata*), y forgath fannog (*Raja montagui*) a'r forgath donnog (*Raja undulata*).

Yn Ardal Cadwraeth Arbennig Bae ac Aberoedd Caerfyrddin, canfuwyd rhywogaethau elasmobranciaid amlaf ym mis Ebrill, Mai a Mehefin ac ni chafwyd unrhyw elasmobranciaid ym mis Hydref. Yn Ardal Cadwraeth Arbennig Pen Llŷn a'r Sarnau, canfuwyd rhywogaethau elasmobranciaid amlaf ym mis Mawrth ac ni chafwyd dim elasmobranciaid ym mis Medi a mis Hydref.

Mae'r data hyn wedi darparu gwybodaeth hanfodol am bresenoldeb rhywogaethau elasmobranciaid a thymorolrwydd o fewn y ddwy Ardal Cadwraeth Arbennig, a byddant yn cael eu defnyddio i ysgrifennu llawysgrif wyddonol i'w chyhoeddi ac i ddeall yn well fuddion ecosystem ehangach y nodweddion cynefin gwarchodedig.

Canfyddiadau Allweddol

- Dadansoddwyd 240 o samplau dŵr misol ar draws ACA PLAS ac ACA CBAE gyda 36 sampl arall wedi'u cymryd ar yr un pryd â BRUVS
- Canfuwyd un ar ddeg o rywogaethau elasmobranciaid, gan gynnwys pob un o'r pedair rhywogaeth ganolog i Brosiect SIARC.
- Canfuwyd maelgwn ar un safle yn ACA PLAS ym mis Mawrth ac ar safle aberol ym mis Mai
- Canfuwyd y ci glas yn y ddwy ACA a hon oedd y rhywogaeth a ganfuwyd fwyaf yn samplau'r BRUVS
- Cafodd y forgath ddu ei chanfod ar un safle yn ACA PLAS ym mis Gorffennaf
- Canfuwyd y ci pigog yn samplau'r BRUVS
- Dim arwyddion tymhorol sylweddol ar gyfer unrhyw rywogaeth ond effeithiodd tymheredd y dŵr yn sylweddol ar y gwaith o ganfod y morgi lleiaf

Introduction

Environmental DNA (eDNA) methods are increasingly used for biodiversity monitoring in research and wider industrial applications (Bruce *et al*., 2021). When organisms move through the ocean, they naturally shed mucus, blood, dead skin cells or faeces which contain trace amounts of genetic material. This genetic material, no longer associated with the animal from which it originated, is known as eDNA. Methods are best established in aquatic systems where eDNA can be filtered out of water samples and then extracted from the filter material (Rees *et al*., 2014). eDNA methods have been shown to complement more conventional survey methods (Bylemans *et al*., 2016) and can outperform them in certain cases (Fediajevaite *et al*., 2021).

Elasmobranchs are one of the most highly threatened taxonomic families (Stein *et al*., 2018) with over one-third of species thought to be threatened with extinction (Dulvy *et al*., 2021). There are 27 elasmobranch species present in coastal waters of Wales, including rare species such as the angelshark (*Squatina squatina*) and tope (*Galeorhinus galeus*), which are listed as Critically Endangered on the IUCN Red List of Threatened Species. Elasmobranchs in the UK are commonly caught in mixed-species fisheries and represent a large proportion of discards at-sea (Silva & Ellis, 2019). The majority of data on populations of British elasmobranch species comes from scientific trawl surveys and historical fishing records (Hiddink *et al*., 2019), with evidence showing that many populations in UK waters have suffered declines (Ellis *et al*., 2005). Targeted fishing for several elasmobranch species is banned under UK law, with obligations to release those species unharmed if accidentally caught during fishing activities (see full list of species here). However, there remains a lack of data on the status of elasmobranch populations in the UK.

The waters around the UK represent a turbid temperate environment, where established non-invasive methods for monitoring elasmobranchs that are commonly used in tropical systems, such as Baited Remote Underwater Video Systems (BRUVS) (Juhel *et al*., 2018; MacNeil *et al*., 2020) and underwater visual census (UVC) (Graham *et al*., 2010), are limited by visibility and therefore not widely used. Recently, environmental DNA methods have been developed and applied to detect elasmobranch species (Boussarie *et al*., 2018; Simpfendorfer *et al*., 2016). Studies have used eDNA data to provide information on the presence of elasmobranch species in a given location (Gargan *et al*., 2017; Weltz *et al*., 2017), the seasonal occurrence of blacktip sharks (Carcharhinus Limbatus) in a bay in Florida (Postaire *et al*., 2020) and to reveal how elasmobranch communities change over a human population gradient in New Caledonia (Bakker *et al*., 2017). eDNA methods represent an opportunity to study the presence of these highly mobile and elusive species without the need to catch or even see them.

As eDNA is no longer associated with the animal it originated from, it can be transported away from where it was released into the environment, raising questions about the utility of eDNA methods for identifying fine-scale species distributions (Deiner & Altermatt, 2014). The notion that eDNA can be transported in aquatic systems has been widely discussed (Barnes *et al*., 2014; Sansom & Sassoubre, 2017). Recent studies have consolidated this by tracking the movement of eDNA along a transect (Ely *et al*., 2021) and determining the distance from the source at which the signal was no longer detected (Deiner & Altermatt, 2014; Murakami *et al*., 2019). Attempting to understand the transport of eDNA in the environment is a relatively new area of research but follows on from studies investigating the transport of larvae in the ocean (Coscia *et al*., 2020; Robins *et al*., 2013). These studies use particle tracking to predict the movement of particles in ocean currents and in the context of eDNA modelling, the methods involved have been applied to predict the origin of eDNA particles, and therefore the location of species, that have been detected in samples (Andruszkiewicz *et al*., 2019; Jenrette *et al*., 2023).

As eDNA is being advected from its origin, it is also being degraded (Harrison *et al*., 2019). The rate of degradation will determine the length of time the eDNA is detectable in a region and the distance that the eDNA will travel away from its origin. Understanding the degradation of eDNA is paramount to understanding eDNA data and unlocking the full potential of eDNA methods for biodiversity monitoring. Many studies have attempted to assess the decay of eDNA particles (e.g. Andruszkiewicz Allan *et al*., 2021; Jo *et al*., 2020). It is thought that eDNA degradation time is fairly consistent across species and that eDNA persists for approximately 48 hours in the marine environment (Collins *et al*., 2018).

In this project, we took water samples from two SACs off the coast of Wales over the course of two independent years, PLAS in 2020-21 and CBAE in 2022-23. Each month, water samples were taken from ten sites in the SAC and we used eDNA methods to investigate the spatial and temporal distribution of elasmobranch communities in the samples. Hydrodynamic models were developed for the SACs and were used to interpret the eDNA detection results, providing information on the potential movement of eDNA particles in the sea before they were detected in our sampling. We also took eDNA samples simultaneously with Project SIARC's BRUVS sampling in the PLAS SAC. These data will provide important information on the presence/absence of elasmobranch species off the coast of Wales, an area in which such information is currently lacking. The results can be used to assess how SAC-designated features are benefiting a wider community of fish and elasmobranch species and inform future management plans.

Methods

1. Study site

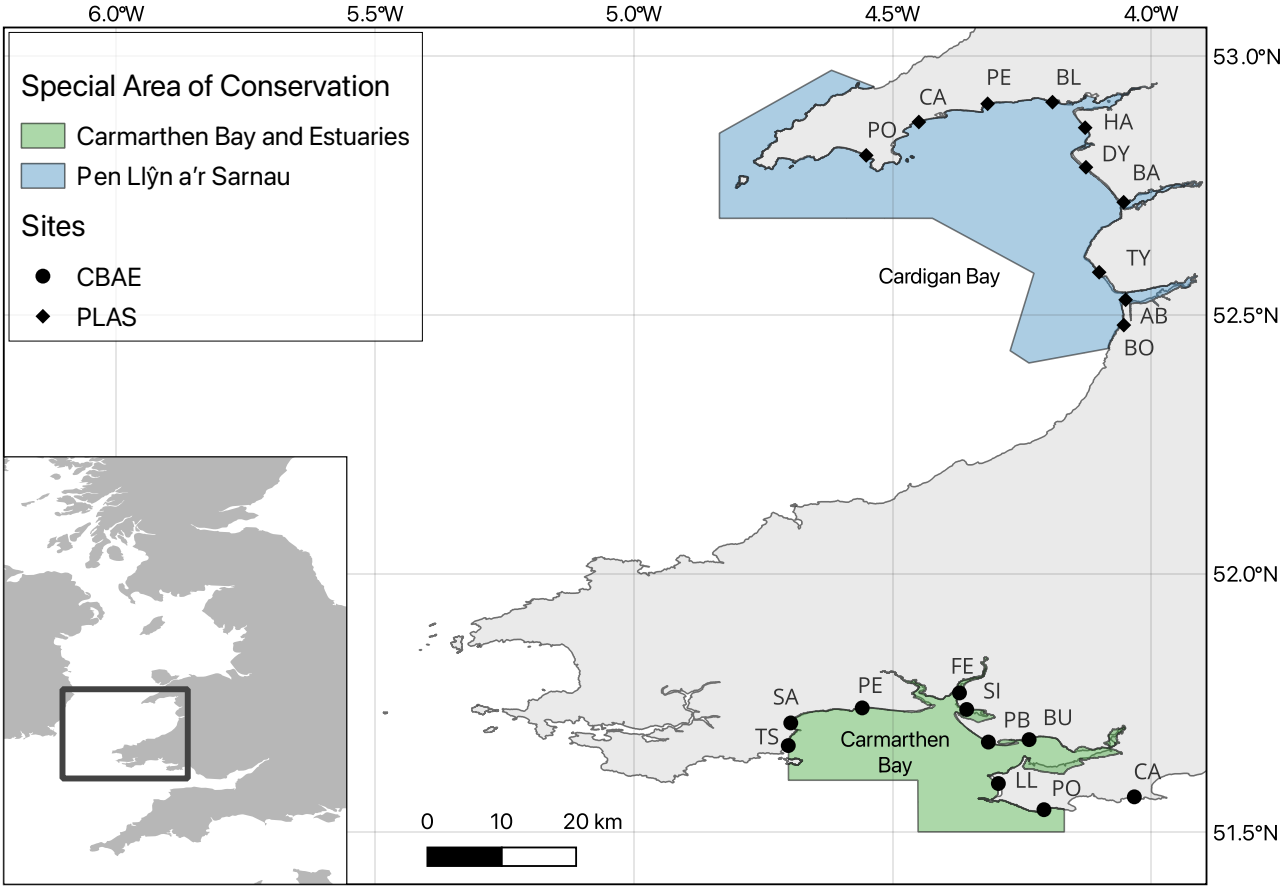
Water samples were taken from the PLAS SAC, Wales (Figure 1), at ten sites every month for a year starting in September 2020 and ending in August 2021, and from the CBAE SAC at ten sites (Figure 1) every month for a year starting in March 2022 and ending in February 2023. Due to time constraints and data availability, analysis for CBAE was run for the first ten months of sampling (March – December). Analysis of the January and February samples is ongoing.

2. Sample collection

The sampling took place over four days each month. After wading to waist-depth (approx. 80cm), collection bags were rinsed in the water and then 6 L of seawater was collected approximately 50 cm above the seabed (at waist height). Samples were stored on ice in a cool box whilst being transferred to the lab. Each sample was then filtered as three 2 L replicates through 0.45 µm Whatman filters using vacuum filtration as per the protocol described below. The filter papers were stored indivually in a preservation buffer and kept at room temperature before DNA extraction and analysis.

During BRUVS surveys, eDNA samples were taken at the BRUVS deployment sites prior to the BRUVS being deployed, to avoid an influx of DNA into the water sample bottles from the mackerel, which is used as bait for the BRUVS. During the BRUVS survey days, a total of three stations were sampled, with three independent replicates of 2 L of sea water collected. During the BRUVS surveys, which took place over a 6-8 hr period, the water samples remained on ice within cooler boxes. Once back at the lab, the samples were filtered as per the protocol described below and individual filters were stored in a preservation buffer and kept at room temperature before DNA extraction.

Figure 1: Map of the PLAS SAC (blue) and CBAE SAC (green) and their respective sampling sites.



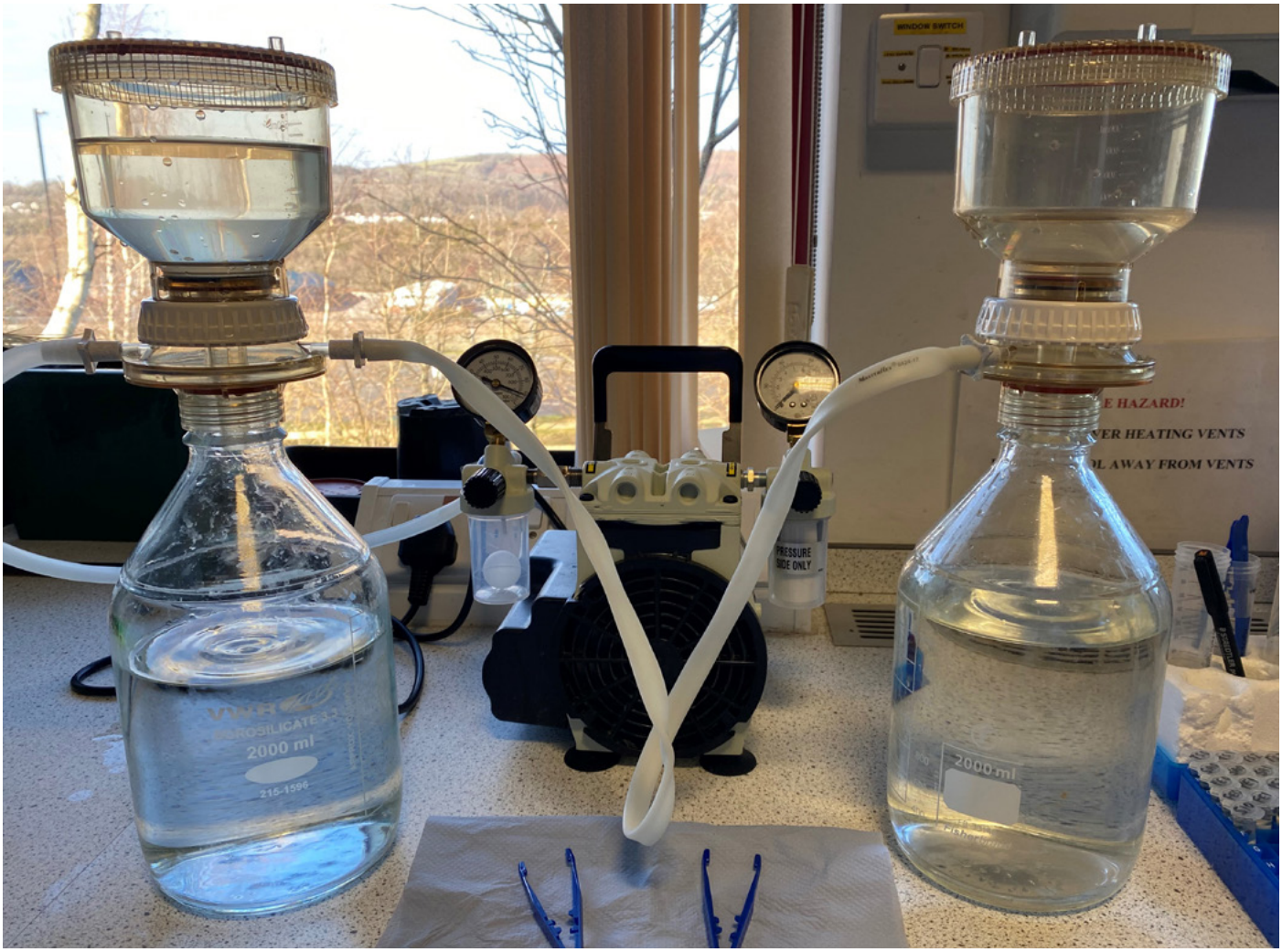
Methods

3. Filtration

Before starting the filtering each day and between filtering each different sample site filter funnels and tweezers were cleaned by soaking in a bucket of 20% bleach for 20 minutes followed by 10 minutes in distilled water. Gloves were worn throughout and changed between samples. A negative of 250 ml of distilled water was filtered through each filter cup before the sample from each site.

Sea water samples were filtered using a two-cup portable filtering station. Two filtering funnel cups allowed for two subsamples to be filtered simultaneously. The filtering funnels each attach to 2L glass bottles, which collect wastewater discharge. Both filter funnels attach to the vacuum pump via tubing (Figure 2). Filter membranes with 0.45 µm pore size (Whatman) were placed onto the filter cups with tweezers. Following this, the water was filtered in three 2 L subsamples. Where the samples were turbid and the filter was getting clogged, the subsample was filtered across two filters labelled A and B. Each filter membrane was folded using clean tweezers and placed inside a pre-labelled 2ml Eppendorf tube. This tube was then filled with preservation buffer (Longmire's lysis buffer) and stored at room temperature for transport to the DNA extraction lab.

Figure 2: Vacuum pump set up for eDNA filtering.



4. DNA extraction

DNA was extracted from each filter following the Spens *et al*. (2015) protocol for filters prepared with Longmire's buffer and using the Qiagen Blood & Tissue Kit (Qiagen). DNA was eluted from the spin columns with 100 µL PCR-grade water and this step was repeated to maximise yield and result in 200 µL of extracted DNA. At this stage, 100 µL from each triplicate was pooled for analysis and the remaining half of each replicate was archived for long-term storage at -20°C.

5. PCR amplification

Amplification of elasmobranch eDNA was done using the Elas02 primers (Taberlet *et al*., 2018) and following the MiFish protocol (Miya *et al*., 2015). The extracted DNA was amplified from the pooled samples in triplicate on a 96-well plate in 12.5 µL reactions consisting of the following; 6 µL KAPA HiFi Hotstart Master Mix (KAPA), 0.7 µL of each primer, 2 µL PCR-grade water and 3 µL of extracted DNA template. Reactions were run on a G-Storm GS1 thermal cycler with an initial denaturation step at 95°C for 2 minutes followed by 40 cycles of 95°C for 30s, 55°C for 15s and 72°C for 15s, and completed with 7 minutes at 72°C. Each plate included a non-template control (NTC) where PCR-grade water was used in place of the DNA template. The amplified products were visualised on a 1.5% agarose gel using GelRed stain (GelRed) and then cleaned using AMPure XP beads (Beckman Coulter), following the manufacturer's protocol and eluted with PCR-grade water.

A second stage PCR was then run to add Illumia index sequences to the PCR products, using the Nextera set A and set B index kits, each product was tagged with unique Illumina tags in the following reaction; 12 µL KAPA HiFi Master Mix, 2.5 µL index primer, 5 µL PCR-grade water and 3 µL PCR product. This was run on a thermal cycler with an initial denaturation at 95°C for 3 minutes followed by 8 cycles of 95°C for 30s, 55°C for 30s and 72°C for 30s, and completed with 5 minutes at 72°C. The products were visualised on a 1.5% agarose gel using GelRed stain and cleaned using AMPure XP beads, following the manufacture's protocol and eluted with PCR-grade water.

Libraries were initially pooled in equal concentrations of 1 µL each and these pooled libraries were run on an Illumina Miseq using a Nanoseq v2 reagent kit (2 x 150 bp) as a quality control run. This resulted in identifying the relative contribution of each individually indexed sample in the pooled library. For the data run, each individual library was pooled at volumes calculated from their relative contribution in the quality control run, to result in equal concentrations being loaded onto the MiSeq. Samples were sequenced using 300-cycle paired-end v2 reagents in 4-month sample batches, resulting in six runs with between 86 and 92 libraries per run.

6. Bioinformatics and data analysis

Sequences were obtained as demultiplexed fastq files from the Illumina MiSeq Reporter software. Demultiplexed sequences were then trimmed, denoised and merged using a DADA2 pipeline in R to produce amplicon sequence variants (ASVs) (Callahan *et al*., 2016). Forward and reverse reads were truncated to 125bp to allow adequate overlap for merging and merged sequences between 150-190bp were retained for analysis. Sequence variants were curated with LULU, the match rate was set at 97 and the minimum relative co-occurrence was set to 0.95. Taxonomy was assigned using Murali *et al* (2018) and a curated classifier that was produced from the metafish (Collins *et al*., 2021) library with the assignment confidence threshold set at 40% (moderate). Data were analysed in R using the "phyloseq" package (McMurdie & Holmes, 2013). After the creation of a phyloseq object, sequences that were more frequent in field negatives than positive samples were identified as contaminants and removed using decontam package in R (Davis *et al*., 2018).

All non-elasmobranch reads were removed from the dataset and only samples containing elasmobranch reads were retained. To limit the impact of sequencing and assignment error, singletons (reads of only 1 for a species in a sample) were removed. Any sequences not assigned to a species and those species thought to be contaminants from alternative lab projects were removed from the dataset. Any remaining species present in the negative control for a given sample were also removed from that sample. Plots were made using "ggplot2" to visualise the detections of each species by site and month. The relationship between detection and water temperature was investigated for the three most abundant species in PLAS and CBAE samples respectively using binomial generalised linear models.

7. Hydrodynamic modelling:

The ocean model (TELEMAC Modelling System v8p2; [www.opentelemac.org]) was applied to simulate the hydrodynamics CBAE SAC to complement similar modelling previously undertaken for PLAS SAC. River flow data and wind estimates were also incorporated into the hydrodynamic model. Values of 15-minute river flow data were obtained from Natural Resources Wales for the following five rivers:

1. Loughor at Tir-Y-Dail (Station 4131, annual mean flow approximately 2 m3/s)
2. Tywi at Capel Dewi (Station 4139, annual mean flow approximately 40 m3/s)
3. Gwili at Glangwyli (Station 4199, annual mean flow approximately 5 m3/s)
4. Dewi Fawr at Glaslyn Ford (Station 4096, annual mean flow approximately 1 m3/s)
5. Taf at Clog Y Fran (Station 4089, annual mean flow approximately 7 m3/s)

The TELEMAC model was run for March 2022 – December 2022, following a 30-day model spin-up period, necessary for model stability. The model output was saved at instantaneous 5-minute temporal resolution, to be used to drive the particle tracking model.

8. Particle tracking modelling:

In this study, theoretical 'particles' were used to represent discrete 'packets' of eDNA via Lagrangian particle tracking. The currents output by the TELMAC-2D model were used to advect the particles within the domain. The simulated particles behaved passively, driven solely by advection from the simulated currents, i.e., no additional diffusive mixing was included. A criterion of minimum water depth of 0.1 m was set – where a particle was advected into water shallower than 0.1 m (or land), the particle returned to its location during the previous timestep. The effect of this was minimised by setting a short hydrodynamic model timestep (5 minutes).

Both particle 'backtracking' and 'forward tracking' were computed. Backtracking simulated potential source locations for eDNA arriving at a sample site at a given a time. Forward tracking simulated where eDNA released from a particular sample site at a particular time could be transported to. Particles were released from the 10 sample sites within CBAE. Each sample site, which had a radius of 200 m (area ~0.125 km^2), was populated by 1000 discrete release locations, with a regular spacing of ~14 m apart. Particles were released from each of the 1000 points at the exact time that each corresponding water sample was taken at that site. This particle release area of 0.125 km2 was designed to account for uncertainty in source/sink location due to the output resolution of the model. This particle release procedure was repeated for each of sample sites 1-10 and for each of the sampling times in March – December 2022. Thus, a total of 10,000 were released from each site over the 10 monthly sampling campaign (10×1,000). Each individual particle was allowed to propagate for 3 days from release, representing the estimated degradation time of eDNA in the system. The position of each individual particle was calculated at each 5-minute interval throughout its 3-day propagation time.

The total dispersal (backwards and forwards, averaged over the 10 months of particles released) was plotted to/from each sample site and presented as density distributions (or heatmaps). The heatmaps are generated by dividing the model domain into a regular 1000x1000 m grid. At each 5-minute timestep, the number of particles within each grid cell is counted and this number converted to a percent (by dividing by total number particles released × timesteps). Thus, the total sum of the distributions in the heatmap is 100% and the figures are useful to illustrate potential particle locations and range of transport, as well as identifying where particles tend to congregate (i.e., highest density in the heatmaps). For further information on the methods and results of this modelling please see here.

Results

eDNA samples

A total of 120 eDNA samples and 120 negative controls were taken and analysed during the CBAE surveys. A total of 1,436,193 sequences were produced. Eight elasmobranch species were identified, and one further amplicon sequence variant (ASV) was assigned to the genera *Raja* and not species due to sequence matches. The eight species detected were tope (*Galeorhinus galeus*), smallspotted catshark (*Scyliorhinus canicula*), nursehound (*Scyliorhinus stellaris*), starry smoothhound (*Mustelus asterias*), thornback ray (*Raja clavata*), blonde ray (*Raja brachyura*), small-eyed ray (*Raja microocellata*) and undulate ray (*Raja undulata*). Smallspotted catshark was detected most often, with 16 detections across seven sites, this was followed by starry smoothhound, which was detected eleven times across five sites (Figure 3 - Elasmobranch detections by month at CBAE). Elasmobranch species were detected most often in June and there were no detections in October (Figure 4 - All elasmobranch detections by month). Water temperature significantly impacted the presence of smallspotted catshark eDNA in water samples, with the probability of detection decreasing with increasing temperature (Figure 5, $z = -2.152$, $p < 0.05$). Water temperature did not significantly impact the detection of starry smoothound or small-eyed ray.

Figure 3: The number of detections in each sampling month for each species in the CBAE SAC samples. Sampling site is indicated by the colour of the block. Months have been plotted in seasonal order to enable comparison between SACs, January and February samples were taken in 2023 and all other samples were taken in 2022.

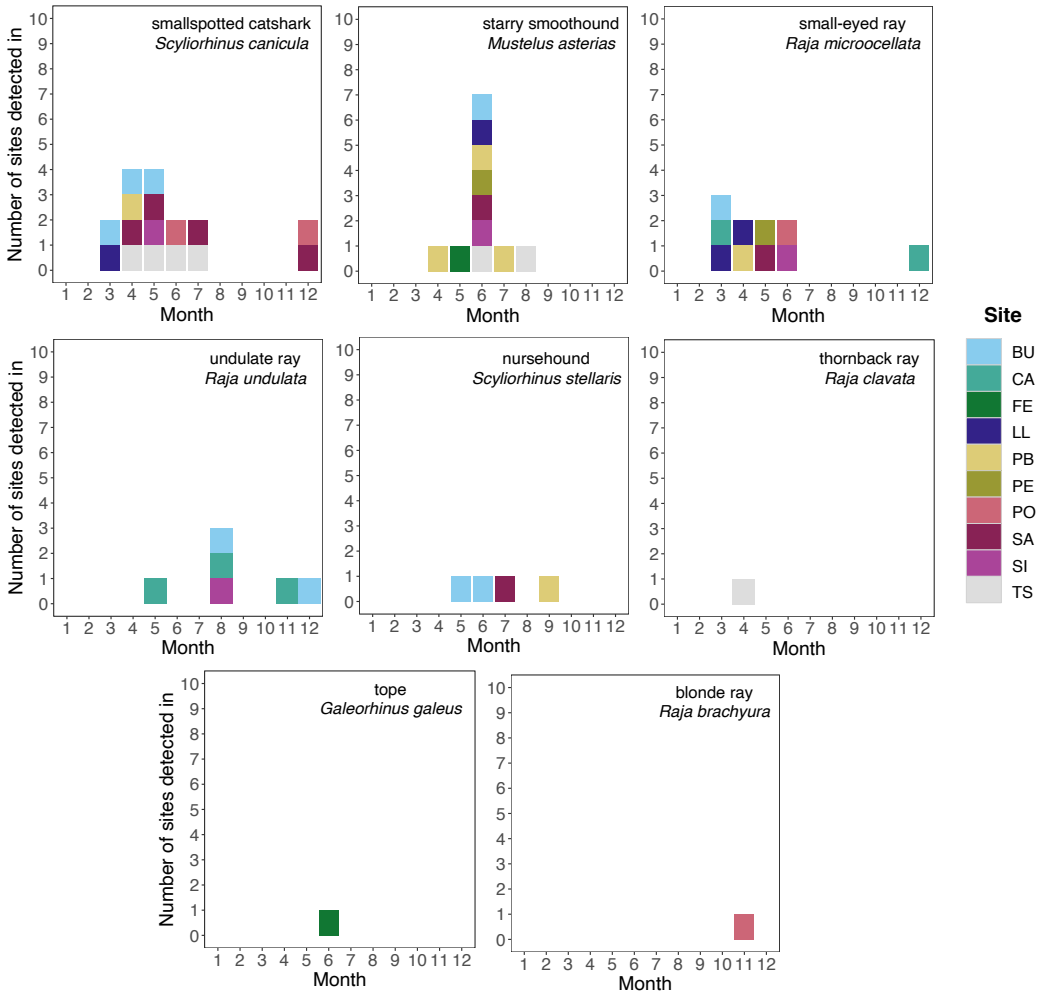


Figure 4: Detections for *Scyliorhinus canicula* was significantly impacted by water temperature at the time of sampling.

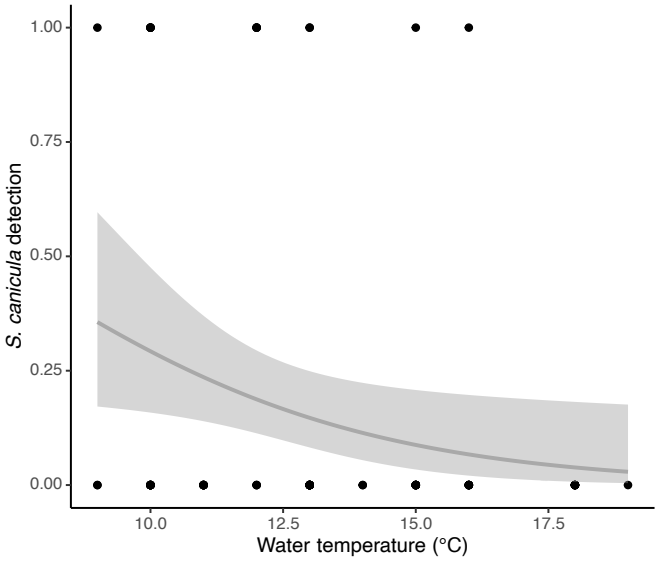
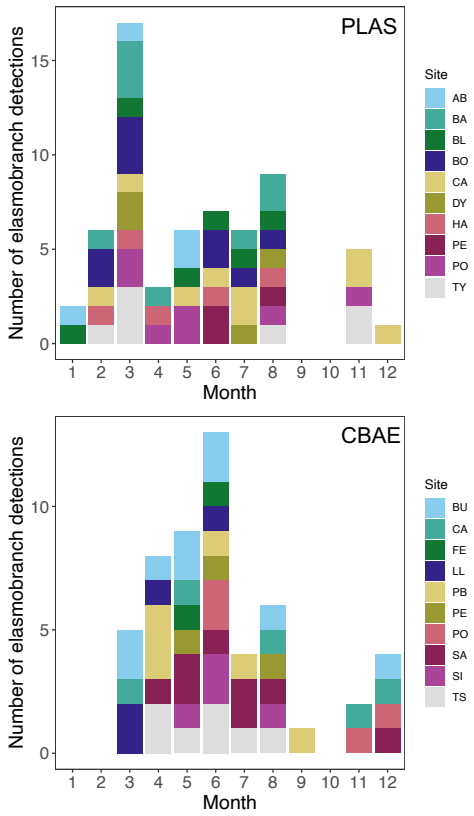


Figure 5: The number of detections of all elasmobranch species by months across the PLAS and CBAE SACs.



Analysis for the 2020/21 monthly surveys in PLAS revealed a total of tenelasmo branch species from 6,177,913 sequences. From 120 eDNA samples and 120 negative controls, three SIARC focal species were detected; the angelshark (*Squatina squatina*), stingray (*Dasyatis pastinaca*) and tope (*Galeorhinus galeus*). The other elasmobranch species detected were; smallspotted catshark, nursehound, thornback ray, blonde ray, starry smoothhound, spotted ray and small-eyed ray. The smallspotted catshark was detected most often, with 17 detections across eight sites and detection in all months except September, October, December and July. This was followed by nursehound with 13 detections across nine sites and detection across the year except for September, October and April (Figure 6 - Elasmobranch detections by month at PLAS). The most detections for elasmobranch taxa occurred in March and no elasmobranch species were detected in September and October (Figure 4 - All elasmobranch detections by month). Angelshark was detected in March at Tywyn and in May at Aberdyfi. There was no statistical significance between the number of detections of any of the three most abundant species and the water temperature at the time of sampling and no significant relationship between the total number of elasmobranch detections and water temperature.

A total of 36 eDNA samples were taken and analysed during the BRUVS surveys alongside 12 negative controls. A total of 784,978 reads were produced for analysis. Six elasmobranch species were detected with tope being detected in 26 out of the 36 samples and smallspotted catshark in 20 of the 36 samples. The SIARC focal species spurdog (*Squalus acanthias*) was also detected in three samples. Three out of the four species that were identified on the BRUVS videos were detected with eDNA, but spotted ray (*R. montagui*) was not detected in the eDNA samples (Figure 7 – Venn). However, there were three species in the eDNA samples that were not identified on the BRUVS videos; undulate ray, thornback ray and spurdog. In the July 2022 sampling in Aberdyfi, BRUVS identified smallspotted catshark, this species was detected in four out of nine eDNA replicates for the sites. When the same species was identified in Barmouth in June 2022, it was also present in nine out of nine eDNA samples. Tope was also identified in the BRUVS samples in Barmouth and was present in every one of the eDNA samples for this location. Contrastingly, nursehound was also seen in the BRUVS videos for that site but was not detected with eDNA.

Figure 6: The number of detections in each sampling month for each species in the PLAS SAC samples. Sampling site is indicated by the colour of the block. Months have been plotted in seasonal order to enable comparison between SACs, September to December samples were taken in 2020 and all other samples were taken in 2021.

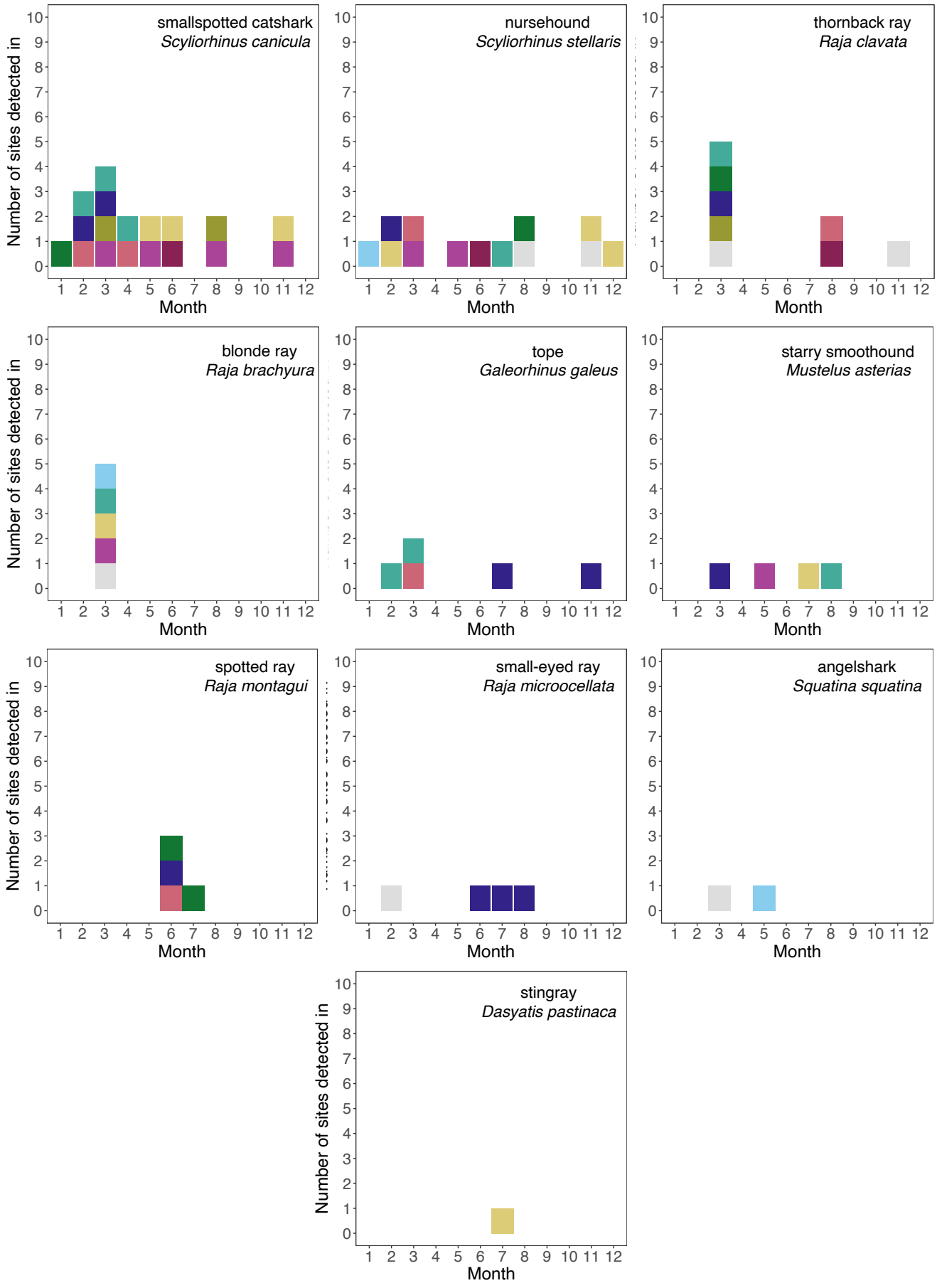


Figure 7: Venn diagram showing the overlap of species detected in each sampling region. Red text indicates species detected in the BRUVS samples that were also identification in the BRUVS videos taken at that location. The spotted ray was identified in the BRUVS video analysis but was not detected with eDNA.

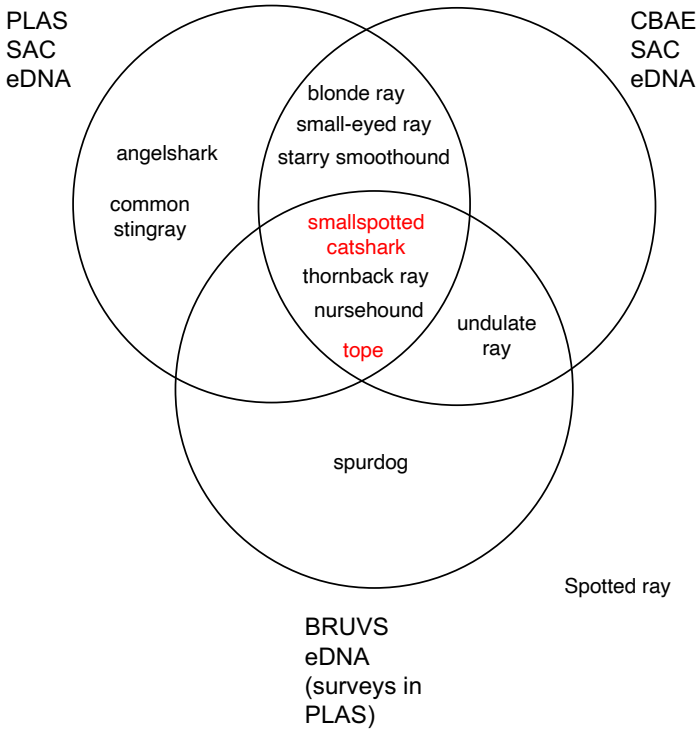
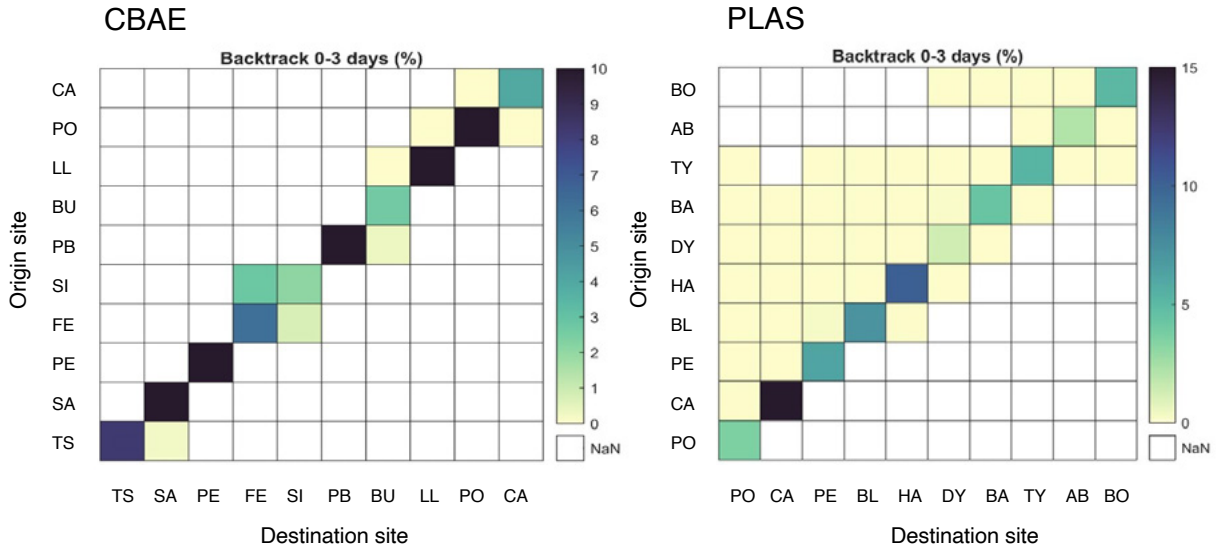


Figure 8: Matrices showing the connectivity of sites in the PLAS and CBAE SACs based on backwards particle tracking for 3 days. The matrix shows the averaged connectivity over the sampling period. The colour scales indicate the number of particles as a percentage of the total particles released from each of the 10 sites (zero connectivity = white, yellow indicates connectivity between the sites, albeit low). The darker colours on the diagonal indicate particles remaining within the same origin/destination (source/sink) site.



Hydrodynamic modelling

Particle tracking in the two SACs from the hydrodynamic models show that PLAS SAC is generally a more mixed environment than CBAE SAC, with more sites being connected to another (Figure 8). The hindcasting shows that for detections in CBAE, the origin of the eDNA is most likely to be from within the vicinity of the sampling site, with the exception of mixing between Ferryside and St. Ishmael. The detection of tope in the Ferryside site (site FE) may have travelled to the site from St. Ishmael (SI) or from further out into Carmarthen Bay (Figure 9). For detections in the PLAS SAC, detections are still most likely to have originated from within the vicinity of the sampling site but there is a greater chance that the eDNA had been advected to the site from a neighbouring site. Backtracking shows that the angelshark eDNA detected at Tywyn (site TY) in March originated from close to the site and close to shore (Figure 10).

Figure 9: Backtrack showing the potential origin of the detected tope eDNA in the CBAE SAC.

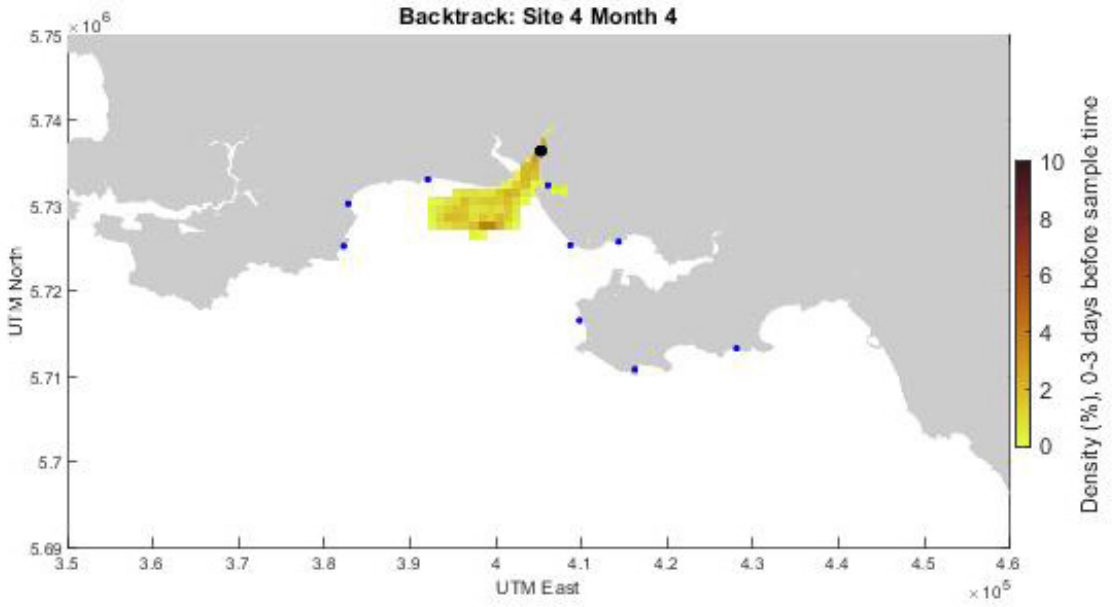
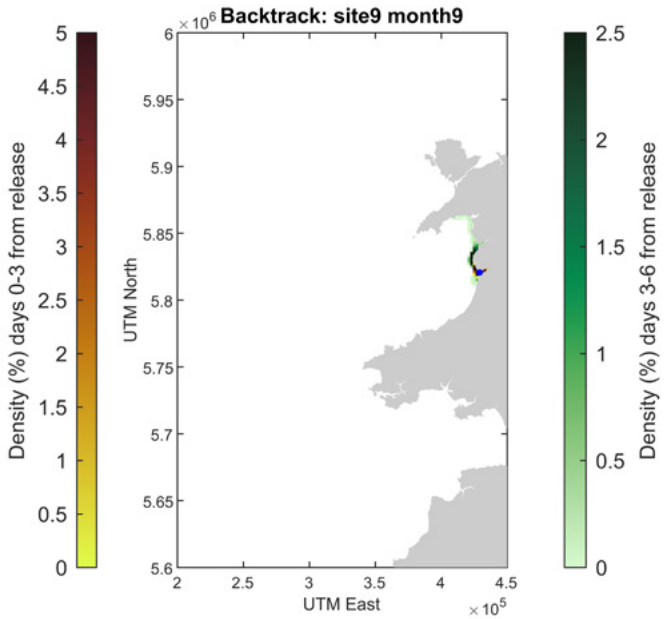


Figure 10: Backtrack showing the potential origin of the detected angelshark eDNA in the PLAS SAC in May.



Next steps

From a total of 276 samples in three sampling regions, we detected a total of nine elasmobranch species. There was at least one positive elasmobranch detection in 115 samples. In PLAS, the most detections occurred in March and in CBAE, June had the most detections. These results demonstrate that eDNA surveys can produce valuable data for monitoring elusive species in temperate water. By sampling over the course of a year, our results represent a comprehensive investigation into the presence of elasmobranch species in the two SACs. The addition of hydrodynamic modelling and subsequent particle tracking gives us a unique insight into the interpretation of our eDNA results.

Environmental DNA is known to be ephemeral in the marine ecosystem, with an estimated persistence time of around 48 hours (Collins *et al*. 2018). To account for this and allow for a conservative estimate to be made, we performed particle backtracking for three days prior to the sampling event. This modelling provides us with an estimate of where eDNA particles may have been released from and therefore the origin of the animal which we detected. Our modelling results showed that sites in CBAE were largely independent of one another and that eDNA sampled at a given site is most likely to have originated from around that site or from one of the neighbouring sites. The water in the PLAS SAC however, is significantly more mixed and eDNA detected at a given site may have originated from one of a number of nearby sites. However, as studies have shown that eDNA degrades at an exponential rate (Strickler *et al*. 2015), it is most likely that eDNA originated near the sampling site. A recent study found that eDNA from fish was detectable for just one hour and that nearly 80% of detections were within 30m of the origin of eDNA (Murakami *et al*. 2019), providing further evidence that eDNA provides a snapshot of the organisms present at the time of sampling.

The majority of species detected were shared across PLAS and CBAE samples. The smallspotted catshark and nursehound are known to be common in the regions and were detected in all regions. Thornback ray was also detected in all the regions despite documented decreases in population in the PLAS region (Whittamore and McCarthy, 2005). Detection of the undulate ray was shared across CBAE SAC and the BRUVS eDNA samples, this species is rarely recorded in the PLAS SAC (where the BRUVS samples were taken) and it was not recorded in the 2020-21 PLAS SAC eDNA samples. This detection may represent ongoing range expansion of the species as waters around the British coast warm but should be treated with caution as the eDNA may have been transported to the sampling site on the water currents.

The detection of angelshark in the PLAS SAC at the estuarine site Aberdyfi and the nearby site Tywyn suggests there may be a hotspot for detecting the species at this site. However, there were just two detections, highlighting its rarity in the region. Particle tracking suggests that the eDNA is likely to have originated near the sampling site. This area does represent a location with a high number of angelshark catch records both historically (Barker et al. 2022) and more recently (J. Davies pers. comms). Future surveys should focus on this region in the spring and summer to investigate whether this site represents an area of interest for the species. There was no detection of angelshark in CBAE SAC despite it being a historical hotspot for the species (Barker et al. 2022). If PLAS SAC remains as one of the last strongholds for the species in the Celtic Sea then it is important that focused research and fisher engagement continues, so that populations can recover and eventually reclaim its historical range.

There was a significant impact of water temperature on the detection of smallspotted catshark in CBAE SAC, with the probability of detection decreasing with increasing water temperature. Little is known about the ecology of this species off the Welsh coast and it may be possible that during warmer months the species moves into deeper, cooler water and is not present around the shoreline in such high abundances, repeated sampling and more in-depth ecological studies would be required to determine if this is a trend for the species.

These results have provided us with information on the spatial and temporal distribution of elasmobranch species in the PLAS and CBAE SACs. Environmental DNA methods are useful for determining the presence of species in a given region and we have achieved that. We must now use the information to target species in order to gain a more in-depth understanding of their ecology. These studies will allow us to ensure that elasmobranch species are adequately protected in the SACs so that populations can recover to historical levels and elasmobranchs can continue to provide essential ecosystem services off the Welsh coast.


Gill