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The Marine Invasive Non-Native Species *Didemnum vexillum*: Loch Creran Survey – September 2019

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Summary

The Loch Creran SAC was established to protect the Priority Marine Features (PMFs) such as marine biogenic reef consisting of the calcareous tube-worm *Serpula vermicularis*, and the horse mussel *Modiolus modiolus*. Loch Creran is also designated as a Marine Protected Area (MPA), conferred to protect the flame shell (*Limaria hians*) beds and other geodiversity features. Loch Creran contains the only example of well-developed serpulid reefs in the MPA network in Europe.

The invasive colonial sea squirt *Didemnum vexillum* (Dvex) was first recorded on an oyster farm in Loch Creran in 2016. Since 2016, Marine Scotland Science (MSS) has undertaken annual monitoring surveys to assess the distribution of Dvex at the oyster farm and in the wider Loch Creran area. In view of Dvex possible impact on the PMFs present in the loch, Scottish Natural Heritage (SNH) has carried out surveys of the subtidal environment, by establishing a diving programme to monitor spread. The surveys described in this report complement ongoing annual surveys of the impacted oyster farm and cover the wider Loch Creran area, incorporating intertidal sections of shore not previously surveyed.

All suspicious samples collected during the shore intertidal survey were tested and only one sample collected was confirmed as Dvex by real time PCR and sequencing. The subtidal survey did not reported any suspicious colonies.

1. Introduction

Detailed shore and boat-based surveys of Loch Creran were carried out by staff from SNH Marine Ecosystems and Sustainable Coasts and Seas teams on 2-4 September 2019. The key purpose of the surveys was to support MSS by surveying for the presence of Dvex to provide up to date information on the potential presence of Dvex in the wider Loch Creran area. Water samples were also taken at a selection of the survey sites for the purposes of environmental DNA (eDNA) analyses by MSS.

This report presents the results of the shore and boat surveys together with DNA analyses of suspicious colonies collected by SNH staff during the surveys.

2. Methods

Shore surveys

Shore surveys broadly followed the method outlined in the previous MSS report <https://data.marine.gov.scot/sites/default/files//SMFS%200910.pdf> and covered additional shoreline to that surveyed in 2016. The shore-based intertidal survey locations are shown in Figure 1. Each survey area was surveyed by a team of two to three surveyors between two hours before and two hours after low tide.

Boat surveys

A Rigid Inflatable Boat (RIB) survey of Loch Creran took place on 2-4 September 2019 to survey for the presence of Dvex on man-made intertidal and subtidal structures within the loch.

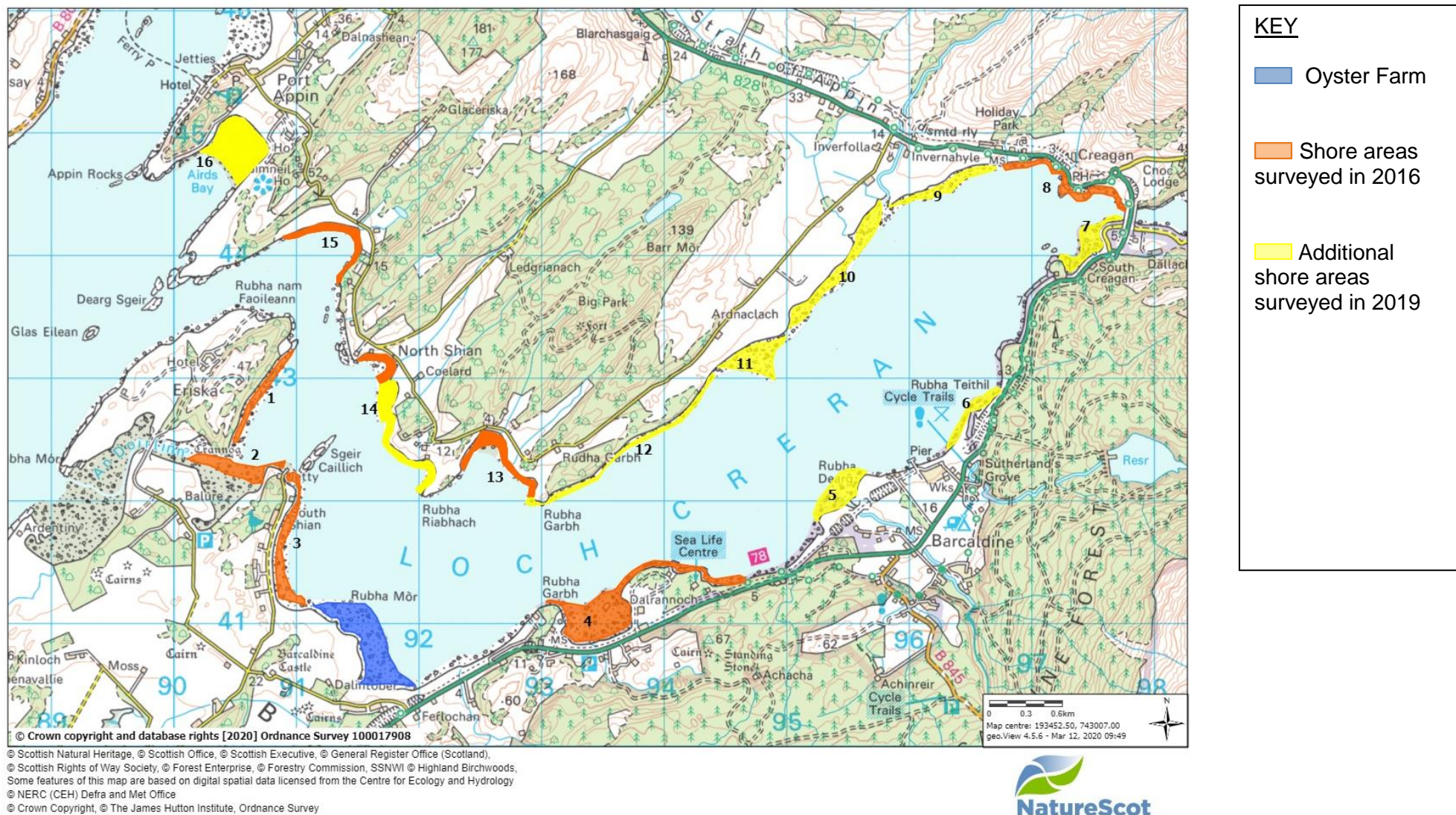
Boat surveys involved repeating the boat survey of 2016 in accordance with the survey methodology in the MSS report <https://data.marine.gov.scot/sites/default/files//SMFS%200911%20-%20update.pdf>.

A combination of visual inspection and a pole-cam was used in the survey. Pole-cam was recorded using a Go-Pro and reviewed in detail at a later date. A range of floating, natural and artificial hard structures were examined. Structures visited during the last (2016) boat survey were re-surveyed where these were still present.

Tissue and water sample collection

Tissue samples of suspicious organisms growing on hard substrates or seaweed were taken at various points around the loch (Table 1) with a view to rule out Dvex and attempt identification to species. Tissue samples were stored at -20°C prior to DNA extraction. Water samples (10 duplicates of 500 ml seawater per site) were collected using sterile bottles and filtered on site using 0.22µm filters (Sterivex). Filters were stored in -20 °C until further processing. The water sample locations are summarised in Table 2. Water samples were collected for eDNA analyses which will be carried out by MSS staff at later date (data not shown in this report).

Figure 1: Loch Creran shoreline surveyed during the 2019 intertidal survey. Areas highlighted in yellow and orange were surveyed in 2019.



Genetic characterization of suspicious tunicate colonies

A piece of tissue of approximately 100mg was excised from the frozen material using individual sterile scalpel. Genomic DNA was extracted using DNeasy blood and tissue kit (Qiagen) according to the manufacturer's protocol and stored at -20°C prior to further analysis.

In house-developed Dvex-species specific real time PCR was designed to target the partial cytochrome oxidase I (COI) gene (Matejusova et al. in prep). Approximately 30ng of genomic DNA was used in 20µl reaction containing 2x PerfeCTa qPCR Toughmix (QuantaBio, VWR), 900nM forward primer, 900nM reverse primer, 50nM probe and sterile water up to the final reaction volume.

Partial COI gene was amplified from all suspicious tissue samples using the Tun-for and Tun-rev2 primers (Stefaniak et al 2009) and cycling conditions previously described by Graham et al (2015). If no product was amplified using the Tun COI primers, additional set of COI gene primer (Folmer 1994) and two sets of 18S primers (Capra et al 2016; Zhan et al 2013), were utilized to obtain DNA fragment for sequencing. Amplified COI and 18S products were purified either using Illustra ExoProStar (VWR) or MinElute PCR purification kit (Qiagen). Approximately 30ng of amplified product was sequenced in both directions using same primers as in the amplification reaction (www.dnaseq.co.uk). Consensus sequences were generated in Sequencher software v. 4.9 (Gene Codes Corporation) and compared to publically available sequences in the NCBI database using the *blastn*.

3. Results

a) Shore surveys

Table 1 summarizes the metadata recorded for each tissue sample collected during the shore survey. Ten suspicious tissue samples were collected from the 16 shore survey locations shown in Figure 1. Photos were taken of some of the samples to assist with identification (see section *Photographs from shore surveys* below).

Table 1

Summary of shore survey tissue samples collected.

Date	time	Shore No.	Tissue sample (Y/N)	Location (lat long WGS84)	MSS tissue sample reference	Photo ID	Description
2/09/19	1455	1	Y	56.533420 - 5.401724	LcDv106	No photo	Growing on seaweed attached to rock
2/09/19	1410	3	Y	56.524670 - 5.399860	LcDv105	Photo IMG_105	On bedrock/boulder, sponge-like
2/09/19	1400-1800	4	Y	56.518845 - 5.343561	LcDv109	No photo	-
2/09/19	1400-1800	5	Y	56.524773 - 5.400346	LcDv112	No photo	No details
2/09/19	1400-1800	7	Y	Between 56.543248 -5.298360 and 56.546953 - 5.290870	LcDv107	No photo	Bryozoan?
3/09/19	1426	12	Y	56.523801 - 5.367403	LcDv113	Photo IMG_4014	Growing on attached <i>Ascophyllum nodosum</i>
3/09/19	1452	12	Y	56.524488 - 5.363967	LcDv108	Photo IMG_4048	Growing on attached seaweed
3/09/19	1504	12	Y	56.525022 - 5.363836	LcDv104	Photo IMG_4058	Growing on attached <i>Fucus vesiculosus</i> ,
3/09/19	1538	12	N	56.528655 - 5.355162	-	Photo IMG_4072	Growing on patch of attached seaweed

3/09/19	1546	12	N	56.529258 - 5.353150	-	Photo IMG_4080	Growing on patch of attached seaweed
3/09/19	1510	13	Y	56.527222 - 5.376667	LcDv110	No photo	Growing on piece of seaweed found lying loose on sandy shore
3/09/19	1400- 1800	16	Y	56.548957 - 5.409857	LcDv111	No photo	Growing on boulder

Table 2

Water sample locations.

Date	Description	Shore No.	Latitude	Longitude
2/09/19	Eriska Hotel pontoon	n/a	56.537395	-5.40510
2/09/19	South Shian	2	56.525846	-5.40081
2/09/19	Scottish Seafarm Pontoon	3	56.521679	-5.40081
2/09/19	Glensanda Pier	4	56.516151	-5.36731
2/09/19	Sealife Centre pontoon	4	56.518554	-5.34536
3/09/19	Barrington's oyster farm	11	56.53575	-5.33736
3/09/19	Port Appin Ferry Pier	n/a	56.553212	-5.41529

Frozen tissue samples and photographs were later examined by MSS and tissue samples were sequenced for the partial COI gene. Table 3 summarises results from genetic analyses of tissue samples. Due to the current incompleteness of the genetic databases and lack of accurately identified reference material for tunicates and bryozoans it was not always possible to provide identification to species. Therefore for purposes of this report, the tissue samples were identified as either Dvex or not Dvex.

Results of the real time PCR analysis showed that only sample LcDv108 was identified as Dvex. All samples were sequenced for the partial COI and/or 18S to confirm species identification where possible. Sequence information derived from both the partial COI and 18S genes, confirmed that only sample LcDv108 (Photo ID IMG 4048) collected at the shore 12 was identified as Dvex.

Table 3

Genetic characterization of tissue samples collected during the survey.

	COI-Tun	COI-Folmer	18S-meta2	18S-Zhan	Dvex specific real-time PCR	Final identification (Dvex or Not Dvex)
LcDv104	no product	Fucus sp	no sequence	no sequence	not Dvex	not Dvex
LcDv105	no product	no product	no product	no product	not Dvex	not Dvex
LcDv106	no product	no sequence	not Dvex	not Dvex	not Dvex	not Dvex
LcDv107	no product	no sequence	not Dvex	not Dvex	not Dvex	not Dvex
LcDv108	Dvex	not Dvex	Dvex	no sequence	Dvex	Dvex
LcDv109	no product	not Dvex	not Dvex	not Dvex	not Dvex	not Dvex
LcDv110	no product	no sequence	no sequence	no sequence	not Dvex	not Dvex
LcDv111	no product	no sequence	not Dvex	not Dvex	not Dvex	not Dvex
LcDv112	no product	not Dvex	not Dvex	not Dvex	not Dvex	not Dvex
LcDv113	no product	no sequence	no sequence	no sequence	not Dvex	not Dvex

Photographs from shore surveys

Figure 3: Shore 3, sample LcDv105, Photo ID IMG_105 location: 56.524670 - 5.399860.



Figure 4: Shore 12, sample LcDv113, Photo ID IMG_4014, location 56.523801 - 5.367403.



Figure 5: Shore 12, sample LcDv108, Photo ID IMG_4048, location 56.524488 - 5.363967.



Figure 6: Shore 12, sample LcDv104, Photo ID IMG_4058, location 56.525022 - 5.363836.

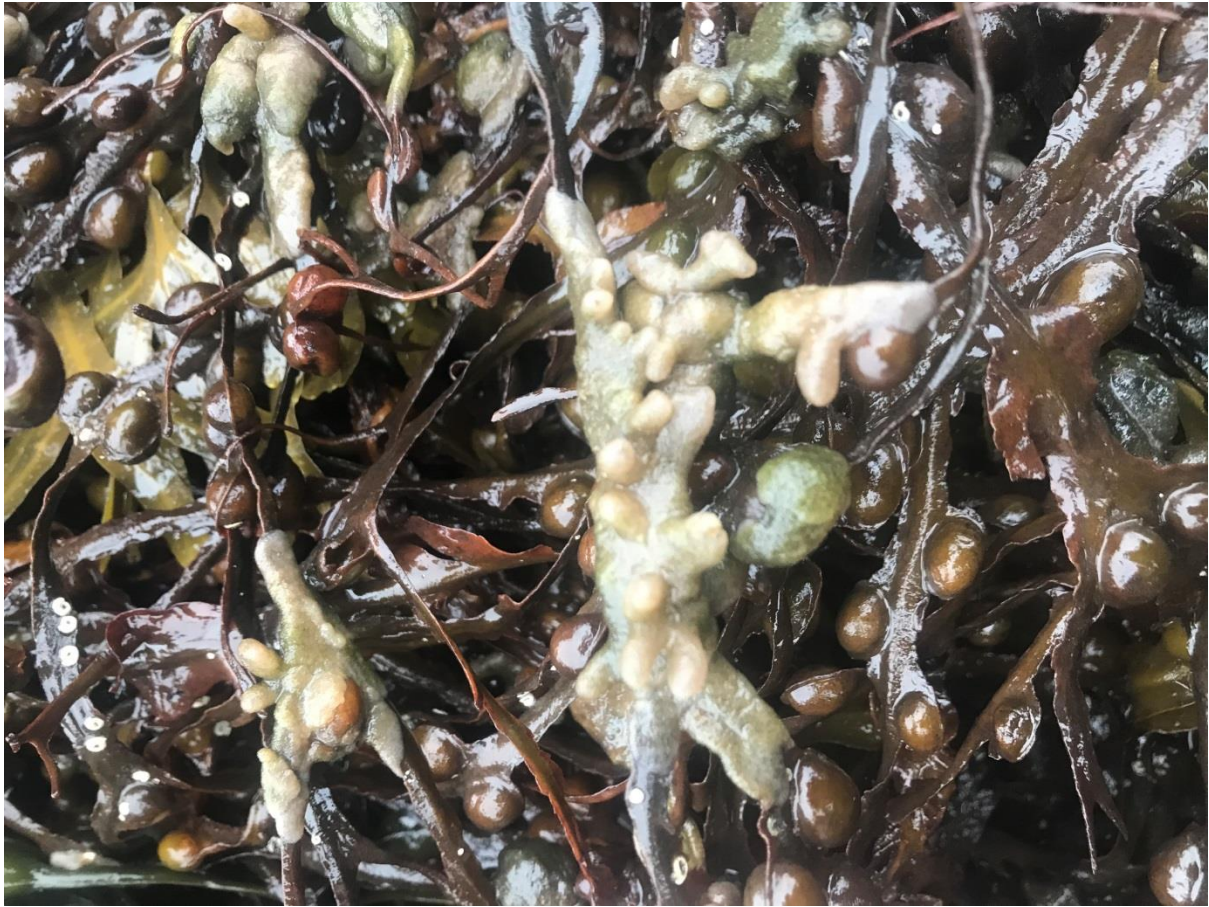


Figure 7: Shore 12, Photo ID IMG_4072, location 56.528655 - 5.355162.



Figure 8: Shore 12, Photo ID IMG_4080, location 56.529258 - 5.353150.



b) Boat surveys

Types of structures that were surveyed are listed in Table 3. Not all structures surveyed in 2016 were still used and present in 2019 but those that were present were re-surveyed. Table 4 provides further details for each structure surveyed.

Table 3

Type and number of structures surveyed within the loch in 2019 survey.

Structure type	No. of structures surveyed
Individual moorings/buoys (M)	31
Vessels plus their moorings/buoys (VM)	11
Rafts (R)	1
Piers (PR)	1
Pontoons (PN)	2
Bridge (B)	1
Not described	7

Table 4

Location and descriptions of structures surveyed. Locations are listed in chronological order of the survey. Numeric values represent new or moved structures visited in 2019. Re-visited 2016 survey locations are listed as alphanumeric survey IDs.

Survey ID (Waypoint)	Date	Time	Latitude	Longitude	Description	Dvex observation
442	02/09/2019	14.05	56.530199	-5.398776	black buoy	-ve
M31	02/09/2019	14.20	56.52835	-5.4041	mooring, orange buoy	-ve
M30	02/09/2019	-	-	-	absent	-
M29	02/09/2019	14.32	56.52723	-5.4055	small mooring	-ve
M27	02/09/2019	14.38	56.52633,	-5.405383	mooring with boat	-ve
M26	02/09/2019	14.51	56.526700	-5.404698	mooring with boat	-ve
444	02/09/2019	14.58	56.527147	-5.401931	buoy covered in caprellids	-ve
445	02/09/2019	15.10	56.527291	-5.401057	buoy covered in caprellids	-ve
446	02/09/2019	15.25	56.520261	-5.397379	buoy	-ve
M33	02/09/2019	15.33	56.518611	-5.398443	mooring with boat	-ve
VM7	02/09/2019	15.43	56.517278	-5.398244	vessel 'Tilleadh' and mooring; buoy covered in caprellids	-ve
VM8	02/09/2019	15.55	56.513718	-5.375828	vessel moored off Caledonian Oysters site; buoy covered in caprellids	-ve
VM10	02/09/2019	16.08	56.51945	-5.3431	vessel 'Louise' and mooring	-ve
PN2	02/09/2019	16.20	56.5190167	-5.3433	pontoon near Sealife Centre	-ve
R2	02/09/2019	16.45	56.519630	-5.342109	raft at Sealife Centre	-ve
451	02/09/2019	16.57	56.529349	-5.323488	buoy	-ve
452	03/09/2019	10.35	56.528291	-5.322911	buoy	-ve

VM6	03/09/2019	10.42	56.527908	-5.322424	vessel 'Serpula' and moorings	-ve
VM5	03/09/2019	10.46	56.528658	-5.319837	vessel 'Staffa Tours' and mooring	-ve
455	03/09/2019	10.52	56.529354	-5.320387	not described	-ve
456	03/09/2019	11.08	56.528678	-5.325993	buoy	-ve
457	03/09/2019	11.14	56.528842	-5.325434	not described	-ve
458	03/09/2019	11.18	56.528693	-5.323596	not described	-ve
459	03/09/2019	11 20	56.528609	-5.323019	not described	-ve
460	03/09/2019	11.24	56.528857	-5.322730	not described	-ve
M14	03/09/2019	11.30	56.529006	-5.322235	Creran moorings	-ve
462	03/09/2019	11.32	56.529146	-5.321739	Creran moorings	-ve
M13	03/09/2019	11.36	56.528972	-5.321171	Creran moorings	-ve
464	03/09/2019	11.38	56.529146	-5.321189	Creran moorings	-ve
465	03/09/2019	11.41	56.529081	-5.320621	Creran moorings	-ve
M11	03/09/2019	11.46	56.529106	-5.319927	yellow mooring off MRC	-ve
PR2	03/09/2019	13.44	56.528554	-5.318233	Barcaldine MRC pier	-ve
470	03/09/2019	13.57	56.529638	-5.315024	not described	-ve
VM4	03/09/2019	14.00	56.531099	-5.317277	vessel 'Legend of Brough' and mooring	-ve
M24	03/09/2019	14.07	56.531313	-5.319134	green navigation buoy	-ve
M20	03/09/2019	14.10	56.533833	-5.313140	Grey mooring	-ve
VM2	03/09/2019	14.16	56.533401	-5.312419	vessel 'SSF' and mooring	-ve
M19	03/09/2019	14.18	56.533858	-5.311482	grey mooring east of MRC	-ve
VM3	03/09/19	14.20	56.534183	-5.313967	vessel 'Felsted' and mooring	-ve
M23	03/09/2019	14.23	56.535672	-5.314555	yellow buoy	-ve

M22	03/09/19	14.28	56.5370167	-5.3117167	creel buoy	-ve
M21	03/09/19	14.50	56.538367	-5.3100167	grey buoy	-ve
B1	03/09/2019	15.10	56.547400	-5.290778	bridge pier	-ve
479	03/09/2019	15.23	56.519630	-5.341884	near R2	-ve
VM10	03/09/2019	15.32	56.519381	-5.343137	vessel 'Louise' and mooring	-ve

No *Dvex* was observed on any surveyed man-made structures. General fouling communities consisting of algae, mussels, barnacles, amphipods, bryozoans and native sea squirts were found on moorings, pontoons, piers, raft and the bridge. Caprellids were present on a number of buoys during day 1 of surveys.

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