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The Marine Invasive Non-Native Species *Didemnum vexillum*: Loch Creran Surveys - October 2016

Scottish Marine and Freshwater Science Vol 9 No 10

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Summary

In September 2016 Marine Scotland Science performed a survey of the Rubha Mor Farm along with a detailed survey of the foreshore around Loch Creran. In this report we present the results of a further survey of the Rubha Mor Farm, surveys of all other oyster farms in Loch Creran, as well as further foreshore surveys.

Staff involved in the surveys, and initials used in this report, are:

Marine Scotland Science (MSS)

Lyndsay Brown	LB
Guillame Hermann	GH
Bill Turrell	BT

Scottish Natural Heritage (SNH)

Jane Dodd	JD
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Table 1

Detailed survey work plan and areas of survey.

Date	Time	Where	What	Who	Low Tide
Saturday 15 th	2.30 – 3pm	MSS	Meet to load up cars and head to Oban	MSS - LB, GH	
Sunday 16 th	Early	Home	Driving second lab car to Oban	MSS - BT	
Sunday 16 th	10.30	Sealife centre carpark	Everyone meeting up before survey work	MSS - LB, GH, BT	
Sunday 16 th	11.00	South Shian Bay – Roger Thwaites' farm	Meet to discuss Dvex and carry out full survey of farm	MSS - LB, GH	12.42 (0.4 m)
THIS REPORT - SECTION 1					
Sunday 16 th	11.00	South Ardnalach – John Barrington's farm	Meet to discuss Dvex and carry out full survey of farm	MSS - BT	12.42 (0.4m)
THIS REPORT - SECTION 2					
Monday 17 th	13.00	Caledonian Oysters – Hugo's farm.	Meet to discuss Dvex and carry out full survey of farm	MSS - LB, GH SNH - JD	13.24 (0.3m)
THIS REPORT - SECTION 3					
Tuesday 18 th	13.30	Creagan Farm – Andrew Hodge and North Shian shoreline survey	Carry out full survey of farm (Andrew will not be there but has given us permission)	MSS – GH SNH – JD	14.08 (0.4m)
THIS REPORT – SECTION 4					
Tuesday 18 th	13.30	Eriska shoreline survey	Further shore survey	MSS – BT, LB	14.08 (0.4m)
THIS REPORT – SECTION 5					
Wednesday 19 th	14.00	All surveyed areas	Collection of live Dvex colonies and water samples	Lyndsay and Guillaume.	14.55 (0.3m)
THIS REPORT – SECTION 6					

Section 1

Sunday 16 October 2016 – South Shian Bay

Narrative

MSS (LB, GH) visited Shian Fisheries Ltd at South Shian (Figure 1.1). They currently rent out part of their site to another business who are managing approximately 100 trestles for Pacific oysters. These lie to the east of the small jetty located in front of the farm house (Figure 1.2). Current site practise to manage bio-fouling includes turning bags on a monthly basis. To the west of the jetty and extending further round the bay lie around 600 unfarmed trestles. These lie on very muddy ground (Figure 1.3). Both areas of trestles were surveyed (Figure 1.4). The furthest set of trestles were not surveyed as the mud was deemed unsafe to walk on. Shian Fisheries mentioned that strong currents flow past the section of managed trestles, while the area of unfarmed trestles is sheltered.

Farmed Trestles

No *D. vexillum* was observed on the farmed trestles or other hard substrates such as rocks, gravel etc. The farmed trestles had very little fouling present on them reflecting effectiveness of the management measures (Figure 1.5). No suspected *D.vex* or other tunicates were observed.

Unfarmed Trestles

On contrary, the unused trestles were heavily fouled with seaweed (Figure 1.6) and six small colonies of *D. vexillum*-like material were observed. One of colony was found growing on a solitary sea squirt (Figure 1.7), one was found growing on a sponge (Figure 1.8) and four were found growing on seaweed (Figures 1.9-1.10). The seaweeds and attached tunicates were removed to avoid detachment and spread, photographed and bagged. Representative samples (in duplicates) were collected for molecular identification. Shian Fisheries were informed of the findings after the survey was finished and was advised of the ongoing molecular verification of *D.vex* presence/absence.



Figure 1.1: Area of Shian Fisheries Ltd within red box.



Figure 1.2: View of farmed trestles (56° 31.519 N, 05° 24.070 W).



Figure 1.3: View of unused trestles ($56^{\circ} 31.539$ N, $05^{\circ} 24.265$ W).



Figure 1.4: Areas of surveys within red boxes.



Figure 1.5: View of farmed trestles with very little fouling (56° 31.520 N, 05° 24.027 W).



Figure 1.6: View of unused trestles heavily fouled with seaweed (56° 31.549 N, 05° 24.252 W).



Figure 1.7: Suspicious colony (LcDv 12, 13, Table 2) growing on solitary sea squirt (56° 31.511 N, 05° 24.362 W).



Figure 1.8: Suspicious colony (LcDv 14, 15, Table 2) growing on a sponge (56° 31.535 N, 05° 24.289 W).



Figure 1.9: Suspicious colonies growing on seaweed (56° 31.522 N, 05° 24.308 W).



Figure 1.10: Suspicious colonies (LcDv 16, 17, Table 2) growing on seaweed (56° 31.944 N, 05° 24.270 W).



Figure 1.11: Unidentified colonial tunicate (LcDv 18, 19, Table 2) growing on seaweed (56° 31.528 N, 05° 24.371 W).

Nucleic acid sequence of partial COI gene was obtained from samples LcDv 12-17 (Table 2). Comparison of obtained sequences to those known from the literature (*D.vex* genotype 1-23) indicated that these tunicates are not *D.vex* but most likely another *Didemnum* species. Identification from sample LcDv 18 and 19 visualized on Figure 1.11 was not possible as *Didemnum vexillum*-specific primers used here failed to amplify a product, suggesting that this sample was not *D.vex* but another unidentified species of tunicate.

Table 2

Summary of Samples for Genetic Analysis (South Shian Bay)

Unique sample identifier	Sample Ref.	Coll. Date	Molecular sample id	Analysis date	GenBank BLAST quick id	Figure
LcDv12	S1 R1	16/10/2016	Sample 1	25/10/2016	<i>D sp.</i>	1.7
LcDv13	S1 R2	16/10/2016	Sample 5	09/11/2016	<i>D sp.</i>	1.7
LcDv14	S2 R1	16/10/2016	Sample 3	25/10/2016	<i>D sp.</i>	1.8
LcDv15	S2 R2	16/10/2016	Sample 7	09/11/2016	<i>D sp.</i>	1.8
LcDv16	S3 R1	16/10/2016	Sample 4	25/10/2016	<i>D sp.</i>	1.10
LcDv17	S3 R2	16/10/2016	Sample 8	09/11/2016	<i>D sp.</i>	1.10
LcDv18	UI 1 R1	16/10/2016	Sample 2	25/10/2016	no PCR product	1.11
LcDv19	UI 1 R2	16/10/2016	Sample 6	09/11/2016	no ID	1.11

Section 2

Sunday 16 October – South Ardnalach Farm

Narrative

MSS (BT) proceeded to South Ardnalach and met with the farm owner and carried out farm and foreshore survey. No *D. vexillum* was found and no samples were taken. The farm was clear of fouling.

The survey consisted of two parts (Figure 2.1):

Part 1 – Foreshore survey (1120 BST – 1200 BST)

A visual survey along the edge of the tide. No *D. vexillum* seen, but a multitude of fouling sponges and sea squirts etc. on the rocky foreshore.

No samples taken as no suspect material seen.

Part 2 – Farm Survey (1213 BST – 1300 BST)

A visual survey of the South Ardnalach farm site. All trestles were inspected. Some bags had fouling from various sponges etc., but no suspect material seen.

No samples taken as no suspect material seen.



Figure 2.1: Map of survey.



Figure 2.2: General view of farm.



Figure 2.3: General view of foreshore.



Figure 2.4: Foreshore Survey: Non-suspect sponge on seaweed 56° 32.06867'N
05° 20.576'W.



Figure 2.5: Foreshore Survey: Sponge on rock – these sponges were common along the foreshore, especially on east facing surfaces ($56^{\circ} 32.06117'N$ $05^{\circ} 20.626667'W$).



Figure 2.6: Foreshore Survey: Sponge on rock – these sponges were common along the foreshore, especially on east facing surfaces ($56^{\circ} 32.0496667'N$ $05^{\circ} 20.657667'W$).



Figure 2.7: Foreshore Survey: Sponge on rock – these sponges were common along the foreshore, especially on east facing surfaces ($56^{\circ} 32.04816667'N$ $05^{\circ} 20.65766667'W$).



Figure 2.7: Farm Survey: Discarded trellis.



Figure 2.8: Farm Survey: General view of farm trellis.



Figure 2.9: Farm Survey: Trestles.



Figure 2.10: Farm Survey: Trestles.



Figure 2.11: Farm Survey: Sponge encrusting trestle frame (56° 32.12116667'N 05° 20.24933333'W)



Figure 2.12: Farm Survey: Sponge encrusting trestle frame (56° 32.12116667'N 05° 20.24933333'W).



Figure 2.13: Farm Survey: Encrusted Bag – algal mat? A frequent occurrence.



Figure 2.14: Farm Survey: Encrusted Bag – algal mat? A frequent occurrence.



Figure 2.14: Farm Survey: Green sponge on bag. Another frequent sight.



Figure 2.15: Farm Survey: Green sponge on bag. Another frequent sight.



Figure 2.16: Farm Survey: Green sponge on bag. Another frequent sight.

Section 3

Monday 17 October 2016– Rubha Mor Farm

Narrative

A full survey of the Caledonian Oyster site was carried out on 17 October 2016 by all members of the survey team (Figure 3.1). The survey started at 1200 BST and continued until 1530 BST. *D. vexillum* was still present on the site and was mainly found on the outer, most seaward trestles. No evidence was observed on the trestles situated higher up the shoreline. In most cases oyster bags and trestles were heavily fouled with seaweed, making it quite difficult to observe the bags in detail. *D. vexillum* colonies were observed in both mat and pendulous forms. Both forms were present on oyster bags, trestles and on seaweed attached to trestles and oyster bags (Figures 3.3 and 3.4). Individual oysters within bags were also observed to be fouled with the mat form (Figure 3.5). Caledonian Oysters staff were tending to bags and whenever *D. vexillum* was observed, the bags were tagged, removed from their holding positions on the trestles and moved to the top of the trestles for easy visibility for subsequent movement and treatment. MSS and SNH staff also tagged bags when *D. vexillum* was observed. One occurrence of *D. vexillum* starting to overgrow another unidentified colonial tunicate was also observed (Figure 3.6



Figure 3.1: Area of survey at Caledonian Oysters within red box .



Figure 3.2: *D. vexillum*-fouled oyster bags (mat form) (56° 30.912 N, 05° 23.360 W).



Figure 3.3: *D. vexillum*-fouled seaweed attached to trestles (mat and pendulous form) (56° 30.914 N, 05° 23.360 W) (LcDv 20, 21, Table 3).



Figure 3.4: *D. vexillum*-fouled oyster bag and trestle (mat form) (56° 30.914 N, 05° 23.360 W) (LcDv 22, 23, Table 3).



Figure 3.5: *D. vexillum* - fouled oyster within oyster bag (56° 30.914 N, 05° 23.360 W).



Figure 3.6: *D. vexillum* beginning to overgrow an unidentified colonial tunicate.



Figure 3.7: *D. vexillum* overgrowing fouling seaweed on an empty trellis.

Material collected for molecular identification and species verification summarized in Table 3 were all identified as *D.vex* by sequencing.

Table 3

Summary of Samples for Genetic Analysis (Rubha Mor Farm).

Unique sample identifier	Sample Ref.	Coll. Date	Molecular sample id	Analysis date	GenBank BLAST quick id	Figure
LcDv20	S1 R1	17/10/2016	Sample 10	09/11/2016	<i>D. vex</i>	3.3
LcDv21	S1 R2	17/10/2016	Sample 9	09/11/2016	<i>D. vex</i>	3.3
LcDv22	S2 R1	17/10/2016	Sample 11	09/11/2016	<i>D. vex</i>	3.4
LcDv23	S2 R2	17/10/2016	Sample 12	09/11/2016	<i>D. vex</i>	3.4

Minilogger Placement – Rubha Mor Farm

Narrative

Two miniloggers were placed on trestles on the Rubha Mor Farm, one on a seaward “wet” trestle, and one on a shoreward “dry” trestle.

Serial Number	Trestle	Latitude	Longitude	Time On Trestle (BST)
SN356477	Wet Trestle	56° 30.658833'N	05° 22.9345'W	1534 18/10/16
SN356484	Dry Trestle	56° 30.663667'N	05° 23.1735'W	1548 18/10/16

Wet Trestle

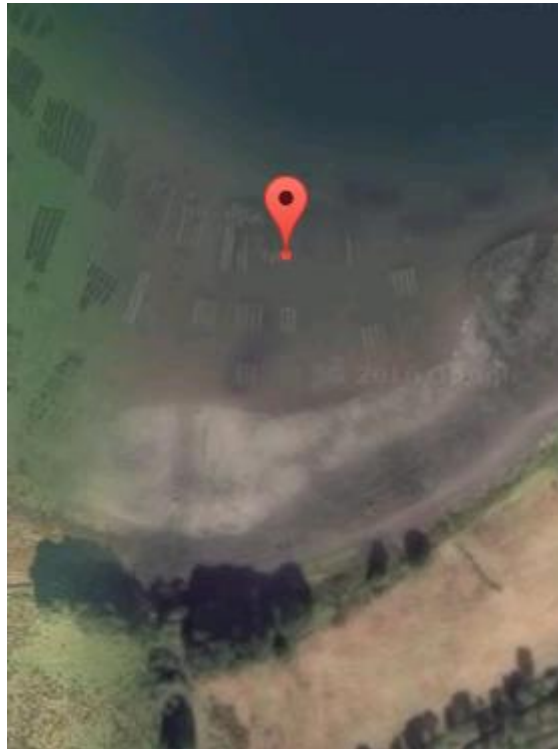


Figure 3.8: Location of minilogger on “wet trestle”.



Figure 3.9: Minilogger on wet trestle.



Figure 3.10: View to shore along wet trestle.

Dry Trestle



Figure 3.11: Location of minilogger on dry trestle.



Figure 3.12: Sighting along Dry Trestle.

Section 4

Tuesday 18 October – Creagan Farm and North Shian Shoreline

North Shian - Narrative

A shoreline survey was carried out along North Shian where three different bays were visited (Figure 4.1). The survey started at 1330 BST and finished at 1530 BST. The three bays were very similar geographically. A central inlet of freshwater from the mainland was surrounded by a rocky and muddy surrounding. Seaweeds and rocks became more frequent the further away from the stream.

The first bay surveyed (Bay 1, $56^{\circ} 33.236$ N, $05^{\circ} 24.268$ W) had some man made features such as metallic railing and concrete blocks.

The second bay (Bay 2, $56^{\circ} 32.050$ N $05^{\circ} 23.285$ W) was the most densely populated with seaweeds.

The third bay (Bay 3, $56^{\circ} 31.748$ N $05^{\circ} 22.502$ W) had a small rocky island accessible at low tide.



Figure 4.1: Shoreline survey locations around North Shian (red boxes) .

Two suspect samples were observed in Bay 3 (Figure 4.1 and Figure 4.2, red circle) where two unidentified colonial ascidians were observed growing on seaweed attached to a man-made concrete block (Figure 4.3 and 4.4).



Figure 4.2: Location of suspect sample in the third bay surveyed in North Shian (red circle)



Figure 4.3: Location of suspect sample on the concrete block (red circle).



Figure 4.4: Suspect samples observed in North Shian (LcDv 28-31, Table 4).

Molecular identification (sequencing of partial COI gene) of suspected material collected from the Bay 3 (Figure 4.1) revealed that these tunicates were not *D.vex* but most likely *Botrylloides*-like species (Table 4).

Table 4

Summary of Samples for Genetic Analysis (North Shian).

Unique sample identifier	Sample Ref.	Coll. Date	Molecular sample id	Analysis date	GenBank BLAST quick id	Figure
LcDv28	S1 UI R1	18/10/2016	Sample 15	09/11/2016	<i>Botrylloides</i>	4.4
LcDv29	S1 UI R2	18/10/2016	Sample 16	09/11/2016	<i>Botrylloides</i>	4.4
LcDv30	S2 UI R1	18/10/2016	Sample 13	09/11/2016	<i>Botrylloides</i>	4.4
LcDv31	S2 UI R2	18/10/2016	Sample 14	09/11/2016	<i>Botrylloides</i>	4.4

Creagan Farm - Narrative

The Creagan Farm, located at the top north east part of Loch Creran was surveyed at 1545 BST (Figure 4.2). No trestles could be found and it has been suggested that the farm is no longer in operation. No *D. vexillum*-looking colonies were observed in the shore surrounding the Creagan Inn hotel and no samples were collected.



Figure 4.2: Shoreline survey at Creagan Farm (red box).

Section 5

Tuesday 18 October 2016 – Eriska Shoreline

Narrative

A shoreline survey was carried out along Eriska, towards, but not reaching, the South Shian site. The survey began at 1330 BST and finished at 1530 BST. This area was much more diverse than the previous sampling sites and had a higher species richness.

A small jetty was surveyed at the beginning of the survey ($56^{\circ} 32.218$ N, $05^{\circ} 24.345$ W) and the shoreline survey followed (end point: $56^{\circ} 31.907$ N, $05^{\circ} 24.248$ W). The ground was quite rocky with lots of boulders dotted around the shore. Most were covered with seaweed. The survey area is shown in Figure 5.1.

No *D. vexillum*-looking colonies were observed during this survey. An unidentified white colonial tunicate was observed growing on seaweed on the shoreline survey ($56^{\circ} 32.218$ N, $05^{\circ} 24.345$ W), and although this was not considered to be *D. vexillum*, it was collected and preserved for molecular testing. No photos of the tunicate were taken.

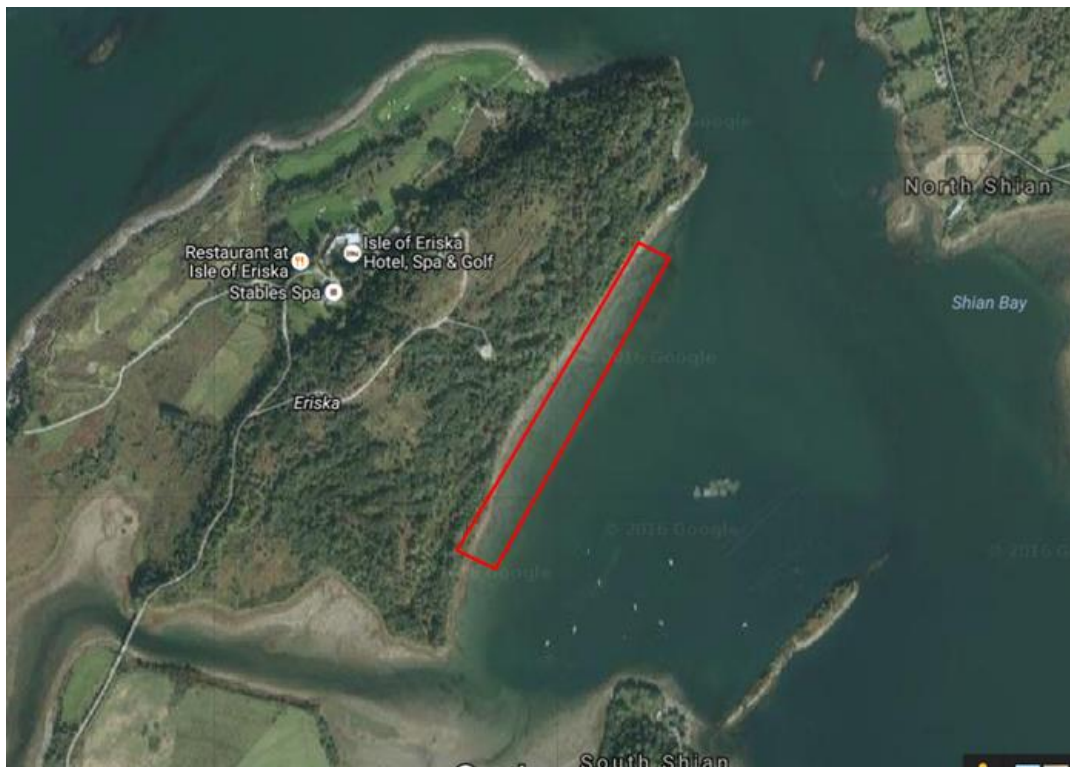


Figure 5.1: Area of survey on Eriska shoreline within red box.



Figure 5.2: Diversity of fouling on small pontoon jetty – northern tip Eriska.



Figure 5.3: Serpulid worms on rocks on rocky Eriska foreshore.



Figure 5.4: Typical view along rocky foreshore – Eriska.



Figure 5.5: Diversity of fouling on boulders – Eriska rocky foreshore.

Sequences obtained from samples 32 and 33 (Table 5) were identical to samples 12-17 collected from South Shian Bay (Table 2) and were identified as most likely another species of *Didemnum* different from *D.vex*.

Table 5

Summary of Samples for Genetic Analysis (Eriska Foreshore).

Unique sample identifier	Sample Ref.	Coll. Date	Molecular sample id	Analysis date	GenBank BLAST quick id	Figure
LcDv32	S1 UI R1	18/10/2016	Sample 17	09/11/2016	<i>D sp</i>	No photos
LcDv33	S1 UI R2	18/10/2016	Sample 18	09/11/2016	<i>D sp</i>	No photos

Section 6

Wednesday 19 October 2016 – Collection of Water Samples from all Survey Areas and Live *D. vexillum* Material from Caledonian Oysters

Narrative

To promote future environmental DNA (eDNA) feasibility work, 6 x 250 ml water samples were collected from all survey areas on 19/10/16. A low water tide was not required for this so sampling began in the morning between high and low tides. Details are in Table 6 and Figure 6.1 shows sampling locations.

Table 6

Details of water sampling locations

Time	Area	Location	Figure 20 reference
10.30	North Shian shoreline	56 33.236 N -05 24.268 W	1
10.40	North Shian shoreline	56 32.050 N -05 23.285 W	2
10.50	North Shian shoreline	56 31.748 N -05 22.502 W	3
11.15	John Barrington's farm	56 32.211 N -05 20.243 W	4
11.30	Creagan, Colin Hodge	56 32.973 N -05 17.944 W	5
11.55	Marine resource Centre	56 31.754 N -05 18.807 W	6
12.10	Sealife Centre	56 31.137 N -05 20.702 W	7
13.00	Thwaites farm (run by Gordon and Nick Turnbull)	56 31.519 N -05 24.070 W	8a
13.10	Thwaites unused farm	56 31.539 N -05 24.265 W	8b
13.40	Caledonian Oysters (Hugo Vajk)	56 30.620 N -05 22.984 W	9



Figure 6.1: Water sampling locations within Loch Creran (red stars).

Live material was collected from Caledonian Oysters after collection of all water samples. This material will be used for future treatment experiments and investigations into genetic diversity at MSS. *D. vexillum*-fouled bags had been previously moved from trestles and placed near the site processing area. Two of these heavily fouled bags were selected and pieces of the wire mesh were cut out from the bags with wire cutters. Both sections were then cut again so that six pieces of *D. vexillum*-fouled mesh were obtained. These were transported back to MSS within closed buckets filled with seawater from the site to keep them fresh. On arrival at MSS, they were placed into holding tanks in a biosecure facility.

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