

# Assessing the effectiveness of early warning systems for the detection of marine invasive non-native species in Scottish waters





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# COMMISSIONED REPORT

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Commissioned Report No. 874

## **Assessing the effectiveness of early warning systems for the detection of marine invasive non-native species in Scottish waters**

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COMMISSIONED REPORT

# Summary

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## Assessing the effectiveness of early warning systems for the detection of marine invasive non-native species in Scottish waters

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### **Keywords**

Marine non-native species; early warning system; survey techniques; detection; marinas; fish farms.

### **Background**

Invasive non-native species (INNS) are considered to be one of the greatest threats to biodiversity, particularly through their interactions with other drivers of change. These species are initially transported through human intervention outside their natural range and across ecological barriers, before becoming established in a new location, where they can have negative impacts on the ecology, as well as serious economic and social impacts. It has been estimated that over 58 non-native marine species are established in the UK, with an estimated cost of at least £40 million per annum to marine-based industries. A number of international and national agreements recognise the negative effects of INNS, reflecting the growing concern by government institutions, wildlife managers, scientific institutions and citizens. For example, the presence of INNS can potentially lead to the failure of a water body to achieve Good Environmental Status under the EU Marine Strategy Framework Directive and, within the 2020 Biodiversity Strategy, the EU committed to halting the loss of biodiversity by 2020 and included a specific target to cover 'tighter controls for invasive alien species'. In a recent European Council Regulation on the prevention and management of the introduction and spread of INNS, three types of intervention are prescribed: prevention, early warning and rapid response, and management.

Prevention of the introduction of INNS is by far the most cost-effective and environmentally favourable of the three types of intervention. However, it is not always effective and INNS may be inadvertently introduced as hitchhikers with aquaculture species, via ballast water or hull fouling. Therefore, detecting introductions at the earliest possible time, when only small populations are present, would provide the best opportunity for a rapid response. The ability to detect INNS introductions, particularly in the marine environment, is poorly developed and often relies on chance sightings. The lack of new technologies for the early detection of INNS was recently highlighted in a list of the top 20 issues currently facing policy makers. This report aims to directly address this issue, by assessing the effectiveness of five sampling techniques in detecting marine epibenthic non-native species (NNS).

## Main findings

- Five early detection techniques were trialled and evaluated for their effectiveness in detecting NNS, these included rapid assessment surveys, settlement panels, scrape samples, *in-situ* photographs and settlement panel photographs.
- The rapid assessment survey provided the most reliable and cost-effective technique for the rapid identification of NNS at a particular site. This technique provided immediate results for the larger, more conspicuous species and enabled the collection of species for subsequent verification, if required.
- The settlement panels and scrape samples were also found to be reliable, particularly in marinas, and could be cost-effective if samples are collected by trained personnel who are already visiting sampling sites for other purposes. Both techniques provided quantifiable data, unlike the rapid assessment survey. The panels could also be deployed over a number of months, extending into early autumn in Scottish waters, thus providing an indication of the species that are able to reproduce at a particular site.
- The *in-situ* and settlement panel photographs were not reliable or cost-effective at detecting NNS. Poor image quality, water clarity and fouling/siltation of the panels prohibited the identification to species level in many cases even for the larger, more conspicuous fauna and flora.
- All the techniques, however, were not without their disadvantages and it is recommended that the rapid assessment survey is used in combination with either the scrape or settlement panel techniques to ensure the highest rate of detection of the NNS at a particular site.
- Marinas were found to be the most successful location for the detection of NNS, although fish farms and monitoring/navigation buoys may provide additional locations for early warning monitoring stations, which could be surveyed for particular species by trained personnel that routinely visit these sites.

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## 1. INTRODUCTION

Invasive non-native species (INNS) are considered to be one of the greatest threats to biodiversity, particularly through their interactions with other drivers of change (Vila *et al.*, 2011, Cook *et al.*, 2013a). These species are initially transported through human intervention outside their natural range and across ecological barriers, before becoming established in a new location, where they can have negative impacts on the ecology, as well as serious economic and social impacts (Mineur *et al.*, 2012). It has been estimated that over 58 marine species are established in the UK (Roy *et al.*, 2012; Minchin *et al.*, 2013), with an estimated cost of at least £40 million per annum to marine-based industries (Williams *et al.*, 2010). A number of international and national agreements recognise the negative effects of INNS, reflecting the growing concern by government institutions, wildlife managers, scientific institutions and citizens. For example, the presence of INNS can potentially lead to the failure of a water body to achieve Good Environmental Status under the EU Marine Strategy Framework Directive. In addition, European countries now have obligations in relation to INNS species and must 'strictly control the introduction of non-indigenous species' (Bern Convention on the Conservation of European Wildlife & Natural Habitats) and 'eradicate those alien species which threaten ecosystems, habitats or species' (UN Convention on Biological Diversity (CBD, 1992)). Within the 2020 Biodiversity Strategy, the EU committed to halting the loss of biodiversity by 2020 and included a specific target to cover 'tighter controls in invasive alien species' (2011). In a new European Council Regulation (2014) on the prevention and management of the introduction and spread of INNS, three types of intervention are prescribed: prevention, early warning and rapid response, and management.

Prevention of the introduction of INNS is by far the most cost-effective and environmentally favourable of the three types of intervention. This has been the focus of a recent INNS horizon-scanning study in the UK (Roy *et al.*, 2014) and the development of biosecurity guidelines (Cook *et al.*, 2014; Payne *et al.*, 2014), which are seen as critical tools in INNS management. Prior to this, measures to control or manage INNS have been predominantly reactive and, for introduced marine species, these measures can be extremely costly. Although numerous examples exist of methods of controlling the spread of marine INNS, very few have resulted in the complete eradication of a species (see review in Bax *et al.*, 2002; Cook *et al.*, 2012).

Prevention is not always effective and INNS may inadvertently be introduced, as hitchhikers with intentionally introduced aquaculture species, via ballast water or hull fouling (Carlton & Geller, 1993; Ruiz *et al.*, 2000; Mineur *et al.*, 2012). The success of intervention is typically inversely related to the size of the population acted upon (Leung *et al.*, 2002). Therefore, detecting introductions at the earliest possible time, when only small populations are present, would provide the best opportunity for a rapid response (Caffrey *et al.*, 2014). However, the ability to detect INNS introductions, particularly in the marine environment, is poorly developed and often relies on chance finds (e.g. *Caprella mutica* on the west coast of Scotland (Willis *et al.*, 2004)). In the majority of cases, the INNS are no longer in the early stages of invasions and, consequently, the use of rapid response techniques would be ineffective at eradicating the species.

The lack of new technologies for the early detection of INNS was recently highlighted in a list of the top 20 issues currently facing policy makers (Caffrey *et al.*, 2014), although deemed as a cost-effective approach to dealing with INNS (Leung *et al.*, 2002). The detection of INNS-specific DNA in water or in the organisms themselves may significantly enhance surveillance programmes in the future, particularly in freshwater systems, which are relatively closed compared to the more open marine environment (Jerde *et al.*, 2011; Dejean *et al.*, 2012). The lack of genetic information available for marine native species and INNS, together with the paucity of equipment and skilled personnel in Europe with the capabilities

of processing the samples, means that the use of DNA markers for marine INNS may not be available in the short to medium term.

Certain detection methods, such as rapid assessment surveys (RAS) (Pederson *et al.*, 2003; Arenas *et al.*, 2006; Ashton *et al.*, 2006; Minchin & Nunn, 2013), settlement panels (Gutierrez *et al.*, 2005; Cook *et al.*, 2011), quadrat scrape samples (Hewitt & Martin, 2001; MacLeod, 2013), still photography (MacLeod, 2013), videography, coring, benthic sleds, beam trawls, traps, plankton netting (Hewitt & Martin, 2001) and citizen science projects (e.g. Shore Thing: MarLIN, 2014), which target locations that are subject to increased maritime activity (e.g. ports and marinas), have been highly effective at identifying new incursions of certain marine INNS. Either singly or in combination, these methods may provide robust early warning systems in the short term, while the molecular techniques are refined.

To date, no systematic assessment has been made to compare (i) the effectiveness of these early warning techniques in detecting a range of marine INNS at a particular location, (ii) whether certain techniques could be used at more offshore locations and associated with other important pathways of INNS introduction, such as aquaculture and (iii) the costs involved with each technique. The aim of this study was to determine the effectiveness and costs associated with RAS, settlement panels (analysed in the laboratory and by digital photography), still photographs of *in-situ* communities and scrape samples taken from a variety of floating structures associated with anthropogenic activities, in detecting fouling, epibenthic non-native species (NNS).

## **2. METHODS**

### **2.1 Study locations**

Two regions on the west coast of Scotland were selected for study: Loch Fyne and the Firth of Lorn (including Loch Creran). These regions have been the focus of previous rapid assessment surveys for NNS (2006 – present) (Ashton *et al.*, 2006; Beveridge *et al.*, 2011) and support extensive recreational vessel and aquaculture activity. Within each region, one marina and one fish farm were selected for assessing the five sampling techniques. Only four techniques were trialled at two oyster farm sites, as the *in-situ* photography technique was only used at sites that remained submerged at all states of the tide (Table 1; Figure 1). In addition, sampling was conducted at two SEPA monitoring buoys and four Northern Lighthouse Board navigation buoys (see Section 2.3 for further details) (Figure 1).



Table 1. Study locations and early warning technique employed: rapid assessment survey (RAS), settlement panels (SP), settlement panel photography (SPP), in-situ photography (ISP), scrape samples (SCR).

Region	Activity Type	Location Name	Latitude/ Longitude	Date of RAS Survey	Average Temp. (°C) (n=2)* (min – max)	EW Technique				
						RAS	SP	SPP	ISP	SCR
Loch Fyne	Oyster Farm	Ardkinglas	56.25 N; 04.97 W	08/10/13	12.1 (5.8 – 15.0)	x	x	x		x
	Fish Farm	Garbara	55.98 N; 05.35 W	11/10/13	11.7 (10.4 – 13.0)	x	x	x	x	x
	Marina	Portavadie	55.87 N; 05.31 W	08/10/13	12.2 (11.0 – 13.8)	x	x	x	x	x
Firth of Lorn	Oyster Farm	South Shian	56.53 N; 05.40 W	07/10/13	12.7 (7.3 – 16.3)	x	x	x		x
	Fish Farm	Dunstaffnage	56.45 N; 05.47 W	10/10/13	13.0 (12.0 – 14.4)	x	x	x	x	x
	Marina	Oban	56.42 N; 05.50 W	10/10/13	13.0 (12 – 13.6)	x	x	x	x	x

\* Temperature data collected using two ibuttons™ at each site, deployed for the same duration as the settlement panels.

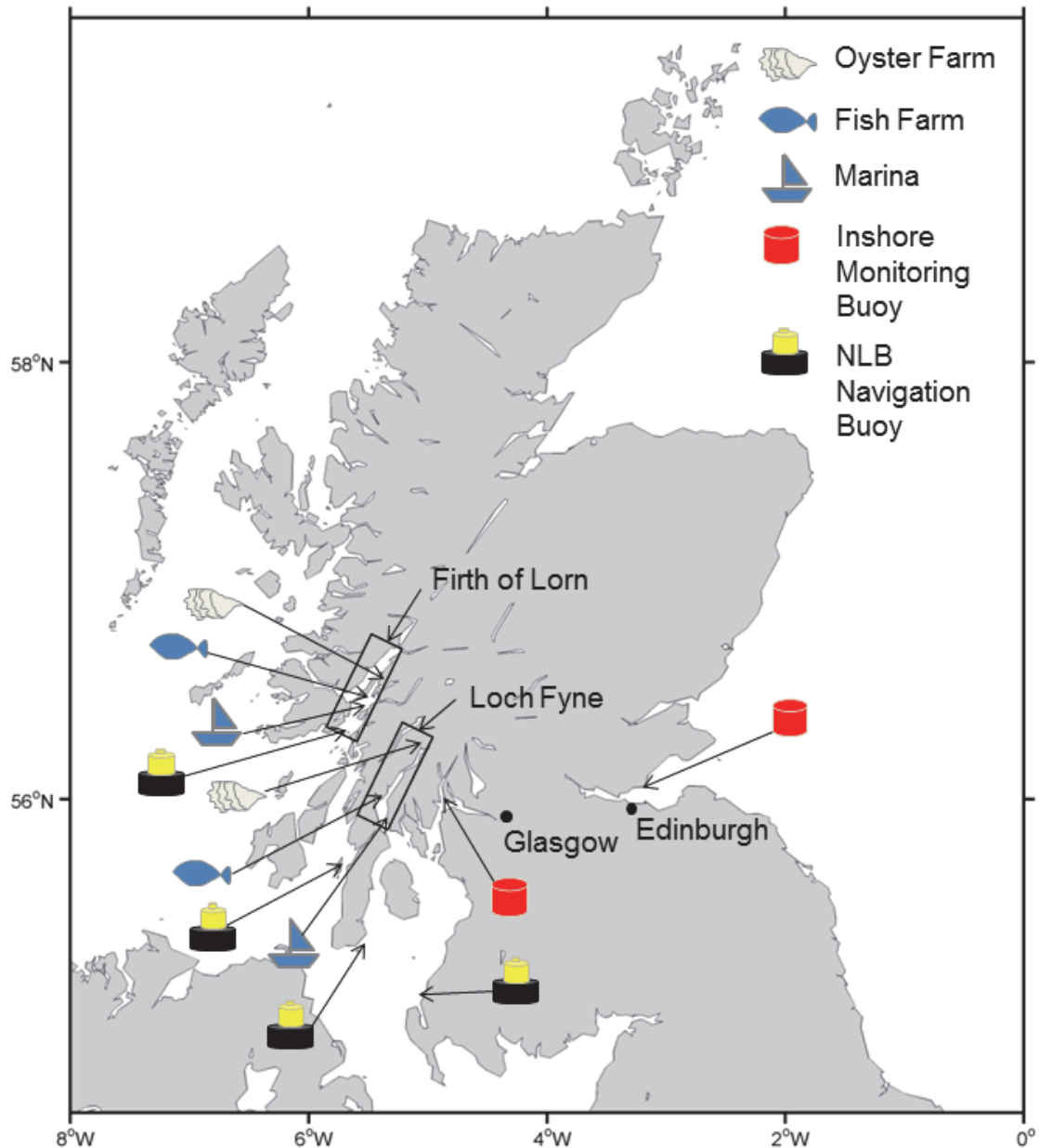


Figure 1. Location of the marinas, fish and oyster farms where the five early warning sampling techniques were assessed. The locations of the inshore SEPA monitoring buoys and NLB navigation buoys are also shown.

## 2.2 Early warning techniques

### 2.2.1 Rapid assessment surveys (RAS)

The rapid assessment survey method was based on Ashton *et al.* (2006) and all the surveys took place in October 2013. Artificial and natural structures, where available, at each location were inspected by two trained personnel for selected target NNS (Table 2) over a period of approximately one hour. The target species were based upon previous survey results (Beveridge *et al.*, 2011) and a review of published and grey literature for the region (Minchin *et al.*, 2013; Minchin & Nunn, 2013; Nall *et al.*, 2015). Additional NNS were recorded if found during the RAS.

Table 2. Target species selected for rapid assessment survey

Species	Taxon	Closest location	Possible pathway	Reference
<i>Asparagopsis armata</i>	red alga	W Scotland	natural drift	Kraan & Barrington, 2005
<i>Asterocarpa humilis</i>	tunicate	Wales, S England, W Scotland	vessel hulls	Bishop <i>et al.</i> , 2013; pers. obs.
<i>Austrominius modestus</i>	barnacle	W Scotland	vessel hulls/ stocking	Beveridge <i>et al.</i> , 2011
<i>Botrylloides violaceus</i>	tunicate	N Ireland, W Scotland	vessel hulls/ aquaculture	Beveridge <i>et al.</i> , 2011
<i>Bugula neritina</i>	bryozoan	SW Scotland	vessel hulls	Ryland <i>et al.</i> , 2011
<i>Bugula simplex</i>	bryozoan	N Scotland	vessel hulls	Nall <i>et al.</i> , 2015
<i>Caprella mutica</i>	amphipod	W Scotland	vessel hulls/ aquaculture	Willis <i>et al.</i> , 2004
<i>Codium fragile</i> subsp. <i>fragile</i>	green alga	W Scotland	aquaculture/ drift	Beveridge <i>et al.</i> , 2011
<i>Colpomenia peregrina</i>	brown alga	W Scotland	drift/oysters	Mineur <i>et al.</i> , 2008
<i>Corella eumyota</i>	tunicate	W Scotland	vessel hulls	Beveridge <i>et al.</i> , 2011
<i>Crassostrea gigas</i>	mollusc	N Ireland, SW Scotland	aquaculture	Miossec <i>et al.</i> , 2009; Solway Firth Partnership, pers. comm.
<i>Crepidula fornicata</i>	mollusc	N Ireland	aquaculture/ stocking	McNeill <i>et al.</i> , 2010
<i>Didemnum vexillum</i>	tunicate	SW Scotland	vessel hulls/ aquaculture	Beveridge <i>et al.</i> , 2011
<i>Heterosiphonia japonica</i>	red alga	W Scotland	vessel hulls	Beveridge <i>et al.</i> , 2011
<i>Sargassum muticum</i>	brown alga	W Scotland	drift/vessels/ equipment	Harries <i>et al.</i> , 2007
<i>Schizoporella japonica</i>	bryozoan	W and N Scotland	vessel hulls	Nall <i>et al.</i> , 2015
<i>Styela clava</i>	tunicate	W Scotland	vessel hulls	Beveridge <i>et al.</i> , 2011
<i>Tricellaria inopinata</i>	bryozoan	W Scotland	vessel hulls	Cook <i>et al.</i> , 2013b
<i>Undaria pinnatifida</i>	brown alga	N Ireland, NW Wales	vessel hulls	Minchin & Nunn, 2013

Floating structures located in the marinas and at the fish farms, together with submerged surfaces, were inspected to a maximum depth of 0.5 m. These structures included pontoons, chains, harbour walls, vessel hulls, fin-fish cage floatation devices, oyster trestles and natural substrates, such as boulders.

When a potential NNS was found, if surveyors were not able to identify it to species level *in situ*, a sample was collected and preserved in ethanol (70%) for later identification under a dissection or high-powered microscope.

### 2.2.2 Settlement panels (SP)

Settlement panels (15 x 15 cm) were constructed using Correx ('corrugated plastic') units, which provided a vertical and horizontal surface for colonisation (Figure 2). Five replicate settlement panels were deployed at each survey location for a period of five weeks at a depth of 1.5 m at the marina and fish farm sites (based on method in Cook *et al.*, 2011), with the intention that three panels would be analysed. This gave allowances for accidental panel loss. At the oyster farm, the panels were attached to the under-carriage of the oyster trestles, so that the bottom edge of the panel was just above the sediment. At each location, the panels were randomly deployed in an area with the greatest local vessel activity – at the marina, the panels were deployed where the visiting vessels were berthed; at the fish farm, the panels were on the cages adjacent to the fish-feed barge; and at the oyster farm they were attached to the trestles in the main production area. The minimum distance between panels was 10 m. Collection of the settlement panels occurred concurrently with the RAS.

Once retrieved, the panels were preserved in ethanol (70%) prior to analysis. Three panels were analysed for the target NNS and any other NNS identified from each location, using dissection microscope and/or high powered microscope where necessary to confirm the identification of each species.



Figure 2. Settlement panel design used at all sampling locations. (Photo: C. Beveridge, SAMS).

### 2.2.3 Settlement panel photography (SPP)

On retrieval, photographs of the settlement panels were taken using a Pentax Optio WG-2 GPS digital waterproof camera (9.9 mega pixels; resolution 72 dpi). The camera was mounted on a 15 x 15 cm quadrat frame at a distance of 30 cm from the surface of the panel to enable comparison of the laboratory-based analysis (Section 2.2.2) with the digital photographs. 'Blind' analysis of the photograph taken from the three panels analysed for NNS in the laboratory from each site was undertaken, without prior knowledge of the source of the image, using Vidana ([www.marinespatialecologylab.org/resources/vidana/](http://www.marinespatialecologylab.org/resources/vidana/), 31/01/2011), which enabled adjustment of the image resolution. Target NNS and any other NNS were identified, where possible.

### 2.2.4 In-situ photography (ISP)

Three digital photographs were taken from random locations within the same general area used for the settlement panel deployment using the same method as in Section 2.2.3. The camera mount and camera were placed so that the upper edge of the mount was at the water line, to capture an *in-situ* photograph of the biofouling community at each survey site (Figure 3).



Figure 3. Photograph showing positioning of camera mount just below the water line on pontoon floats in Portavadie marina, Loch Fyne (Photo: C. Beveridge, SAMS).

### 2.2.5 Scrape samples (SCR)

Three replicate scrape samples were collected immediately following the ISP at each survey site, using the camera mount as the reference quadrat (0.0225 m<sup>2</sup>) and a 10 cm wide paint scraper. The method was based on Macleod (2013), whereby the scraper was used to remove all the biofouling biomass from a given area of submerged structure using the quadrat for reference. This enabled the efficiency of the scrape sample to be directly compared with the *in-situ* photography technique. At the marinas, scrapes were taken from the vertical sides of the pontoon floats; at the oyster farm sites, from the oyster trestles and surrounding large boulders, where present; and at the fish farm sites, from the circular cage floatation devices and the feed barge. All the biomass removed from the structures was captured within a fine-mesh net (1 mm mesh size) before transfer to a storage container, where it was preserved in ethanol (70%).

For analysis, all the material was rinsed thoroughly through a 1 mm sieve prior to the identification of the target NNS.

In addition, to determine the most effective number of scrape samples required for future monitoring work, ten scrape samples were randomly collected from the marina in the Firth of Lorn on 28 May 2014, following the method outlined previously. The samples were then analysed for the presence of the target NNS, and any additional NNS were recorded.

## **2.3 Additional sampling locations**

### *2.3.1 Inshore SEPA monitoring buoys*

Two inshore Scottish Environment Protection Agency (SEPA) monitoring buoys were identified as suitable locations for the assessment of the five techniques: Gunnet Ledge Buoy (Firth of Forth, east coast of Scotland) and Dunoon Buoy (Clyde Estuary, west coast of Scotland) (Figures 1 & 4). SEPA personnel visit both these sites on a regular basis for the collection of both biological and physical environmental data (M. Blackburn, SEPA, pers. comm.). Four settlement panels at the Dunoon and Gunnet Ledge sites were deployed by SEPA personnel on the 9<sup>th</sup> and 20<sup>th</sup> September 2013, respectively, at a depth of 1.5 m, with the intention that three would be analysed. Prior to deployment, thicker ropes were added to each panel by SEPA staff due to the more exposed nature of these buoys. In addition, heavier weights were added to the Gunnet Ledge panels, as the stronger current at this location meant that the panels were unable to hang vertically in the water column with the weight initially provided.

Only three vertical panels were retrieved from the Gunnet Ledge Buoy after four weeks of deployment by SAMS and SEPA personnel (17<sup>th</sup> October 2013). The horizontal panels and weights were missing and the remaining vertical panels showed signs of abrasion, so no further analysis was undertaken. No RAS, photographs or scrape samples could be taken due to the poor sea state and the high sides of the boat used to sample this buoy (7.9 m catamaran) but a small scrape sample was opportunistically obtained using a 50 ml plastic sample tube attached to an extension pole. All four panels were successfully retrieved from the Dunoon Buoy after seven weeks of deployment (28<sup>th</sup> October 2013) by trained SEPA personnel. No RAS, settlement panel or *in-situ* photographs, or scrape samples could be taken due to the sea conditions.



*Figure 4. Inshore SEPA monitoring buoys: Gunnet Ledge (left) and Dunoon (right).*

### *2.3.2 Offshore Northern Lighthouse Board (NLB) navigation buoys*

Four scrape samples were collected from four NLB navigation buoys (Figure 5) on the south and west coasts of Scotland between September and December 2013 using a 15 x 15 cm quadrat (0.0225 m<sup>2</sup>) (Table 3). The buoys were chosen based on their location (i.e. close to the study locations used for assessing the other techniques and activities) and on the maintenance schedule of the NLB. A half-day training course was given to the crew of the MV Polestar on scrape sample collection and a protocol was provided (see Annex 1). The crew proceeded to collect the scrape samples from pre-selected navigation buoys during routine maintenance operations, in which the buoys are lifted from the water and placed on board the service vessel. Once collected, the samples were placed in plastic bags provided, labelled and immediately frozen rather than preserved in formalin or ethanol, due to concerns about having these chemicals stored on board the vessel.

On return to the laboratory, the samples were defrosted overnight and rinsed prior to sorting. All target NNS were recorded using a dissection and/or high-powered microscope, to confirm identification where necessary. The freezing method of preservation was not found to adversely affect the identification process, with the exception of the soft-bodied polychaetes. As there is a distinct paucity of information on non-native polychaetes in the UK and they were not included on the target list (Table 2), this method was deemed satisfactory for the identification of target species for this study.



Figure 5. Retrieval of Northern Lighthouse Board navigation buoy for routine maintenance (Photo: A. Macleod, SAMS)

Table 3. Locations of the NLB navigation buoys used to collect scrape samples for NNS

Site	Location
Patersons Rock, Mull of Kintyre	55.283N; 5.547W
Cath Sgeir (Gigha)	55.667N; 5.617W
Bono Rock (near Easdale)	56.017N; 5.683W
Milleur Point, Loch Ryan	55.017N; 5.083W

## 2.4 Data analysis

Prior to analysis, the data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Bartlett's test; Zar, 1996). If necessary, the data were transformed to meet statistical assumptions (Underwood, 1997). Significant treatment effects ( $p < 0.05$ ) were further investigated to identify the source of the differences (Tukey's multiple comparison test). For the data analysis, the number of NNS was assessed using a Generalised Linear Model with Location (Loch Fyne or Firth of Lorn) (2 levels) as a random factor, Activity (Oyster Farm, Fish Farm or Marina) (3 levels) as fixed factors and Treatment/Technique Type (RAS, SP, SPP, ISP or SS) (5 levels) as a fixed factor. All GLM and post hoc tests were performed using MINITAB, Release 14 for Windows.



### 3. RESULTS

A total of nine species were identified from the target list of 19 NNS, including seven sessile or semi-sessile animal species and two macroalgal species (Table 4). From the target list, the sessile animals not found using any of the detection techniques assessed were *Botrylloides violaceus*, *Bugula neritina*, *Crassostrea gigas*, *Crepidula fornicata*, *Didemnum vexillum* and *Styela clava*. The macroalgal species on the target list that were not found were *Asparagopsis armata*, *Colpomenia peregrine*, *Sargassum muticum* and *Undaria pinnatifida*. No significant difference was observed between the two locations surveyed (Firth of Lorn and Loch Fyne) (GLM,  $df=1$ ,  $F=0.51$ ,  $p>0.05$ ).

#### 3.1 Comparison between NNS detection techniques

Overall, a significant difference was found between the five techniques in their ability to detect NNS (GLM,  $df=4$ ,  $F=70.86$ ,  $p<0.001$ ). The RAS detected significantly greater numbers of NNS than either the settlement panel or the *in-situ* photography (Tukey Test  $p<0.05$ ). No significant difference was found between the other techniques (Figure 6).

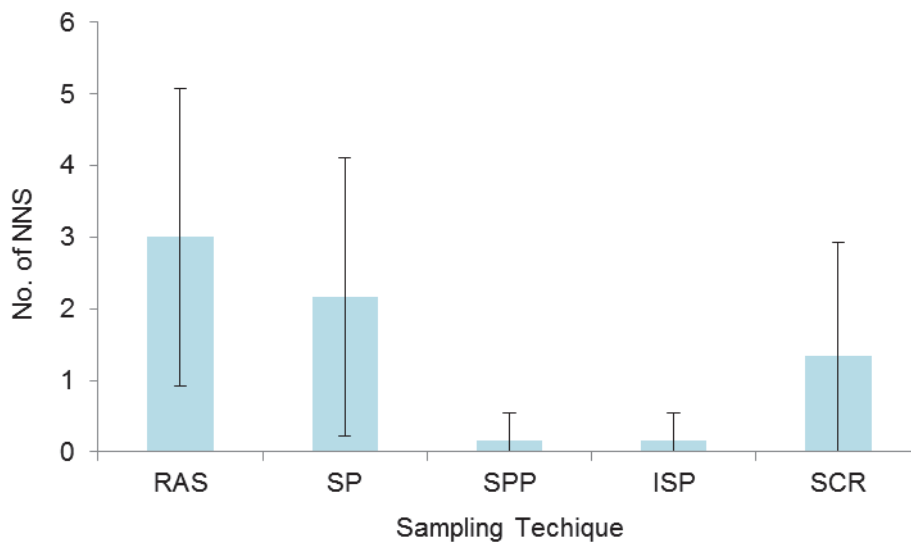


Figure 6. Number of NNS (mean  $\pm$  SE) detected by the five sampling techniques ( $n=6$ ): rapid assessment survey (RAS), settlement panels (SP), settlement panel photography (SPP), in-situ photography (ISP) and scrape samples (SCR)

Table 4. Non-native species identified in the survey locations used for comparing the five early warning techniques: rapid assessment survey (RAS); settlement panels (SP), settlement panel photography (SPP), in-situ photography (ISP), scrape samples (SCR).

	Marina										Fish Farm										Oyster Farm									
	Loch Fyne					Firth of Lorn					Loch Fyne					Firth of Lorn					Loch Fyne					Firth of Lorn				
	RAS	SP	SPP	ISP	SCR	RAS	SP	SPP	ISP	SCR	RAS	SP	SPP	ISP	SCR	RAS	SP	SPP	ISP	SCR	RAS	SP	SPP	ISP	SCR	RAS	SP	SPP	ISP	SCR
<b>Solitary ascidians</b>																														
<i>Corella eumyota</i>	√	√				√	√			√	√	√																		
<i>Asterocarpa humilis</i>	*	*				√	*			√	*	*																		
<b>Barnacles</b>																														
<i>Austrominius modestus</i>	√	√				√	√														√					√	√		√	
<b>Erect bryozoans</b>																														
<i>Bugula simplex</i>	√	√																												
<i>Tricellaria inopinata</i>	√	√			√	√	√				√	√																		
<b>Encrusting bryozoans</b>																														
<i>Schizoporella japonica</i>					√																									
<b>Crustaceans</b>																														
<i>Caprella mutica</i>	√	√	√		√	√	√			√	√	√				√														
<b>Algae</b>																														
<i>Codium fragile</i> subsp. <i>fragile</i> .						√					√																			
<i>Heterosiphonia japonica</i>					√																									
<b>TOTAL NNS</b>	<b>5</b>	<b>5</b>	<b>1</b>	<b>0</b>	<b>4</b>	<b>6</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>3</b>	<b>4</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>

\*Possibly juvenile *Asterocarpa humilis*, but unable to verify due to lack of research on this life stage.

### 3.2 Comparison between activity types

A mean total of 5.5 NNS ( $\pm 0.71$  SD) was found at the marina sites compared to mean total of 2.5 NNS ( $\pm 2.12$  SD) and 1.0 NNS ( $\pm 0$  SD) at the fish farm and oyster farm sites respectively. A significant difference was observed between these three activity types in the detection of NNS (GLM,  $df=2$ ,  $F=129.96$ ,  $p<0.001$ ), with a significantly greater number of NNS detected at the marina locations compared with the oyster farms (Tukey Test  $p>0.05$ ). No significant difference was found between the NNS detection rate at the marina and fish farm sites, or the fish farm and oyster farm sites (Figure 7).

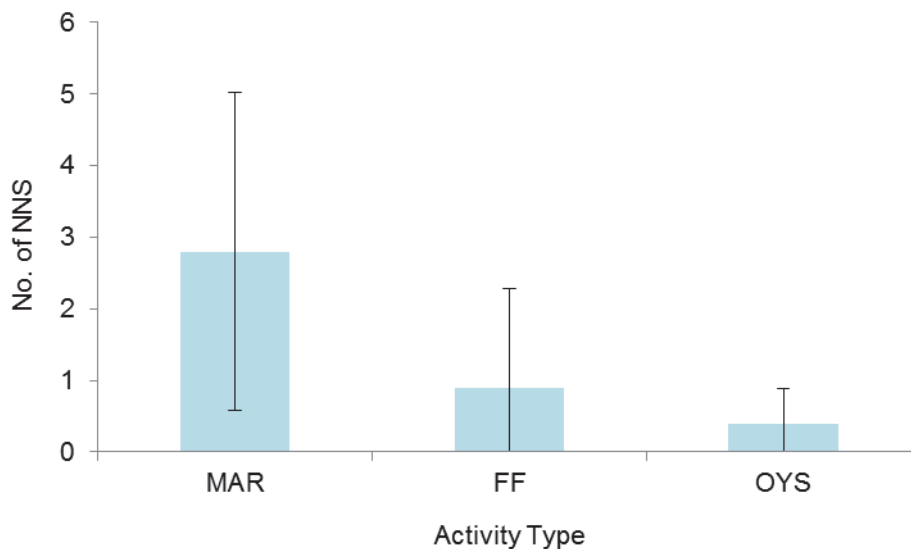


Figure 7. Number of NNS (mean  $\pm$  SD) detected by the five techniques at the three activity types ( $n=10$ ): marina (MAR), fish farm (FF) and oyster farm (OYS).

### 3.3 Interaction between techniques and activity types

A significant interaction was seen between activity type and detection technique (GLM,  $df=8$ ,  $F=21.88$ ,  $p<0.001$ ).

#### 3.3.1 Rapid assessment surveys

The RAS found a total of seven NNS: the ascidians *Corella eumyota* and *Asterocarpa humilis*, the barnacle *Austrominius modestus*, the bryozoans *Bugula simplex* and *Tricellaria inopinata*, the caprellid amphipod, *C. mutica* and the alga *Codium fragile* subsp. *fragile* (Figure 8). The majority of these species were identified on-site at the survey location, with samples of the bryozoans and the alga being verified at the laboratory.

The RAS found all six of these NNS in the marina in the Firth of Lorn and five in the marina in Loch Fyne. There was large variation between the Firth of Lorn and Loch Fyne fish farm sites, with four and one NNS respectively. Only one NNS, the barnacle *A. modestus*, was recorded at the oyster farms.

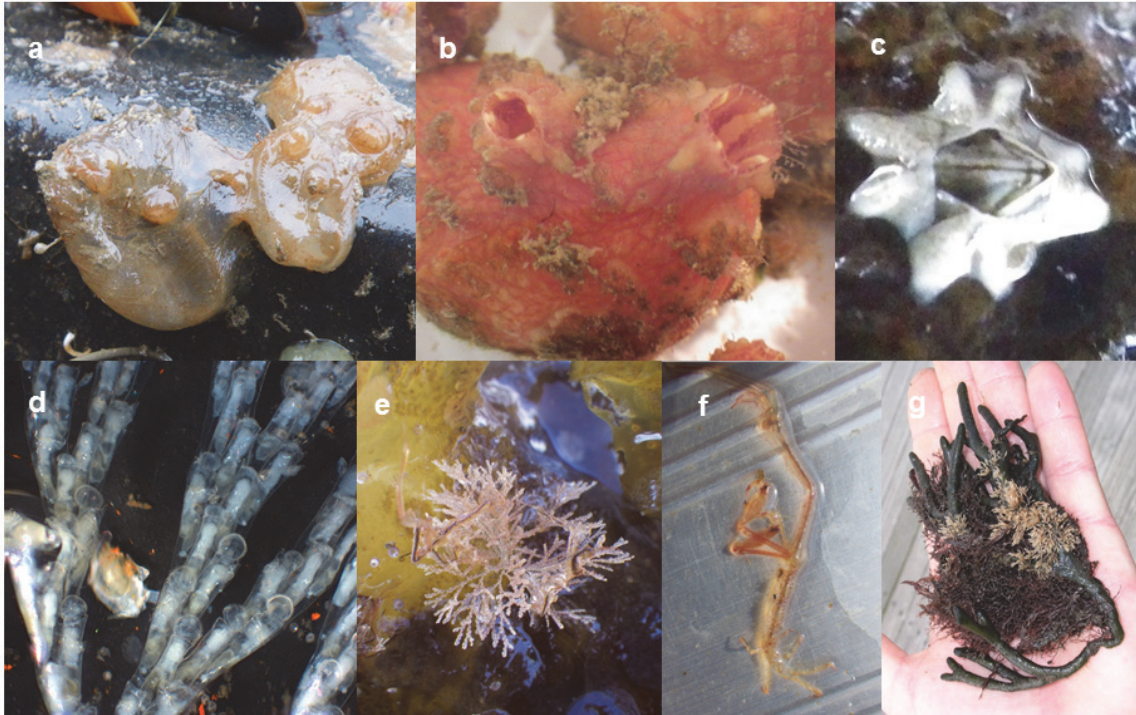


Figure 8. Non-native species found during the RAS: (a) *Corella eumyota*, (b) *Asterocarpa humilis*, (c) *Austrominius modestus*, (d) *Bugula simplex*, (e) *Tricellaria inopinata*, (f) *Caprella mutica* and (g) *Codium fragile subsp. fragile* (Photos: C. Beveridge and E. Cook, SAMS).

### 3.3.2 Settlement panels

The SP detected a total of five NNS: *C. eumyota*, *A. modestus*, *B. simplex*, *T. inopinata* and *C. mutica*. A sixth NNS, the ascidian *Asterocarpa humilis*, may have also been found on the settlement panels but, as the juvenile stage of this species (as found on the panels) has not been verified to date, this ascidian was not included in the analysis.

The SP detected the highest number of NNS in the two marinas surveyed: four species in the Firth of Lorn and five species in the Loch Fyne marina. The species that remained undetected by the SP, compared to the RAS, in the marinas were the ascidian *A. humilis* and the alga *C. fragile subsp. fragile*. At the fish farms, the SP detected three NNS at the fish farm in Loch Fyne, compared with the four species that were recorded on the RAS. Again, the alga *C. fragile subsp. fragile* was not detected on the SP. At the farm in the Firth of Lorn, the SP failed to detect the one NNS, *C. mutica*, which had been found at this site during the RAS. At the oyster farm sites, the SP only detected one NNS at the farm in the Firth of Lorn, *A. modestus*, which was also found at both sites during the RAS.

### 3.3.3 Settlement panel photography

The SPP detected one NNS, *C. mutica*, which was recorded on the settlement panels deployed in the marina in Loch Fyne. This species was found during the RAS at this site and in the laboratory analysis of the settlement panels. Ascidians, bryozoans and a barnacle were recorded from the photographs taken from the panels but identification to species level was not possible using this technique.

### 3.3.4 In-situ photography

The ISP detected one NNS, *C. mutica*, from the fish farm in the Firth of Lorn. This NNS was also detected by the RAS at this site, but not by any of the other techniques. No other NNS were recorded from any other survey site by ISP.

### 3.3.5 Scrape samples

The SCR samples detected five NNS which were also found by the other techniques: *C. eumyota*, *A. humilis*, *A. modestus*, *T. inopinata* and *C. mutica*. Two additional NNS were found in the marina in Loch Fyne using this technique – the encrusting orange bryozoan *Schizoporella japonica*, and the filamentous red alga *Heterosiphonia japonica* (Figure 9). The SCR technique detected three of these species in the marina in the Firth of Lorn and four in the marina in Loch Fyne. No NNS were found in the SCR samples collected at the two fish farms. At the oyster farm sites, this technique recorded one NNS at the Firth of Lorn site, *A. modestus*, which was also found by the RAS and SP.

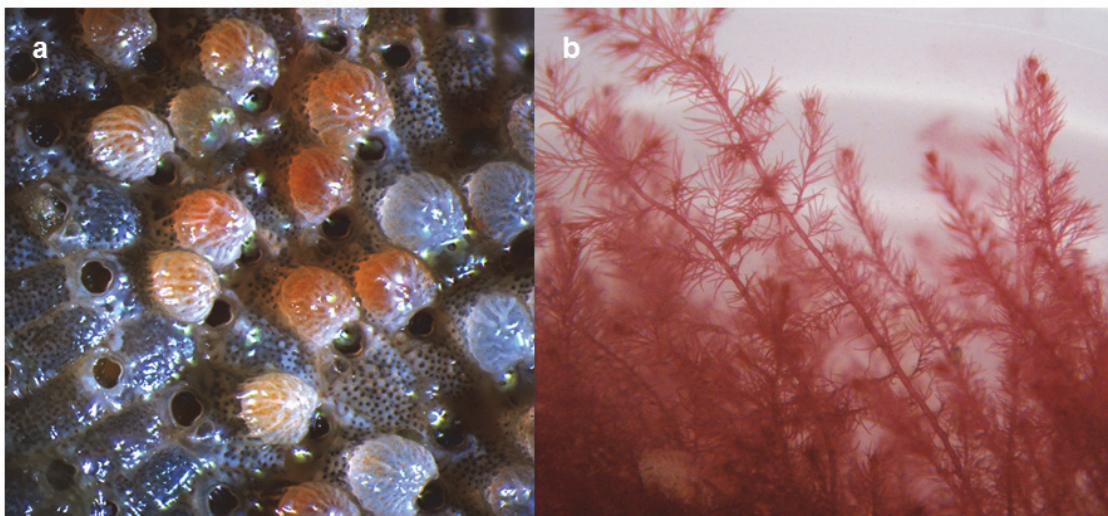


Figure 9. Two additional NNS found by the scrape sample technique: (a) *Schizoporella japonica* and (b) *Heterosiphonia japonica*. Photos: C. Beveridge, SAMS

The additional 10 scrape samples contained a total of seven NNS in the marina in the Firth of Lorn (Table 5) compared to the three species previously found at this site based on three scrape samples. The results showed that, for this site, the appropriate minimum number of scrape samples to effectively detect the seven recorded NNS was four scrape samples (Figure10).

Table 5. NNS recorded in each of the 10 scrape samples randomly collected from the Firth of Lorn marina.

Species	Scrape No.									
	1	2	3	4	5	6	7	8	9	10
<i>Caprella mutica</i>	√	√	√	√	√			√		√
<i>Corella eumyota</i>	√	√	√					√		√
<i>Heterosiphonia japonica</i>	√	√		√	√	√	√		√	√
<i>Schizoporella japonica</i>	√		√	√		√	√		√	
<i>Asterocarpa humilis</i>		√	√		√					√
<i>Tricellaria inopinata</i>			√							
<i>Austrominius modestus</i>				√						
<b>TOTAL</b>	<b>4</b>	<b>4</b>	<b>5</b>	<b>4</b>	<b>3</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>4</b>

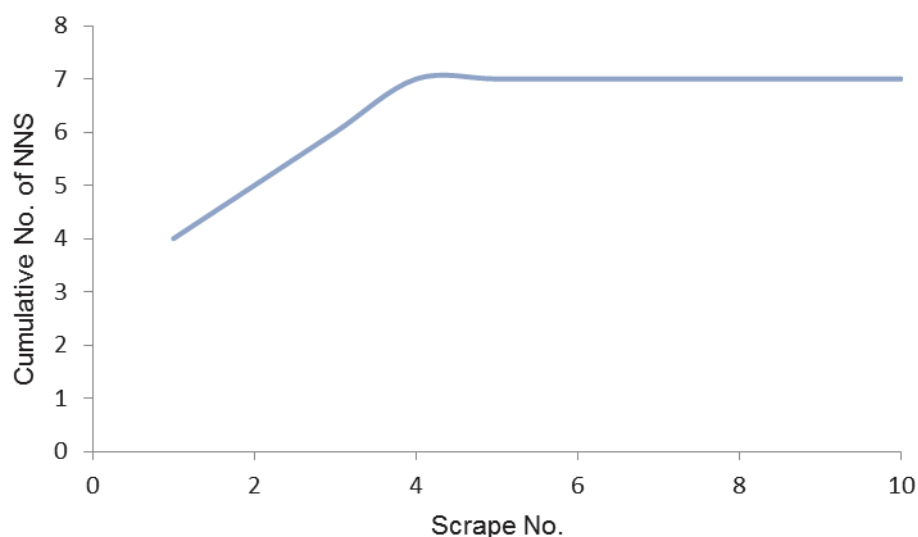


Figure 10. Cumulative number of NNS sampled by increasing number of randomly collected scrape samples in the Firth of Lorn marina.

### 3.3.6 Additional sampling locations

#### Inshore SEPA monitoring buoys

Two NNS were found on the monitoring buoys – the bryozoan *T. inopinata* and the caprellid *C. mutica*. The bryozoan was recorded in a quick scrape sample that was collected from the Gunnet Ledge Buoy, located in the Firth of Forth, on the east coast of Scotland. *C. mutica* was recorded on two panels recovered from the Dunoon Buoy, located in the Clyde Estuary, on the west coast. In addition to the NNS found on the panels deployed from the Dunoon Buoy, a number of native species were recorded: keel worms (*Pomatoceros* sp.), barnacles (*Verruca stroemia* and *Balanus balanus*), encrusting bryozoans (*Membranipora membranacea* and *Celleporella hyaline*), hydroids (*Obelia* sp. and *Tubularia* sp.), diatoms, the brown filamentous alga *Giffordia* sp., native caprellids (*C. linearis*) and the amphipod *Jassa falcata*.

### NLB navigation buoys

Two NNS were found on the NLB navigation buoys that were sampled by trained NLB crew members – the tunicates *Corella eumyota* and *Styela clava*. *C. eumyota* was recorded on both the Bono Rock buoy (near Easdale, west coast) and the Milleur Point buoy (near Loch Ryan). *S. clava* (1 individual, 9 cm in length) (Figure 11) was only recorded at the Milleur Point buoy. Both NNS were found on the lower section of the buoy, known as the 'skirt'.



Figure 11. The clubbed tunicate *Styela clava* (Photo: C. Beveridge, SAMS)

### **3.4 Comparison of costs between the sampling techniques**

The costs for the five sampling techniques used in this study are shown in Table 6. The costs were calculated on a per-site basis and, where SAMS staff were involved, these included sites which could be reached in one day. For sites located at distances of greater than 50 miles from Oban, additional travel and subsistence costs would have to be included.

Based on the costs presented, the settlement panels collected by trained government agency staff during routine monitoring programmes and analysed at SAMS, were shown to be the least costly of the five techniques, followed by the RAS and the scrape samples, if collected by trained personnel who are already 'in the field'. The deployment and retrieval of settlement panels and the collection of scrape samples by SAMS staff experienced in NNS identification did prove to be the most costly of the five techniques when considered alone. However, experience has shown that RAS, SP and scrape samples can be successfully carried out at the same site visit, thus considerably reducing the costs. In addition, this study has shown that having staff experienced in NNS identification involved in the collection of the samples improved the likelihood of sighting new, previously unrecorded NNS.

Table 6. Estimated costs for each early warning sampling technique: rapid assessment survey (RAS), settlement panels (SP), settlement panel photography (SPP), in-situ photography (ISP) and scrape samples (SCR). Cost-effectiveness is calculated as the success at detecting NNS (total number of NNS identified) divided by the financial costs of the technique.

Technique	Tasks	Personnel Involved	Cost (£)	Total no. NNS identified	Cost-effectiveness
RAS	<i>Costs based on one day round trip*</i> Includes: travel and subsistence for 2 persons; consumables for preservation of samples; some laboratory analysis if required	Staff experienced in NNS identification	£1,307	7	£187
SP (1)	<i>Costs based on x5 SP from one site</i> Includes: travel and subsistence for deployment and retrieval by SAMS staff; consumables for panels; laboratory analysis of panels and preservation	Staff experienced in NNS identification	£2,310	5	£462
SP (2)**	<i>Costs based on x5 SP from one site</i> Includes: consumables for panels; laboratory analysis of panels	Staff trained in SP deployment and retrieval employed by government agency (e.g. SEPA, SNH)	£914	5	£183
SPP/ISP	<i>Costs in addition to SP (1 or 2) and based on x5 SP from one site</i> Includes: analysis of photographs***	Staff experienced in NNS identification	SP (1 or 2) + £884	1	1) £3,194 2) £1,798
SCR (1)	<i>Costs based on x5 scrapes taken at one site</i> Includes: travel and subsistence for scrape collection by SAMS staff; consumables for scrapes; laboratory analysis and preservation	Staff experienced in NNS identification	£2,064	6	£344
SCR (2)	<i>Costs based on x5 scrapes taken at one site</i> Includes: consumables for scrapes; laboratory analysis and preservation	Staff trained in scrape sample collection by government agency (e.g. SEPA, SNH) or other companies (e.g. Northern Lighthouse Board)	£1,393 <sup>^</sup>	6	£232
Combined RAS/SP/SCR	<i>Costs based on RAS, x5 SP and x5 scrapes from one site</i> Includes: travel and subsistence for deployment and retrieval by SAMS staff; consumables for panels; laboratory analysis of panels, and preservation	Staff experienced in NNS identification	£3,997	6	£666

\*Costs based on one day round trip, which could involve 1-3 sites depending on distance from Oban and location of site. \*\* Costs for training staff involved in deployment and retrieval of panels not included as would only be relevant for initial use of the SP. \*\*\* Costs do not include camera equipment (approx. £200 per camera), as assumed this equipment would be available. <sup>^</sup> Not including initial training costs (depending on initial level of taxonomic knowledge of staff; 2 day SAMS CPD NNS course approx. £300) and £100 charge per site by NLB for scrape collection from navigation buoys, as this depends on number of buoys to be sampled.



## 4. DISCUSSION

### 4.1 Comparison between techniques

#### 4.1.1 Rapid assessment survey

The most reliable and one of the most cost-effective techniques for the rapid identification of NNS was the RAS. This technique provided immediate results for the larger, more conspicuous species and enabled the collection of species for subsequent verification, if required. The RAS also had a significantly lower cost per site (approximately £1,307) compared to previous RAS costs, when large teams of taxonomic experts were employed, with costs of between £1,966 to £2,482 per site (e.g. US surveys; see review in Campbell *et al.*, 2007). The current study used two highly trained personnel and a target list of species, which was compiled prior to the survey through a detailed review of published and grey literature for the region. This study supports the conclusions of a recent study by Minchin & Nunn (2013) that this methodology is effective in the detection of certain NNS.

This RAS technique, however, is not without its disadvantages. It can only provide a point-in-time snapshot of the situation at a particular site, it is not quantifiable (i.e. the sites are sampled as a whole with no replication, resulting in a list of NNS present), and it relies heavily on highly trained personnel, who have taxonomic expertise in the field. It is also typically restricted to species that can be sampled from structures within 'arms-reach' of the surface (e.g. intertidal zones, ropes, fenders or sides of floating pontoons), thus restricting this survey to a depth of < 1 m on subtidal structures. In addition, the RAS did not detect two NNS, the orange bryozoan *Schizoporella japonica* and the red filamentous alga *Heterosiphonia japonica*, which were identified by the 'scrape' technique. *S. japonica* is relatively new to the west coast of Scotland, first recorded in Portavadie Marina in ~2011 (Ryland *et al.*, 2014), and may well have only been present in low abundance and thus not recorded in the RAS. *H. japonica* has previously been recorded on the west coast of Scotland (Moore & Harries, 2009). An abundance of native red filamentous algae typically grows on the floating pontoons in marinas and this NNS may have been missed in the RAS, due to misidentification as one of these native species.

#### 4.1.2 Settlement panels

The settlement panels were also shown to be reliable at detecting the majority of NNS found in the RAS, particularly in the marinas where a number of NNS are known to be present and well established. Interestingly, the panels detected the settlement of NNS relatively late in the season (October). Previously, panel deployment has taken place in the summer months (e.g. July to September) to maximise the chances of detecting NNS (Cook *et al.*, 2011). This finding suggests that these panels could effectively be used over an extended period of time at high risk sites. In addition, this technique can provide replicated, quantitative sampling from differing depths and sub-regions of a site over specific time periods if required (Campbell *et al.*, 2007; Cook *et al.*, 2011).

The settlement panel technique was also as cost-effective in detecting a NNS as the RAS, particularly if the panels are deployed and collected by trained personnel (typically 1-2 people), who are routinely visiting the site as part of a monitoring or maintenance programme. The cost per settlement panel (£183 or £462 depending on collection procedure) was also comparable to previous studies, which averaged approximately £117 per panel (excluding personnel time, travel and subsistence and data analysis) (see review in Campbell *et al.*, 2007).

The probability of a Type II error (or a 'false negative'), where the survey technique fails to detect a species that is present in a region, was greater when using the settlement panels compared to the RAS. At sites with potentially lower abundances or more sparse

distributions of NNS, it is possible that the sample size used in this study (3 panels) was too small to detect the NNS present and/or the panels were deployed in a location or at a time that was unsuitable for sampling for the target NNS. In the case of the non-native barnacle, *Austrominius modestus*, settlement panels did record this species at the oyster farm in Loch Fyne and at the two marinas. Yet, the panels failed to detect the same species at the other oyster farm site in the Firth of Lorn and the two fish farm sites. This suggests that local or temporal environmental conditions may have influenced the settlement patterns of this species at the different sites. The panels also failed to detect the green alga *Codium fragile* subsp. *fragile* at two sites where it was found by the RAS. This alga can reproduce sexually or parthenogenetically and this typically occurs in late summer to early autumn (Trowbridge, 1998). Panel deployment, therefore, may have been too late in the year to detect this particular species.

The use of this technique also requires extensive taxonomic expertise of both the juvenile and adult stages of each target NNS, which does not always exist. For example, the compass sea squirt *Asterocarpa humilis*, originally from the Southern Hemisphere, was first recorded in northwest Europe in 2005 (Camaret-sur-Mer Marina, Brittany, France), before spreading northwards to the south coast of England (Bishop *et al.*, 2013) and most recently to the west coast of Scotland (Oban Marina, 2013, pers. obs.). Detailed taxonomic descriptions are available for the adult specimens (Bishop *et al.*, 2013) but the juvenile stages still require description (Bishop, pers. comm.) and so were recorded here as 'unverified'.

#### 4.1.3 Scrape samples

The scrape sample technique was similar to the settlement panels in overall reliability and cost-effectiveness at detecting the NNS compared with the RAS, at all the sites except the two fish farms. Reliability could potentially have been improved if four scrape samples had been analysed instead of three at each site. In addition, the laboratory analysis of the scrape samples enabled a more detailed examination of the material collected and as a result, was able to detect two NNS that were not detected by any of the other methods tested, namely *S. japonica* and *H. japonica* in Portavadie marina, Loch Fyne. This technique also provides replicated, quantitative sampling, which can be undertaken by trained personnel that are visiting the site for monitoring or maintenance reasons. In contrast to the settlement panels, this technique does not require two visits to each site, thus reducing the cost of this method. It also allows sampling of epibenthic fouling communities from structures where it would be either prohibited to attach settlement panels (e.g. navigation buoys) or impractical in their current design (e.g. in strong tidal currents). For example, it is assumed that the exposed nature of the Gunnet Ledge Buoy led to the settlement panels either being lost or damaged whilst attached to the buoy.

This technique failed to detect the three NNS found by the RAS and the settlement panels at the fish farm site in Loch Fyne (*Corella eumyota*, *Tricellaria inopinata* and *Caprella mutica*), although all three of these species were recorded using this technique at the two marina sites. This suggests that the cage floatation devices may not be the most appropriate location on the fish farms for scrape sample collection. The curved, smooth profile, shallow draught and increased exposure to wave action of these structures may not have provided these species with optimal conditions for growth. Structures such as feed barges may provide a more appropriate substrate for sample collection.

#### 4.1.4 In-situ and settlement panel photographs

The *in-situ* and settlement panel photographs were the least reliable and had the lowest cost-effectiveness of all the techniques assessed. For both techniques, only one NNS, the large amphipod *C. mutica*, was detected from the photographs. Males can grow to over 40 mm in length, they have distinctive dorsal spines and typically stand erect from the fouling

community, so can be easily distinguished from native caprellid species and other fouling epibiota (Willis *et al.*, 2004). These two techniques failed to detect other conspicuous species that were identified by the other techniques, such as *C. eumyota* and *A. modestus*. For the *in-situ* photographs, reduced water clarity tended to obscure the image and, together with high community complexity, prevented the majority of species from being identified to species level. For the panel photographs, image quality was affected by residual water and siltation of the panel surface which prevented accurate species identification.

#### 4.1.5 Undetected NNS on target list

A number of NNS on the target list remained undetected by all five techniques. Four species have previously been recorded in marinas in the Clyde area, south of Loch Fyne: the colonial ascidians *Botrylloides violaceus* and *Didemnum vexillum*, the solitary ascidian *Styela clava* and the macroalga *Sargassum muticum* (Ashton *et al.*, 2006, Beveridge *et al.*, 2011). The latter two species have also been recorded in the Firth of Lorn (see review by Harries *et al.*, 2007; Cook *et al.*, 2013a) and *S. clava* was recorded in a scrape sample taken from the NLB navigation buoy at Milleur Point, Loch Ryan. In the case of the colonial ascidians, these two species have only been found in specific areas in the Firth of Clyde and may not yet have spread northwards to Loch Fyne and the Firth of Lorn. *Styela clava* and *S. muticum* have been found within 5 km of both the fish farm and marina study sites in the Firth of Lorn. The former was recorded by divers and beam trawl from subtidal sites (Cook *et al.*, 2013a; Beveridge, pers. obs.), which suggests that it is now spreading via natural dispersal on the west coast of Scotland, so would not necessarily be found in areas of anthropogenic activity. *Sargassum muticum*, is also typically spread by natural fragmentation and drift, although vessels have also been implicated in its spread (Harries *et al.*, 2007). Attached plants are generally found in intertidal rock pools and in the sub-littoral environment to a depth of a few metres (Andrew & Viejo, 1998). Unattached, floating specimens have previously been found in enclosed marinas in the Firth of Clyde (Ashton *et al.*, 2006; Beveridge *et al.*, 2011); however, to date, *S. muticum* has not been found attached to floating pontoons or settlement panels. Intertidal rocky shore surveys may therefore provide a more reliable detection technique for this species.

The remaining NNS on the target list, including the macroalgae *Undaria pinnatifida*, *Asparagopsis armata* and *Colpomenia peregrina*, *Crassostrea gigas* and *Crepidula fornicata* have not been found to date in Scottish marinas (Ashton, 2006; Beveridge *et al.*, 2011). The macroalgae *A. armata* and *C. peregrina* have been previously reported from the west coast of Scotland but only from natural subtidal habitats in the region. The Pacific oyster, *C. gigas*, has also been recorded in Scottish waters, although this was further south in the intertidal zone of the north coast of the Solway Firth (C. Beveridge, pers. obs.) and in the Firth of Forth (Smith *et al.*, 2014), so lower surface seawater temperatures may be restricting the spread of this species northwards at present (Cook *et al.*, 2013a). *Crassostrea gigas* has been recorded in marinas on the south coast of England (J. Bishop, pers. comm.) and the Isle of Man (E. Cook, pers. obs.), but in Scotland it is highly likely that this species will spread northwards by natural dispersal of the pelagic larval phase. As in the case of *S. muticum*, targeted intertidal rocky shore surveys may prove more reliable in their detection than the techniques assessed in this study.

The two final species on the target list have yet to be reported in Scotland. *Undaria pinnatifida* was included in this study due to the potential for rapid northwards spread. This species has been recorded in marinas on the south coast of England (Arenas *et al.*, 2006) and, more recently, attached to floating pontoons in Liverpool docks, north-west England (S. Brown, pers. comm.) and in Carrickfergus marina, Northern Ireland (Nunn & Minchin, 2013). In the case of *C. fornicata*, there is potential for accidental introduction via aquaculture activities (Cook *et al.*, 2013a). Both species have previously been recorded using the RAS technique (Arenas *et al.*, 2006; Nunn & Minchin, 2013; J. Bishop, pers. comm.) and are

large, relatively conspicuous NNS. It is likely, therefore, that as they were not recorded in the current study, the probability of either species being established in the study area is low.

## 4.2 Comparison between activities

### 4.2.1 Marinas

Hull fouling on vessels (both commercial and recreational) and aquaculture activities are two of the most important vectors associated with the introduction of NNS to a new region (Minchin *et al.*, 2013). Marinas and ports typically have high volumes of vessel traffic from a wide variety of ports of origin (Ashton *et al.*, 2006; Ware *et al.*, 2014), they are generally relatively sheltered by breakwaters and/or seawalls from the prevailing wind and, as a consequence, provide ideal refuges for NNS (Ashton *et al.*, 2006). The results of this study found that the marinas surveyed had significantly higher numbers of NNS compared with the oyster farm sites. The latter are typically sited in more remote locations and are subjected to fewer vessel movements than the marinas, which may account for the lower numbers of NNS.

### 4.2.2 Aquaculture activities

Aquaculture activities are also found in relatively sheltered environments and, in some cases, have accidentally introduced NNS to on-growing sites as an unintentional consequence of stock movements (Minchin *et al.*, 2013).

#### Oyster farm sites

Oyster farms now purchase their spat from UK hatcheries, rather than from abroad as was the practice in the past (Minchin *et al.*, 2013), thus primarily minimising the risk of introducing disease, but also NNS. The oyster farm sites surveyed provided a relatively hostile environment for many NNS because a) they experienced the greatest fluctuation in temperature relative to the other sites (>10 °C difference recorded between the maximum and minimum temperatures over the study period) due to their intertidal nature, and b) they were located close to rivers so may have been exposed to lower salinities. Interestingly, the only NNS found at both oyster farms was the barnacle *A. modestus*, which has a pelagic larval phase and is found extensively in intertidal habitats throughout the west coast of Scotland.

#### Fish farm sites

The fish farm sites experience a greater volume of vessel traffic than the oyster farms. They are visited both by the routine daily maintenance vessels, which are typically located relatively close to the fish farm, and by larger supply vessels, which periodically supply the farm with food or stock and originate from further afield. One NNS was found at both fish farms, the amphipod *C. mutica*. This species has previously been found in high densities on the fish farm in the Firth of Lorn and other sites throughout the west coast of Scotland (Ashton *et al.*, 2007), suggesting that these sites provide ideal conditions for this species to thrive (Cook *et al.*, 2006). Additional NNS were found at the fish farm site in Loch Fyne, namely *C. eumyota*, *T. inopinata* and *C. fragile* subsp. *fragile*, which is the first time that these species have been recorded on a fish farm in the UK. However, these species were not found at the fish farm site in the Firth of Lorn, even though they were detected in the marina. This suggests that fish farms may not be as reliable a site for the early detection of NNS compared with marinas.

### 4.2.3 Monitoring and navigation buoys

As only one or two techniques were assessed using the SEPA monitoring buoys and NLB navigation buoys, at sites outwith the two sampling regions, their overall effectiveness cannot be effectively compared with the other activities. It is important to note that NNS were

found on both types of buoy, and that the tunicate *S. clava* was only found on a NLB buoy and not at any other site sampled in this study. This buoy was adjacent to Loch Ryan, where a large population of *S. clava* is known to exist (Minchin *et al.*, 2013). The flora and fauna collected by the NLB crew and preserved as frozen samples were in good condition, with the exception of the polychaetes which disintegrated on defrosting.

### 4.3 Conclusions and recommendations

The RAS was the most reliable and cost-effective technique in detecting NNS at all the sites surveyed, followed by the settlement panel and scrape sampling techniques. This supports the findings of previous RAS surveys throughout the UK (Arenas *et al.*, 2006; Ashton *et al.*, 2006; Minchin & Nunn, 2013; Nall *et al.*, 2015) and suggests that this method could become a UK-wide standard and incorporated into future surveillance strategies. It could also be used at both intertidal and subtidal sites for a wide range of maritime activities including ports, harbours, marinas and aquaculture sites.

The two photographic techniques proved highly unreliable in detecting NNS in this study. Although cheaper than the other techniques, it is recommended that this technique is only used to survey for large, conspicuous NNS (such as the tunicate *S. clava* and the oyster, *C. gigas*) in combination with the collection of material for verification.

None of the techniques evaluated were able to detect all the NNS that were found, so a combination of the RAS (in conjunction with a target list of species) and either the settlement panels or the scrape samples would provide the most reliable and quantifiable technique for NNS detection in the short to medium term (see Table 7 for a summary of the advantages, disadvantages and potential improvements that could be made to each of the sampling techniques). For the longer term early detection of NNS, the use of specific DNA markers in water or in the organisms themselves may significantly enhance surveillance programmes in the future.

Marinas were found to be the most successful location for the detection of epibenthic NNS, although fish farms and monitoring/navigation buoys may provide additional locations for early warning monitoring stations. Marinas are typically located in sheltered bays or man-made enclosures, many are adjacent to major international ports and they provide safe access to floating pontoons, which can be easily surveyed for NNS. Over 200 marinas exist around the UK coastline (Foster, 2013) and it is recommended that marinas located in areas at high risk for the introduction of NNS (e.g. in regions of high international/national vessel activity), should be surveyed by trained personnel on an annual basis.

These techniques and others will only be successful in the early detection of new NNS if experienced personnel are conducting the RAS and analysing the samples. Thus, the training of future NNS taxonomists is paramount to the success of any future UK early warning system. Personnel trained in either settlement panel deployment and/or scrape sample collection that are already visiting sites for routine monitoring or maintenance of a structure could significantly reduce the analysis costs and thus provide a more cost-effective early warning system.

The techniques assessed in this study are primarily used to detect epibenthic NNS. Techniques for sampling soft sediment (e.g. diver-operated or surface-deployed corers), the seabed (e.g. benthic sleds, beam trawls and traps) and the water column (e.g. plankton nets) for NNS have been successfully employed in extensive sampling campaigns in Australia and New Zealand (Hewitt & Martin, 2001), but were not assessed as part of this study and still require further investigation in UK coastal waters.

To conclude, European countries now have obligations to strictly control the introduction and to prevent the spread of INNS under a number of policies, including the MSFD, the 2020 Biodiversity Strategy and the recent Regulation on the prevention and management of the introduction and spread of INNS. A network of monitoring sites throughout Scotland and the UK, that are regularly surveyed using techniques such as the rapid assessment survey, in combination with a 'target list' based on a horizon scanning exercise, could be an effective method of rapidly recording new introductions of marine INNS in the short to medium term, whilst the molecular DNA techniques are further refined.

Table 7. Summary of advantages, disadvantages and potential improvement for the five early warning techniques assessed: rapid assessment survey (RAS), settlement panels (SP), settlement panel photography (SPP), in-situ photography (ISP) and scrape sampling (SCR).

Technique	Advantages	Disadvantages	Potential Improvements
RAS	<p>Rapid, immediate results obtained (unless verification required).                      &gt;80% of NNS established at the site identified.                      Typically 1–3 sites sampled per day.                      Most cost-effective of all the techniques assessed.</p>	<p>Requires highly trained staff in wide range of taxa, including NNS.                      Only identify species established at the time of the RAS.                      Requires relatively sheltered sites with easy access to submerged structures, which is not always possible at all sites.</p>	<p>To conduct annual RAS in the summer (July – Sept) at sites subject to high commercial and recreational vessel movements, with the potential to increase RAS frequency (2-3 per year) at high risk sites.</p>
SP	<p>Relevant staff can be easily trained to deploy and retrieve panels whilst undertaking other routine monitoring work.                      Inexpensive to construct.                      Could be used for intensive monitoring at high risk sites, in conjunction with RAS.</p>	<p>Requires two trips to a site for deployment and retrieval at specified interval.                      Requires skilled NNS experts for identification of juvenile stages.                      Incomplete understanding of juvenile stages of some NNS, preventing confirmation of species identity.                      Less reliable than RAS in detecting NNS.                      Unlikely to detect highly mobile benthic species or species with seasonal trends.                      Potential for loss of panels at more exposed sites.</p>	<p>Greater understanding of NNS spawning behaviour and timing.                      Improved skills development in identification of juvenile stages of NNS.                      Improved knowledge regarding the placement of settlement panels throughout a site to improve detection rates.</p>
SPP	<p>Less expensive than laboratory analysis of SP.                      Photos can be rapidly assessed, with NNS identified within a short period of time.                      Good for identifying large, conspicuous NNS, if image quality is good.</p>	<p>High probability of poor image quality.                      Difficult to identify with accuracy majority of NNS using this technique.                      Difficult to identify less conspicuous species once SP has become heavily fouled (&lt; 6 weeks in summer months).</p>	<p>Improvements in techniques to reliably provide high image quality.                      Increased numbers of photographs taken for each panel, including macro-photos of unusual or dominant species.                      Requirement to only take photographs of panels deployed for ≤ 6 weeks, particularly in summer.</p>

ISP	<p>No requirement for settlement panels. Good for identifying large, conspicuous NNS, if image quality is good. Photos can be rapidly assessed, with NNS identified within a short period of time.</p>	<p>High probability of poor image quality. Difficult to identify with accuracy majority of NNS using this technique. Difficult to identify less conspicuous species on heavily fouled surfaces.</p>	<p>Improvements in techniques to reliably provide high image quality (e.g. image stacking using multiple images). Increased numbers of photographs taken for each area sampled, including macro-photos of unusual or dominant species.</p>
SCR	<p>Excellent for detecting small, inconspicuous NNS that may not be detected using the other techniques. Staff from other organisations can be easily trained to take scrape samples, whilst undertaking other routine monitoring work.</p>	<p>Most expensive technique, due to time required to process the typically greater quantities of material collected. Requires smooth, flat surface for sample collection, which is not always available. Can only reliably collect samples from surface to depth of 0.5 m, when surface to be sampled is submerged.</p>	<p>Refinement of collection system to allow samples to be obtained at greater depths. Improved knowledge regarding scrape sampling locations throughout a site to improve detection rates.</p>



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## ANNEX 1: PROTOCOL FOR SCRAPE SAMPLING PROVIDED TO CREW MEMBERS OF THE NORTHERN LIGHTHOUSE BOARD VESSEL MV POLESTAR, IN ADDITION TO SHORT TRAINING SESSION

### Aim

To take two random samples (15 x 15 cm) from the 'top wall' and two from the 'skirt' (Figure 1). Although specific areas of the buoy are to be sampled, ensuring that no deliberate choices are made about which patch of fouling to remove ensures that samples are taken randomly and will on balance represent the organisms which are there. There are a number of ways to do this; the most straightforward will be to take a sample from the part of the buoy which is most fore and most aft as the buoy is landed on the deck in a random fashion.



Figure 1: Showing the various parts of the buoy.

### Before arriving at the Buoy

- 1) Please prepare bags and labels detailing: the date, the navigation buoy, and location of the area sampled on the buoy (Top or Bottom).

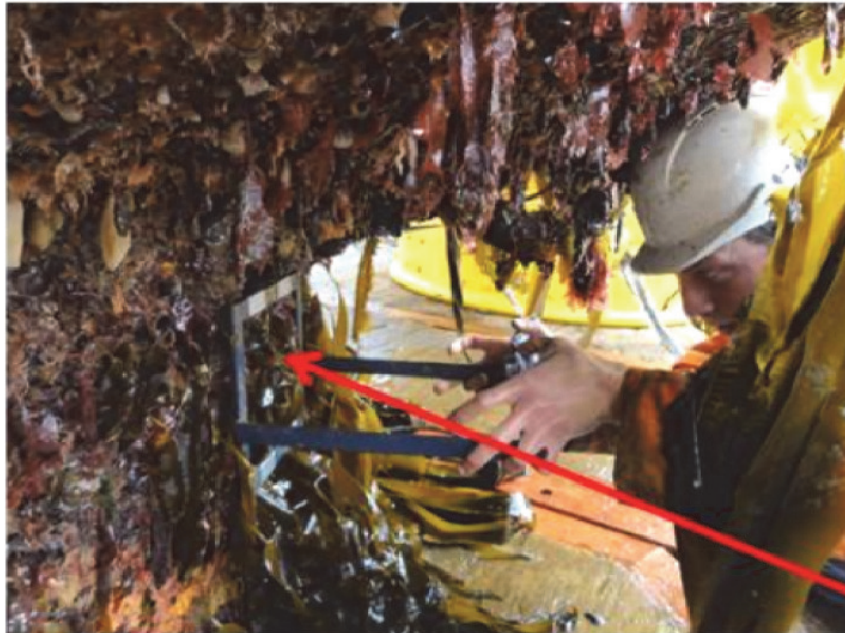
### During Buoy maintenance

- 2) Samples must be chosen randomly from the zones specified on the buoy (Figure 2)
- 3) Using the white reference squares as a guide, scrape an area 15cm by 15cm, catching all the contents including large kelp within the area sampled.
- 4) Place all the contents into the zip lock bag provided along with the label removing excess air from the bag.

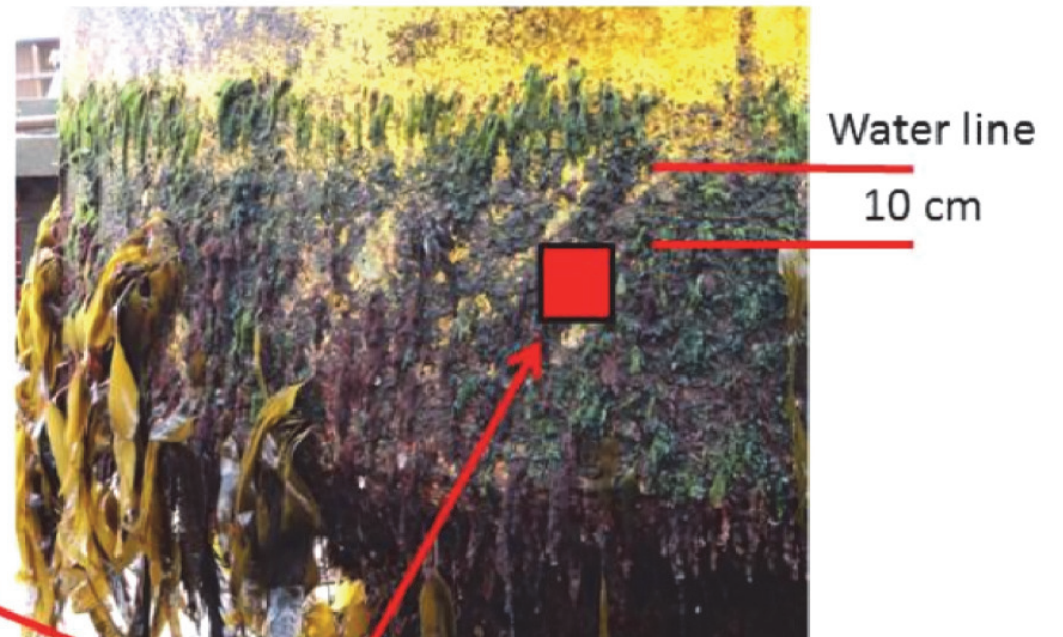
### After buoy maintenance

- 5) Double the bag and place the samples in the freezer for later collection.

Skirt Sample



Top Wall Sample



Sample Area

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