Research Article

The value of regular monitoring and diverse sampling techniques to assess aquatic non-native species: a case study from Orkney

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Abstract

A monitoring programme for marine and brackish water non-native species (NNS), initiated by Orkney Islands Council, has produced comparable data from multiple sites since 2012. Sampling was performed at both natural habitats and areas under anthropogenic influence, such as marinas, and has included rapid assessment, wall scrape, settlement panel, benthic grab and phytoplankton samples, from which 15 NNS and 12 cryptogenic species have been recorded, of which three NNS (*Boccardia proboscidea*, *Asterocarpa humilis* and *Melanothamnus harveyi*) and one cryptogenic (*Ctenodrilus serratus*) represent new records for Orkney. A historical bibliographic and database review, conducted also within this study, shows these results to represent 71% of all non-native and 60% of all cryptogenic species ever found to have been identified for Orkney (total 41 non-native or cryptogenic species). The most widespread non-native species found in the present study were red algae (*Melanothamnus harveyi* and *Bonnemaisonia hamifera*), the bryozoan *Schizoporella japonica* and the Japanese skeleton shrimp (*Caprella mutica*). Many of the benthic non-native species recorded were found in multiple sample types but some of the smaller species were missing from rapid assessment samples. Additional methods and locations would be necessary to produce a complete inventory of non-native species in Orkney, as evidenced by comparison with records from other sources. The programme has provided a valuable baseline, including new Orkney records for some non-native species. Continuity and comparability of future surveys will be essential to monitor changes in the distribution and abundance of current non-native species and for tracking new arrivals.

Key words: port biological survey, ballast water management, fouling, cryptogenic species, non-indigenous species, alien species, introduced species

Introduction

Non-native species (hereafter NNS), also referred to as non-indigenous or introduced species, are a global issue (Bax et al. 2002). In Great Britain, approximately 10–12 new non-native species are estimated to be established annually in terrestrial and aquatic environments (GB NNSS 2015). Non-native species have been defined as fauna or flora that have been introduced to a new area by either human-mediated methods such as aquaculture (Grosholz et al. 2015), shipping (Seebens et al. 2016) or leisure craft (Darbyson et al. 2009), or by long range dispersal such as rafting on human-made objects (Carlton 1989; Carlton et al. 2017). A sub-set of NNS can become invasive (pests) if, once in their new environment, they spread rapidly leading to detrimental environmental or economic impacts or cause harm to human health (GB NNSS 2015). Cryptogenic species have been defined as those that are neither demonstrably native nor non-native and whose origins are unknown (Carlton 1996).

The Orkney Islands Council Ballast Water Management Policy (OIC BWMP) was implemented to a large extent in response to international concerns over non-native species in ballast water. To control the spread of marine non-native (including invasive) species due to shipping, the International Convention for the Control and Management of Ships Ballast Water and Sediments 2004 was established (IMO 2004, hereafter "The Convention", ratified September 2017). The Convention sets global standards for ballast water exchange and treatment, both of which are mitigation measures to reduce the transfer of harmful aquatic organisms and pathogens from one region to another (IMO 2004). The Convention also includes a requirement of all member states to "monitor the effects of ballast water management in waters under their jurisdiction" (IMO 2009). The monitoring of the environment receiving the ballast water has been generally interpreted to mean any shipping port or harbour area which receives ballast water discharge (Hewitt and Martin 2001; HELCOM 2013; Awad et al. 2014). Although the programme was instigated in response to concerns over ballast water, there are several other known vectors of NNS. Biofouling on the hulls of commercial and recreational vessels is one important example (Williams et al. 2013; Ferrario et al. 2017; Marić et al. 2017; Ulman et al. 2017). To effectively monitor the effects of The Convention and to provide early warning of any newly arriving NNS, it is vital to know the baseline of marine organisms (native, non-native and cryptogenic) in the port environment.

The Baseline Port Surveys for Introduced Marine Species were developed in Australia by Hewitt (1996) and the protocols were revised in 2001 by Hewitt and Martin (2001). Over 19 countries have since applied similar port baseline surveys (Campbell et al. 2007). Following the revised guidance from Hewitt and Martin (2001), the Scapa Flow harbour area baseline survey and the subsequent marine NNS monitoring programme for the Scapa Flow harbour area were developed as integral parts of the "Ballast Water Management Policy for Scapa Flow" (BWMP) by the Orkney Islands Council Harbour Authority (OICHA 2017). Scapa Flow is a large (324.5 km^2) deep water sheltered anchorage in the southern part of the Orkney Islands (Figure 1B) with a long history (since 1980's) as a location for ship-to-ship oil transfers at anchor. A designated Special Area of Conservation (SAC), Loch of Stenness (SAC EU code UK0014749), the largest saline lagoon in the UK, is connected to Scapa Flow by a narrow channel at Brig O'Waithe. The OICHA BWMP and the NNS baseline survey and monitoring plan are integral for ensuring the protection of the Loch of Stenness SAC from NNS. The planning process for the Scapa Flow harbour baseline survey and monitoring programme commenced in early 2012. After a full consultation and advice from national bodies and experts, the baseline survey for Scapa Flow and monitoring plan for the Scapa Flow harbour area was finalised in 2012. The baseline survey in the Scapa Flow harbour area was completed in 2013, prior to the adoption of the BWMP in December 2013. The on-going annual marine NNS monitoring programme in Scapa Flow commenced in 2014. In addition to the Scapa Flow baseline survey and monitoring programme, Orkney moorings for visiting yachts and other sites have been surveyed separately from the programme, to fully understand the presence and distribution of NNS in the Orkney Islands, Supplementary material Table S1.

The main objective of this paper is to bring together records of NNS and cryptogenic species from the OICHA BWMP monitoring programme and from additional surveys, in order to determine the prevalence of these species in Orkney and to assess the effectiveness of the different methods used in the programme. Records include (A) scrape sample data from Orkney moorings for visiting yachts (2012–2017), (B) the Scapa Flow harbour area port baseline survey data from 2013, (C) the on-going Orkney non-native species monitoring data from 2014 to 2017 and (D) other surveys as described in Supplementary material Table S1. In addition, sources (bibliography, databases) of Orkney NNS historical records have been extensively reviewed in order to provide context for the more recent records from the programme which are reported in this study.

Materials and methods

Review of historical introduction records

Historical records of Orkney NNS and cryptogenic species were reviewed from other sources. The introduction histories of the species have been researched to illustrate the significance of the Orkney records in global and national contexts. This included a review of published and unpublished literature, including Marine Nature Conservation Review (MNCR) reports, and searches of relevant databases, such as NBN and the Orkney Biodiversity Records Centre. The history of introductions outside of the native range of the relevant species was reviewed through the published literature and summarised as part of the process of record collation.

Study area

As part of the OICHA BWMP, a selection process for monitoring sites was undertaken. This involved mapping all human-made structures within Scapa Flow and Loch of Stenness, as well as modelling the ballast water discharge pattern. During the OICHA BWMP planning, it was highlighted that no new structures, such as buoys, were allowed to be placed in Scapa Flow or Loch of Stenness, as these could potentially act as stepping stones for NNS. Therefore, it was paramount that all current human-made structures were considered in the selection process. In total, nine structures were chosen for the 2014–2017 on-going NNS monitoring programme. These comprised four intertidal sites (Loch of Stenness sluice gates, Brig O' Waithe Bridge, Bu Point concrete outflow pipe from the waste water treatment facility and Moaness pier), two sub-tidal sites (The Hurdles sub-marine defence netting and the SMS Cöln shipwreck sunk in 1919) and three navigation buoys (The Grinds, Vanguard and Gutter Sound). In addition to the sampling of human-made structures, it was recommended that soft sediment samples be collected from the seabed beneath the ship-to-ship transfer locations and that plankton tow samples be collected from three areas within Scapa Flow.

Annually, Orkney Harbour Authority deploys 18 moorings for visiting yachts. Each spring, these moorings are placed in sheltered locations around the Orkney Islands and left for the summer to enable visiting leisure craft to tie up. At the end of the summer, the moorings are removed from the water. As these moorings and their associated buoys are part of an on-going Harbour Authority activity, the decision was taken to use the buoys as "settlement panels". Every autumn during 2012–2017, when the buoys and moorings were removed from the water and prior to them being cleaned, samples were collected for NNS monitoring.

The three Orkney marinas, Kirkwall, Stromness and Westray, were included in the 2014–2017 annual NNS monitoring programme. Kirkwall Marina is north facing, Stromness Marina is within Scapa Flow and Westray Marina is on the island of Westray, which is one of the northern islands; Westray Marina is a seasonal marina, only operational during summer, with pontoons removed from the water each winter.

In addition to these survey sites, others have been added opportunistically as appropriate. Other mooring or navigational buoys (Ore Bay and Hatston mooring buoys, Royal Oak buoy and Riddock Shoal navigation buoy) have been sampled for NNS when maintenance schedules have enabled access. Ore Bay and Hatston mooring buoys were sampled on the quay; Royal Oak and Riddock Shoal buoys were examined on the deck of the maintenance vessel. Opportunistic pier and harbour wall surveys have been conducted (Burwick Pier, Houton Pier, Kirkwall Pier and Harbour, Scapa Pier, Tingwall Pier, Flotta Piers and Flotta Jetty) to establish a better understanding of the distribution and presence of NNS in Orkney.

All sampling locations are presented in Figure 1 with site numbers in Supplementary material Table S1.

Sampling methods

Sampling methods used at each location are presented in Supplementary material Table S1.

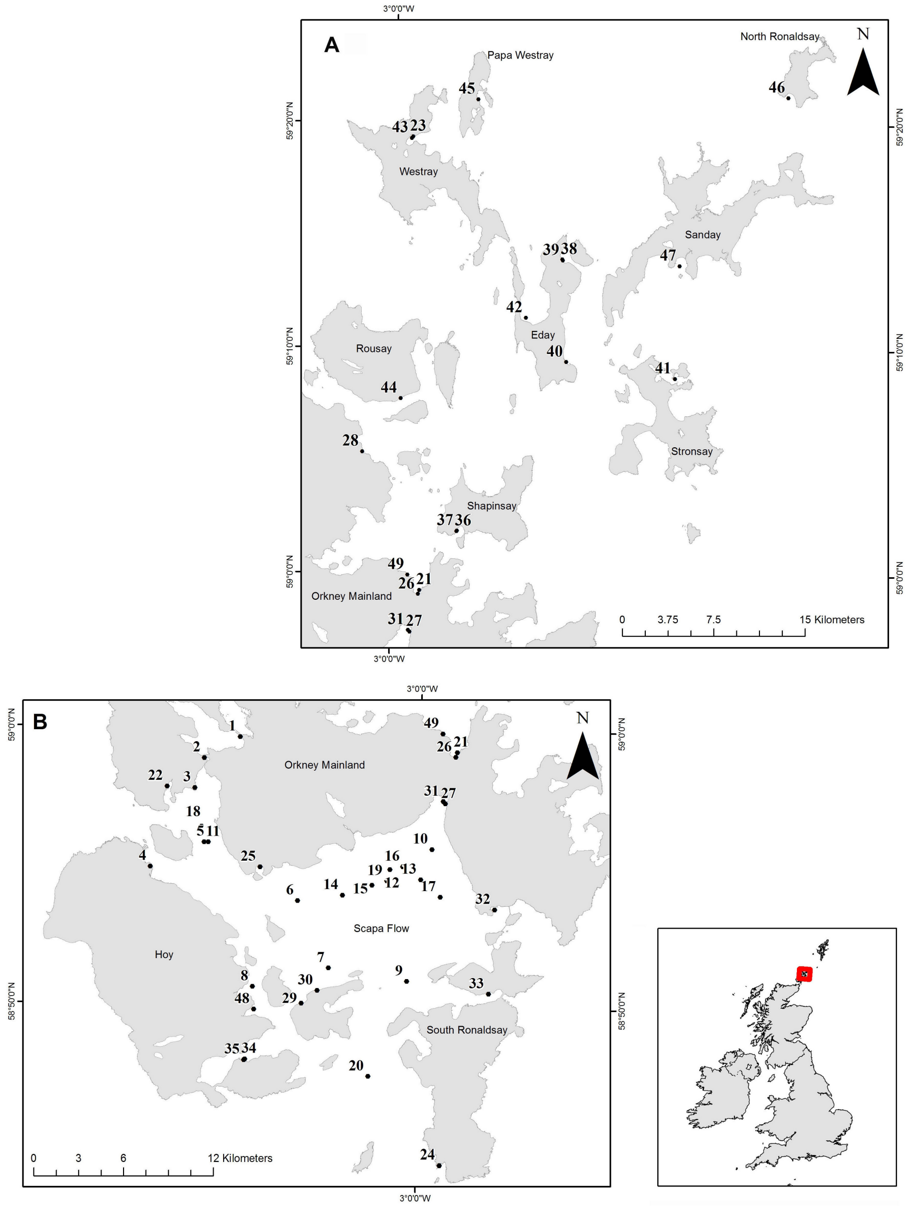

Figure 1. Locations of the monitoring sites. A: North of Orkney Mainland and northern isles, B: Scapa Flow and southern isles. For corresponding site names refer to Supplementary material Table S1.

Rapid assessment surveys

A rapid assessment methodology (Hewitt and Martin 2001; Pederson et al. 2003), comparable to recently published methods (Arenas et al. 2006; Nall et al. 2015; Bishop et al. 2015a), was used at all sites in the Scapa Flow harbour area, apart from sediment, sub-tidal and plankton sampling sites (Supplementary material Table S1). At marina sites, floating pontoons, ropes, fenders, buoys and small ladders were inspected and samples collected of all marine NNS or suspected NNS found. At intertidal, pier and harbour wall sites, a set area (approximately 20 m^2) was searched rigorously for a set time (maximum 30 mins) and samples of all suspected marine NNS were collected. The navigation and mooring buoys were fully searched and samples of all suspected marine NNS were collected.

Table 1. Taxonomic breakdown of non-native and cryptogenic species in the Orkney Islands.

Phylum	NNS	Cryptogenic
Cnidaria	1	
Annelida	1	2
Arthropoda	3	5
Mollusca	3	4
Bryozoa	3	2
Chordata	2	7
Ochrophyta	2	
Rhodophyta	5	
Chlorophyta	1	
Myzozoa		1

Hard substratum sampling

All intertidal monitoring sites were selected to include at least one human-made structure which was suitable for collection of scrape samples. At these sites, two scrape samples of 15 cm × 15 cm were collected and all material placed into a sampling container.

Scrape samples were also collected from harbour and pier walls, from navigation and mooring buoys and (2017 onwards) from marinas.

Plankton tows

Phytoplankton and zooplankton tows were taken from a static boat. For each phytoplankton sample, a 20 m drop net trawl was taken using a 50 cm diameter net with a 20 μm mesh, as recommended by Hewitt and Martin (2001). Zooplankton sampling methods were identical to phytoplankton sampling but using a 100 μm mesh (Hewitt and Martin 2001). Three replicate samples were collected for each plankton sample type at each site.

Benthic samples

In 2013, benthic samples were collected using a Remotely Operated Vehicle at two sites (between STS2 and STS3 and Between STS3 and STS4). A small core sampler of approximately 3.5 cm diameter and 10 cm depth was used to collect three replicate samples at each location.

From 2015 onwards, methods for benthic sampling were aligned with standard benthic sampling (Davies et al. 2001) and with the Scottish Environment Protection Agency's benthic sampling methods (O'Reilly 2012). Samples were collected using a 0.1 m^2 van Veen grab deployed from a vessel, with three replicate samples collected at each site and sieved over a 0.5 mm mesh.

Remotely Operated Vehicle surveys

Remotely Operated Vehicle (ROV) surveys were conducted in 2013 at two sub-tidal sites, The Hurdles and SMS Cöln. The surveys at these sites took a minimum of 30 minutes, during which both video and still images were taken. No physical samples were collected during these surveys.

Settlement panels

Correx (corrugated plastic) settlement panels were made in accordance with recommendations from The Marine Aliens II Consortium (Cook et al. 2011). The settlement panels have a vertical panel of 18 cm × 15 cm and a horizontal panel of 15 cm × 15 cm, with two connecting panels of 15 cm × 8 cm. A small weight was attached to the inside of the panel to keep it vertical in the water and the panels were lightly sanded to roughen the surface. The panels at Loch of Stenness, Brig O'Waithe and Bu Point were hung from a small buoy, immersed in water at all times, approximately 1 m below the water surface and left *in situ* for 6–9 weeks. The settlement panels on the marinas were hung between the marina pontoons: at Kirkwall and Stromness Marina, they were at 1 m below the water surface; at Westray Marina, they were at 0.5 m below the water surface. The settlement panels were left in the marinas for 9–14 weeks.

Temperature and salinity

Seawater temperature and salinity were measured at most of the sampling locations (Supplementary material Table S1). Water temperature was recorded in the field using a Cole-Parmer Traceable® Waterproof thermometer. TinyTag© temperature loggers have been deployed in the marinas and on the navigation buoys since summer 2015, recording temperature every 30 minutes. In 2013 and 2014, salinity samples were collected and sent to a specialist laboratory for analysis. From 2015 onwards, salinity samples were analysed in-house at Orkney Harbour Authority, using a YSI Pro30 Conductivity salinity meter.

Laboratory processes

All samples were preserved in 4% buffered formaldehyde solution, with the exception of phytoplankton samples, which were preserved using Lugol's Iodine.

Samples were processed and identified by Fugro EMU Ltd in 2012 and 2013, and by APEM Ltd from 2014 to 2017. Rapid assessment samples were sieved over a 4.0 mm mesh and the more conspicuous biota extracted and identified. Other samples were rinsed over a 0.5 mm mesh and the retained material examined in full under a stereo-microscope. Countable animals were extracted and enumerated. Encrusting or attached colonial organisms were extracted as representative portions of each taxon and recorded qualitatively. Some samples were subsampled due to large numbers of amphipods of the genus *Jassa*. The methodology outlined in the North-east Atlantic Marine Biological Analytical Quality Control Scheme Processing Requirements Protocol (Worsfold et al. 2010) was followed using a "quarteriser" (Proudfoot et al. 2003). Non-subsampled taxa were extracted in full with the sample then subsampled to one-quarter for the identification of *Jassa* species. Collected biota were identified to the lowest practicable taxonomic level, usually species. Nomenclature followed the World Register of Marine Species (WoRMS 2018), or more recent literature, as appropriate. Samples sorted by APEM were subject to internal Quality Assurance procedures. Identifications of specimens of some species were confirmed by experts in their respective groups.

Voucher specimens of some of the NNS and cryptogenic species from the monitoring programme have been deposited with the National Museums of Scotland (NMS) (fauna) and with the Royal Botanic Garden Edinburgh (RBGE) (algae) collections. Reference numbers are included in the species descriptions where applicable. Voucher specimens of all NNS and cryptogenic species from the monitoring programme are currently in the OICHA's own voucher specimen collection in Orkney; plans have been made to deposit a complete duplicate collection in the relevant institutions in the near future.

Results

The mean salinity was equivalent to seawater at all but two of the sites; brackish water was recorded at Loch of Stenness and Brig O'Waithe (Supplementary material Table S1). The TinyTag© temperature loggers have enabled continual recording of seawater temperatures in Orkney between 2015 and 2017. The lowest recorded seawater temperature was 3.7 °C at Kirkwall Marina in February 2016 and the highest seawater temperature was 15.9 °C, also at Kirkwall Marina, in August 2017.

Records of 21 non-native (NNS) and 20 cryptogenic marine or brackish water species have been made from Orkney and surrounding waters (Table 2). Of these, 15 NNS and 12 cryptogenic species have been recorded through the OIC Harbour Authority monitoring programme, of which three NNS (*Boccardia proboscidea*, *Asterocarpa humilis* and *Melanothamnus harveyi*) and one cryptogenic (*Ctenodrilus serratus*) represent new records for Orkney.

Numbers of NNS and cryptogenic species recorded from each monitoring station are shown in Figure 2.

A summary of each non-native or cryptogenic species recorded by Orkney Harbour Authority in 2012–2017 is provided below with a corresponding number to Table 2. It details Orkney records, wider distribution and arrival history, or evidence for cryptogenic status.

Species accounts

MYZOZOA

(16) *Karenia mikimotoi* (Miyake and Kominami ex Oda) Gert Hansen and Ø. Moestrup, 2000

Status in U.K. – cryptogenic.

During the surveys considered here, *Karenia mikimotoi* has been recorded from Scapa Flow on a single occasion, from a phytoplankton sample in 2015.

Table 2. Year of first record of non-native and cryptogenic species in the Orkney Islands. A. Records found in this study, B. Records from review of bibliography and databases (not recorded during the present study). Species in bold are first records for Orkney.

No.	Phylum	Species	NNS / Cryptogenic	Origin	OICHA Records	Year of first record
A. Records found in this study						
1	Phaeophyceae	*Colpomenia peregrina*	NNS	NW Pacific	20	1960–1987 (Nall et al. 2014)
2	Rhodophyta	*Asparagopsis armata*	NNS	Australasia	2	1960–1987 (Nall et al. 2014)
3	Rhodophyta	*Bonnemaisonia hamifera*	NNS	NW Pacific	56	1960–1987 (Nall et al. 2014)
4	Rhodophyta	*Dasysiphonia japonica*	NNS	NW Pacific	21	2011 (W.G. Sanderson)
5	**Rhodophyta**	***Melanothamnus harveyi***	**NNS**	**NW Pacific**	**12**	**2014 (This study)**
6	Chlorophyta	*Codium fragile* ssp. *fragile*	NNS	NW Pacific	30	1891 (Provan et al. 2008)
7	**Annelida**	***Boccardia proboscidea***	**NNS**	**NW Pacific**	**1**	**2014 (This study)**
8	Arthropoda	*Caprella mutica*	NNS	NW Pacific	141	2006 (Ashton 2006)
9	Diptera	*Telmatogeton japonicus*	NNS	NW Pacific	1	2015 (L. Johnson)
10	Mollusca	*Potamopyrgus antipodarum*	NNS	Australasia	9	1938 (ICIT 2004)
11	Bryozoa	*Tricellaria inopinata*	NNS	NW Pacific	27	2012 (Nall et al. 2014)
12	Bryozoa	*Bugulina simplex*	NNS	NW Atlantic	2	2012 (Nall et al. 2014)
13	Bryozoa	*Schizoporella japonica*	NNS	NW Pacific	61	2011 (Ryland et al. 2014)
14	**Chordata**	***Asterocarpa humilis***	**NNS**	**Southern Ocean**	**20**	**2014 (This study)**
15	Chordata	*Corella eumyota*	NNS	Southern Ocean	33	2012 (Nall et al. 2014)
16	Myzozoa	*Karenia mikimotoi*	Cryptogenic		1	2006 (Davidson et al. 2009)
17	**Annelida**	***Ctenodrilus serratus***	**Cryptogenic**		**2**	**2015 (This study)**
18	Arthropoda	*Jassa marmorata*	Cryptogenic		15	2012 (Nall et al. 2014)
19	Arthropoda	*Monocorophium acherusicum*	Cryptogenic		28	1997 (Murray et al. 1999)
20	Arthropoda	*Monocorophium insidiosum*	Cryptogenic		26	1997 (ICIT 2004)
21	Arthropoda	*Crassicorophium bonellii*	Cryptogenic		2	1995 (Murray et al. 1999)
22	Bryozoa	*Bugulina fulva*	Cryptogenic		26	2012 (Nall et al. 2014)
23	Chordata	*Ascidiella aspersa*	Cryptogenic		73	1994 (Thorpe 1998)
24	Chordata	*Ascidiella scabra*	Cryptogenic		52	1994 (Thorpe 1998)
25	Chordata	*Botrylloides leachii*	Cryptogenic		28	1995 (Murray et al. 1999)
26	Chordata	*Botryllus schlosseri*	Cryptogenic		40	1995 (Murray et al. 1999)
27	Chordata	*Diplosoma listerianum*	Cryptogenic		17	1997 (Murray et al. 1999)
B. Records from review of bibliography and databases (not recorded during the present study)						
28	Arthropoda	*Austrominius modestus*	NNS	Australasia		2017 (S.J. Hawkins)
29	Chordata	*Botrylloides violaceus*	NNS	NW Pacific		2012 (Nall et al. 2014)
30	Cnidaria	*Diadumene lineata*	NNS	NW Pacific		1994 (Thorpe 1998)
31	Mollusca	*Magallana gigas*	NNS	Australasia		1991 (Nall et al. 2014)
32	Mollusca	*Mya arenaria*	NNS	NW Atlantic		1938 (ICIT 2004)
33	Phaeophyceae	*Sargassum muticum*	NNS	NW Pacific		2015 (D. Mayes)
34	Annelida	*cf. Aphelochaeta marioni*	Cryptogenic			1997 (Murray et al. 1999)
35	Arthropoda	*Crassicorophium crassicorne*	Cryptogenic			1995 (Murray et al. 1999)
36	Chordata	*Molgula manhattensis*	Cryptogenic			1995 (Murray et al. 1999)
37	Bryozoa	*Conopeum seurati*	Cryptogenic			1994 (Thorpe 1998)
38	Mollusca	*Nototeredo norvagica*	Cryptogenic			1917 (R. Winkworth)
39	Mollusca	*Psiloteredo megotara*	Cryptogenic			1950–1955 (R. Rendal))
40	Mollusca	*Teredo navalis*	Cryptogenic			2015 (Want et al. 2017)
41	Mollusca	*Teredora malleolus*	Cryptogenic			1956 (R. Rendal)

However, it has frequently been identified in Scottish waters and was responsible for significant blooms recorded in 1999, 2003 and 2006 in Orkney and surrounding waters (Davidson et al. 2009). It was described from Japan and has a near cosmopolitan distribution. Based on this, Streftaris et al. (2005) regarded it as non-native in Europe and Minchin (2007a) likewise regarded it as non-native in Ireland. Hégaret et al. (2008) consider that relocation of bivalve molluscs for aquaculture represents a potential vector for its spread and van den Bergh et al. (2002) likewise suggest

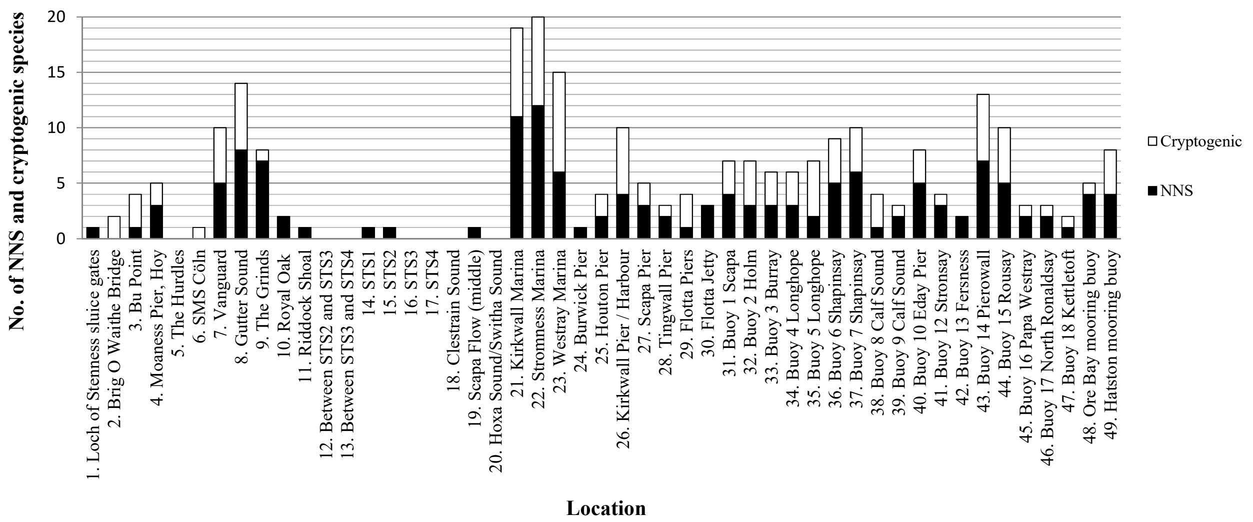

Figure 2. The total number of non-native and cryptogenic species recorded at each monitoring location (2012–2017).

that transplantation of Japanese oysters to France from 1966 onwards may have aided its invasion. Transport in ballast water represents another potential vector. However, contrary to these opinions, Gómez (2008) simply regarded *K. mikimotoi* as cosmopolitan and likely transported by water currents. Given this conflict we herein regard it as cryptogenic pending further investigation.

PHAEOPHYCEAE

(1) *Colpomenia peregrina* Sauvageau, 1927

Status in U.K. – non-native.

Colpomenia peregrina ("oyster thief") has been frequently recorded through the monitoring programme, at several sites each year since 2014. It was recorded at five new sites in 2016: Westray and Stromness marinas and at Burray, Shapinsay and Stronsay Visitor Yacht Moorings. In both 2016 and 2017, it was most frequently found in rapid assessment samples but was also present in a few scrape samples in 2017.

Import of oysters from the U.S.A. has frequently been cited as the vector for the introduction of *C. peregrina* to Europe (Blackler 1964; Eno et al. 1997). However, a recent molecular study (Lee et al. 2014) suggests that European populations were derived from the north-west Pacific rather than America. Cotton (1908) believed that it may have reached Britain via natural dispersal from previously introduced French populations but did not discount vessel transport or transport on imported oysters as possible vectors. It is thought to have arrived in Britain in 1906–1907 and was already present in two discrete locations, Swanage and Torquay, and in relatively high abundance when it was discovered; it had not been detected at Swanage in early 1906 (Cotton 1908). Following its first known occurrence in the U.K., it quickly spread through natural dispersal and was first recorded from the Orkney Islands in 1940 (Lund 1949). More recently, Want et al. (2017) recorded it fouling marine renewable energy structures in Orkney.

RHODOPHYTA

(2) *Asparagopsis armata* Harvey, 1855

Status in U.K. – non-native.

There have been two records of *Asparagopsis armata* ("harpoon weed") from the monitoring programme, from buoys at Holm and Shapinsay, both from 2013. The species is believed to have been introduced to Europe from southern Australia or New Zealand in the 1920s (Ní Chualáin et al. 2004) and has otherwise been recorded in Orkney at Kirkwall and Skatelan Skerry by Wilkinson (1975) and Maggs and Stegenga (1999).

(3) *Bonnemaisonia hamifera* Hariot, 1891

Status in U.K. – non-native.

Bonnemaisonia hamifera ("Bonnemaison's hook weed") was introduced to Europe from Japan and was first found in southern England in 1890 (Maggs and Stegenga 1999); it has been recorded from the monitoring programme each year since 2012, at many sites from both rapid assessment and scrape samples. It has previously been recorded from Orkney by Wilkinson (1975), who found it at Skatelan Skerry, Burray Ness and the Bay of Kirkwall; Nall et al. (2015) summarised other Orkney records from (Marine Nature Conservation Review (MNCR) surveys.

(4) *Dasysiphonia japonica* (Yendo) H.-S. Kim, 2012

Status in U.K. – non-native.

The filamentous red alga *Dasysiphonia japonica* ("siphoned Japan weed") has been recorded at several sites within Scapa Flow and four locations in the Northern Isles. It was first recorded in 2015 and was common in 2016, mainly in scrape samples. It is considered a fairly new introduction to Orkney; it was first recorded by W.G. Sanderson in 2011, during a survey dive at Gutter Sound, Scapa Flow (specimens identified by C. Moore).

The vector for the introduction to Europe from Japan is considered to be with Pacific oyster, *Magallana gigas* (Thunberg, 1793), importations (Sjøtun et al. 2008) but, within Europe, dispersal has probably been partly through shipping movement (Sjøtun et al. 2008). It was first recorded in the U.K. in 1999 at Milford Haven in Wales and in Scotland in 2004 at Alturlie Point, Moray Firth (Sjøtun et al. 2008). Since then, it has been recorded in Loch Laxford, Sutherland in 2009 (Moore et al. 2010), Ullapool, Wester Ross in 2010 (Moore et al. 2011) and Shetland (Collin et al. 2015). Want et al. (2017) recorded it growing on renewable energy structures in Orkney.

(5) *Melanothamnus harveyi* (Bailey) Díaz-Tapia and Maggs, 2017

Status in U.K. – non-native.

The red seaweed *Melanothamnus harveyi* ("Harvey's siphon weed") has been recorded each year since 2014, from rapid assessment and scrape samples. It has been recorded at four locations within Scapa Flow, at Scapa, Burray and Longhope visitor yacht moorings and at Vanguard navigation buoy, and has also been recorded in four locations north of Orkney Mainland.

It was introduced to the south coast of England before 1908 (Maggs and Stegenga 1999) and although known from Loch Ryan in south-west Scotland since at least the late 1980s (Maggs and Hommersand 1990; Nall et al. 2015) and from St. Andrews, Fife since 1992 (Maggs and Stegenga 1999), the first confirmed records of this species in North Scotland were made in Kinlochbervie and Scrabster harbours in 2012 (Nall et al. 2015). The records presented here from 2014 are the first from Orkney.

RBGE voucher specimen reference number: E00884773.

CHLOROPHYTA

(6) *Codium fragile* ssp. *fragile* (Suringar) Hariot, 1889

Status in U.K. – non-native.

Codium fragile ssp. *fragile* ("green fingers") has been recorded each year of the monitoring programme since 2014, mainly from rapid assessment samples but also from a scrape sample in 2017. It has been found throughout Scapa Flow and at two sites outside Scapa Flow. Wilkinson (1975) previously reported the subspecies (as *C. f.* ssp. *tomentosoides* (van Goor) Silva, 1955) from Orkney at Burray Ness on the east coast of Burray and it was reported from renewable energy structures in Orkney by Want et al. (2017) but according to Provan et al. (2008) it has been present in Orkney since at least 1891. It is otherwise widespread and well established throughout the U.K. (Minchin et al. 2013).

ANNELIDA

(7) *Boccardia proboscidea* Hartman, 1940

Status in U.K. – non-native.

One record of the burrowing worm *Boccardia proboscidea* was made from the monitoring programme, from Scapa Pier, in 2014.

Boccardia proboscidea has recently become recognised as a global invader (Simon et al. 2010; Kerckhof and Faasse 2014; Jaubet et al. 2015; Spilmont et al. 2018). There are unpublished records from throughout Britain and Ireland and a published record from Skye (Hatton and Pearce 2013), suggesting that the species has long been established in British waters. The record presented here from 2014 is the first from Orkney.

(17) *Ctenodrilus serratus* (Schmidt, 1857)

Status in U.K. – cryptogenic.

There are two records of this species from the monitoring programme, from Kirkwall Marina in 2015 and from a scrape sample from Stromness Marina in 2016.

Ctenodrilus serratus has a worldwide distribution and is frequently found in aquaria (Fauvel 1927). It was genetically demonstrated to be truly amphi-Atlantic in distribution by Westheide et al. (2003), who concluded that the distribution must have been through dispersal and considered anthropogenic dispersal among the possibilities. It has been reported from the Plymouth area (Marine Biological Association 1957) and listed from the English Channel by Dauvin et al. (2003). It is found primarily in the vicinity of port facilities (*unpublished data*) and it is here considered to be cryptogenic in the U.K. The records presented here from 2015 and 2016 are the first from Orkney.

CRUSTACEA

(18) *Jassa marmorata* Holmes, 1905

Status in U.K. – cryptogenic.

Jassa marmorata has been recorded from several sites from the monitoring programme, each year since 2013, mainly from scrape samples. It is less common in the samples than the congeneric *J. herdmani* (Walker, 1893), which is considered native.

Conlan (1990) concluded that *J. marmorata* has a cosmopolitan distribution and further stated that the worldwide distribution of the species would make suspect any native species of *Jassa* from heavily populated areas. Historic confusion, especially between *J. marmorata* and *J. falcata* (Montagu, 1808), has led to the distributions of many species being misinterpreted. Establishing the origin of *J. marmorata* is therefore problematic. Chapman (2000) considered it to be introduced to the north-eastern Atlantic from a native range of the north-western Atlantic but this is not certain. Due to these uncertainties, and in keeping with Marchini and Cardeccia (2017), we here consider it cryptogenic in the U.K.

(19) *Monocorophium acherusicum* (Costa, 1853)

Status in U.K. – cryptogenic.

Monocorophium acherusicum has been widely recorded from the monitoring programme at several marina and pier sites, each year since 2014, from settlement panel and occasionally scrape samples.

Chapman (2000) stated that *M. acherusicum* is perhaps the most widely distributed and widely introduced estuarine invertebrate in the world with a potential native range in the north-west Atlantic and introduced in all other locations, including the north-east Atlantic. However, Marchini and Cardeccia (2017) were of the opinion that, although potentially native to the North Atlantic, the true origins of *M. acherusicum* may never be known since it is now so widespread. Here we regard it as cryptogenic in the U.K.

(20) *Monocorophium insidiosum* (Crawford, 1937)

Status in U.K. – cryptogenic.

Monocorophium insidiosum was first recorded from the monitoring programme in 2013 and has been recorded from many sites since 2015, from both settlement panel and scrape samples.

Monocorophium insidiosum often associates with artificial habitats and is considered non-native in several regions (Chapman 2000; Heiman et al. 2008; Marchini and Cardeccia 2017). Chapman (2000) considered it native to the north-west Atlantic but introduced in all other regions and Marchini and Cardeccia (2017) stated that it has become so widely distributed that its original range is unknown. We herein regard it as cryptogenic in the U.K., in keeping with Minchin (2007a) who reported it as cryptogenic in Ireland.

NMS voucher reference number: NMS.Z.2017.144.62

(21) *Crassicorophium bonellii* (H. Milne Edwards, 1830)

Status in U.K. – cryptogenic.

Crassicorophium bonellii has a widely reported distribution but the identity of the species is problematic and it is possible that a species complex is involved (Marchini and Cardeccia 2017). Chapman (2007) considered that *C. bonellii* was indistinguishable from *Monocorophium acherusicum*. In the current sampling programme *C. bonellii* has been found at Vanguard in 2013 and from a scrape sample at Gutter Sound in 2016 but it has also been recorded in MNCR surveys in 1995 and 1997 (Murray et al. 1999).

Its widespread nature and association with human vectors makes establishing the native range of the species problematic (Marchini and Cardeccia 2017) and we consider it to be a cryptogenic species in the U.K.

(8) *Caprella mutica* Schurin, 1935

Status in U.K. – Non-native.

The "Japanese skeleton shrimp", *Caprella mutica*, was first recorded in British waters from Oban, Scotland in July 2000 but it is unknown how long it was present prior to its discovery (Willis et al. 2004). In Europe it has been present since at least 1995 (Platvoet et al. 1995; Ashton et al. 2007) and it is now very widespread along Scottish coasts (Ashton et al. 2007). The first Orkney records were made on mooring lines in 2006 (Ashton 2006) and it has recently been recorded fouling marine renewable energy structures in Orkney (Want et al. 2017). Ship hull fouling or recreational boats were cited by Ashton (2006) as a possible means of introduction to Orkney. Subsequently, it was recorded from the HMS Vanguard navigation buoy in 2008 (J. Kakkonen, *pers. obs.*) and has been consistently recorded in high abundance from scrape, settlement panel and rapid assessment samples throughout Orkney each year since the start of the programme in 2012.

NMS voucher reference number: NMS.Z.2017.144.63.

INSECTA

(9) *Telmatogeton japonicus* Tokunaga, 1933

Status in U.K. – non-native.

Larvae of non-biting midges, Chironomidae, have been consistently recorded from samples throughout the monitoring programme but they are not identified to species since pupae or adults are usually required to provide a definitive identification. The non-native *Telmatogeton japonicus* was recorded in Co. Clare, Ireland in 1999 by Murray (2000) and in Wales by Murray (2013). Langton and Hancock (2013) provided records of two *Telmatogeton* species from Scotland: *T. japonicus* from St Kilda and the closely related *T. murrayi* Sæther, 2009 from Shetland. This latter species was described from Iceland based on material originally identified as *T. japonicus* by Murray (1999) but the taxonomic status of the species is uncertain and requires investigation (D. Murray, *pers. comm.*).

Telmatogeton japonicus was first recorded in Orkney in 2015 by L. Johnson (identity confirmed by P.H. Langton), based on adult specimens. Pupae from scrape samples collected in Gutter Sound in 2017 under the current sampling programme were also identified as belonging to *T. japonicus* using Langton and Visser (2003). It is, however, acknowledged that the pupa of *T. murrayi* is currently unknown and, since *T. murrayi* is recorded from Shetland (Langton and Hancock 2013), it is conceivable that the material could belong to that species. Nevertheless, based on the available information, in keeping with the previous Orcadian record, the present pupal records are retained as *T. japonicus* pending a formal review of *T. murrayi*.

Adults of *T. japonicus* are relatively short lived whilst the larvae are commonly regarded as members of the fouling community, indicating that larval transport is the species' most likely dispersal mechanism over large distances. Failla et al. (2015) state that long-range movements of chironomids would be nearly impossible without human or animal assistance and the introduction to Europe is thought to have occurred with shipping from Japan (Brodin and Andersson 2008; Raunio et al. 2009; Failla et al. 2015). Dispersal within Europe may be aided by offshore structures such as navigation buoys and windfarm pilings (Kerckhof et al. 2007; Brodin and Andersson 2008); *T. japonicus* was not recorded on such structures in Orkney by Want et al. (2017) but sampling within a particular season would have been necessary to obtain the pupae required for identification.

MOLLUSCA

(10) *Potamopyrgus antipodarum* (Gray, 1843)

Status in U.K. – non-native.

The "New Zealand mud snail", or "Jenkin's spire shell" *Potamopyrgus antipodarum* has been recorded from scrape samples at Loch of Stenness, each year since 2013 and also at The Grinds navigation buoy in 2014. It was mentioned as having been part of the Loch of Stenness invertebrate community since 1938 in a report commissioned by Scottish Natural Heritage (ICIT 2004).

Potamopyrgus antipodarum is widespread throughout the British Isles (Kerney 1999) and has become one of the most common freshwater snails in Britain (Heppell 2008).

BRYOZOA

(11) *Tricellaria inopinata* d'Hondt and Occhipinti-Ambrogi, 1985

Status in U.K. – non-native.

The bryozoan *Tricellaria inopinata* has been recorded each year since 2014 from scrape, settlement panel and rapid assessment samples. It has been found at Gutter Sound and The Grinds navigation buoy, Stromness and Kirkwall marina and in Fersness Bay and Pierowall visiting yacht moorings.

Cook et al. (2013) document the European distribution of *T. inopinata* demonstrating a wide U.K. range along southern and western coasts as well as eastern Scotland. The first Scottish records were made in 2006 from marinas in Troon and Clyde and it has since been recorded in eight other marinas in Scotland (Cook et al. 2013). Its occurrence in marinas indicates that recreational yachting may play a role in the dispersal of this species but Bishop et al. (2015b) also note that it is present on natural shores indicating natural dispersal on a more localised scale probably also occurs. Cook et al. (2013) did not include any northern Scottish marinas in their review of the Scottish distribution of *T. inopinata* but it was recorded from Orkney in August 2012 by Nall et al. (2015). The species is probably of Pacific origin (possibly NE) but its precise native range remains uncertain (Dyrynda et al. 2000; Cook et al. 2013).

(22) *Bugulina fulva* (Ryland, 1960)

Status in U.K. – cryptogenic.

Bugulina fulva has been recorded at several, mainly marina, sites in the monitoring programme, each year since 2015, from scrape, settlement panel and rapid assessment samples. Following Hayward and Ryland (1998), Ryland et al. (2011) and Porter et al. (2017), we consider it cryptogenic in U.K. waters based on its frequent occurrence in docks and harbours.

(12) *Bugulina simplex* (Hincks, 1886)

Status in U.K. – non-native.

Bugulina simplex was found at Kirkwall Marina in 2014 and 2015, and had also been recorded there in 2012 (Nall et al. 2015), but has not been recorded since. It is otherwise known from Shetland (Collin et al. 2015), Peterhead, Somerset and Hartlepool (Bishop et al. 2015b), Northern

Ireland (Porter et al. 2017) and Wales (Ryland et al. 2011). Ryland et al. (2011) believed that it was under-recorded in Britain and indicated that its strong seasonality may reduce the likelihood of detection in some months.

(13) *Schizoporella japonica* Ortmann, 1890

Status in U.K. – non-native.

Schizoporella japonica was first reported in British waters from Holyhead in July 2010 (Ryland et al. 2014) but Loxton et al. (2017) found an archived sample from Plymouth that backdated the introduction to at least November 2009. It has a near continuous distribution in Scotland (Loxton et al. 2017) and has been present in Orkney since at least May 2011 (Ryland et al. 2014) where it is also found on human-made structures (Want et al. 2017). It has been recorded in scrape and rapid assessment samples from several monitoring programme sites each year since 2014.

ASCIDIACEA

(23) *Ascidiella aspersa* (Müller, 1776)

Status in U.K. – cryptogenic.

Ascidiella aspersa ("fluted sea squirt") has been recorded from many monitoring programme sites, each year since 2012, from scrape, settlement panel and rapid assessment samples. It has also been recorded from renewable energy structures in Orkney (Want et al. 2017).

This species has been listed as "introduced" in the Mediterranean (López-Legentil and Legentil 2015) and is considered invasive in Japan and elsewhere (Nishikawa et al. 2014). Its type locality is southern Norway (Skagerrak) and it is widely considered to be native to northern Europe: however, it should be regarded as cryptogenic in the U.K. until its native range can be confirmed.

NMS voucher reference number: NMS.Z.2017.144.117.

(24) *Ascidiella scabra* (Müller, 1776)

Status in U.K. – cryptogenic.

Ascidiella scabra has been recorded from many monitoring programme sites, each year since 2012, from scrape, settlement panel and rapid assessment samples.

Genetic studies (Nishikawa et al. 2014) have confirmed the distinction between this species and *A. aspersa*, to which it is very similar and the two species share the same type locality. It is likewise considered invasive in Japan but has been listed as cryptogenic in the Mediterranean (López-Legentil and Legentil 2015). It should be considered cryptogenic in British waters until its origin has been determined.

NMS voucher reference number: NMS.Z.2017.144.120.

(14) *Asterocarpa humilis* (Heller, 1878)

Status in U.K. – non-native.

The "compass sea squirt" *Asterocarpa humilis* has been recorded at several monitoring programme sites, each year since 2014, from rapid assessment and scrape samples. It has been found on the Gutter Sound navigation buoy and mooring buoys in Ore Bay, it has also been found in both Stromness and Kirkwall marinas.

Asterocarpa humilis was first recorded in England in 2009, in Wales in 2011 (Bishop et al. 2013) and in Scotland (Kerrera Marina, Oban) in 2013 (Nall et al. 2015). The records presented here from 2014 are the first from Orkney.

(15) *Corella eumyota* Traustedt, 1882

Status in U.K. – non-native.

The "orange tipped sea squirt" *Corella eumyota* has been recorded at many monitoring programme sites, each year since 2014, from scrape, settlement panel and rapid assessment samples. Within Scapa Flow, it has been recorded at The Grinds and Gutter Sound navigational buoys and from mooring buoys in Ore Bay; it has also been recorded from all three marinas in the Orkney Islands: Stromness, Kirkwall and Westray.

The first U.K. record was from 2004 on the south coast of England, at Brighton Marina, Gosport Marina and Weymouth Harbour (Arenas et al. 2006). It was first recorded in Scotland in 2009, on the west coast and its first record in Orkney was in 2012 (Nall et al. 2015). More recently, Want et al. (2017) provided records from Orkney from renewable energy infrastructure.

NMS voucher reference number: NMS.Z.2017.144.112.

(25) *Botrylloides leachii* (Savigny, 1816)

Status in U.K. – cryptogenic.

Botrylloides leachii has been recorded at several monitoring programme sites, each year since 2014, from rapid assessment and scrape samples.

It is considered cryptogenic in Europe (López-Legentil and Legentil 2015).

(26) *Botryllus schlosseri* (Pallas, 1766)

Status in U.K. – cryptogenic.

Botryllus schlosseri ("star ascidian") has been recorded at several monitoring programme sites, each year since 2012, from settlement panel, rapid assessment and scrape samples, and has also been recorded from renewable energy structures (Want et al. 2017).

It is considered cryptogenic in Europe (López-Legentil and Legentil 2015).

(27) *Diplosoma listerianum* (H. Milne Edwards, 1841)

Status in U.K. – cryptogenic.

Diplosoma listerianum has been recorded at several monitoring programme sites, most years since 2012, most recently from scrape samples.

It has also been recorded from renewable energy structures (Want et al. 2017). It is difficult to identify with certainty and is likely to have been present more widely but recorded as Didemnidae. A record from 2012 was listed as *D. listerianum/spongiforme*.

It is listed as non-native in the Mediterranean (Airoldi et al. 2015; López-Legentil and Legentil 2015) and as invasive in the Netherlands (Gittenberger 2007). Molecular studies by Pérez-Portela et al. (2013) identified cryptic species and evidence of anthropogenic introductions but did not determine the native range. The species should be considered cryptogenic in the U.K. *Diplosoma spongiforme* (Giard, 1872) is likewise also considered here as cryptogenic in Europe. Both *D. spongiforme* and *D. listerianum* were recorded from Orkney by Want et al. (2017) growing on marine renewable energy structures.

NMS voucher reference number: NMS.Z.2017.144.110.

Discussion

The present study recorded 15 NNS and 12 cryptogenic species through the monitoring programme, of which three NNS (*B. proboscidea*, *A. humilis* and *M. harveyi*) and one cryptogenic (*C. serratus*) represent new records for Orkney. According to a comprehensive review of historical introduction records, these results represent 71% of all non-native and 60% of all cryptogenic species ever recorded from Orkney and surrounding waters (total 21 non-native (NNS) and 20 cryptogenic marine or brackish water species). We have considered the definition of cryptogenic species to include those that have been treated as possibly non-native in the past, or for which there is some evidence of potential introduced origin. Technically, Carlton's (1996) definition would include all species that have yet to be proven native but this could represent most of the biota of a region.

Potential impacts

Many of the NNS known from Orkney may have an impact in their new environment. Populations of the ascidians *Asterocarpa humilis* and *Corella eumyota* and the bryozoan *Tricellaria inopinata* may negatively impact the abundance of other sessile filter feeders and they have the potential to become significant foulers of aquaculture nets or water intake pipes (Dyrynda et al. 2000; Arenas et al. 2006; Bishop et al. 2013; Cook et al. 2013). The red alga *Bonnemaisonia hamifera* may become dominant and outcompete local species (Katsanevakis et al. 2014). High densities of *Caprella mutica* have been known to cause dense biofouling on artificial surfaces, including fish farm nets, and it is known to display aggressive behaviour towards other crustaceans (Willis et al. 2004; Shucksmith et al. 2009). In many areas where the non-native green seaweed *Codium fragile* ssp. *fragile* has been introduced, it has displaced native species (Chapman

1998; Katsanevakis et al. 2014). Non-native populations can become a nuisance by growing in very high densities and causing obstruction and smothering on shellfish beds (Chapman 1998; Katsanevakis et al. 2014). For many other non-native and cryptogenic species, however, the possible impacts are not known and, as there are no known impacts from the NNS in Orkney, we have avoided labelling any as "invasive". The clear distinction between the different categories of invasive/non-native/cryptogenic species require extensive work, which is beyond the scope of the current study.

Taxonomic breakdown and geographical origins

The taxonomic breakdown of non-native and cryptogenic species recorded here from Orkney is presented in Table 1. The high proportion of cryptogenic species for Annelida, Arthropoda and Chordata (all of which belonged to the Ascidiacea) highlights the need for more research into the origins of species in these phyla, compared, for example, to the better-studied Mollusca. It is also likely that the number of cryptogenic species has been underestimated amongst the Annelida and, especially, for poorly studied groups, such as Nematoda, which were not identified through the OICHA programme. The lack of cryptogenic benthic algae in the list is possibly a result of the expertise-bias of the current authors. One cryptogenic phytoplankton (holoplanktonic) species, *Karenia mikimotoi*, has been recorded from the surveys on a single occasion and sampling in subsequent years did not detect it. It may not have established or persisted in the ecosystem or may be in such low abundance that the initial detection was a chance occurrence. It is also possible that a population had temporarily expanded its range to Orkney but has since contracted or moved away as previously reported by Davidson et al. (2009).

Tsiamis et al. (2018) divided the origins of non-native species in Europe into 14 broad biogeographical units, based on the biogeographic provinces established by Spalding et al. (2007). Not all of these are relevant to cool temperate northern European waters. The native ranges of the non-native species recorded from Orkney are summarised in Table 2. The primary origin is the temperate North Pacific. There are also several temperate southern hemisphere introductions.

Regional comparisons of NNS

This list of NNS present in the area is not considered exhaustive but it reflects current knowledge and practicalities of this study. This compares with 11 NNS recorded from Shetland (Collin et al. 2015). Nall et al. (2015) provided numbers of NNS within each of the 11 regions in Scotland. However, they considered only fully marine fouling species, having restricted their study to rapid assessment surveys. They show only nine of these species for Orkney and give the highest numbers for their Clyde Sea and West Scotland regions. Similar approaches have been taken in other areas such as English marinas, where surveys (Bishop et al. 2015a) focussed on a list of target species that could mostly be identified *in situ*. The higher numbers of species recorded in this paper most likely result from the inclusion of brackish water and smaller macrofaunal species (e.g. *Boccardia proboscidea*, *Potamopyrgus antipodarum*) overlooked in other studies. Brackish water species are an important component of NNS concerns, globally (Bij de Vaate et al. 2002) and it is recommended that regional assessments account for these habitats. Full lists of NNS and cryptogenic species are available for few comparatively sized (to Orkney) regions. Ashelby (2005) recorded 14 NNS and three cryptogenic species from Harwich Harbour and the Stour and Orwell estuaries. Sixty-one non-native, including eight cryptogenic species are listed for the Belgian coast (Kerckhof et al. 2007); 66 (NNS and cryptogenic species) were listed for the Wadden Sea (Buschbaum et al. 2012) but this was revised to 88 in the North Sea and 34 in the German Baltic (Zettler et al. 2018), again, for NNS and cryptogenic species combined.

Temporal changes

Two NNS (*Bonnemaisonia hamifera* and *Caprella mutica)* and three cryptogenic species (*Ascidiella aspersa, Botryllus schlosseri* and *Diplosoma listerianum)* were first recorded from Orkney in the first year of the OICHA programme (2012) and can be considered to have been present for several years prior. Others were recorded only sporadically or may have been misidentified or recorded at higher taxonomic levels in early years, such that their true date of introduction cannot be assessed. A large suite of species were first recorded in 2014, when the first marina sites were surveyed through the programme. Some of these were subsequently found elsewhere, suggesting arrival at marinas through recreational vessels, followed by natural local spread. Those species that had their first records after the origin of sampling in marinas may have been missed in the earliest years, either because they are difficult to identify or were present only in low numbers. It has therefore not yet been possible to confirm the date of introduction to Orkney for any species.

Sampling methods and species recorded outside of the programme

Of all species records reviewed, six non-native (*Austrominius modestus* (Darwin, 1854), *Botrylloides violaceus* Oka, 1927, *Diadumene lineata* (Verrill, 1869), *Magallana gigas* (Thunberg, 1793), *Mya arenaria* Linnaeus, 1758 and *Sargassum muticum* (Yendo) Fensholt, 1955) and eight cryptogenic species (cf. *Aphelochaeta marioni* (Saint-Joseph, 1894), *Crassicorophium crassicorne* (Bruzelius, 1859), *Molgula manhattensis* (De Kay, 1843), *Nototeredo*

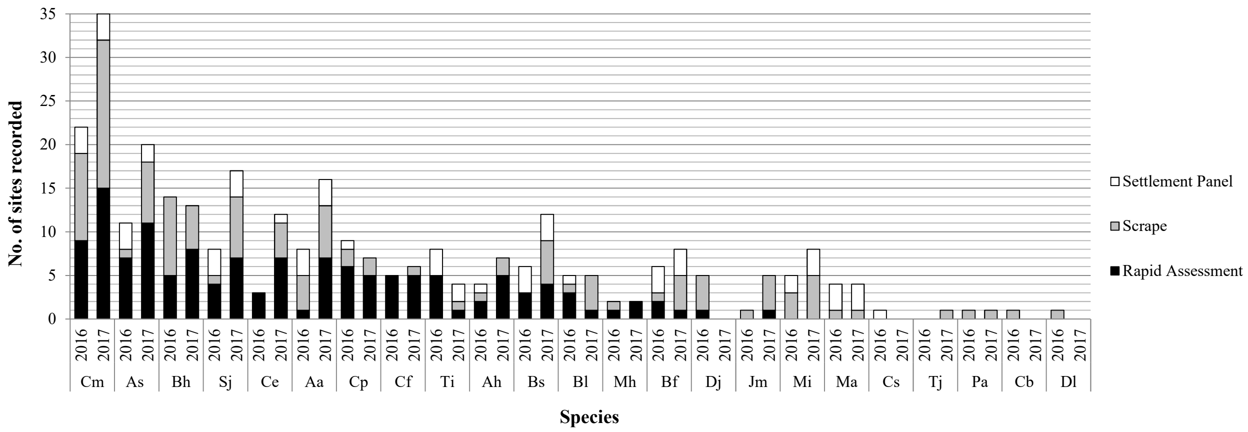

Figure 3. The total number of sites for which each non-native or cryptogenic species has been recorded for each sampling method for 2016 and 2017. NB: Only species recorded in these two years are reported in this figure. Abbreviations: Cm: *Caprella mutica*, As: *Ascidiella scabra*, Bh: *Bonnemaisonia hamifera*, Sj: *Schizoporella japonica*, Ce: *Corella eumyota*, Aa: *Ascidiella aspersa*, Cp: *Colpomenia peregrina*, Cf: *Codium fragile* ssp. *fragile*, Ti: *Tricellaria inopinata*, Ah: *Asterocarpa humilis*, Bs: *Botryllus schlosseri*, Bl: *Botrylloides leachii*, Mh: *Melanothamnus harveyi*, Bf: *Bugulina fulva*, Dj: *Dasysiphonia japonica*, Jm: *Jassa marmorata*, Mi: *Monocorophium insidiosum*, Ma: *Monocorophium acherusicum*, Cs: *Ctenodrilus serratus*, Tj: *Telmatogeton japonicus*, Pa: *Potamopyrgus antipodarum*, Cb: *Crassicorophium bonellii*, Dl: *Diplosoma listerianum*.

norvagica (Spengler, 1792), *Psiloteredo megotara* (Hanley in Forbes and Hanley, 1848), *Teredo navalis* Linnaeus, 1758, *Teredora malleolus* (W. Turton, 1822) and *Conopeum seurati* (Canu, 1928)) were not recorded by the OICHA sampling programme. As expected, rapid assessment samples recorded mainly large, conspicuous species, such as ascidians and algae. However, as an effort was made to record all non-native species from the samples, most of the species of interest have been found in such samples. Some of the smaller annelids, arthropods and molluscs (e.g. *Ctenodrilus serratus*, *Monocorophium insidiosum*, *Potamopyrgus antipodarum*) were found only in quantitative scrape or settlement panel samples. Settlement panels were found to be ineffective in the intertidal sites, where the panels were hung on buoys in the near shore environment; the panels were deployed for a relatively short time (6–9 weeks) and, due to their location close to the shore, could have been "brushed" clean by seaweeds at times. Due to this, the species recorded from the panels comprised one sessile species, *Mytilus edulis* Linnaeus, 1758, and several mobile amphipod species (e.g. *Gammarus zaddachi* Sexton, 1912), which were able to shelter in the honeycomb structure on the sides of the panels. This contrasts with the marinas, where the settlement panels have been very successful in detecting both NNS and cryptogenic species. The distribution of NNS and cryptogenic species records across sampling types is illustrated in Figure 3.

Want et al. (2017) studied the biodiversity of biofouling communities on artificial structures on harbours, marinas and marine renewable energy devices (MRED) in Orkney. They concluded that different biofouling communities are present at different habitat types. Although biofouling was dense on MREDs, no NNS were recorded on the devices; NNS were only recorded on the marinas and harbours, habitats which are well known to be "hot-spots" for NNS (Arenas et al. 2006; Foster et al. 2016; Ferrario et al. 2017, Ulman et al. 2017).

Of the species found only outside of the OICHA programme, the majority (summarised below) are large, conspicuous species that were recorded *in situ*, through surveys that extended to other sites. A few are infaunal species that would require more extensive sampling of soft substrata. The species are discussed below.

The only Orkney record of *Sargassum muticum* ("Japanese wireweed") known to date was in 2015 from Warbeth, west coast of Orkney by D. Mayes (confirmed by Prof. M. Wilkinson). The specimen was unattached and after extensive shoreline survey no other specimens were found (J. Kakkonen, *pers. obs.*); it may have either drifted on ocean currents or been entangled on a passing vessel and drifted off and onto the shore. Recreational shipping is a potential vector, through fouled yacht hulls and entanglement on propellers (Critchley et al. 1983; Murray et al. 2011; Engelen et al. 2015).

A single individual of *Austrominius modestus* ("Australasian barnacle", "Darwin's barnacle") was recorded from Stromness Marina by S.J. Hawkins in August 2017. An extensive search in the nearby area on the same day recorded no further individuals. Surprisingly, given the widespread nature of this species in Scotland (Nall et al. 2015) and in Britain in general (Southward 2008), this was the first Orkney record. This is perhaps even more surprising since *A. modestus* has been known to occur in Shetland since at least 1977 (Hiscock et al. 1978). It has not yet been recorded from the monitoring programme but the rapid assessment samples and settlement panels will be crucial in monitoring its potential establishment and spread.

The ascidian, *Molgula manhattensis*, was recorded from the west coast of Mainland Orkney in 1995 and Sanday in 1997 (Murray et al. 1999). Although *Molgula* have been recorded with reasonable regularity from the monitoring programme, their identity is uncertain. Based on haplotype diversity, Haydar et al. (2011) concluded that *M. manhattensis* is native to the north eastern U.S.A. and is non-native in the Black Sea, Japan and San Francisco Bay. However, they regarded it as cryptogenic in Europe and this status is followed here.

The "orange cloak sea squirt" *Botrylloides violaceus* has been recorded once in Orkney (Kirkwall Marina; Nall et al. 2015) but not by the monitoring programme. It originates in the northwest Pacific (Carver et al. 2006; Minchin et al. 2013) and was first recorded in Great Britain from marinas on the south coast of England in 2004 (Arenas et al. 2006) and in Ireland in 2006 (Minchin 2007b). Its spread has possibly been assisted by recreational shipping (Murray et al. 2011).

The "orange-striped green anemone", *Diadumene lineata* is considered a prolific invader native to Japan or Hong Kong that now has a near world-wide distribution (Hancock et al. 2017). It has not yet been recorded from the monitoring programme but was found at Finstown in 1994 and North Shapinsay (records from NBN, reported by Nall et al. 2015). Nall et al. (2015) report other historical records of the species in western and northern Scotland and Collin et al. (2015) report a single record from Shetland but did not find it in their surveys. Northern Scotland would be at the northern limit of the species' reported range (records summarised by Hancock et al. 2017) and it is possible that it either cannot tolerate the cooler winter water temperatures or that it has yet to establish for some other reason.

There is a record of *Aphelochaeta marioni* from Shapinsay Sound, by the MNCR in 1997 (Murray et al. 1999). This species has been recorded from estuarine sediments (not sampled in the programme) throughout northern Europe, as one of the most common and characteristic species of the habitat. The traditional use of the name (previously as "*Tharyx marioni*") for a cirratulid with moniliform mid body segments and a strongly expanded posterior end, without obvious acicular chaetae was retained by Rayment (2007) and the NMBAQC Scheme (Worsfold 2009) but with a caution that the species did not fit the original description and that the name would change with future research. Blake and Göransson (2015) described a new species, *T. maryae* Blake and Göransson, 2015, from western Sweden, which resembles the concept of "*A. marioni*" commonly used up to that time, except that it has acicular chaetae on a few posterior chaetigers. It is possible that most "*A. marioni*" records represent *T. maryae* and that the acicular chaetae have been missed. We consider the species to be cryptogenic due to its prevalence near ports and absence from remote areas. *Aphelochaeta marioni* is also listed as cryptogenic by Wolff (2005) and by Gollasch and Nehring (2006). Further work will be required to determine its true identity, if it is non-native and, if so, its origin.

Bousfield and Hoover (1997) summarised the distribution of *Crassicorophium crassicorne* as holarctic and subarctic. As it is not clear whether human mediated transport has been involved in the current distribution, we consider it to be cryptogenic. It has not been found in the monitoring programme but was recorded at three locations in Scapa Flow, many locations on the north coast of Mainland Orkney and on Sanday, Stronsay and Shapinsay during MNCR surveys carried out in 1995 to 1997 (Murray et al. 1999). It has also been recorded abundantly in OICHA sandy core sampling (J. Kakkonen, *pers. obs.*) as well as monitoring samples at several local fish farms but the OICHA monitoring programme has not sampled its preferred habitat of infralittoral mixed sediments.

The "Pacific oyster" *Magallana gigas* has not been found in the monitoring programme but was previously recorded in 1986 and again in 1991 near the island of Hunda, Scapa Flow by the Conchological Society (Nall et al. 2015). These sporadic records suggest it is not currently established in Orkney and support the contention of Nall et al. (2015) that it may fail to establish due to a requirement for higher summer water temperatures for spawning.

The “sand gaper” or “soft shell clam”, *Mya arenaria*, is considered one of the oldest marine introductions to Europe. It is native to Atlantic North America and is thought to have been introduced to Europe in the 16th, or possibly as early as the 13th century (Petersen et al. 1992), a view corroborated by Essink and Oost (2017). It has not been found in the monitoring programme, probably as its preferred estuarine sediment habitat is not currently included in the scope of the programme but has been known in the area since at least 1830 (Rendall 1956). More recently, it has been found by MNCR surveys (Thorpe 1998) and it has been recorded regularly in OICHA sandy core sampling (J. Kakkonen, *pers. obs.*).

Four shipworm species are known from Orkney. *Nototeredo norvagica* was recorded by Rendall (1956) from near Stromness in 1917 and in Birsay in 1950–1955. *Psiloteredo megotara* was recorded (Rendall 1956) from Birsay and Black Craig. *Teredo navalis* was recorded from Orkney by Want et al. (2017). *Teredora malleolus* was recorded (Rendall 1956) from Skaill, Sandwick. The origins of shipworm species remain uncertain. *T. navalis* is the most likely to be non-native; *N. norvagica*, *P. megotara* and *Teredora malleolus* may have European origins but should be considered cryptogenic pending further research (Borges et al. 2014). As these are wood boring species, specific survey methods would be required to find them and it is unsurprising that they have not been found in the monitoring programme.

Conopeum seurati is a widely introduced bryozoan worldwide and is usually considered native to the North Atlantic and Mediterranean (e.g. Lu et al. 2007; Mead et al. 2011; Kasaei et al. 2017). Wyatt et al. (2005), however, regarded *C. seurati* as cosmopolitan and Hayward and Ryland (1998) speculated that it may have been introduced to Britain within historical times but without stating a likely origin. Given this conflicting information, we here regard it as cryptogenic. It was recorded from Orkney by Murray et al. (1999).

Relative abundance of NNS and habitat preferences

The most widespread NNS detected through the programme were the algae *Melanothamnus harveyi* (12 sites), *Bonnemaisonia hamifera* (9 sites), *Dasysiphonia japonica* (7 sites), *Colpomenia peregrina* (9 sites), and *Codium fragile* ssp. *fragile* (8 sites), the bryozoan *Schizoporella japonica* (7 sites) and the skeleton shrimp *Caprella mutica* (9 sites). In this study, the majority of non-native and cryptogenic species were recorded from hard artificial substrata (Figure 2). This is comparable with other studies (Ruiz et al. 2009; Mineur et al. 2012; Cook et al. 2015; Ulman et al. 2017), which showed artificial hard substrata in marinas and navigation buoys to be hot spot locations for detecting non-native and cryptogenic species. However, some of the species noted (*Boccardia proboscidea, Crassicorophium* *crassicorne, Mya arenaria*) are likely to have been more common on softer substrata and others (Teredinidae) would require specific surveys of submerged wood habitats. Of the 49 sites surveyed between 2012 and 2017, seven sites have no records of non-native or cryptogenic species (Figure 2).

The Hurdles is a sub-tidal site which was surveyed in 2013 using a remotely operated vehicle, during which only video footage was collected; as only large conspicuous biota may be recorded in this way, it is unsurprising that no NNS or cryptogenic species were found. Clestrain Sound and Hoxa Sound/Switha Sound are zooplankton sampling sites; few NNS or cryptogenic zooplankton are known and they are yet to be found in Orkney. Between STS2 and STS3, Between STS3 and STS4, STS3 and STS4 are sub-tidal sediment sites. They all represent relatively low diversity, full salinity communities, which are not known to support many NNS or cryptogenic species. It is possible that more soft substratum species (including NNS and cryptogenics) may be present in other areas, particularly in more variable salinity (Nehring 2006). Non-native species were recorded in all of the soft sediment sub-tidal samples collected using diver operated hand corers, Petit ponar bottom samplers and hand dredges from the Port of Rotterdam GiMaRIS (2014), as well as from other sub-tidal soft sediment samples from other port environments (Ashelby 2005; Inglis et al. 2005; GiMaRIS 2017). These studies generally sampled shallow, often estuarine, habitats near heavily built up ports: Rotterdam (GiMaRIS 2014), Vlissingen (GiMaRIS 2017), Lyttelton (Inglis et al. 2005), Felixstowe/Harwich (Ashelby 2005). The current study area, Scapa Flow, is a natural deep water sheltered area with low anthropogenic influence. Although no non-native or cryptogenic species were recorded from the sub-tidal soft sediment in Scapa Flow, it is vital to include this habitat in the sampling programme to ensure all possible habitats are monitored for non-native and cryptogenic species.

Conclusion and recommendations for future studies

The OICHA sampling programme has proved to be highly significant in maintaining records of NNS on hard substrata in Orkney. It has demonstrated the continued presence of these species over several years and identified the most important fouling organisms. The programme has been able to monitor the range expansion of species within the Orkney Islands and has recorded the presence of three NNS new to Orkney (*B. proboscidea*, *A. humilis* and *M. harveyi*), demonstrating the value of regular, repeated monitoring in the assessment of NNS status. Continuation of the programme, and others like it, may prove to be an important means of detecting the dates and sites of first arrival of future incursions, as well as any long-term changes to interactions between native species and NNS, as has been noted in other studies (Bishop et al. 2015b). The inclusion of brackish water sites, full-analysis settlement panels and scrapes from intertidal hard substrata has allowed records of many species that would have been missed by rapid assessments of marinas alone (see Figure 3).

A fuller account of NNS in Orkney could be made possible by increased sampling of soft substrata and more coordinated integration of records from other sampling programmes in the region. The cost effectiveness and importance of different survey methods are discussed by Lehtiniemi et al. (2015). Future surveys will provide more material for museum donation, with a view to allow access to voucher material of all species recorded from the programme, eventually. In addition, future surveys are planned to incorporate molecular analysis of both water samples and individual specimens. It will be useful to maintain a collection of DNA barcodes to assist with the resolution of future taxonomic problems (Dias et al. 2017). Nationally, further work is required to determine the status of cryptogenic species and to establish coordinated sampling and record integration programmes in different regions.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Details of monitored sites including GPS coordinates (Lat, Long), salinity, type and survey methods used. A. Orkney moorings for visiting yachts (2012 – 2017), B. The Scapa Flow Harbour area baseline survey (2013), C. The on-going Orkney non-native species monitoring programme (2014 – 2017), D. Other additional surveys conducted, the year of survey indicated by the site name.

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