Marine Pollution Bulletin 141 (2019) 332–342

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Monitoring of sessile and mobile epifauna – Considerations for non-indigenous species

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ARTICLE INFO

Keywords:
Management
Aquatic NIS
Sampling methods
Habitat collector
Fouling plates

ABSTRACT

The present study aimed to develop monitoring methods for shallow water sessile and mobile epifauna with the main focus on enhancing the chance of early detection for new non-indigenous species (NIS) invasions. The field sampling was conducted between June and September in 2012, in the Archipelago Sea (Finland). The tested monitoring methods included baited traps that capture organisms and habitat collectors that provide habitat and refuges for organisms, as well as fouling plates. Catch efficiency of a trap/collector was defined as the number of NIS and all species caught, including their abundances. The American collector with oyster shells (habitat collector) caught the highest number of NIS, and their use is recommended in all places, where oyster shells are easily accessible. Sampling of all habitats of interest between 1 and 2 m depth is recommended with at least three habitat collectors per site.

1. Introduction

Since the late 20th century, the problem of the spread of aquatic non-indigenous species (NIS) through global shipping has become more and more evident (Carlton et al., 1990; Ruiz et al., 1997; Reise et al., 1998). The transferred NIS can cause multiple impacts ranging from food web alteration and devastation of regional fisheries to introduction of new pathogens into local ecosystems (David et al., 2007; David and Gollasch, 2015). One of the steps to manage NIS introductions and prevent the impacts of NIS in invaded ecosystems includes established monitoring programs that enable early detection, rapid management responses and monitoring NIS abundances (Lodge et al., 2006; Bax et al., 2008; Anderson, 2009; Lehtiniemi et al., 2015). Surveys provide information on the species present, which enables the monitoring for future changes, as well as identification of the areas prone to establishment of NIS (Lehtiniemi et al., 2015).

The monitoring of NIS is required through several international or regional agreements, such as the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) of the International Maritime Organization (IMO) (IMO, 2004; David et al., 2013), as well as the Biodiversity Strategy and the Marine Strategy Framework Directive (MSFD) of the European Union (EU) (EC, 2008, 2011; Ojaveer et al., 2014; Lehtiniemi et al., 2015). Furthermore, EU Regulation 1143/2014 on invasive alien species (IAS) entered into force on January 1, 2015 (EC, 2014). It declares that member states must execute a surveillance system to monitor NIS in order to prevent the distribution of certain harmful NIS into or within the EU.

Monitoring of regional waters is usually conducted by national authorities that have agreed to apply monitoring programs designed by international organisations, such as the Baltic Marine Environment Protection Commission (HELCOM) and the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) (Lehtiniemi et al., 2015). However, mainly due to resource limitations, monitoring programs and species datasets are often inaccurate, discontinuous and out of date (Delaney et al., 2008).

Currently, there is no NIS-targeted monitoring program in the Baltic Sea (HELCOM, 2017a), and the observations on the presence or absence of NIS usually originate from other monitoring programs (ICES, 2014; HELCOM, 2017b). The Joint Harmonised Procedure by HELCOM and OSPAR is solely aimed at NIS monitoring, but only in port environments to provide data for granting exemptions under the BWM Convention (HELCOM and OSPAR, 2013). In addition, there are no general guidelines for monitoring mobile and sessile epifauna, even though certain methods from the Joint Harmonised Procedure can be utilised, such as baited traps and fouling plates (HELCOM, 2017a).

The relative importance of developing monitoring for mobile and sessile epifauna originates not only from the lack of such protocols

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https://doi.org/10.1016/j.marpolbul.2019.02.055
Received 9 July 2018; Received in revised form 18 January 2019; Accepted 24 February 2019
Available online 05 March 2019

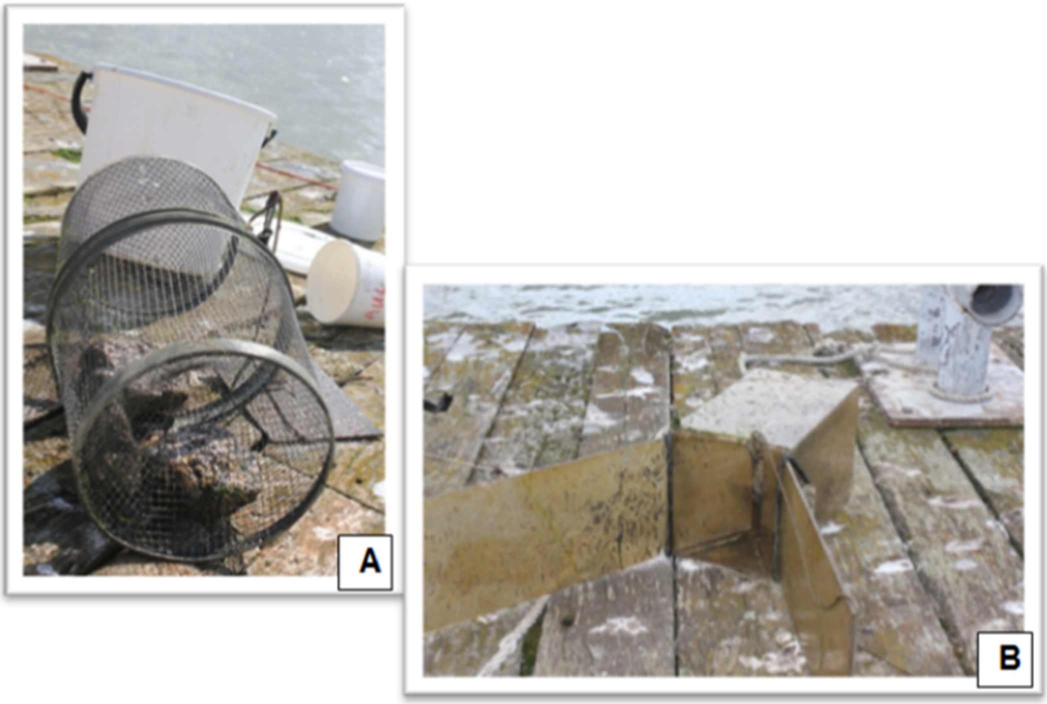

Fig. 1. The Baited traps. Gee's minnow trap (A) and Goby trap (B).

(HELCOM, 2017a), but also from potential interactions with native species and possible habitat alteration (Sellheim et al., 2010; Leclerc and Viard, 2017). The communities of mobile and sessile epifaunae consist primarily of top-down controlling predators, such as fishes and crustaceans, as well as mobile macroinvertebrates and fouling assemblages (Leclerc and Viard, 2017). Fouling communities (e.g. tunicates, bryozoans and mussels) tend to inhabit various substrates and surfaces, which can result in new habitats in the form of shells and skeletons that provide attachment surfaces and refuges for mobile organisms (Sellheim et al., 2010). Mobile and sessile shallow water species often have characteristics of successful invasive species, as some of them are euryhaline and most of them are able to utilize artificial structures that are generally associated with the most NIS-stressed areas, e.g. ports, marinas and coastal industrial sites (Nurkse et al., 2015; Leclerc and Viard, 2017).

In addition to abiotic factors such as temperature and salinity, habitat selection for epifaunal species, such as crabs and shallow water fishes is usually driven by the balance between food availability and a need of finding sheltered refuges (Werner et al., 1983; Grabowski et al., 2005). Estuarine environments are prone to invasions due to the diversity of food sources and habitats, including seagrass beds, oyster and rock reefs, salt marshes and sandy or muddy bars (Zimmerman et al., 1989; Grabowski et al., 2005). Several epifaunal species prefer complex habitat structures with variable surface features and vertical vegetation, due to the reduction of prey capture efficiency by predators and improved feeding quality (Savino and Stein, 1989; Zimmerman et al., 1989; Layman and Smith, 2001).

Overall, the development of an effective and appropriate monitoring program requires multiple considerations, such as sites and habitats of interest, monitoring methods, timing and cost-effectiveness of sampling (Rozas and Minello, 1997; Hewitt and Martin, 2001; Hayek and Buzas, 2010). Due to the high proportion of NIS that are considered to be transported via ship ballast water, the majority of marine NIS surveys should be located in port or shore areas with a high likelihood of new invasions and NIS present (Hewitt and Martin, 2001). As space and time windows for sampling are often relatively limited in port environments due to intensive ship traffic, utilisation of passive sampling methods (e.g. traps) is preferred over trawls and gillnets to monitor sessile and mobile organisms (HELCOM and OSPAR, 2013). Traps can be either baited traps that capture organisms or artificial habitat collectors that provide refuges for organisms (Roche et al., 2009; Fowler et al., 2013). The advantages and disadvantages of passive sampling techniques in the detection of estuarine shallow water fauna have been widely studied (He and Lodge, 1990; Kubecka, 1996; Rozas and Minello, 1997; Layman and Smith, 2001). Passive sampling equipment are often selective in species, have relatively low catching efficiency and are unable to provide density estimates of the species caught (Rozas and Minello, 1997).

However, passive traps are relatively cost-efficient and they can be easily applied and replicated even in ports with high traffic frequency (Rozas and Minello, 1997; Hewitt and Martin, 2001). Passive sampling methods include also artificial habitat collectors (Roche et al., 2009; Fowler et al., 2013). Instead of capturing organisms, individuals can move freely in and out of the collectors that contain artificial habitat structures to provide refuges for the organisms. Additionally, sampling for fouling organisms can be significantly enhanced with utilisation of fouling plates within a site (Freestone et al., 2013; Maraffini et al., 2017). Similarly to traps and collectors, fouling plates are passive sampling equipment, primarily designed to provide attachment surfaces for sessile organisms (deRivera et al., 2005; Maraffini et al., 2017). Overall, fouling plates can serve as a standardized and easily repeatable sampling method for present invertebrate communities with relatively minor workload.

The purpose of the present study was to test different types of passive sampling methods in various habitat types and depths to develop monitoring protocols for aquatic NIS and especially sessile and mobile epifauna, corresponding to the EU MSFD requirements. The monitoring should be applicable also in other temperate, shallow and sheltered coastal regions with similar habitats. The monitoring recommendations are expected to provide information on the distribution, abundance and changes in abundances of NIS locally. More importantly, the developed monitoring program should enhance early detection of new NIS invasions.

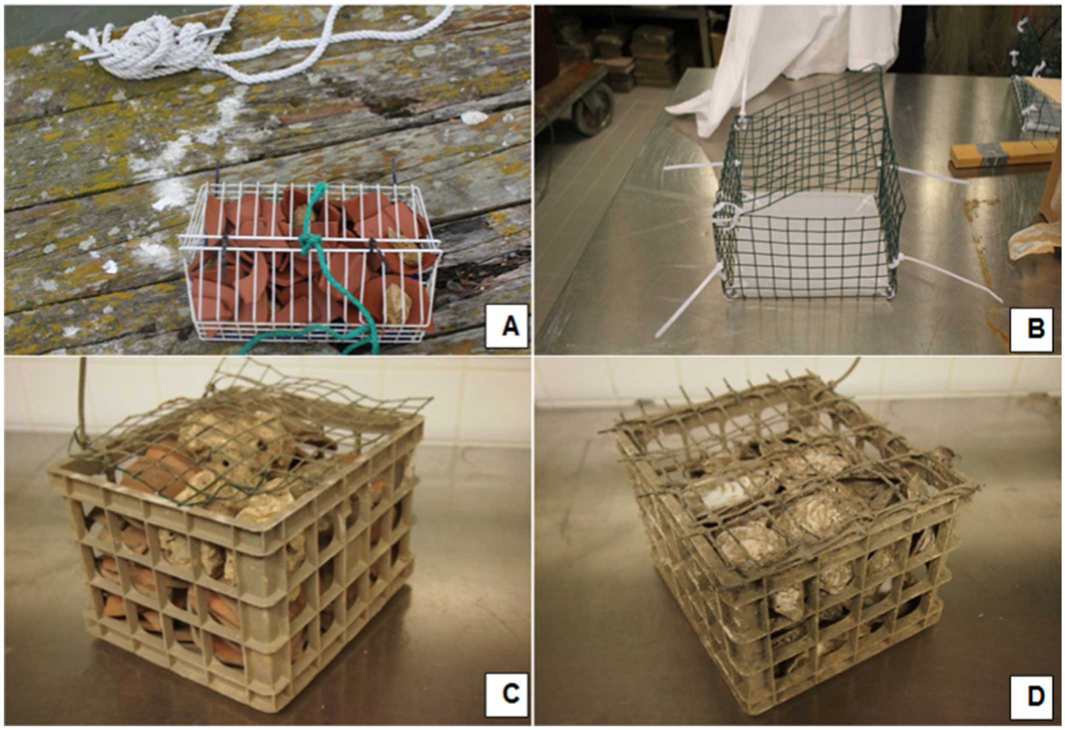

Fig. 2. The collectors. Basket collector (A), metal net collector (B), American collector (C) and American collector containing oyster shells (D).

2. Materials and methods

2.1. Traps, collectors and fouling plates

The catching efficiency of two different traps and four different collectors was compared in the present study. The selection of traps and collectors was based on experiences from pilot studies conducted by the University of Turku and the Finnish Environment Institute, as well as earlier research (Roche et al., 2009; Fowler et al., 2013). The traps were baited traps that capture organisms, whereas the habitat collectors provided habitat and refuges for organisms (Fowler et al., 2013).

The baited traps included Gee's minnow traps (42 × 23 cm diameter with 6.4 mm mesh size, Fig. 1A) and Goby traps (16,3 × 17,5 × 30 cm solid plastic frame, Fig. 1B), whereas the collectors included basket collectors (20 × 10 × 12 cm, self-made with 1 × 7 cm mesh and a solid bottom, baskets purchased from domestic appliance stores, Fig. 2A), metal net collectors (19 × 19 × 15 cm with 1–2 cm^2 ranging mesh, including a solid bottom, Fig. 2B) and American collectors (19 × 22 × 16 cm with 2 × 2 cm mesh, Fig. 2C). Basket, metal net and American collectors contained flowerpot and gardening hose pieces, as well as decorative rocks to create gaps and refuges for organisms. Additionally, the catching efficiency of a fourth collector, an American collector containing autoclaved oyster shells (Fig. 2D) was compared to the previously mentioned traps. This collector is commonly used in the monitoring of Harris' mud crabs *Rhithropanopeus harrisii* (Gould, 1841), a globally successful invasive species (Roche and Torchin, 2007; Roche et al., 2009; Fowler et al., 2013).

In addition, PVC plastic fouling plates (15 × 15 cm) were placed at each sampling site (except site 10) at 1 m, 2 m and 3 m depths (Table 1) to monitor the presence of fouling organisms and to compare their catching efficiency with the traps and collectors.

2.2. Study area and experimental design

The study was conducted between June and September in 2012, at the Southwestern archipelago of Finland, Northern Baltic Sea. The traps and collectors were placed in different habitat types and depths to determine where NIS are most commonly present. The ten sampling sites in the present study included the following habitat types; muddy shores (sites 4–6), sandy bottoms (sites 7–10) and sea beds dominated by bladderwrack seaweeds (sites 1–3) (Fig. 3). Overall, the experimental layout was uneven, as there were not enough traps and collectors for all depths in all sites (Table 1). In addition, the American collectors with oyster shells were deployed only at three sites and checked only in July and September as they were used in other monitoring surveys simultaneously.

2.3. Field sampling and sample preparation

The deployment was conducted with boat, and each trap and collector was tied with rope onto a buoy or pier structure on the surface. The fouling plates were adjusted to the aforementioned depths with a rope, which was tied to a buoy on the surface, and a brick as a weight at the bottom. An illustration of the layout of the fouling plates is presented in the Joint Harmonised Procedure (HELCOM and OSPAR, 2013).

Organism richness and abundance was recorded from the traps and collectors approximately every two to four weeks, depending on the site and weather. The traps and collectors were placed back to same sample location after retrieval, and therefore same traps and collectors were retrieved several times over the study period. This also enabled the evaluation of the impact of soak time per the number of attracted taxa. Traps and collectors were rapidly lifted and placed into a larger container with water. The traps and collectors were opened and emptied, and all of the habitat structures within the habitat traps were rinsed to collect all organisms present. The frames of the traps and collectors were also rinsed to collect the attached sessile organisms.

After retrieval, artificial habitat structures were placed back into the collectors, and all organisms present were collected and taken to a laboratory (University of Turku) for organism identification and calculation of abundances. Certain taxa, such as amphipods (*Gammarus* sp.) and gastropods (*Hydrobia* sp.) were only identified to the genus level. Up to 20 *Hydrobia* sp. were counted individually, whereas snail abundances were estimated in samples containing > 20 snails.

Table 1
Layout of the traps, collectors and plates at each site and depth.

Site	Habitat type	Fouling plates	Gee's trap	Goby trap	Basket collector	Metal net collector	American collector	American collector with oyster shells
1	Seaweed	1, 2, 3 m	2 m	2 m	1 m	2, 3 m	1, 2, 3 m	N/A
2	Seaweed	1, 2, 3 m	2 m	2 m	3 m	1, 2 m	1, 2, 3 m	N/A
3	Seaweed	1, 2, 3 m	2 m	2 m	N/A	1, 2, 3 m	1, 2, 3 m	N/A
4	Muddy	1, 2, 3 m	2 m	2 m	1, 2, 3 m	1, 2 m	1, 2, 3 m	×3 at 2 m
5	Muddy	1, 2, 3 m	2 m	2 m	2 m	1, 2, 3 m	1, 2, 3 m	×3 at 2 m
6	Muddy	1, 2, 3 m	2 m	2 m	N/A	1, 2, 3 m	1, 2, 3 m	N/A
7	Sandy	1 m, 2× at 2 m, 3 m	2 m	2 m	N/A	1, 2, 3 m	1, 2, 3 m	N/A
8	Sandy	1, 2, 3 m	2 m	2 m	N/A	1, 2, 3 m	1, 2, 3 m	N/A
9	Sandy	1, 2, 3 m	2 m	2 m	N/A	1, 2, 3 m	1, 2, 3 m	N/A
10	Sandy	N/A	N/A	N/A	N/A	2 m	×2 at 2 m	×3 at 2 m

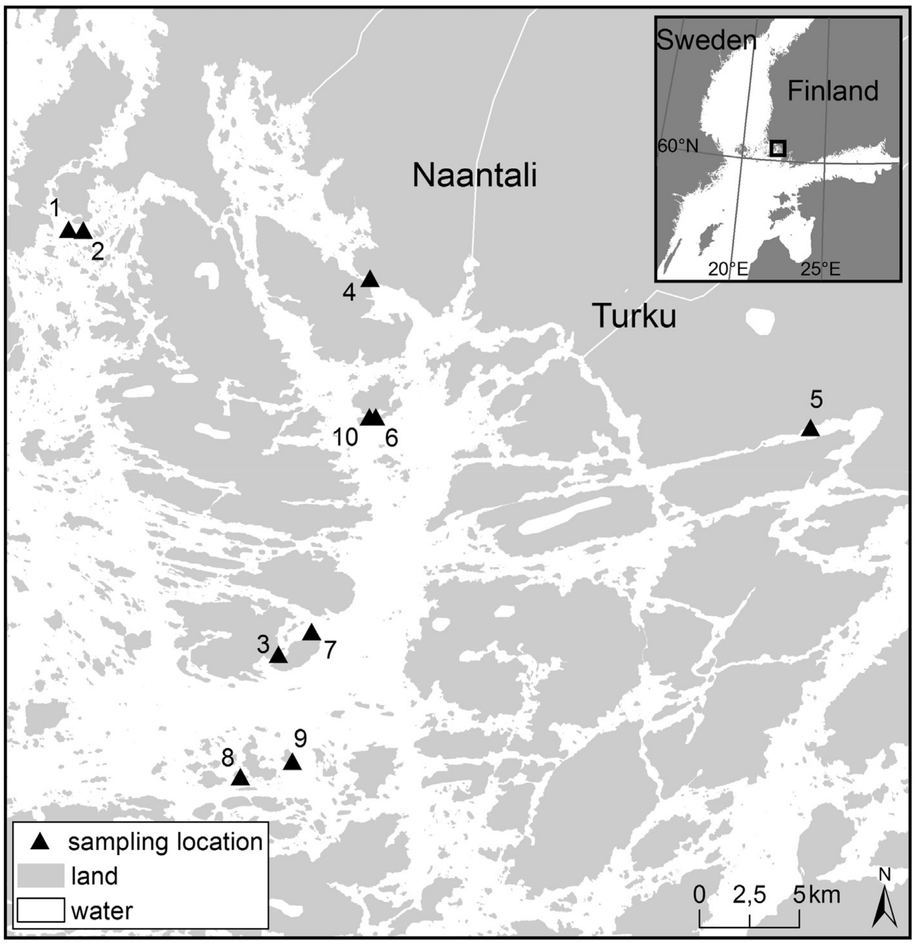

Fig. 3. The study area. Sampling area and sampling sites in the Archipelago Sea, Northern Baltic Sea.

Soak time of the fouling plates was approximately 50 days, as they were deployed around mid-June and retrieved around mid-August. After retrieval, the plates were frozen in re-sealable plastic bags. Species identification and calculation of relative abundances of the species from the plates was done using plastic film moulds (15 × 15 cm) that were divided into 100 squares (1,5 × 1,5 cm). All organisms were identified from the plates, and relative organism abundances were calculated from five randomly pre-determined squares. These steps were conducted on both sides of each plate.

2.4. Results analysis

The catching efficiency of every trap and collector was measured as the NIS richness and abundance, as well as the total (native + invasive) species richness and diversity, which was determined with Shannon-Wiener (S-W) index, as follows;

$$(H') = -\sum_{i=1}^{R} pi \ \ln \ pi$$

where pi is the proportion of a species of all organisms in a sample.

The presence and abundance of the bay barnacle, *Amphibalanus improvisus* (Darwin, 1854) and the bryozoan *Electra crustulenta* (Pallas, 1766) were disregarded from the S-W index calculations since their abundances were not determined. *A. improvisus* and *E. crustulenta* were present in all samples and at all sites. Organisms from the fouling plates were identified and counted from the five randomly pre-determined squares (1,5 cm^2), but these results were not applied in the statistical analyses. The purpose behind the deployment of fouling plates was to determine whether the plates attracted organisms that the traps and collectors were not able to collect. However, as there were no replicates for the plates within sites and no additional species were found from the plates in comparison to traps and collectors, they were excluded from the statistical analyses. Species richness calculated from the plates was averaged to each habitat type, depth and site.

Rarefaction curves were created for total species and NIS richness separately to measure sufficient sampling effort in terms of the number of traps/collectors used and the number of days deployed (soak time) to catch all species or NIS present. The rarefaction curve related to soak time was drawn from the whole data set, whereas the curve related to number of traps/collectors was drawn only for American collectors, metal net collectors and Gee's minnow traps on sites 3 (seaweed), 5 (muddy) and 8 (sandy) at 2 m depth due to the uneven experimental layout, and to exclude the impact of depth, habitat and trap/collector type on species accumulation. The rarefaction curves were computed with EstimateS, version 9 (Colwell, 2013), using chao1 estimator.

2.5. Statistical analysis

The response variables to measure catch efficiency were NIS richness and abundance, total species richness, and the calculated S-W index value. Differences in catch efficiency were studied between the traps and collectors, sampling depths, habitat types, soak times (covariate), sampling months and the interactions of habitat types and sampling depths. The interactions of traps/collectors and habitat types, traps/collectors and sampling depths, as well as traps/collectors, sampling depths and habitat types were left out of the analysis, since all traps and collectors were not applied in all depths or at all habitat types. Non-significant interaction effects were removed with the aid of the Akaike Information Criterion (Littell et al., 2006).

The data was tested for normality and homogeneity of variance by visual observation and the Levene's test. Square root transformation was applied for the NIS abundance dataset to obtain a parametric dataset. Effect of the variables on catch efficiency was tested with a mixed-model ANOVA (SAS Institute 9.3). Differences between statistically significant variables were further tested with Tukey-Kramer's pairwise comparisons, and the adjusted *p*-values are shown in the results.

3. Results

3.1. NIS abundance and richness

Overall, six NIS were detected in the present study; bay barnacle *Amphibalanus improvisus* (Darwin, 1854), dark false mussel *Mytilopsis leucophaeata* (Conrad, 1831), round goby *Neogobius melanostomus* (Pallas, 1814), New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1853), rock shrimp *Palaemon elegans* (Rathke, 1837) and Harris mud crab *Rhithropanopeus harrisii* (Gould, 1841). Overall, the study methods attracted all invasive mobile and sessile organisms of the area in comparison to earlier observations and studies from the Archipelago Sea (LUOMUS, 2019).

The compared traps and collectors caught significantly different numbers of NIS (Table 2), as the American collector with oyster shells caught the most NIS and the goby trap caught the fewest (Fig. 4A). The American collector with oyster shells was also the only sampling tool that attracted all NIS found in the study. In relation to NIS abundances, American collectors with oyster shells caught also significantly more individuals of NIS than any other trap (Fig. 4B). The goby traps attracted the lowest numbers of NIS.

Habitat types did not significantly influence NIS richness or abundance. Significant differences were, however, detected in NIS richness between different depths (Table 2), with higher richness at 1 and 2 m depths than 3 m (Fig. 5A). However, NIS abundances caught did not differ significantly between different depths (Fig. 5B).

NIS richness and abundance in the traps and collectors increased significantly throughout the study period, with the lowest numbers detected in June and the highest in September (Fig. 6). The increases were significant after every month of the study. Soak time of the traps had a significant positive correlation with NIS richness (*Pearson*, $R^2 = 0.087$, $p < 0.001$), but not with NIS abundances (Table 2).

The interaction of habitat types and sampling depths impacted NIS richness (Table 2). In muddy and seaweed habitats, significantly more NIS were caught in 1 and 2 m, whereas NIS richness was not influenced by depth at sandy habitats.

Table 2
Results of the statistical analyses conducted between the measured variables in NIS abundance and richness.

Variables	NIS richness				NIS abundance			
	df1	df2	F	*p*	df1	df2	F	*p*
Type of trap	5	294	14.57	< **0.001**	5	293	20.97	< **0.001**
Depth	2	320	13.85	< **0.001**	2	326	2.44	0.089
Habitat type	2	4.08	0.02	0.983	2	5.58	0.01	0.987
Month	3	312	26.3	< **0.001**	3	316	38.74	< **0.001**
Soak time	1	328	13.11	< **0.001**	1	335	0.23	0.630
Habitat ∗ depth	4	319	4.1	**0.003**	4	325	0.4	0.808

3.2. Total species richness and the S-W diversity index

A total of 44 species was detected in the present study, but the tested traps and collectors caught significantly different numbers of species (Table 3). Gee's minnow traps and goby traps caught the lowest numbers of species, but overall, significant differences in catch efficiency varied widely between the traps and collectors (Fig. 7A). The calculated species diversity varied between the traps and collectors, with the lowest values in goby traps, which differed significantly from American collectors with oyster shells, metal net collectors and American collectors (Fig. 7B).

Total species richness and diversity varied significantly between the sampled depths (Fig. 8). Significantly more species were found at shallower depths (1 and 2 m) (Fig. 8A). Similarly, the calculated S-W index values were significantly lower at 3 m than at the shallower depths. Total species richness and S-W index values were significantly highest at sandy habitats in comparison to muddy and seaweed habitats (Fig. 8B, Table 3).

The interaction of habitat type and depth had significant effect on species diversity. S-W index values did not differ significantly between sampling depths in sandy habitats, but decreased significantly at 3 m in muddy and seaweed habitats in comparison to shallower depths. In addition, species richness decreased also significantly in seaweed habitats at 3 m depth.

Total species richness varied significantly throughout the study months (Table 3). The lowest number of species was detected in June, which was significantly less than the number of species caught in July or August. However, sampling month did not have effect on the detected species diversity. Soak time of the traps and collectors did not have significant effect on the total species richness, but had a significant correlation with the S-W index values (*Pearson*, $R^2 = 0.035$, $p = 0.007$, Table 3).

3.3. Fouling plates

The catching efficiency of the fouling plates was relatively low throughout the study. Only three different NIS and 11 different native species were found from the fouling plates after retrieval, including all sampling sites and depths. The fouling plate samples did not contain any additional NIS or native species that the traps or collectors would have not already caught. Even on average, the plates attracted only 3.8 total species and 1.03 NIS per plate. *A. improvisus* was attracted by several plates as it is abundant in the entire Baltic Sea. Regarding the other two NIS attracted by the plates, *R. harrisii* was recorded only once

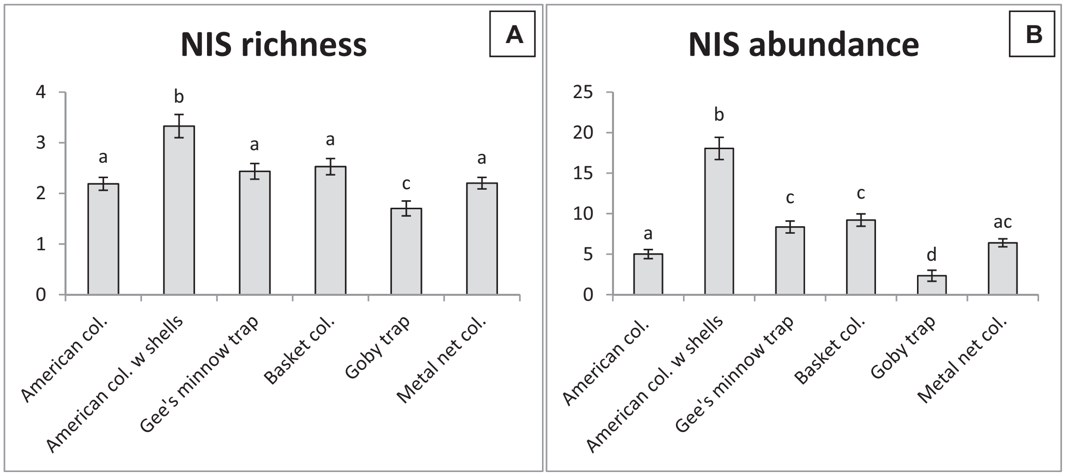

Fig. 4. Catch efficiency of different traps and collectors in NIS richness (A) and abundance (B). Different letters indicate significant pairwise differences between the collectors and traps. Number of replicates per trap/collector in the same order as columns above; 63, 18, 46, 38, 56, 142.

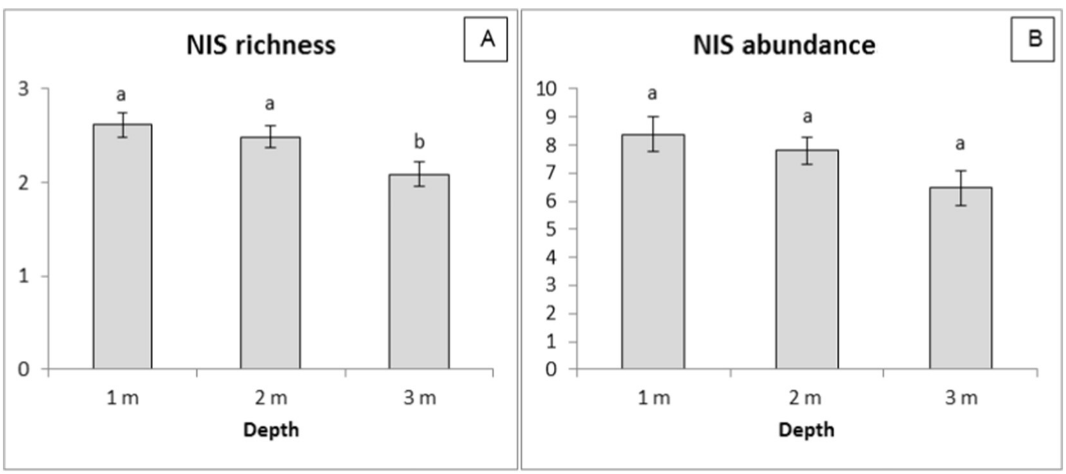

Fig. 5. NIS richness (A) and abundance (B) detected at different depths. Different letters indicate significant pairwise differences between the depths. Number of replicates per depth in the same order as columns above; 82, 208, 73.

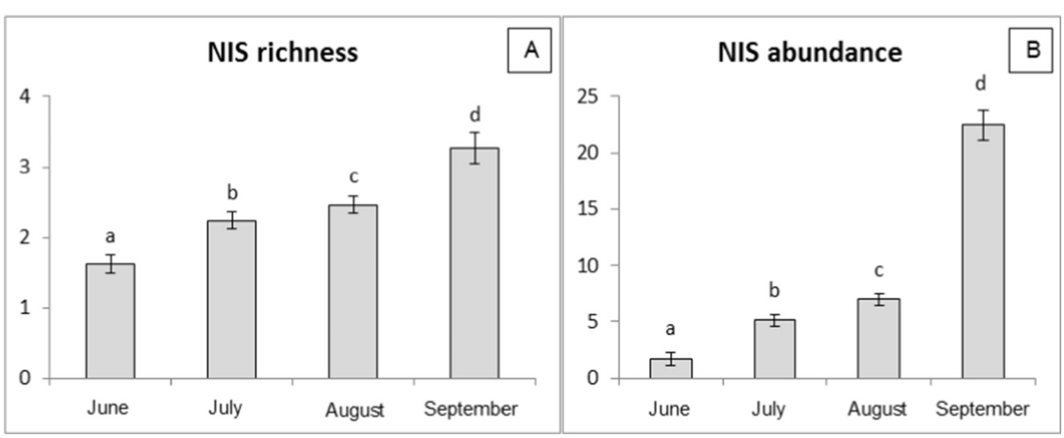

Fig. 6. NIS richness (A) and abundance (B) detected during different months. Different letters indicate significant pairwise differences between the study months. Number of replicates per month in the same order as columns above; 81, 131, 126, 25.

and *M. leucophaeata* from four plates.

3.4. Cumulative frequency

The number of different NIS attracted in the study was detected within eight days of soak time, whereas all species (native and non-indigenous) were detected within 11 days of soak time (Fig. 9A). In relation to number of traps, the average number of caught species and NIS increased with increasing number of traps (Fig. 9B).

Table 3
Results of the statistical analyses conducted between the measured variables in species richness and the S-W index values representing species diversity.

Variables	Species richness				S-W Index			
	df1	df2	F	*p*	df1	df2	F	*p*
Type of trap	5	67.7	6.07	**< 0.001**	5	273	9.56	**< 0.001**
Depth	2	64.7	19.38	**< 0.001**	2	329	12.65	**< 0.001**
Habitat type	2	7.12	10.12	**0.008**	2	9.16	18.13	**< 0.001**
Month	3	288	4.66	**0.003**	3	310	1.42	0.238
Soak time	1	295	0.45	0.505	1	305	7.29	**0.007**
Habitat * depth	4	61.5	2.11	0.091	4	330	4.35	**0.002**

4. Discussion

4.1. Catch efficiency of the traps and collectors

Planning of any environmental monitoring program should include several important factors, such as decisions on monitoring sites, habitats and methods (Rozas and Minello, 1997), as well as duration, timing (Hewitt and Martin, 2001) and sampling frequency (Hayek and Buzas, 2010), to ensure that population-level changes in the targeted species or communities can be detected. Further, the selection of sampling equipment is one of the most important components in the development of a sampling program (Rozas and Minello, 1997). Catch efficiency of the sampling equipment, in turn, plays a crucial part in the sampling process, as it affects the accuracy and representativeness of the data.

The present study aimed to determine the most appropriate sampling practices to monitor invasive mobile and sessile epifauna in temperate, sheltered and shallow coastal waters. The traps and collectors tested in the present study have been used previously (Layman and Smith, 2001; Roche et al., 2009; Fowler et al., 2013; Forsström et al., 2015, 2018), but their catch efficiencies have not been evaluated. American collector with oyster shells was the most efficient trap type by attracting not only the most NIS in the present study, but also the greatest abundances of NIS in comparison to the rest of the traps and collectors. The other traps and collectors attracted five out of six NIS (other habitat collectors), or four out of six NIS (baited traps). However, the catch efficiency of goby traps was the poorest in terms of both NIS richness and abundance.

In reality, the statistical superiority of the American collectors with oyster shells compared to the catch efficiency of other traps and collectors can be considered even more impressive as they were only applied at three different sites and at one depth (2 m). Even though the

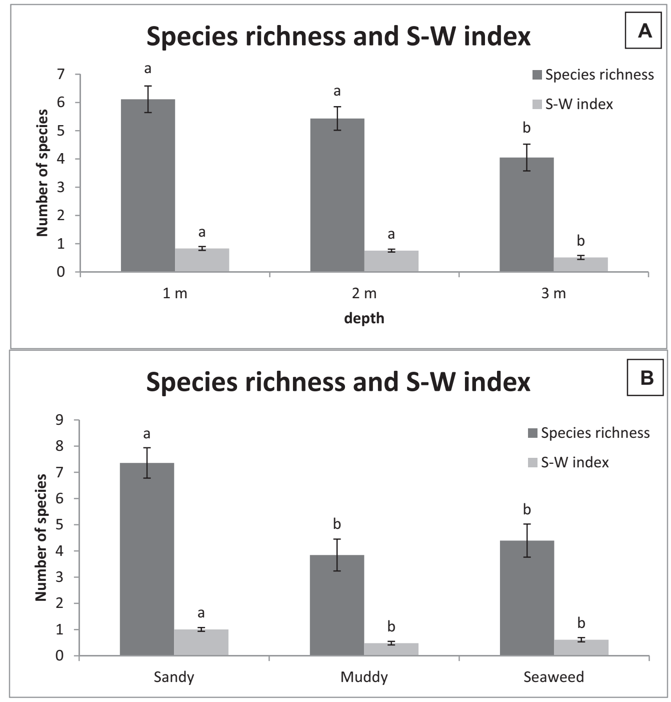

Fig. 8. Species richness and diversity at different depths (A) and habitats (B). Different letters indicate significant pairwise differences between the depths and habitats. Number of replicates per habitat in the same order as columns above; 122, 151, 90.

catch efficiency of the American collectors with oyster shells was not significantly higher in terms of total species richness and the calculated species diversity, the main focus of this study was determining the catch efficiency of NIS. The higher catch efficiency of American collectors with oyster shells was probably due to heterogeneous structure that the crate and oyster shells together provide as attachment surfaces for sessile species, as well as habitats and refuges for mobile species (Fowler et al., 2013).

Interestingly, sampling with a combination of different traps and collectors can result in better catch efficiency and reduce the selectivity problem that comes with using only certain type of traps (Kubecka, 1996). For example, all NIS detected in the present study were caught with a combination of just the regular American collectors and basket collectors. Nevertheless, American collectors with oyster shells were

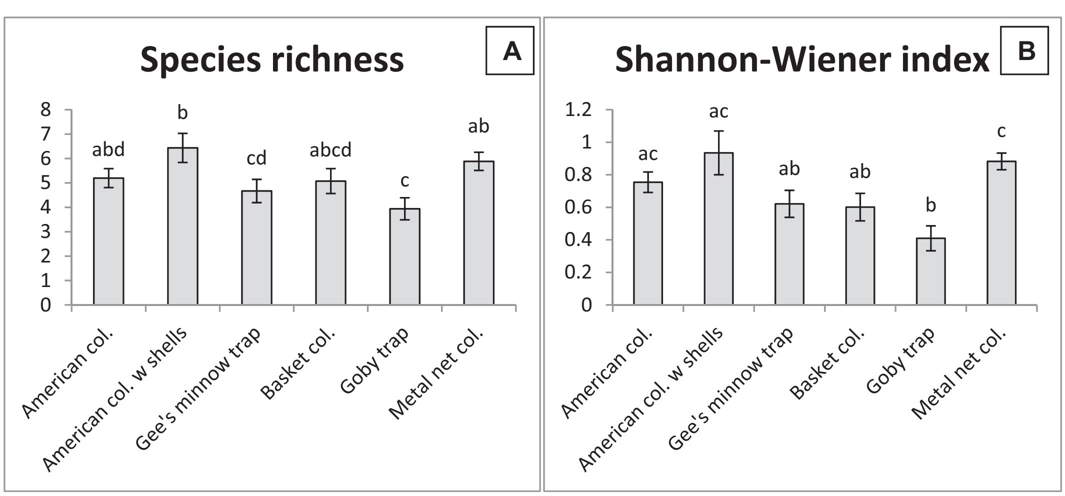

Fig. 7. Catching efficiency of different collectors and traps in the detected species richness (A) and diversity (B). Different letters indicate significant pairwise differences between the traps.

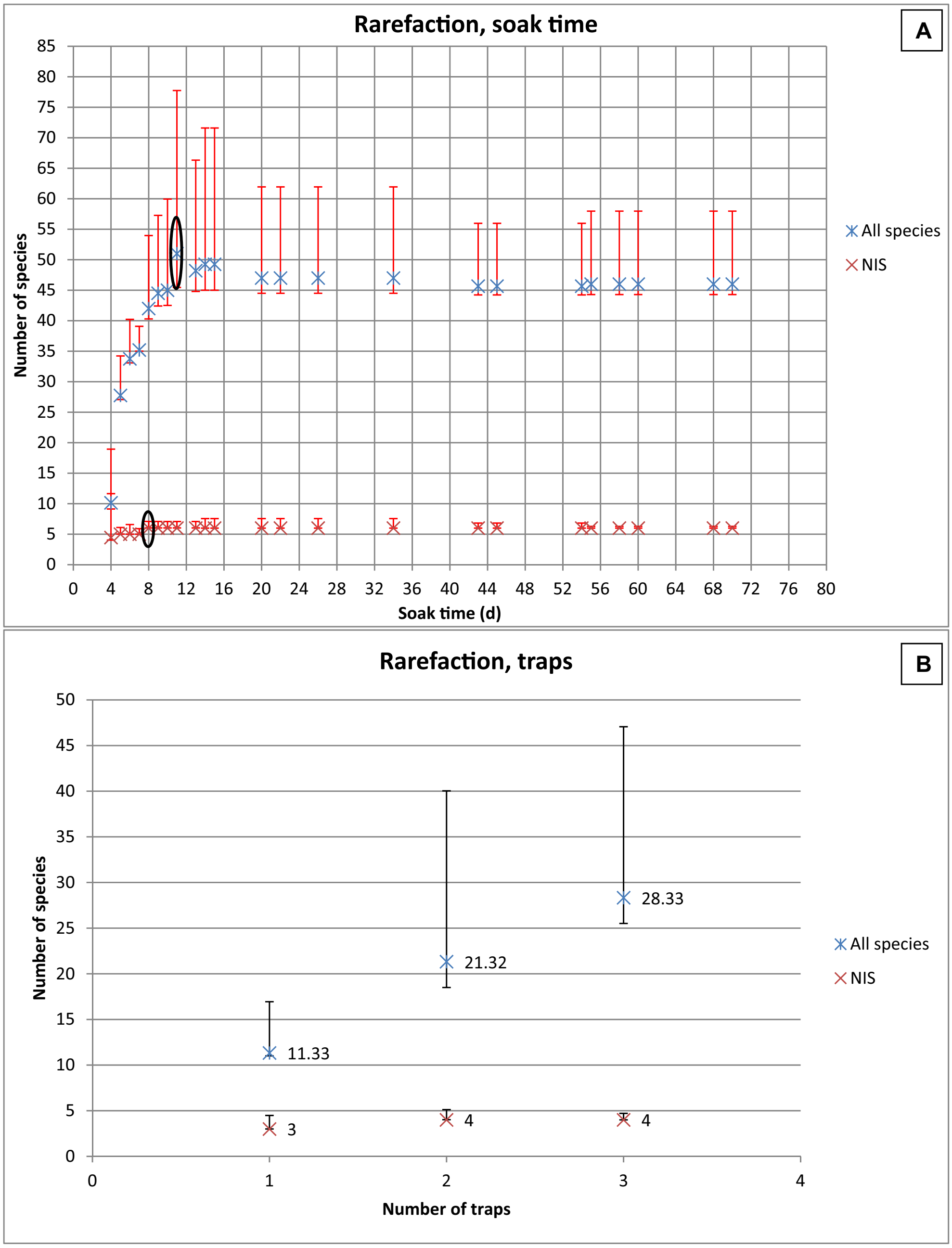

Fig. 9. Rarefaction curves in relation to total species and NIS richness within the soak time of a trap/collector in days (A) and by one, two and three traps per sample location (B). Error bars demonstrate Chao 95% confidence intervals (lower and upper boundaries). Dark circles in panel A demonstrate the number of days when all species and NIS were detected (according to Chao 95% CI lower boundary).

superior to all other traps and collectors in attracting NIS abundances, and this measure was hard to match even with different combinations of other traps and collectors.

No additional species or NIS were found from the fouling plate samples in comparison to habitat collectors. Therefore, deployment of fouling plates is not considered necessary in the Northern Baltic Sea. However, deployment of the plates is recommended in a situation where only baited traps are available. Fouling plates might be also useful in more species-rich areas, as all collectors may not provide enough attachment surfaces for more diverse fouling communities. Earlier studies on fouling plates (deRivera et al., 2005; Canning-Clode et al., 2011; Gartner et al., 2016) also strongly support this assumption. For example Canning-Clode et al. (2011) found 40 species from fouling plates deployed at a tropical site (Panama canal), in comparison to only 16 species detected from samples at a temperate site (Virginia's Eastern shore). Similarly, a broad-scale experiment conducted by deRivera et al. (2005) studied invasion patterns of aquatic NIS along the West Coast of North America by monitoring sessile epifauna, as well as coastal crabs and fishes all the way from San Diego (California) to Kachemak Bay (Alaska). They discovered that NIS richness decreased significantly with increasing latitude, although native and cryptogenic species did not follow the latitudinal pattern. This pattern was found particularly true to tunicates and bryozoans (deRivera et al., 2005). In addition, even though fouling plates may fail to present the diversity of the sampled organisms, they can be beneficial when included into a larger monitoring program with diversity of sampling methods (Gartner et al., 2016). This is especially true in a situation where baited traps are the only available method for the sampling of mobile epifauna, as these traps are not designed to provide attachment surfaces for sessile species (deRivera et al., 2005).

As the catch efficiency of American collectors with oyster shells was the highest, their use is highly recommended in all places, where oyster shells are easily accessible. After all, oyster shells played a crucial part in attracting NIS, as for example the regular American collectors had identical frame and they attracted significantly less NIS with different habitat materials as contents. However, if oyster shells are being used outside of their native range, they need to be autoclaved before deploying them to prevent the potential transfer of NIS from the oyster shells. Even though habitat collectors and fouling plates are passive sampling methods, they share the benefit of enabling sample collection on different temporal scales (Cangussu et al., 2010). Furthermore, the collectors and fouling plates are simple to create and deploy, do not cause harm to native species, do not require trapping permission for most areas, and can be monitored at several sites simultaneously with a minimal force of labour.

The collectors need to be filled with reasonably accessible contents to provide habitat and refuges for organisms. The American, basket and metal net collectors contained flower pot and gardening hose pieces, as well as decorative rocks. Based on the observations made during the study, flower pot pieces provided refuges for organisms but failed to provide attachment surfaces for sessile species as they became slimy and crowded by epibiont algae. Gardening hose pieces offered reasonably good refuges for small fishes, whereas decorative rocks failed to provide organisms with refuges or attachment surfaces. Fouling organisms were found mainly attached to the plastic frames of the American collectors. Therefore, more tests on potential content materials of the collectors are needed when oyster shells are not available, as well as developing a standardized volume range for oyster shells or other content materials within a single collector.

4.2. Habitat and depth variability

Shallow estuarine waters are prone to new invasions of NIS due to their proximity to major shipping pathways (Glasby et al., 2007). These areas also provide a wide range of potential habitats to many small fish species and decapod crustaceans, gastropods and bivalves (Rozas and Minello, 1997; deRivera et al., 2005; Cangussu et al., 2010; Birdsey et al., 2012), and it is possible that NIS in a new location also take advantage of novel habitats. For instance, depending on geographical location, invasive Harris mud crabs (*Rhithropanopeus harrisii*) utilize rocks and woody debris (Roche and Torchin, 2007), sandy sea beds with different types of detritus, as well as mud and rocky bottoms with seagrass vegetation (Fowler et al., 2013; Forsström et al., 2015). Similar patterns have been detected for sessile invertebrates that colonize a wide range of available substrates (Cangussu et al., 2010).

Habitat type had no significant effect on NIS richness or abundance in the study. Nevertheless, sampling the most dominant habitat types within a sampling area is recommended, as some NIS can have more restricted habitat requirements (Bax et al., 2008; David and Gollasch, 2015). More importantly, stratified habitat mapping can be used to identify areas of interest, such as shipping pathways and potential discharge sites of ballast water (Glasby et al., 2007; Hayek and Buzas, 2010; Lehtiniemi et al., 2015).

As the highest NIS richness's and abundances were detected at shallower depths, sampling between 1 and 2 m depth is suggested. Nonetheless, planning of sampling depths may require some reconsideration in other regions, as certain organisms can prefer even shallower areas due to abiotic factors, e.g. relatively high water turbidity (Leppäkoski and Olenin, 2000). Furthermore, certain species may also prefer deeper areas, and their distribution is not necessarily restricted to two metres (Kornis et al., 2012; Fowler et al., 2013; Nurkse et al., 2015). This perspective can be also supported by the findings here, as most New Zealand mud snails were caught at 3 m. Some NIS can also have a wider depth range in more species-rich environments where their habitat selection is influenced more by tides and competition for space (Carlton et al., 1990; Roche et al., 2009; Kornis et al., 2012).

4.3. Timing and sufficient sampling effort

NIS richness and abundance increased significantly after every month of the study, with the highest numbers during September. This finding suggests that sampling should be conducted at the end of the summer, when the seasonal succession of these organisms is at its highest, although this assumption naturally may not hold true in all geographical regions. Establishment and introductions of NIS in the northern Baltic Sea most likely depend on seasonal weather fluctuations (Leppäkoski and Olenin, 2000), as water temperature and sea ice coverage can determine the survival of many species, whereas NIS invasions are year-round threats in less temperate areas (Cangussu et al., 2010). The Joint Harmonised Procedure by HELCOM and OSPAR suggests two separate sampling events to monitor NIS in ports due to seasonal succession patterns of certain species (plankton): one during spring bloom and another at the end of the summer (HELCOM and OSPAR, 2013). If the establishment of targeted NIS seasonally fluctuates, an extended sampling period starting from early summer could enhance the chance of early detection when environmental circumstances become more preferable for NIS establishment after winter. Monitoring mobile and sessile epifauna does not have to be conducted several times a year, as these species are more long-lived than seasonally occurring planktonic species (Lehtiniemi et al., 2015).

NIS richness increased significantly with increasing soak time of a trap but correlated negatively with species diversity. However, there is a possibility that this outcome was influenced by other factors, as the traps with the longest soak time were all retrieved during September, which was the most successful month in terms of NIS richness and abundance. Furthermore, increasing soak time only increased the total richness, and the species accumulation curves indicated that no new species were detected after 11 days and no new NIS after 8 days. Therefore, a soak time of 7 to 14 days can be considered sufficient. However, sampling of more species-rich environments may become problematic with prolonged soak times due to potentially high densities of fouling organisms (Cordell et al., 2008). Moreover, some variation in the soak times of the traps and plates can be beneficial due to species competition and turn-over. For example, certain sessile species will be found in the first few days after a fouling plate has been deployed, and can be entirely absent later, whereas other species can be found only on plates with significantly longer soak times (Cangussu et al., 2010).

In the assessment of sufficient sampling effort with respect to the number of traps per sample location (here, a single depth within a sampling site), our results are conservative. Due to the uneven statistical design of the study, these data should be considered preliminary, but it is possible to catch several native species and NIS even with one collector, as long as it is placed in a well-chosen location and retrieved often enough. However, a more representative and reliable sample of the species present is obtained with two or three collectors, and utilisation of at least three collectors is suggested since the chance of losing collectors is also possible. In addition, as the tested traps and collectors were unevenly distributed along the study area, more research is needed to conclude the number of deployments/retrievals during the study period to collect all species present. This aspect also very likely varies between latitudes and different coastal areas due to prevailing abiotic factors.

Previously, Hewitt and Martin, 2001 recommended that a minimum of 15 passive traps should be applied per site, as well as deployed and retrieved on a periodic basis. This recommendation was meant for baseline port surveys and is not always practical at natural coastal habitats. Based on the results of this study, fewer collectors can be used at each site if they are efficient and monitoring locations are well-chosen. Deployment of three habitat collectors per sampling site is considered sufficient for a routine coastal survey. Deployment of a set of fouling plates can be considered in areas with higher species pools, as the plates can attract several tunicates and bryozoans also in temperate regions (deRivera et al., 2005) that habitat collectors may not attract. In addition, depths of individual fouling plates can be adjusted above the seabed and certain sessile species can also attach underneath the plates, which is not possible with habitat collectors. If sampling deeper areas (e.g. in more species-rich regions), the recommendation can be also understood as three collectors per the sampled depth.

4.4. Conclusions

The increasing demand of harmonised monitoring methods to sample non-indigenous sessile and mobile epifauna originates from the need to match the requirements of international legislations (EC, 2008, 2014), as well as to obtain comparable and reliable results of the NIS present, their abundances, spread and impacts on other species (Lehtiniemi et al., 2015; Whomersley et al., 2015). Findings of the present study provide reasonable frames on how to monitor mobile and sessile epifauna regardless of the location. Due to regional differences in abiotic factors, attempts to create a universal monitoring protocol are not considered highly appropriate. Certain monitoring characteristics and methodologies can be however recommended to any temperate and sheltered coastal regions based on the study. Sampling with similar equipment to American collectors with oyster shells is recommended. Utilisation of other habitat collectors should aim to provide similar conditions for the targeted organisms, including attachment surfaces, habitats and refuges. Altogether, habitat collectors share the benefit of not endangering native species, as they do not capture organisms. Due to the same reason, authorities conducting the sampling do not need a permission to place habitat collectors in most areas, or check them on a regular basis. However, no sampling equipment is completely unbiased and the chance of missing species during a monitoring event is always present, especially with NIS as they may occur in low densities after first invasion (Rozas and Minello, 1997; Lehtiniemi et al., 2015).

Acknowledgements

Implementation of the present study was enabled by the Finnish Ministry of Environment and European Regional Development Fund (VIPU project), as well as the Interreg Baltic Sea Region Programme 2014–2020 for the Completing management options in the Baltic Sea Region to reduce risk of invasive species introduction by shipping (COMPLETE), and their funding is highly acknowledged. The assistance of Riikka Puntila-Dodd (Finnish Environment Institute), Mikael Von Numers, Timo Rantanen and Dmitry Kuznetsov (University of Turku) in field surveys as well as with the study maps is also greatly appreciated.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.02.055.

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