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Sampling native and non-native mobile epifauna with baited traps and habitat collectors – Port of Gdynia case study (southern Baltic Sea, Poland)

by

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Abstract

The effectiveness of two artificial habitat collectors, crab condo (HC1) and habitat crate (HC2), providing a refuge for small mobile fauna, was tested along with two commercial baited traps, Chinese box trap (BT1) and Gee's Minnow trap (BT2) recommended for only single deployments under a harmonized survey of the Baltic and the North-East Atlantic. Our objective was also to determine whether a multi-deployment of baited traps in the growing season increases the diversity and abundance of collected mobile epifauna. Nineteen species of benthic mobile epifauna, including six non-indigenous species (NIS), were collected between May and October 2014 using all tested types of traps in the Port of Gdynia (southern Baltic Sea). Crustaceans, represented by 16 taxa, constituted the group with the highest diversity and abundance. Our study showed that HC1 and HC2 are more effective gear than BT1 and BT2, as both species richness (including NIS) and abundance were higher. Furthermore, the double deployment of BT1 and BT2 increased the diversity and abundance of the captured fauna. The use of artificial habitat collectors as an additional method to the already recommended baited traps for mobile epifauna monitoring in ports should be considered and the number of baited trap deployments should be increased during the growing season.

Key words: port survey, monitoring methods, sampling techniques, macrozoobenthos, non-indigenous species, Baltic Sea

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Introduction

Effective assessment of biodiversity is essential for successful management of marine ecosystems (Painting et al. 2020). Reliable monitoring should be carried out on an appropriate spatial and time scale, using relevant methods that are effective in terms of time, cost and detection. However, this is not always possible. In specific locations, representative results can only be obtained by modifying commonly used monitoring methods. These include ports, where sampling is particularly difficult. Many methods, such trawling, the use of gillnets or diving are not always possible due to challenging sampling conditions or daily port operations. It should be noted that ports and adjacent waters are considered to be the first places where non-indigenous species (NIS) occur and "hotspots" that have great potential for being both their donors and receivers (Drake & Lodge 2004; Bishop & Hutchings 2011). This is due to the fact that the most important pathway for marine NIS introductions is shipping (David et al. 2013; Katsanevakis et al. 2013). Newcomers arrive both in ballast water and sediment, as well as in fouling communities on ships' hulls (Gollasch et al. 2002; Hewitt et al. 2004a; Coutts & Dodgshun 2007; Drake 2015; Castro et al. 2017). Outside their natural range, they usually continue to pose a threat on a global scale (Gollasch et al. 2002; Chandra et al. 2008; Costello et al. 2010; Ojaveer et al. 2014), although their effects, especially economic consequences, can be more evident at the local level (Vilà et al. 2010).

All the facts point to an urgent need to monitor seaports, as well as to the continuous development of efficient and cost-effective passive sampling methods that are approved by port authorities. In recent years, a harmonized survey has been developed for Baltic Sea ports to grant exemptions for ballast water management in accordance with the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWMC) under Regulation A-4 (HELCOM 2013). This monitoring protocol is specifically dedicated to NIS detection and involves sampling of several groups of organisms: phytoplankton, zooplankton, and benthic fauna – fouling (hard substrate) organisms, infauna (soft-bottom benthos), and mobile epifauna. Benthic fauna represents an important and usually the most abundant and most diverse group among NIS introduced to the coastal regions (Nunes et al. 2014). In the Baltic Sea, zoo- and nektobenthic species constitute up to 65% of all NIS with established populations (Ojaveer et al. 2017). This group therefore requires particular attention during port surveys in order to provide solid data on taxonomic composition, which is a prerequisite not only for granting exemptions from BWMC under Regulation A-4, but also for assessing the environmental status based on the newly-introduced NIS descriptor for the purpose of the Marine Strategy Framework Directive (EC 2008; David et al. 2014; Ojaveer et al. 2014; Olenin et al. 2016; EC 2017; Gollasch et al. 2020). The detailed port survey protocol outlined in the HELCOM-OSPAR Joint Harmonized Procedure (JHP) for BWMC A-4 exemptions (HELCOM 2013) recommends the use of two baited traps (Fukui box trap and Gee's Minnow) deployed once in midsummer to sample mobile epifauna. However, due to the use of mesh size 1.3 cm and 6.4 mm, respectively, it is possible to capture only relatively large animals (e.g. decapods, demersal fish). Therefore, we have recognized the need to improve the recommended methods with additional types of traps to collect more effectively also smaller mobile epifauna (e.g. amphipods, isopods). Representatives of this group usually require a refuge, so it seems reasonable to use that trait while planning what type of gear to engage during the survey. As a result of this line of thinking, we chose two types of traps from among non-commercial artificial habitat collectors, which provide a habitat for colonization and are known for their effectiveness in mobile fauna collection (Roche et al. 2008; Fowler et al. 2013; Hewitt & McDonald 2013). Moreover, it seems highly unlikely to collect a representative sample of any fauna during only one visit in the field, and this issue was also addressed during our study.

We have established the following objectives for our study: (1) to compare the effectiveness of artificial habitat collectors and baited traps recommended in JHP for monitoring mobile benthic epifauna in marine ports; and (2) to ascertain whether multiple deployments of baited traps during the growing season affect the number of species caught during the survey and their abundance. Furthermore, we have also determined the diversity of mobile epifauna (with special consideration of non-indigenous species) in the Port of Gdynia.

Materials and methods

Study area, sampling gear and sample preparation

Our study was conducted in the inner and outer part of the Port of Gdynia (western part of the Gulf of Gdańsk, the southern Baltic Sea, Poland), between May and October 2014. The depth was 10 m, whereas salinity varied from 7.0 to 7.1 and temperature from

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9.3 to 22.8°C.

We used four gear types to collect benthic mobile fauna. Two of them are commercially available baited traps recommended by HELCOM (2013) for mobile fauna sampling during port surveys: Chinese box trap (BT1, 60 × 45 × 20 cm, with 1.3 cm mesh size, Fig. 1A) and Gee's Minnow trap (BT2, 42 × 23 cm, with 6.4 mm mesh size, Fig. 1B). The next two were self-made non-baited artificial habitat collectors: crab condo (HC1, 2013; 25 × 50 mm PVC tubes, closed at one end with 0.5 mm mesh, arranged in a 3 × 3 matrix, surrounded by 2 mm mesh, Hewitt & McDonald 2013; Fig. 1C) and habitat crate (HC2, 22 × 16 × 18.5 cm plastic crate filled with autoclaved oyster shells; Roche et al. 2008; Fowler et al. 2013, Fig. 1D). As a single deployment (n) of BT1 and BT2 (both with a dead European flounder *Platichthys flesus* as a bait) we defined three replicates of each baited trap placed at the sampling site for 48 h. In the case of HC1, a single deployment (n) refers to three collectors deployed for four weeks, while for HC2 – three collectors deployed for eight weeks. After retrieving traps BT1 and BT2, the catch was immediately preserved in 4% formaldehyde solution. Collectors HC1 and HC2 were carefully removed and then placed (separately) in a cool box filled with in situ water before being transported to the laboratory, where the whole catch was rinsed out

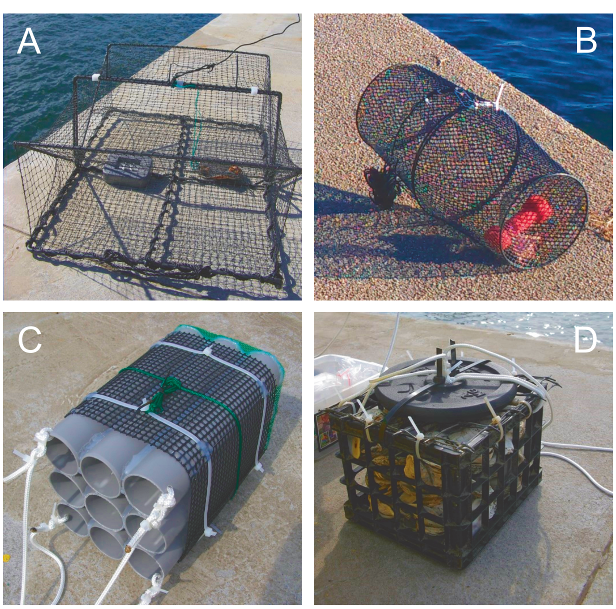

Figure 1

Types of traps used for mobile epifauna sampling from May to October 2014 in the Port of Gdynia: A – Chinese box trap (BT1), B – Gee's Minnow trap (BT2), C – crab condo (HC1) and D – habitat crate (HC2)

of the traps, and then preserved in 4% formaldehyde solution. All organisms were then identified to the species level.

Comparison of the effectiveness of different types of sampling gear

The effectiveness of different types of sampling gear was tested in the inner part of the port. For collectors HC1 used between June and July (single deployment, n = 1), the total number of species and the total abundance of fauna obtained with traps BT1 and BT2, deployed in the same time frame (single deployment of each type of trap every month, n = 2), were taken into account. Similarly, for collectors HC2 used between May and July (single deployment, n = 1), the total number of species and their total abundance sampled with traps BT1 and BT2, deployed in the same time frame (single deployment in May, June and July, n = 3), were compared. In addition, differences in species diversity between the baited traps and habitat collectors (BT1 + BT2 vs. HC1 and BT1 + BT2 vs. HC2) were determined with the Shannon–Wiener index (Spellerberg & Fedor 2003) as follows:

$$H' = -\sum_{n=1}^{S} (p_i \log p_i)$$

where p_i is the proportion of a given species relative to the total number of individuals found in a sample. To indicate the statistical difference in species diversity between the traps (BT1 + BT2) and collectors (HC1 or HC2), the Kruskal–Wallis statistical test was used at $p < 0.05$ significance. The analyses were carried out using Statistica 13.1 PL.

Comparison of the effectiveness of single vs. multiple deployment of baited traps

The effectiveness of the multiple deployment of baited traps in the collection of mobile fauna in relation to a single exposure was tested in the outer part of the port. We have compared the total number of species and the total abundance obtained with BT1 and BT2 deployed in July (n = 1) with those exposed from July to August (n = 2), July to September (n = 3) and July to October (n = 4).

Diversity of mobile epifauna in the port

Diversity of the mobile epifauna occurring in the Port of Gdynia was determined based on samples collected with all applied sampling gear types as described in the previous sections. In addition, the mobile epifauna was also obtained by a single deployment of HC1 (August) and HC2 (September–October) collectors in the outer part of the port.

Results

During our study, the benthic mobile epifauna in the Port of Gdynia was represented by 19 species, including six non-indigenous species (Table 1). Malacostraca (Crustacea), represented by 16 species, was the group with the highest diversity and the highest abundance. The proportion of non-native crustaceans among all collected in our study was 0.31. The use of trap HC2 allowed the detection of species new to the Polish coastal waters, the amphipod *M. nitida* (Normant-Saremba et al. 2017) and the tanaid *S. vanhaareni* (Brzana et al. 2019).

The smallest number of species was caught with baited traps BT1 and BT2 (five and six, respectively), and the largest with artificial habitat collectors HC1 and HC2 (12 and 15, respectively). Two species, *Idotea chelipes* and *Neogobius melanostomus*, were found in all types of traps. Two other species, *Neomysis integer* and *Praunus flexuosus*, were found only in trap HC1, while another five species, *Heterotanais oerstedii, Sinelobus vanhaareni*, *Lekanesphaera rugicauda*, *Gammarus salinus*, and *Tenellia adspersa*, were found only in trap HC2.

When comparing the diversity of mobile epifauna collected in the inner part of the port between June and July, using different types of traps, we found that more species were caught with HC1 than with BT1 or BT2, i.e. respectively three times and almost twice as many. On the other hand, the abundance was more than twice and four times higher in HC1 than in BT1 and BT2, respectively. Similarly, the diversity of mobile epifauna collected in the inner part of the port between May and July, using HC2, was three and two times higher compared to BT1 or BT2 (Table 2). The abundance was also almost five and eleven times higher in HC2 than in BT1 and BT2, respectively. In addition, habitat collectors were more efficient in sampling NIS than baited traps.

The Shannon–Wiener index calculated on the basis of species richness (see Table 2) for both baited traps

Table 1

Mobile epifauna species collected in the Port of Gdynia by different types of traps (symbols are explained in the text), from May to October 2014. "+" – present, n – the number of deployments, non-indigenous species are marked in bold

Species	Baited trap		Habitat collector	
	BT1 n = 7	BT2 n = 7	HC1 n = 2	HC2 n = 2
Crustacea				
Neomysis integer (Leach, 1814)			+	
Praunus flexuosus (Müller, 1776)			+	
Heterotanais oerstedii (Krøyer, 1842)				+
Sinelobus vanhaareni Bamber, 2014				+
Idotea chelipes (Pallas, 1766)	+	+	+	+
Jaera albifrons Leach, 1814			+	+
Lekanesphaera rugicauda (Leach, 1814)				+
Apocorophium lacustre (Vanhöffen, 1911)			+	+
Leptocheirus pilosus Zaddach, 1844			+	+
Melita nitida Smith, 1873			+	+
Gammarus zaddachi Sexton, 1912			+	+
Gammarus salinus Spooner, 1947				+
Gammarus tigrinus Sexton, 1939			+	+
Palaemon adspersus Rathke, 1837	+	+		+
Palaemon elegans Rathke, 1837	+	+		
Rhithropanopeus harrisii (Gould, 1841)		+	+	+
Gastropoda				
Tenellia adspersa (Nordmann, 1845)				+
Actinopterygii				
Neogobius melanostomus (Pallas, 1814)	+	+	+	+
Nerophis ophidion (Linnaeus, 1758)	+	+	+	
Number of all species	5	6	12	15
Number of NIS	**2**	**3**	**4**	**5**

Table 2

The number of species and total observed abundance of mobile epifauna collected in the inner part of the Port of Gdynia by different types of traps (symbols are explained in the text), from May to July 2014. Non-indigenous species are marked in bold, n – number of deployments.

Species number/ abundance	June–July			May–July		
	BT1 n = 2	BT2 n = 2	HC1 n = 1	BT1 n = 3	BT2 n = 3	HC2 n = 1
Number of all species	3	5	9	3	5	10
Number of NIS	**1**	**1**	**2**	**1**	**1**	**4**
Abundance of all species	16	8	35	18	8	87
Abundance of NIS	**1**	**2**	**9**	**1**	**2**	**20**

(BT1 + BT2) varied from 0.382 to 0.555 and from 0.807 (HC1) to 0.868 (HC2) for habitat collectors. There was a statistically significant difference ($p < 0.05$) in species diversity between samples caught by baited traps and habitat collectors.

Multiple sampling with baited traps conducted in the outer part of the port demonstrated an increase in the number of species caught, from three with a single deployment to four with double, triple and quadruple deployment. On the other hand, that total abundance increased from 5 to 75 individuals (Figure 2).

Discussion

During the research conducted in the Port of Gdynia, a relatively high species diversity of mobile epifauna was observed, especially when considering the prevailing environmental conditions, including the presence of potentially harmful chemical elements and compounds (Radke et al. 2013), underwater noise (Klusek et al. 2014), as well as water and sediment dynamics (causing periodic movement or burial of some organisms) in the port basins. On the other hand, ports with a large surface of quays, covered with biofouling (providing refuge), offer perfect habitat for this group of organisms. The ratio of non-native to native mobile epifauna species recorded in the Port of Gdynia amounted to 1:3.2, which is higher than that observed in estuaries and lagoons, i.e. 1:5, (Reise et al. 2006).

Of the 19 recorded taxa, 84% were crustaceans, a species-rich taxonomic group, which also occur in a similar proportion among the mobile benthic fauna from other regions of the southern Baltic Sea (Zettler & Daunys 2007; Zettler et al. 2007; Masłowski 2010; Janas & Kendzierska 2014; Brzana & Janas 2016;

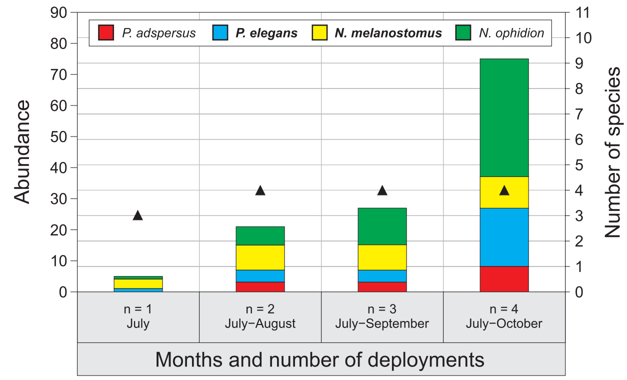

Figure 2

Abundance (bar) and the number (triangle) of mobile epifauna species collected in the outer part of the Port of Gdynia in 2014 by multiple deployment (n) of baited traps (BT1 + BT2) from July to October 2014. Non-indigenous species are marked in bold.

Dąbrowska et al. 2016). The proportion of non-native crustaceans among all those collected in our study is also close to that recorded outside the Port of Gdynia (Janas & Kendzierska 2014). In addition to crustaceans, two representatives of ichthyofauna, the native straightnose pipefish *N. ophidion* and the non-native round goby *N. melanostomus* as well as the lagoon sea slug *T. adspersa* were also found. The presence of the latter species in traps was somewhat surprising, because it is usually associated with communities dominated by hydrozoans, whose hydroids are used to attach eggs and as a food source (Chester 1996).

Based on the species richness, abundance and Shannon–Wiener index, it can be concluded that habitat collectors are more effective when sampling mobile epifauna compared to baited traps recommended by HELCOM (2013). They were also more effective for NIS. Outinen et al. (2019) came to the same conclusions based on research conducted in the Archipelago Sea (Finland) between June and September in 2012. The effectiveness of shelter traps was also confirmed by studies conducted by Veldhuizen (2000), Fowler et al. (2013) and Hewitt and McDonald (2013). Artificial habitat traps have proved particularly effective in catching small crustaceans, including isopods and amphipods, i.e. fauna using macrophytes, stones, as well as man-made structures as a refuge from predators (Viejo 1999; Reichert & Beermann 2011; Sedano et al. 2020). In ports like Gdynia, these organisms seem to hide near the quays, as well as among stones filling gabions and other structures used to stabilize the bottom against sediment movement. To get them out of these hideouts, an alternative shelter should be provided, e.g. an artificial habitat collector. However, settling in this type of trap by mobile fauna requires time, which is why artificial habitat collectors (HC1 and HC2) must be exposed for a longer time than baited traps (BT1 and BT2), which attract organisms in a relatively short time (Slack-Smith, 2001). In addition, in the latter case, the large mesh size means that smaller organisms (e.g. isopods, amphipods) can freely leave the trap after consuming the bait. Therefore, longer exposure may also reduce the efficiency of capturing smaller species.

The habitat crate worked better than the crab condo, i.e. oyster shells provided better habitat and shelter than PVC tubes. However, as shown by Outinen et al. (2019), the effectiveness of the habitat crate may also be affected by the type of material used for filling. Oyster shells seem to be a better substrate than e.g. ceramic pieces (Outinen et al. 2019). This is not surprising as oyster reefs create excellent habitats that are colonized by a large variety of benthic fauna (Ruesink et al. 2005). This also applies to *M. nitida*, a North American amphipod, which was found for the first time in Polish waters during our studies (Normant-Saremba et al. 2017). This species is commonly associated with oysters both in its native and non-native regions (Watling & Maurer 1972; Faasse & van Moorsel 2003). It is worth mentioning that in our study *M. nitida* was not found in any other sampling gear, contrary to *S. vanhaareni* that was also found in fouling communities on PVC plates (Brzana et al. 2019). Therefore, the use of habitat collector HC2 allowed for rapid detection of a new species in the port. This type of information is extremely important for various reasons, e.g. it may indicate ineffective prevention or help to take immediate management action on a new NIS.

In early summer (May–July), habitat collectors also enabled the collection of a few to several times more mobile epifauna than baited traps. This, in turn, may increase the chance of detecting additional species and the possibility of sampling several individuals of the same species, which may be important for taxonomic identification of new NIS. This was the case with *M. nitida*, where only three of the 13 individuals collected were males with clear species characteristics (Normant-Saremba et al. 2017). A larger number of individuals collected for one species may also provide more information, e.g. on size and sex structure.

Based on our data it can also be concluded that multiple exposure of baited traps in summer/early autumn (July–October) may be more effective in collecting Baltic mobile fauna than single exposure in summer peak. Although Lehtiniemi et al. (2015) suggested that monitoring of mobile epifauna does not have to be conducted several times, we collected one species more (*P. adspersus*) already in the second deployment (in August) compared to a single deployment (July, summer maximum). On the one hand, it is only one more species, but on the other, it could also be a new species and its detection is one of the main objectives of monitoring. This also seems to be of particular importance in regions with low macrobenthos biodiversity, like the southern and northern Baltic Sea (Zettler et al. 2014). No further increase in the number of species with three or four trap deployments can be simply accidental, because this number may even vary between samples collected two days apart in the same month, as shown in similar studies carried out with the Chinese box trap (BT1) in the port of Liepaja (Latvia) in 2014 (HELCOM 2015). The results of the survey carried out in the latter port also confirmed that multiple deployment (more than once) during the growing season can lead to an increased number of species caught, i.e. diversity of mobile fauna collected with BT1 was more than twice as high in October 2014 (nine species) compared to May 2014 (HELCOM 2015). So far, there is no literature concerning such a comparison with regard to monitoring of marine ports, but many studies on biodiversity stress the importance of a sufficient number of samples to be analyzed in order to find all species occurring in the studied area (e.g. de-la-Ossa-Carretero 2009; Van Hoey et al. 2010; Painting et al. 2020). In the case of the Baltic Sea region, the sampling period should be adjusted to the growing season, which varies spatially in duration and timing. In some parts, extending the sampling period to early autumn may enhance the chance of sampling adult individuals of species that breed later as well as an early detection of new coming species. This may also be relevant for determining whether a new NIS occurred in the ecosystem only accidentally (single record), as in the case of *Palaemon longirostris* recorded in the Port of Gdynia not only in July, but also in August and October 2018 (AquaNIS 2020).

Similarly to Outinen et al. (2019), our study indicates high sampling efficiency of artificial habitat collectors compared to baited traps. Admittedly, they require a long exposure and time-consuming analysis if many organisms are caught, but the effectiveness of the mobile epifauna collection (even with fewer deployments) favors their use in port monitoring. For this reason, artificial habitat collectors were already implemented in some countries where a national program of port/coastal water monitoring was conducted, e.g. Australia, New Zealand (Hewitt et al. 2004b; Hewitt et al. 2009). Based on the obtained results, we conclude that both the use of artificial habitat collectors (especially the habitat crate with oyster shells as a filling material), as an additional tool to the already recommended baited traps for mobile epifauna sampling in Baltic ports, as well as increasing the number of baited traps' deployments should be considered in the harmonized port survey protocol developed by HELCOM (HELCOM 2013). At this stage, however, these considerations should be approached in general terms, as detailed recommendations require more representative studies confirmed by statistical analysis.

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