Marine Pollution Bulletin 109 (2016) 350–355

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

# Can environmental DNA (eDNA) be used for detection and monitoring of introduced crab species in the Baltic Sea?

Tiia Forsström [a,*], Anti Vasemägi [b,c]

[a] Section of Ecology, Department of Biology, University of Turku, FIN 20014 Turku, Finland
[b] Section of Genetics and Physiology, Department of Biology, University of Turku, FIN 20014 Turku, Finland
[c] Department of Aquaculture, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, 51014 Tartu, Estonia

ARTICLE INFO

*Article history:*
Received 9 March 2016
Received in revised form 17 May 2016
Accepted 21 May 2016
Available online 31 May 2016

*Keywords:*
eDNA
Brackish water
*Rhithropanopeus harrisii*
Biological invasions
Detection

ABSTRACT

The need to detect and monitor introduced marine species has increased with the increasing number of marine invasions. To complement standard detection and monitoring techniques, new approaches using environmental DNA (eDNA) have recently been developed. However, most of the eDNA work has focused on vertebrate species in spatially limited freshwater habitats while benthic invertebrates in coastal environments have received much less attention. Here, we evaluated the suitability of the eDNA approach for detecting benthic, hard-shelled, crustacean mud crab species in a brackish water environment. We demonstrated for the first time that eDNA from an introduced mud crab *Rhithropanopeus harrisii* can be successfully amplified in aquarium water samples and detected in the brackish water environment. However, the detection rate was rather low. This suggests that in contrast to freshwater vertebrates, it may be more challenging to develop a highly sensitive eDNA method for detecting crustacean species in a marine environment.



## 1. Introduction

Introduced species are considered one of the most important threats to the structure and functioning of coastal ecosystems creating a need for the early detection and monitoring of the non-native species (Ruiz et al., 1997; Bax et al., 2003; Molnar et al., 2008). However, the detection of aquatic organisms using traditional methods, especially at low densities, can be challenging and ineffective. This makes traditional detection and monitoring techniques often problematic due to difficulties associated with taxonomic identification, non-standardized sampling procedures, and the invasive nature of some survey techniques. To overcome some of the limitations of traditional detection and monitoring methods of aquatic species, new approaches using environmental DNA (eDNA) have recently been developed (Ficetola et al., 2008).

Using environmental DNA, identification of species is achieved by detecting DNA fragment(s) that animals release into the water. DNA in water may originate from various sources, including feces, skin cells, epidermal mucus, urine and saliva. During recent years, eDNA has been successfully used to assess biodiversity and species abundance in a wide range of aquatic organisms, including various fish species, amphibians, and marine mammals (Ficetola et al., 2008; Goldberg et al., 2011; Rees et al., 2014 and references therein; Takahara et al., 2012; Thomsen et al., 2012a,b). The majority of these studies have used two alternative molecular genetic approaches. First, species-specific primers that amplify certain DNA fragment only in a single target species can be used to detect species in combination with gel electrophoresis or quantitative real-time PCR (qPCR) methodologies (Dejean et al., 2012; Ficetola et al., 2008; Thomsen et al., 2012b; Tréguier et al., 2014). Alternatively, more universal primers that amplify certain DNA fragment in multiple species can be used together with next generation sequencing (NGS) approaches that enable in silico identification of several species based on DNA sequence information (Deiner et al., 2015; Evans et al., 2016; Kelly et al., 2014).

eDNA approach has often been claimed to be more efficient in detecting species than traditional detection methods (Dejean et al., 2012; Keskin, 2014; Thomsen et al., 2012a) representing an exciting new tool for the detection of recently introduced species and monitoring species dispersal (Blanchet, 2012; Comtet et al., 2015; Keskin, 2014; Piaggio et al., 2014; Takahara et al., 2013). However, most of the earlier studies have focused on vertebrate species, i.e. fish and amphibians (Dejean et al., 2012; Ficetola et al., 2008; Takahara et al., 2013; Thomsen et al., 2012a), which are known to secrete body mucus containing extracellular DNA (Livia et al., 2006). On the other hand, the applicability of eDNA approach for detection of benthic invertebrate species with an exoskeleton has received much less attention (Tréguier et al., 2014). Furthermore, the majority of the published studies have focused on spatially limited freshwater environments such as lakes, ponds and streams (Roussel et al., 2015). However, unlike most freshwater environments, the detection of eDNA in seawater is expected to be more challenging due to the larger volume of water and currents. At the same time the introductions of non-native species in

* Corresponding author.
*E-mail address:* ttfors@utu.fi (T. Forsström).

http://dx.doi.org/10.1016/j.marpolbul.2016.05.054
0025-326X/

marine environments have increased rapidly due to shipping activities (Bax et al., 2003; Ruiz et al., 1997) with zoobenthos being the dominant introduced species group in many sea areas (Gollasch, 2006; Streftaris et al., 2005). Thus, there is an increasing need for effective and reliable detection and monitoring methods of marine introduced species. However, before eDNA methodology can be routinely applied for screening introduced invertebrate species in marine or brackish water environment, more information about its performance, sensitivity and reliability is needed.

Globally, one of the most widely distributed brachyuran crab species is the North American white-fingered mud crab *Rhithropanopeus harrisii* (Roche and Torchin, 2007). *R. harrisii* has invaded over 20 countries, two oceans, ten seas, and fresh-water inland reservoirs across four continents (Roche and Torchin, 2007). It arrived in the southern parts of the Baltic Sea and rapidly spread along the southern Baltic coast (Bacevičius and Gasiunaite, 2008; Demel, 1953; Kotta and Ojaveer, 2012; Wolff, 1954). It was first recorded in the Archipelago Sea along the southwestern coast of Finland in 2009, and since then the population has continued to spread along the coast (Fowler et al., 2013). In its introduced range, *R. harrisii* have been found in a variety of habitats ranging from soft sand and mud sediments to hard bottom habitats dominated by macroalgae *Fucus vesiculosus*. In the Baltic Sea, *R. harrisii* has been found to act as an effective predator decreasing species richness and the diversity of the native species (Forsström et al., 2015).

In this study, we evaluated the suitability of the eDNA approach for detecting benthic, hard-shelled, crustacean mud crab species. We designed species-specific primers and probes for the target species *R. harrisii* and conducted aquarium experiment to assess i) the amount of eDNA released in the water by *R. harrisii* and ii) the persistence of eDNA in the water after the removal of the target species. In addition to aquarium experiment, we evaluated the applicability and sensitivity of the eDNA method in the natural brackish water environment of the Baltic Sea.

## 2. Materials and methods

### 2.1. Aquarium experiment

To assess the rate of eDNA released by *R. harrisii*, we placed four individuals in separate clean plastic aquariums containing only 10 L of tap water mixed with Instant Ocean® sea salt mix at 5.6 psu salinity. This corresponds to the salinity in the area where the *R. harrisii* individuals used in the experiment were collected one week prior to the start of the experiment. The four male *R. harrisii* served as biological replicates and varied little in size; carapace width of 18.78–21.55 mm. We kept the aquariums at a temperature of 17 °C with a light regime of 10 L: 14D. The *R. harrisii* individuals were in the aquariums for 8 days with sera® viformo food ad libitum, after which we removed the *R. harrisii* from the aquariums.

We collected water samples from each of the four aquariums before adding the *R. harrisii* to the aquariums, to test for potential contamination prior to the start of the experiment. To evaluate the accumulation of the eDNA, we sampled the water at day 1, 3, 5 and 8 after adding the *R. harrisii*. To characterize the persistence of *R. harrisii* DNA in the water after the individuals were removed from the aquariums, we sampled the water at day 1, 2, 3, 5 and 7 after the removal of the *R. harrisii*. During each sampling, we collected 15 mL of water in a 50 mL falcon tube and immediately added 1.5 mL of sodium acetate 3 mol/$L^{-1}$ and 33 mL of ethanol (99.5%) to the samples (Ficetola et al., 2008). We collected two replicate water samples from each aquarium on each sampling day. All the samples were stored at −20 °C until the DNA extraction. During and after the experiment we also collected negative control samples in similar way as described above, except that the aquarium water was replaced with tap water to test for potential contamination.

### 2.2. Field validation

Water from natural water bodies usually contains organic material and DNA from a large number of species, which may influence the detectability of the target DNA. Therefore, to test for the detectability of *R. harrisii* using the newly developed eDNA assay, we sampled water from a sheltered bay near the Archipelago Research Institute in Seili, Nauvo (N 60° 14′ E 21° 60′) where *R. harrisii* is readily observed (TF pers. obs.). We collected water samples of near bottom sediment to maximize the detection probability of the target species as *R. harrisii* is a benthic species. We sampled 15 mL of water near the bottom sediment with sterile syringes by snorkeling. Altogether, 21 samples were collected from seven sites (three biological replicate samples per site) from different parts of the bay at a depth of 1 to 1.20 m. One of the sample replicates was lost during DNA extraction. Each sample was treated as described above.

### 2.3. Baltic Sea samples

To further test the usefulness of the newly developed eDNA assay for detecting *R. harrisii*, we collected water samples in sixteen different locations around the Archipelago Sea and in one location at Lake Littoinen (Fig. 1). *R. harrisii* is known to occur in ten sampling locations, while in four sites *R. harrisii* occurrence is not known and in two sites *R. harrisii* does not occur (TF pers. obs.). As an additional negative control, we also sampled Lake Littoinen near the Archipelago Sea where *R. harrisii* is absent. In each site, we sampled 15 mL of water from just below the water surface (three biological replicates per site) in falcon tubes and treated the samples as described above. The samples were taken below the water surface to evaluate whether the eDNA assay works using the simplest sampling procedure which could be easily implemented for large scale detection and monitoring of *R. harrisii*.

### 2.4. DNA extraction

We treated and extracted DNA from all of the collected samples in a similar, randomized manner. To recover the precipitated DNA and/or cellular remains, we centrifuged the samples at 4100 rpm (2481 $\times g$) for 40 min at 6 °C. After the centrifugation, we discarded the supernatant and the obtained pellet was subjected to DNA extraction using Macherey-Nagel® NucleoSpin Tissue kit (product number 740952.250) and following the manufactures protocol with the final elution step of 50 μL for each sample.

### 2.5. qPCR

For quantitative real-time PCR (qPCR), a species specific primer set CrabqF1 5′TTTAGCTGCTGCTATTGCTCA3′ CrabqR1 5′ GAAACACCTGCTAAATGTAAGGAGA3′ was designed to target a small (75 bp) fragment of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) of *R. harrisii* using sequence from GenBank (GenBank accession no. FJ517417) and checked against all other *R. harrisii* sequences in GenBank at the time. The specificity of the primers was evaluated by comparing the sequences to other crab species (*Dyspanopeus sayi*, *Eurypanopeus depressus*, *Pilumnus floridanus*, *Pseudocarcinus gigas*, *Xantho hydrophilus*, *Scyra compressipes*, *Lophozozymus pulchellus*, *Actaea semblatae*, *Lybia* sp., *Eriocheir leptognathus*) including two crab species that have occasionally been encountered in the Archipelago Sea (*Eriocheir sinensis*, *Carcinus maenas*). The primers were designed to have at least 6 mismatches with non-target species. As a probe we used Roche Universal ProbeLibrary probe number 92 (CAGGAGCC) labelled with fluorescein 5′ (FAM) and 3′ Black Hole Quencher. A single clean PCR product was initially observed after standard PCR and 2.5% agarose gel electrophoresis (data not shown). Subsequently, qPCR was carried out in a 10 μL reaction volume containing 5 μL of ABI TaqMan Universal Master Mix II, 1 μL of each primer, 1 μL of probe and 2 μL of