Fisheries Research 191 (2017) 41–48

Contents lists available at ScienceDirect

Fisheries Research

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

Full length article

On the way for detecting and quantifying elusive species in the sea: The *Octopus vulgaris* case study

Q. Mauvisseau[a], M. Parrondo[a], M.P. Fernández[b], L. García[b], J.L. Martínez[c], E. García-Vázquez[a], Y.J. Borrell[a,*]

[a] Department of Functional Biology, University of Oviedo, Calle Julián Clavería S/N, 33006, Spain
[b] Centro de Experimentación Pesquera, Dirección de Pesca Marítima, Gobierno Del Principado De Asturias, Gijon, Spain
[c] Sequencing Unit, Edificio Severo Ochoa, C/Julian Claveria S/N, University of Oviedo, Oviedo 33006, Spain

ARTICLE INFO

Article history:
Received 22 August 2016
Received in revised form 2 February 2017
Accepted 28 February 2017
Handled by George A. Rose

Keywords:
Environmental DNA
Detection
Fisheries
Octopus vulgaris
Quantification

ABSTRACT

Environmental DNA (eDNA) can be a powerful method for assessing the presence and the distribution of aquatic species. We used this tool in order to detect and quantify eDNA from the elusive species *Octopus vulgaris*, using qPCRs (SybrGreen protocol). We designed species-specific primers, and set up an experimental aquarium approach to validate the new molecular tool in different controlled conditions. Field validation was conducted from sea water samples taken from 8 locations within an octopus fishery area in the Cantabrian Sea during February–March 2016. A significant positive correlation between the total biomass (g of *O. vulgaris* within thanks) and the amount of *O. vulgaris* eDNA detected (p-value = 0.01261) was found in aquarium experiments. The species was also detected by PCR in 7 of the 8 water samples taken at sea, and successfully quantified by qPCR in 5 samples. This preliminary study and innovative method opens very promising perspectives for developing quick and cheap tools for the assessment of *O. vulgaris* distribution and abundance in the sea. The method could help in a close future for quantifying unseen and elusive marine species, thus contributing to establish sustainable fisheries.

1. Introduction

Growth strategies in the marine sector aim at strengthening the potential for sustainable development in coasts, seas and oceans of Europe. Innovation in all sectors of the blue economy could help to develop all the potential for growth, employment and significant environmental benefits. Local fisheries represent a good snapshot of the lifestyle of many European communities and guaranteeing their future is necessary. They must be scientifically managed to ensure their sustainability over the long term and their benefits for the community. Well-managed fisheries are the source of sustainable incomes and generate several other economic activities. To drive commercial fisheries towards sustainability while improving the management of small-scale fisheries and aquaculture practices is currently a serious need worldwide (De Melo et al., 2016). Despite many efforts, nearly a third of commercial fisheries globally have already collapsed and only a few of the ocean's fish stocks have been rigorously assessed (Vasilakopoulos et al., 2014; Smith et al., 2014). Overfishing affects many marine species and destroys marine ecosystems by removing species performing essential functions and/or spawning individuals (Howarth et al., 2014).

The common Octopus, *Octopus vulgaris* (Cuvier, 1797) is an elusive coastal cephalopod living between the surface and a depth of about 100–150 m (González et al., 2015). The lifetime of the species is estimated to be two years for both males and females (Otero et al., 2007; González et al., 2015). Its biology and ecology are relatively well known (Mangold and Von Boletzky, 1973; Otero et al., 2009; Perales-Raya et al., 2014; Hermosilla et al., 2010, 2011; González et al., 2015), although there are still regional knowledge gaps (González et al., 2015). This cephalopod is the most harvested octopus's species from the Atlantic and the Mediterranean Sea (Hermosilla et al., 2011) with more than 43,000 t in the world in 2014 (FAO, 2016). Most important fisheries of *O. vulgaris* occur in the east Atlantic (Guerra, 1981) and it is the major cephalopod species in the Cantabrian coast (Spain) (González et al., 2015). This species is of highest interest for the fisheries in that region (Fernández-Rueda and García-Flórez, 2007), as well as in other areas of the east Atlantic. However, to date its population abundance and microscale distribution are largely unknown (González et al., 2015). Short lifespan and single breeding of cephalopods means that time available to assess and respond to changes in

* Corresponding author.
E-mail address: borrellyaisel@uniovi.es (Y.J. Borrell).

http://dx.doi.org/10.1016/j.fishres.2017.02.023
0165-7836/

population biology is too short when compared with conventional multi-age fish population (Boyle and Rodhouse, 2005).

Environmental DNA (eDNA) method is a revolutionary tool for detecting aquatic species (Ficetola et al., 2008; Jerde et al., 2011; Thomsen et al., 2012a; Wilcox et al., 2016). It consists of the detection of DNA molecules left by living organisms in the environment (Laramie, 2013; Wilson and Wright, 2013; Thomsen and Willerslev, 2014; Mächler et al., 2016). It is recognized as an effective non-invasive method for detecting species present in very low abundance (Dejean et al., 2011; Jerde et al., 2011; Fukumoto et al., 2015; Dougherty et al., 2016; Evans et al., 2016; Boothroyd et al., 2016; Wilcox et al., 2016) or undetected by other conventional methods (Janosik and Johnston, 2015; Smart et al., 2015; Eichmiller et al., 2016b; Shaw et al., 2016; Valentini et al., 2016; Yamanaka and Minamoto, 2016). Low persistence of eDNA allows the detection of species actually or recently present in the aquatic environment (Dejean et al., 2011; Thomsen et al., 2012a; Barnes et al., 2014; Piaggio et al., 2014). For all the reasons above, the eDNA method is more sensitive than traditional methods for the detection of an aquatic species (Valentini et al., 2016; Wilcox et al., 2016). On the other hand, current tools employed for estimating the abundance of fish populations only give an estimation of their relative abundance (Lacoursière-Roussel et al., 2015). Estimating real population abundance is essential for a sustainable management of marine species, especially when they are subject to fisheries (e.g. Beddington et al., 2007; Hare et al., 2016). Therefore, developing the eDNA method for quantifying the absolute abundance (biomass) of marine populations will be a revolutionary tool for fisheries and population management, as well as for control and management of invasive and endangered aquatic species (Lacoursière-Roussel et al., 2015; Eichmiller et al., 2016a).

The present study aimed at developing a method for population assessment of *O. vulgaris* based on eDNA. Species-specific primers were designed for this purpose. An experimental approach in aquarium conditions was set up to confirm primers' specificity and validate qPCR methodology under controlled conditions. The method was also assayed from in situ water samples obtained at different locations in the Cantabrian Sea. This pioneer study is the first step towards the use of eDNA-based methodology for assessing the sustainability of common octopus fisheries.

2. Material and methods

2.1. Specific primers design

Species-specific primers targeting the mitochondrial cytochrome oxidase subunit I gene (COI) were designed for *O. vulgaris*. Coding sequences for the COI region were retrieved from the GenBank database and aligned using Bio Edit (Hall, 1999) and MEGA (Tamura et al., 2013). Primers were designed using the Primer3 website (Koressaar and Remm, 2007; Untergasser et al., 2012) and tested *in silico* using NCBI website and GenBank database. The chosen primers amplify a 204 bp (base pair) fragment of the COI region (Forward 5′-TGTTACAGCTCACGCATTTGTT-3′ and Reverse 5′- CCGGTACCTGCACCTCTTTC-3′). The primers were also tested *in vitro* by PCR from DNA extracts of 22 individuals of *O. vulgaris* and one *Eledone cirrhosa* individual (Lamarck, 1798). All the individuals used for tests were previously barcoded using the complete COI gene and blasted in the BOLD database with species identifications with more than 99%. Catch statistics for octopods in ICES (2014, 2016) waters do not always distinguish between these two major species. This has been pointed out by ICES (2014, 2016) as a serious problem in order to compile reliable data regarding octopods catches and efforts (González et al., 2015).

2.2. DNA extraction and PCR amplification from tissues samples

DNA from tissue samples was extracted using the QIAGEN® Kit QIAamp DNA Mini Kit with Tissues Protocol following the manufacturer's instructions. DNA was stored at −20 °C. PCRs were conducted in 20 μl total volume with Green Go Taq Flexi Buffer (1x) PROMEGA®, NgCl2 (2.5 mM), dNTPs (0.5 mM), 0.2 μM of each primer, 0.1 U of Go Taq G2 Flexi Polymerase PROMEGA®, 1 μl of DNA and H2O. The PCR programme included an initial 5 min denaturation at 95 °C, 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and elongation at 72 °C for 30 s. PCR products were visualized using electrophoresis on 2% agarose gel stained with SimplySafe™.

2.3. Aquarium experiments

A lab experiment was set up in order to monitor the eDNA detection in a controlled ecosystem available within the CEP (Centro de Experimentación Pesquera, Gijón, Spain). Five individuals of *O. vulgaris* (total of 4170 g) were put in a tank with 170 l of marine water. One of the 5 octopus escaped of the tank at the end of the first week. The rest of octopus (4 individuals, total of 3260 g) remained in the 170 l tank during the next weeks of the experiment. The water flow of this tank was 1.2 l/min. Other two individuals of *O. vulgaris* (total of 1100 g) were put in a tank with 130 l of marine water. This way in both mesocosms will be some octopus's interactions. The water flow of this last tank was 1.5 l/min. The marine water was obtained from a coastal site in Gijon (Asturias, Spain). Octopus were feed *ad-libitum* with pieces of various crustacean and *Micromesistius poutassou* (Risso, 1827). Tanks were clean once per week just after taking the water samples. Remain of food debris and faecal matter were removed with a nest and a pump. A small part of the water in the tanks were changed during each process. The temperature and the pH were monitored at least twice a week during the 5 week of the experiment using the pH meter WTW ProfiLine pH 197. Water temperature ranged from 12.1 to 15 °C (13.26 °C mean ± 0.89 SD) and pH ranged from 7.9 to 8.11 (7.98 mean ± 0.06 SD). Water quality was confirmed similar in the different tanks. Water samples of 1 l were taken once a week from each tanks and frozen until the filtration and DNA extraction.

2.4. Study area in the Cantabrian Sea and sampling protocol

A total of 8 samples (1L each) were taken from an artisanal fishing vessel by an observer on board from different fishing places in the Cantabrian Sea (Fig. 1). Water samples were taken at different depths in the sea using a 1.7 bottle Niskin KC Denmark. All the water samples were stored at −20 °C before filtration. The *O. vulgaris* individuals used in the experimentation and in primers tests were caught by fishermen near the water sampling area (Fig. 1).

2.5. DNA extraction from water samples and PCR amplification

Water samples were unfreezing at room temperature before filtration. We disinfected all lab equipment prior to filtering with a 10% bleach solution and ethanol at 100% and after that rinsed with distilled water. The water was vacuum filtered through Supor® 200 PES Membrane Disc Filters (Pall Corporation, Life Sciences, USA) with 0.2 μm pore size and 47 mm diameter. DNA was extracted using the PowerWater® DNA isolation Kit (MOBIO Laboratories, USA) following the manufacturer's instructions. DNA was stored at −20 °C.

PCR were conducted in 20 μl total volume with Green Go Taq Flexi Buffer (1X) PROMEGA®, NgCl2 (2.5 mM), dNTPs (0.5 mM), 0.2 μM of each primer, 0.1 U of Go Taq G2 Flexi Polymerase