Biochemical Systematics and Ecology 56 (2014) 68–74

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Detection of invasive freshwater fish species using environmental DNA survey

Emre Keskin*

Ankara University, Agricultural Faculty Department of Fisheries and Aquaculture, Turkey

ARTICLE INFO

Article history:
Received 17 March 2014
Accepted 3 May 2014
Available online 25 May 2014

Keywords:
eDNA
Invasive species
Carassius gibelio
Clarias gariepinus
Oreochromis niloticus
Pseudrasbora parva

ABSTRACT

Invasive species are one of the most significant problem in freshwater ecosystems. Most common non-native freshwater species in Turkish freshwater fish fauna are Prussian Carp (*Carassius gibelio*), North African Catfish (*Clarias gariepinus*), Nile Tilapia (*Oreochromis niloticus*) and Topmouth Gudgeon (*Pseudorasbora parva*).

Recent studies showed that environmental DNA could be used to detect target species inhabiting the ecosystem with higher precision and less effort compared to traditional field surveys. In this study, eDNA approach was used to investigate non-native freshwater fish species from fifteen different locations of Upper Sakarya Basin. eDNA was successfully extracted from the water samples of locations where the species were visually observed. Mean amplification rate of eDNA was calculated as 77.03%.

This study is the first environmental DNA study used in detection of four of the most common invasive freshwater fish species. Results clearly indicating that eDNA surveys could be used as an important molecular tool to monitor invasive fish species in freshwater ecosystems.

1. Introduction

Alien species and biological invasions are considered as one of the most serious problems in conservation of ecosystems and biodiversity (Gozlan et al., 2005). Four of the most common non-native freshwater fish species in Turkish freshwaters were Prussian Carp (*Carassius gibelio*), North African Catfish (*Clarias gariepinus*), Nile Tilapia (*Oreochromis niloticus*) and Topmouth Gudgeon (*Pseudorasbora parva*) (Ekmekçi and Kırankaya, 2006; Emiroğlu, 2011). Recent advances in molecular biology quicken the development of effective DNA based methods that could be used in detection and monitoring of alien fish species. Environmental DNA (eDNA) method is the most recent approach in identification and monitoring of aquatic invasive species.

eDNA could be defined as the mitochondrial or nuclear DNA that was detached from an organism and released into the environment. There are many sources of eDNA including gametes, feces, mucus and shed skin. eDNA can be found in both cellular and extracellular form (Pilliod et al., 2012). eDNA found in the freshwater systems was diluted and transported by hydrological mechanisms such as currents. Diluted eDNA can be degraded as a result of exposure to UVB radiation, pH, heat and nucleases. It can only last for 7–21 days in the aquatic environment (Dejean et al., 2011).

Recent studies based on collection and analysis of environmental DNA as a tool in molecular ecology resulted in a promising success with improved rates of detection sensitivity for freshwater organisms (Taberlet et al., 2012; Thomsen et al.,

* Ankara Üniversitesi Ziaat Fakültesi, Su Ürünleri Mühendisliği Bölümü, 06110 Dışkapı, Ankara, Turkey. Tel.: +90 3125961722.
E-mail address: keskin@ankara.edu.tr.

http://dx.doi.org/10.1016/j.bse.2014.05.003
0305-1978/

2012). eDNA approach was successfully applied on detection of amphibians (Ficetola et al., 2008; Goldberg et al., 2011), fish (Dejean et al., 2011; Jerde et al., 2011; Takahara et al., 2012) and crustaceans (Thomsen et al., 2012). The most important common outcome of these studies was the fact that eDNA approach is successful even if the target species are at low densities.

The aim of this study is to detect alien invasive fish species (*C. gibelio*, *C. gariepinus*, *O. niloticus* and *P. parva*) found in Upper Sakarya Basin using eDNA approach and making molecular identification at species level from DNA extracted from water samples. To achieve this, species specific primers were designed to amplify mini barcode regions from mitochondrial cytochrome oxidase I (COI) gene as this is the first eDNA study targeting *C. gibelio*, *C. gariepinus*, *O. niloticus* and *P. parva*.

2. Materials and methods

2.1. Study area

Upper Sakarya Basin was selected as the study area because of previously published monitoring studies in this region have already reported occurrence of invasive species which were also specified as target species (*C. gariepinus*, *C. gibelio*, *O. niloticus* and *P. parva*) in this study. Fifteen sampling stations (Fig. 1) with distinct ecological and hydrological properties were specified. Population density information based on traditional monitoring study (Emiroğlu, 2011) were given in Table 1. All stations were surveyed again to determine the current status of populations.

2.2. Sampling

Water samples were taken from 15 different stations, at 2 different seasons (February and July) as triplicates (without negative and positive controls). In each sampling, 2 L of water were collected into sterile containers and carried to the laboratory on ice in order to filter the water samples. Three types of controls were used during the entire water sampling and transport process (Goldberg et al., 2013). These were negative field controls, negative transport controls and negative equipment controls which contains deionized water samples. All controls were treated same with the actual samples. Positive control samples were taken from another pond in which none of the target species exist.

Dissolved oxygen, pH, temperature and conductivity were measured to construct a relationship among eDNA concentrations and effects of seasonal changes on water parameters.

2.3. Molecular analysis

Water samples were filtered through Sterivex-GP (Millipore, MA) unit with a membrane pore size of 0.22 μm. After the filtration, membrane pores holding the eDNA were directly used for DNA extraction. DNA extractions were conducted using

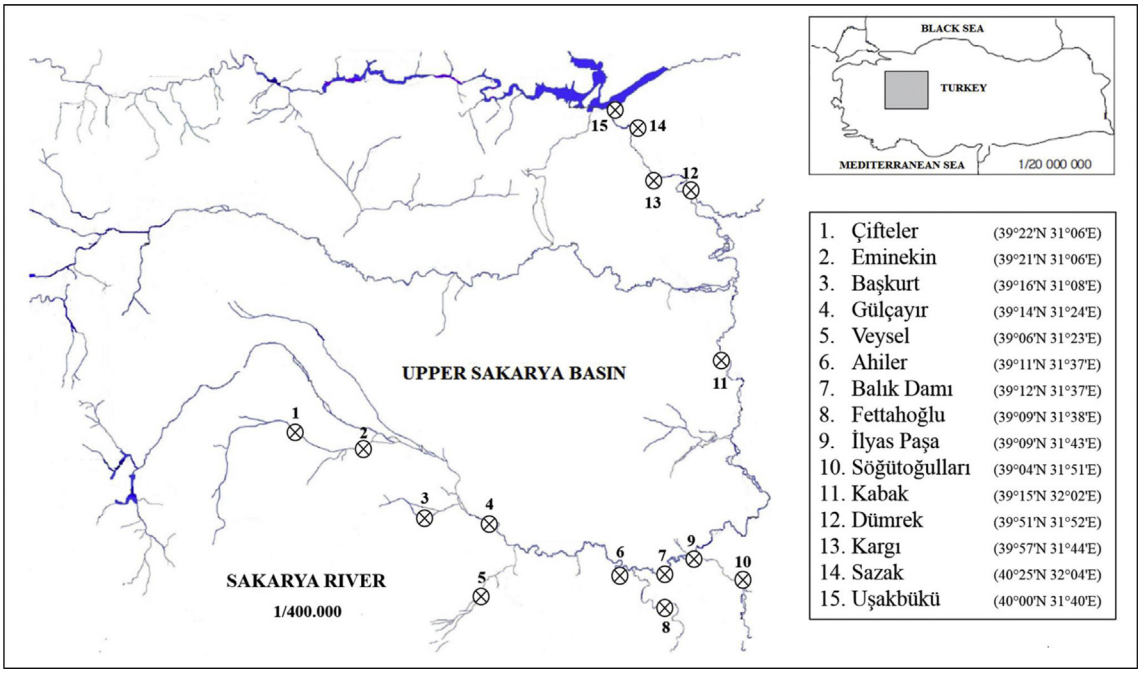

Fig. 1. Locations of sampling stations on Sakarya River.