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The use of environmental DNA of fishes as an efficient method of determining habitat connectivity

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ABSTRACT

This study demonstrated the use of environmental DNA (eDNA) to determine habitat connectivity for migration of fishes between the sea and river. Environmental DNA is DNA fragments released by fishes in water, which can be used as a species-specific marker of the presence/absence of the target species. A year-round water sampling regime at 15 sites on the Yodo River, Japan, was conducted to determine whether three major man-made barriers on the river inhibited the migration of fishes using species-specific detection of DNA fragments from three target migrant species, temperate seabass, *Lateolabrax japonicus*, flathead grey mullet, *Mugil cephalus*, and ayu, *Plecoglossus altivelis altivelis*. The presence/absence of eDNA from target species was consistent with known patterns of species' seasonal migration. The detection of the DNA of temperate seabass and flathead grey mullet at sites upstream of the dam closest to the river mouth indicated successful upstream migration of these species via a fish ladder bypassing the dam. On the other hand, DNA of these two species was not detected from the upstream side of the two remaining dams, which are not equipped with fish ladders. Ayu is the only species among the three target species with a land-locked population in Lake Biwa located at the headwater of Yodo River. Ayu DNA was detected at most of the sites in the freshwater area during the warm months; however, in the coldest month of February, eDNA was only detected in the uppermost site of Yodo River at the southern tip of Lake Biwa. The eDNA we detected at this site suggests that it was derived from juvenile ayu spending their winter months in the lake. These results suggest that the eDNA analysis presented here can accurately track the seasonal migration of fishes in a river, demonstrating its application as an indicator of habitat connectivity for fishes in association with man-made barriers in a river. The sampling of eDNA involves merely scooping a tank full of water; therefore, it is a simple, rapid, and cost-effective method for long-term monitoring of habitat connectivity associated with the construction of barriers in a river.

1. Introduction

Most animals utilize multiple habitats during their life cycles (Noss, 1991), and the connectivity among habitats is essential for the growth and survival of the species. Known as the River Continuum Concept, river ecosystems are maintained by a series of complex physical, chemical, and biological interactions (Vannote et al., 1980). From an ecological point of view, the transportation of nutrients from marine habitats to freshwater habitats via upstream migration of fishes plays an essential role in a river ecosystem (Cederholm et al., 1999). Sufficient connectivity allows animals to sustain their life cycles (Noss, 1991); however, once barriers are inserted, their life cycles and the accompanying nutrient transportation can be disrupted. Therefore, adequate connectivity among habitats is an important precondition for maintaining diversity of fishes as well as river ecosystem functions.

Artificial barriers such as dams sometimes inhibit free migration of fishes (Agostinho et al., 2008; Dugan et al., 2010; Katano et al., 2006). Proper and sufficient environmental impact assessment before the construction of barriers could reduce negative impacts on the resident fish community. While the assessment of fish migration before and after barriers were constructed is essential, the post-construction phase often requires relatively long-term monitoring which can be difficult due to time and budget constraints. In particular, monitoring is inevitable in the case of dams that are equipped with fish ladders set as a precondition for the construction of the dams. Therefore, the adoption of quick and

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cost-effective methods is required to facilitate adequate assessments for ecological monitoring dealing with artificial barriers in a river.

There are a number of methods used to examine the distribution and migration of fishes, including mark and recapture, passive integrated transponder (PIT) tags, acoustic telemetry, and radio telemetry (e.g., Cooke et al., 2013; Cunjak et al., 2005; Pegg et al., 1997). However, the marking of a large number of individuals in the mark and recapture method is labor-intensive. For the application of telemetry techniques, large loss of transmitters often occurs. To acquire sufficient data for statistical analysis, the large number of transmitters that's needed is sometimes financially prohibitive. In recent years, techniques using eDNA analysis have advanced rapidly for determining species presence (Takahara et al., 2013; Thomsen et al., 2012a,b), species abundance (Pilliod et al., 2013; Takahara et al., 2012), and even for estimating community composition (Kelly et al., 2014; Minamoto et al., 2012) in aquatic environments. The detection probability of fish using eDNA is higher than that of conventional assessment methods such as direct observation and capture (Dejean et al., 2012; Jerde et al., 2011; Takahara et al., 2013), and the method could cut cost per sample in comparison with traditional morphological identification (Jerde et al., 2011; Sigsgaard et al., 2015).

Our study is the first to determine the practicality of the eDNA analysis as an indicator of habitat connectivity, focusing on dams on a river, and the presence/absence of fish ladders. We applied the eDNA analysis to determine habitat connectivity between the sea and a river by examining eDNA distribution derived from three target species along the Yodo River and its three tributaries in Japan. The three target species consisted of two marine fishes, namely temperate seabass, *Lateolabrax japonicus*, and flathead grey mullet, *Mugil cephalus*, and an amphidromous freshwater fish ayu, *Plecoglossus altivelis altivelis*. The Yodo River has three major man-made barriers on its mainstem. The objective was to determine whether the barriers affected the migration of fishes between the sea and the river. We will discuss the merits and possible deficiencies in the use of eDNA analysis in assessing habitat connectivity in lotic systems.

2. Materials and methods

2.1. Field survey

The current study was conducted along the mainstem of the Yodo River, draining ca. 8240 km^2 of catchment area in the Kansai region, western Japan. The Yodo River water system supplies water resources to a large resident population of ca. 14 million people. The Yodo River flows out from Lake Biwa and connects to Osaka Bay downstream. We set 12 water sampling sites along the mainstem of the Yodo River (from river mouth to Lake Biwa; S01 to S12) and the remaining three sites (ST01 to ST03) along three major tributaries (Fig. 1). The 12 sites along the mainstem were set 6–10 km apart. ST01 is located on a tributary, Ohkawa River, ca. 1 km downstream from a branching point from the Yodo River. ST02 and ST03 are located along the other two tributaries, Katsura and Kizu rivers, ca. 0.5 and 0.8 km upstream from their branching points in the Yodo River, respectively.

We conducted water sampling at the 15 sites in 1 day and repeated it monthly from July 2012 to June 2013. During each sampling, water collection was finished within 8–9 h. We collected one full tank (2 L) of river water at each site using a plastic bucket and a funnel. Concurrently, water temperature (°C), pH, and electrical conductivity (EC; mS/cm) were measured on site with water quality sensors (AD-5612A, A&D, Tokyo, Japan; pHep5 and DiST6, HANNA, Chiba, Japan, respectively). All sampling equipment including the plastic bucket, funnel, and plastic tanks were flushed thoroughly twice with river water at each site by following the same protocol in the previous studies (Fukumoto et al., 2015; Takahara et al., 2012, 2013) to reduce the risk of contamination. We transferred the samples in a cooler box to the Research Institute for Humanity and Nature, Kyoto, Japan. The water tank used for water collection was doubly sealed (i.e. with an inner cap and an outer cap), and was locked tightly while in the cooler box. In all sampling, no breakage of the 180 water tanks or their caps was observed on arrival at the research institute.

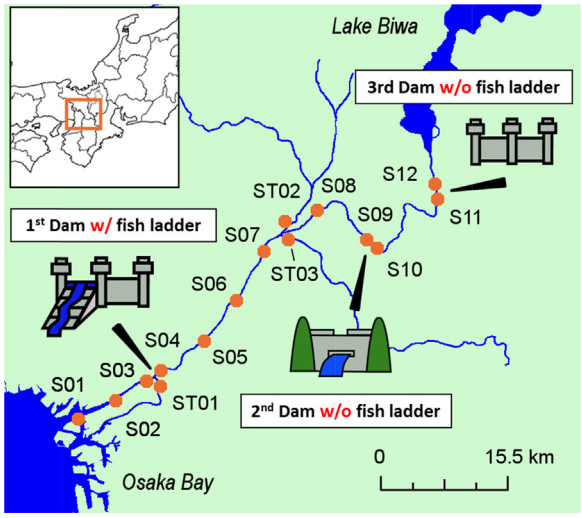

Fig. 1. The spatial arrangement of sampling sites, located on the Yodo River system in the Kansai region, Japan.

We conducted further molecular analyses at the Research Institute for Humanity and Nature. First, water samples were filtered using GF/F glass fiber filter (nominal pore size = 0.7 μm; diameter = 47 mm; GE Healthcare Japan Corporation, Tokyo, Japan). To prevent clogging, a single 2-L sample was divided into three equal volumes (ca. 667 mL each), which were filtered separately. One negative control sample was taken by filtering 2 L of reverse osmosis ultrafiltrated water to assess the occurrence of undesirable cross contamination during the filtering and the DNA extraction processes. Filter funnels were flushed every time with a large amount of tap water and rinsed with reverse osmosis ultrafiltrated water between different samples to prevent cross contamination (Fukumoto et al., 2015; Takahara et al., 2012, 2013). Filtered samples were stored in a 50 mL conical tube (i.e., with the three glass filters from a single sample places in one tube) and the frozen at −20 °C before the subsequent DNA extraction process.

2.2. DNA extraction

DNA was extracted from glass fiber filters and purified using the protocol described by Fukumoto et al. (2015). Briefly, DNA was first extracted by the standard phenol–chloroform method using a three-step purification, namely phenol, phenol–chloroform–isoamyl alcohol, and chloroform–isoamyl alcohol. DNA was collected by ethanol precipitation using a 1/10 volume of 3 M sodium acetate (NaAc; pH 5.2) as a salt. The DNA pellet was re-dissolved into 200 μL of distilled water and then further purified using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer, except for a modification in the final step of elution of DNA from the filter column. We eluted DNA using 100 μL AE buffer, though the original manual specified 200 μL.