Evaluation of an Environmental DNA Method as a Potential Tool for Monitoring Salmonid Fishes in the Wild

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There are a variety of migratory fish species in the North Pacific, including Pacific salmonids (*Oncorhynchus* spp.). While there is no doubt that they play an important role in the ecosystem and commercial fisheries, it is difficult to obtain a comprehensive understanding of their migration routes and spatio-temporal distribution precisely, partly due to the huge efforts and costs required for collecting such data with conventional research tools.

Environmental DNA (eDNA) is a new ecological tool to identify species in the wild. eDNA was first developed for detecting the presence of invasive bullfrog in France (Ficetola et al. 2008). Since then, it is increasingly recognized as a new molecular tool for detecting organisms in the wild (Takahara et al. 2013; Sigsgaard et al. 2015). How efficiently it can be applied for monitoring migratory salmonid species, however, is still not well understood. Although previous studies tried to figure out the relationship between the eDNA concentration and a biomass nearby (e.g., Takahara et al. 2012), these results might not be directly applicable to salmonid fish species because of the wide varieties of their body size and of habitat they use, such as headwaters, mid- to downstream of a river, and the ocean.

Fig. 1. Sakhalin taimen (*Parahucho perryi*) captured in Hokkaido, Japan. The photo was taken by H. Mizumoto.

To evaluate the potential of eDNA as a tool for monitoring salmonid fish species in the wild, we conducted an aquarium experiment on Sakhalin taimen (*Parahucho perryi*) (Mizumoto et al. 2018). Sakhalin taimen is a large and rare salmonid species distributed in Sakhalin, Russia, and Hokkaido, Japan (Fig. 1). They were historically distributed in many rivers in these areas, but they experienced a sharp decline of populations in almost all these areas presumably due to human activities (Rand 2006; Fukushima et al. 2007, 2011). It takes them a long time to reach sexual maturation (Yamashiro 1965), and the persistence of their populations is susceptible to environmental changes (Fukushima et al. 2011). They also require a vast area of watersheds and estuarine habitats during their life cycle, migrating from a river mouth to spawning grounds in headwaters for reproduction and migrating back to the sea for wintering and foraging.

We first developed an eDNA detection system for Sakhalin taimen, making primers and a probe for this species based on DNA sequences in NCBI database. *In silico*, we compared available DNA sequences in NADH dehydrogenase subunit 2 (ND2) from species belonging to genera *Hucho*, *Oncorhynchus*, *Salvelinus*, *Salmo*, *Brachymystax*, *Coregonus*, *Prosopium*, *Stenodus* and *Thymallus*. We then tested the species specificity of designed primers and the probe by trying to amplify DNA from tissue samples of Sakhalin taimen, chum salmon (*O. keta*), pink salmon (*O. gorbuscha*), sockeye salmon (*O. nerka*), rainbow trout (*O. mykiss*), masu salmon (*O. masou*

masou), Dolly Varden (*Salv. malma malma*), white-spotted charr (*Salv. leucomaenis leucomaenis*), and brown trout (*Salmo trutta*), using qPCR. The result clearly suggested the species specificity of the primers and the probe to DNA from Sakhalin taimen only (Fig. 2).

Fig. 2. Validation of species-specificity of new primers by tissue-DNA based PCR and qPCR (cited from Mizumoto et al. 2018). (a): A gel image showing results of an electrophoresis of standard PCR products. The expected size of band was 124 bp. 1: distilled water (negative control), 2: *Parahocho perryi*, 3: *Oncorhynchus keta*, 4: *Oncorhynchus nerka*, 5: *Oncorhynchus gorbusha*, 6: *Oncorhynchus mykiss*, 7: *Oncorhynchus masou masou*, 8: *Salvelinus malma malma*, 9: *Salvelinus leucomaenis leucomaenis*, 10: *Salmo trutta*, L: a DNA ladder of 100 bp intervals. (b): Amplification plots of qPCR. X-axis represents the number of PCR cycles and Y-axis represents the strength of fluorescence. The same tissue-oriented DNA templates above were applied to the standard PCR (a) and to the qPCR (b). These species cover all coexisting salmonid species with Sakhalin taimen at least within their potential distribution in Japan.

Following the confirmation of the species specificity of our eDNA detection system for Sakhalin taimen, aquarium experiments were conducted using Sakhalin taimen with different ages and sizes (Mizumoto et al. 2018). In two types of tanks of different sizes (40L-tank and 2000L-tank, hereafter), we put Sakhalin taimen with age 0+, 1+, 2+ and > 20+. The average fork lengths were approximately 28mm for 0+, 100mm for 1+, 184mm for 2+, and 800mm for > 20+ at the beginning of the experiments. Inlet water was drawn from the nearby Kashiwagi River (42°53'25.7"N, 141°32'19.4"E) and fed into the 40L- and 2000L-tanks at the rates of 4.0 and 12.9 L·min⁻¹, respectively. No recycling of water was used during the experiments. All equipment was sterilized by spraying bleach solution (10 or 20% sodium hypochlorite) and rinsed with DNA-free water before the experiments. To mitigate the effect of human disturbances, all the tanks were covered by plastic sheets. After 3–5 days of fish introduction, we collected two 1L water samples per sampling period from the outlet of each tank for eDNA analyses. Water samples were filtered immediately after the samplings with glass-membrane filters with a mesh size of 0.7 µm (Whatman GF/F, GE Healthcare Japan, Tokyo, Japan). Every time before filtering tank water, we filtered 500 mL pure water with the same type of filter to serve as a negative control. To prevent cross-contamination, all filtration equipment was sterilized and carefully rinsed with pure water in each filtration. No detection of eDNA from any negative control was observed throughout the experiments.

Fig. 3. Relationships between the log-transformed eDNA concentration (log₁₀ DNA copies per 2 μL template DNA samples) per fish and their log-transformed age (a), average Fork Length (FL) (b) and Body Weight (BW) (c). $n = 66$ in all analyses and correlations were statistically significant in all cases ($p < 0.001$). (a): $y = 1.85x + 1.04$, $r = 0.87$. (b): $y = 2.80x - 4.69$, $r = 0.89$. (c): $y = 0.95x - 0.07$, $r = 0.89$. (modified from Mizumoto et al. 2018)

Fig. 4. Relationships between the log-transformed eDNA concentration adjusted to the total body weight of fish at each group and their log-transformed age (a) and average fork length (FL) (b). None of the correlations were statistically significant (a: $y = -0.11x + 3.58$, $r = -0.11$, $p > 0.05$, b: $y = -0.13x + 3.85$, $r = -0.091$, $p > 0.05$), indicating constant eDNA concentration among different age- and FL-groups when their total body weights were adjusted. $n = 66$ in both analyses. (Mizumoto et al. 2018)

We found positive relationships of eDNA concentration with fish age, fork length, and body weight (Fig. 3). They indicate a clear association between the biomass of Sakhalin taimen and eDNA concentration, at least under the controlled environment with flowing river water. In addition, the body size effect was cancelled out when the total body weight per tank was adjusted (Fig. 4). The latter strongly suggests that the eDNA concentration can be a reasonable indicator of biomass, in terms of body weight, nearby.

Currently we are applying this tool to identify the potential presence of Sakhalin taimen in > 120 river systems in Hokkaido, Japan. Although the results are still tentative, we found eDNA from this endangered species in several river systems in Hokkaido (Mizumoto et al. unpublished data). While they do not cover all the river systems with recent visual observation of Sakhalin taimen, they include a few river systems without any previous record of this species. Although further investigation is necessary to evaluate the possibilities of false positive, as well as false negative, for the precise mapping of spatial distribution of the endangered species, the above studies suggest a great potential of eDNA as a tool for monitoring salmonid fish species in general. In fact, the eDNA detection systems have also been developed for chum salmon and masu salmon in our lab. While their applicability to the monitoring survey is still ongoing, we are positive about identifying eDNA from headwaters to the open ocean, including the Bering Sea for chum salmon. However, spatio-temporal scales of eDNA detection likely varies among ecosystems, and cross-testing with conventional catch surveys would be necessary at each environment to understand the ecology of eDNA *per se* in the near future.

Fig. 5. Detected species diversity by MiFish metabarcoding at each station. Circles on each map indicate detected fish species number. Both size and color reflect the species number (Yamamoto et al. 2017).

An interesting option for eDNA surveys is eDNA metabarcoding. This is a method to estimate organism compositions in the wild based on the Next Generation Sequencing technology to identify the owners of DNA fragments collected as eDNA. For fish species, for example, we recently developed effective fish-universal primers for eDNA metabarcoding known as MiFish (Miya et al. 2015). Using MiFish primers, we found eDNA from more than 120 fish species in Maizuru bay, a species-rich coastal sea in Japan, based on a six-hour survey for collecting water samples (Fig. 5, Yamamoto et al. 2017). We applied it to coastal areas around Hokkaido and in a middle of Bering Sea, resulting in a wide variety of species identification including chum salmon, Chinook salmon, etc. (Araki et al. unpublished data). These results, together with a rapid development of molecular techniques, indicate that eDNA is a promising tool for monitoring salmonid fish species throughout their whole life histories in the near future.

REFERENCES

- Ficetola, G.F., C. Miaud, F. Pompanon, and P. Taberlet. 2008. Species detection using environmental DNA from water samples. *Biol. Letters*. 4: 423–425.
- Fukushima, M., S. Kameyama, M. Kaneko, K. Nakao, and S.E. Ashley. 2007. Modelling the effects of dams on freshwater fish distributions in Hokkaido, Japan. *Freshwater. Biol.* 52(8): 1511–1524.
- Fukushima, M., H. Shimazaki, P.S. Rand, and M. Kaeriyama. 2011. Reconstructing Sakhalin taimen *Parahucho perryi* historical distribution and identifying causes for local extinctions. *Trans. Am. Fish Soc.* 140: 1–13.
- Miya, M., Y. Sato, T. Fukunaga, T. Sado, J.Y. Poulsen, K. Sato, T. Minamoto, S. Yamamoto, H. Yamanaka, H. Araki, M. Kondoh, and W. Iwasaki. 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *Roy. Soc. Open Sci.* 2: 150088.
- Mizumoto, H., H. Urabe, T. Kanbe, M. Fukushima, and H. Araki. 2018. Establishing an environmental DNA method to detect and estimate the biomass of Sakhalin taimen, a critically endangered Asian salmonid. *Limnology*. 19: 219–227.
- Rand, P.S. 2006. *Hucho perryi*: IUCN (International Union for the Conservation of Nature) 2010 red list of threatened species, version 2010.3. IUCN. Available at: iucnredlist.org. (September 2010).
- Sigsgaard, E.E., H. Carl, P.R. Møller, and P.F. Thomsen. 2015. Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. *Biol. Conserv.* 183: 46–52.
- Takahara, T., T. Minamoto, H. Yamanaka, H. Doi, and Z. Kawabata. 2012. Estimation of fish biomass using environmental DNA. *PLoS ONE*, 7: 3–10.
- Takahara, T., T. Minamoto, and H. Doi. 2013. Using Environmental DNA to Estimate the Distribution of an Invasive Fish Species in Ponds. *PLoS ONE*, 8: e56584.
- Yamamoto, S., R. Masuda, Y. Sato, T. Sado, H. Araki, M. Kondoh, T. Minamoto, and M. Miya. 2017. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Sci. Rep.* 7: 40368.
- Yamashiro, S. 1965. Age and growth of the ITO (*Hucho perryi*) in northeastern Hokkaido. *Bull. Japan. Soc. Sci. Fish.* 31: 1–75.