HOKKAIDO UNIVERSITY

Title	Understanding the current distribution and biomass of an endangered salmonid species, Sakhalin taimen, by using environmental DNA [an abstract of entire text]
Author(s)	水本, 寛基
Description	この博士論文全文の閲覧方法については、以下のサイトをご参照ください。 https://www.lib.hokudai.ac.jp/dissertations/copy-guides/
Degree Grantor	北海道大学
Degree Name	博士(農学)
Dissertation Number	甲第13157号
Issue Date	2018-03-22
Doc URL	https://hdl.handle.net/2115/70199
Type	doctoral thesis
File Information	Hiroki_Mizumoto_summary.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

博士論文の要約

博士の専攻分野名称：博士（農学）　　氏　名：Hiroki MIZUMOTO

学位論文題名

Understanding the current distribution and biomass of an endangered salmonid species, Sakhalin taimen by using environmental DNA

（環境DNAを用いた絶滅危惧種イトウの分布域・生物量の解明）

In the world, over 25,000 species are listed in the International Union for Conservation of Nature (IUCN) Red List as the endangered species (IUCN 2017). Many kinds of threats are considered as the key factors of their extinction (e.g. loss or degradation of habitat, illegal trade, invasive species or human activity), and 85% of endangered species listed by the United States Fish and Wildlife Service (USFWS) faced to the threat of habitat degradation or loss (Wilcove et al. 1998). The importance of understanding the distribution or biomass of endangered species is the first and a crucial step for their conservation and these are common sense among the conservation ecologists. However, for monitoring endangered species, it is often very difficult to determine their distribution because they are rare in the wild by definition, and because it requires taxonomic skills to identify species with traditional sampling (e.g. casting nets or backpack electrofishing in the case of fishes).

The environmental DNA (eDNA) technique has recently developed as a potential solution for detecting organisms in the wild, especially for rare species. This technique was first developed for detecting an aquatic vertebrate in 2008 (Ficetola et al. 2008). By using an eDNA technique, we can identify wild organisms without capturing or observing visually because this technique is based on detection of DNA captured in environmental

media (e.g. water for aquatic species) as evidence of the presence of target species (Takahara et al. 2012; Thomsen et al. 2012; Sigsgaard et al. 2015; Spear et al. 2015). This technique is very useful for monitoring rare species because of the difficulty in finding and capturing such species in the wild (Jerde et al. 2011; Fukumoto et al. 2015; Janosik et al. 2015; Laramie et al. 2015; Bellemain et al. 2016; Pfleger et al. 2016). In addition, positive correlations between eDNA concentrations and biomass of target species that are reported by previous studies (Takahara et al. 2012; Pilliod et al. 2013; Eichmiller et al. 2014; Maruyama et al. 2014; Evans et al. 2016; Lacoursiere-Roussel et al. 2016; Doi et al. 2017). Furthermore, this technique was applied to monitor the fish migration or seasonal change of distribution in the river systems (e.g., Yamanaka and Minamoto 2016).

Sakhalin taimen *Parahucho perryi* (Brevoort), target species in the present study, is a rare salmonid species. This species is known to be one of the largest salmonids in the world and a keystone species in river ecosystems (Fukushima et al. 2011). They were historically distributed in the Far Eastern Russia and northern Japan, but their population sizes have declined drastically in almost all these areas, most likely due to human activities (Fukushima 2006; Rand 2006; Fukushima et al. 2007, 2011). They require long time for sexual maturation (Esteve et al. 2009; Fukushima et al. 2011), and therefore the persistence of their populations is susceptible to environmental changes such as river modifications, deforestations and loss of coastal wetlands (Fukushima 2006; Fukushima et al. 2011; Rand 2013). They use a whole area of watersheds and estuarine habitats through their life history, migrating from a river mouth to spawning grounds in headwaters for reproduction and back to the sea for wintering and foraging. The species is currently classified as critically endangered (CR) in the IUCN Red List (Rand 2006). Unfortunately, however, basic ecological information such as current distribution or

population status, which is essential for their conservation, are still not well understood.

Here, I have four chapters in my study. In Chapter 1, I developed species-specific primers and a probe for detecting eDNA from Sakhalin taimen. Furthermore, using the eDNA detection system, I examined relationships between the eDNA concentration and biomass of the species with different age and body size groups in aquarium tanks. In this chapter, I tested an ability of an eDNA technique for detecting Sakhalin taimen and estimating biomass of them in aquarium experiments. For comprehensively understanding the applicability of this tool to estimate the distribution and biomass of target species, it is important to elucidate the relationship across the different ages and sizes. The biomass-eDNA relationship across the different developmental stages was indicated in a few previous studies (e.g., Ficetola et al. 2008; Maruyama et al. 2014), but to my knowledge, this is the first study examined the relationship covering over 20 years of age difference, 29 times of FL difference and 23200 times of BW difference of the target species. More importantly, I found that the eDNA-based biomass estimation can be reliable for 3–80cm of fish if the fish biomass is estimated as their body weight, supporting that the eDNA concentration can be a good indicator of fish biomass regardless of their age or FL. The species-specific primers and probe that I developed successfully detected Sakhalin taimen eDNA both from juveniles and from adults. And I confirmed that there was no amplification of DNA from any co-occurring salmonid species in Japan (Fig. 3). This is evidence of species-specificity of this tool, indicating that it is ready to be tested in field-oriented eDNA studies for evaluating the natural distribution of Sakhalin taimen in Japan, just as shown in previous studies for other aquatic species (Jerde et al. 2011; Fukumoto et al. 2015; Janosik et al. 2015; Laramie et al. 2015; Bellemain et al. 2016; Pfleger et al. 2016).

In Chapter 2, I aimed to detect eDNA from Sakhalin taimen and to estimate their abundance in a natural river environment. I successfully detected their eDNA in Karibetsu River, one of the largest tributaries of the Sarufutsu River system in spring, 2015 and 2016. During both years of the study, there were some days without eDNA detection, even though Sakhalin taimen were recorded on those days by the sonar imaging system. However, our previous research showed that detectability of eDNA technique was strongly affected by water volume because eDNA concentrations decreased when water volume increased (Mizumoto et al. 2017). Unfortunately, the spawning season of Sakhalin taimen is wholly during the season of snow melt runoff, so the discharge of the river increased in their spawning season (Fukushima 1994, 2001). As the result of high discharge of the river caused by snow melt runoff, the detectability of eDNA may go down on the middle season of their spawning. In fact, days when no eDNA was detected were in the middle of the spawning season, and the water levels at those days were very high compared with the beginning and end of the spawning season (Fig. 12). I also tested the presence of PCR inhibitors in the samples of these non-detection days by mixing these samples and synthetic DNA, but there was little difference between mixed samples and synthetic DNA. It suggests that there was few effect of PCR inhibitors. In 2015, there was a significantly negative correlation between the eDNA concentration and the number of counted fish on the point of the sonar system ($p < 0.05$). In 2016, on the other hand, there was not significant, but positive correlation between these two parameters ($p = 0.23$). Activities of fish around noon are generally higher than those in morning because water temperature becomes higher (Rand and Fukushima 2014). And the difference of their activities might affect to the relationship between the eDNA concentration and the number of counted fish because eDNA concentration was higher at higher temperature

condition in the case of *Salvelinus fontinalis* (Mitchill) (Lacoursière-Roussel et al. 2016). In addition, when these parameters were averaged for five sampling days, there was positive significant correlation between these parameters ($p < 0.05$) (Fig. 11b). These results suggest that a snapshot survey is not enough for estimating biomass of target species. In comparison between aquarium experiments and field surveys focusing on the result of Ohmagari branch for comparing the eDNA concentrations, α_R was estimated as 0.86. This result suggests that considering river discharge during sampling, the biomass estimation method in aquarium experiments by using eDNA is applicable to the natural river surveys in this case. The reason why I got this result, my aquarium experiments, so to speak, were semi-natural conditions because the rearing water was continued overflowing in my aquarium experiments like the natural river flow. Furthermore, adult Sakhalin taimen that was around the sampling site of Ohmagari branch was staying there for mating, it helped to make the conditions similar to the aquarium experiments. According to the results of chapter-1 and 2, I demonstrated that the eDNA concentration estimated from natural river samples supposed to reflect the biomass around sampling sites with considering river discharge during the sampling periods.

In Chapter 3, I tried to estimate the distribution of Sakhalin taimen allover Hokkaido with my new eDNA tool. As the results of my field surveys, I successfully detected eDNA of Sakhalin taimen from 12 of 125 rivers, including 8 rivers for which Sakhalin taimen were reported as unknown or extinct in a previous study (Fukushima et al. 2011). However, there were positive detections from negative controls in two of eight locations. These results suggest that eDNA detection will be a good indicator for detecting the Sakhalin taimen population, as reported for rare species in other studies (Pilliod et al. 2013, Doi et al. 2016, Baldigo et al. 2017). On the other hand, I need to continue careful

sampling and analysis protocols to avoid cross-contaminations because of the sensitivity of this tool. I did not detect their eDNA from several regions where stable populations of Sakhalin taimen were reported in a previous study (Fukushima et al. 2011). At present, it is difficult to evaluate whether these populations went extinct or not, just based on the fact of no eDNA detection. It is also noteworthy that there may be many factors in natural environments that decrease the detectability of this tool (e.g., water temperature, water volume, and population size). For example, many adult Sakhalin taimen were observed upstream of location M during their spawning seasons despite no detection of their eDNA. This presumably means false negative caused by some environmental factors. On the other hand, I also need to consider the possibility of false positives with this highly sensitive DNA detection method. For example, cross-contaminations in filtration, extraction or qPCR steps were mainly taken as the reasons for the contaminations in location H and K. In this chapter, however, negative controls for qPCRs worked accurately and there were few risks for cross-contaminations because detected eDNA had quite low concentrations.

In Chapter 4, I tried to reveal seasonal migration of Sakhalin taimen and its mechanism focusing on the presence/absence information of their prey fish by using eDNA. In qPCR analyses, I found seasonal gradations of eDNA concentrations of Sakhalin taimen especially in Sarufutsu River system and these gradations are assumed to reflect their seasonal migration (e.g., spawning migration in spring, juveniles drift downstream in summer). However, the migration pattern of Sakhalin taimen was not consistent between studied river systems. I successfully detected fish fauna and its seasonal changes cyclopaedically by using the NGS analysis (Table 6). The fish compositions co-occurred with Sakhalin taimen did not clearly explain their migration

pattern. However, some possibilities for explaining migration of Sakhalin taimen depends on the presence/absence of their prey species still remained. As I mentioned in chapter 1, detectability of this tool was strongly affected by water volume and biomass (Mizumoto et al. 2017). Comparing these two river systems, Bekanbeushi River system had larger and longer watershed than Sarufutsu River system. Given this, the detectability of fish compositions in Bekanbeushi River system is probably lower than in the of Sarufutsu River system. Furthermore, the distances among the sampling locations may be too long to reveal detailed migration patterns because eDNA will be degraded in a few hundred meters (Mizumoto et al. *unpublished data*). In addition, some fish species co-occurred with Sakhalin taimen at high frequency were observed from their stomach contents in previous studies by using stomach pump or isotope analyses (Edo et al. 2005; Honda et al. 2014). Future studies based on a more detailed sampling design and a longer sampling period will be needed to resolve these issues and reveal ecological interactions among Sakhalin taimen and other fish species in these ecosystems.

In conclusion, I successfully developed a new monitoring tool for simultaneously estimating the current distribution and biomass of Sakhalin taimen. In addition, I was able to apply this approach to address questions on the ecology of Sakhalin taimen, including their interactions with prey fish communities. Moreover, the eDNA method may be useful for assessing the distribution and biomass of other endangered fish species given the noninvasive, objective, and rapid approach of this technique. If so, eDNA surveys may help provide critical information for the conservation of many rare endangered fishes, especially in parts of the world where little is known about their distribution or ecology.