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Keynote Lecture

Diet analysis of juvenile fishes associated with zooplankton

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Abstract

Studying the diet of larval and juvenile fishes is important in fisheries science for the maintenance and improvement of fish stocks. We present a case study to propose a precise method of diet analysis in juvenile fishes. The stomach contents of juvenile chum salmon collected from the Pacific coast of northern Japan were analyzed by both morphological observation and a DNA-based (DNA barcoding) method using the mitochondrial cytochrome c oxidase subunit I (COI) region as a DNA marker. Results indicated that the DNA-based method has advantages in detecting a higher number of prey species, but also disadvantages, e.g., the occurrence of unknown taxa due to a lack of available sequence information in public gene databases, and the detection of secondary prey. This study suggests that the feeding habit (diversity of stomach contents) of juvenile fishes can be more precisely understood by using a DNA-based method in addition to normal morphological observation. It is necessary to note the shortage of sequence data in public gene databases when diet analysis of marine animals is conducted using the COI region, which is frequently used as a molecular marker with DNA barcoding.

Key words: zooplankton, chum salmon, diet analysis, DNA barcoding, COI

INTRODUCTION

Fish larvae and juveniles feed on zooplankton, mainly copepods (Islam et al. 2006, Mitsuzawa et al. 2017). The relationship between the larvae or juvenile fish and their zooplankton diet is frequently studied to understand their ecology and food webs (Planque et al. 2014). Diet analyses is an important and classical research theme in fisheries science. Generally, fish stocks are closely linked to mortality in the early life stages of fishes (Houde 1987). Inadequate feeding success in these stages results in decreases in predator avoiding ability and growth rate, which leads to increased mortality (Hjort 1914, 1962). Therefore, understanding what kind of organisms the larvae and juveniles feed on is important.

Conventionally, prey organisms of fish are identified by morphological observation of contents in the digestive tract, including the stomach. However, this morphological method presents some problems in terms of the accuracy of prey organism identification, such as misidentifications and bias of identification due to differences in the digestibility and/or indigestibility of soft- or hard-bodied prey items (Randall 1967, Carreon-Martinez 2011). DNA barcoding (a method of species identification based on DNA sequences), has been utilized as a diet analysis method since the 2000s using genomic DNA extracted from the contents of stomach, gastrointestinal tracts, and scat in marine organisms, including fishes (Casper et al. 2007, Chow et al. 2011, Berry et al. 2015). This method may solve identification problems found with the morphological method. We introduce a case study of diet analysis in juvenile fishes. To understand the stomach contents of the juveniles in detail and to propose a more precise method of diet analyses in juvenile fishes, the stomach contents of juvenile chum salmon were analyzed by both morphological observation and a DNA-based method. This paper is the summary of Sakaguchi et al. (2017a), and the summary formed a part of our presentation "Brackish-water copepod faunas in Kuroshio Current region, and a diet analysis of juvenile fishes associated with zooplankton including

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copepods" in the 13th international Kuroshio science symposium.

CHUM SALMON IN TOHOKU REGION, NORTHERN JAPAN AND DIET ANALYSIS OF CHUM SALMON JUVENILES

Chum salmon (*Oncorhynchus keta*) is a very important fish in the fishery of the Tohoku region, northern Japan. Chum salmon juveniles migrate out to sea after hatching in rivers. They spend about four years in the ocean, then they return to the river to spawn. In Japan, a chum salmon hatchery program has been in operation for 130 years. This program comprises salmon that are artificially cultured until they are juvenile, and then are released to the river. However, the salmon fishery in Iwate Prefecture of Tohoku region has experienced problems. The homing rate of adult salmon has decreased significantly since the early 2000s, despite the fact that the number of released juvenile chum salmon has remained constant since the mid-1980s (Ogawa and Shimizu 2012). Additionally, the number of released juveniles decreased after 2011 due to the Great East Japan Earthquake and Tsunami on 11 March 2011, causing a potential decrease in the return rate (Watanabe et al. 2015). In chum salmon, the mortality of the juveniles is the highest across all of the life stages, and the degree of the mortality affects the stocks (Kaeriyama 1986, Seki 2005). It is therefore important to reduce the juvenile mortality for the maintenance and improvement of chum salmon stock. The relationship of feeding success to the mortality in juvenile chum salmon needs to be understood in detail to improve this situation.

Sakaguchi et al. (2017a) analyzed stomach contents in 89 individuals of juvenile chum salmon from three bays (Miyako, Yamada and Kamaishi Bays) in the Iwate Prefecture. The stomach contents were observed under microscopes for morphological identification. After that, the gene cytochrome oxidase I (COI) region in the mitochondrial DNA was PCR-amplified using the genomic DNA extracted from these stomach contents as templates. The prey organisms of the stomach contents were identified based on the resultant COI sequences using blast analysis. The number of identified prey taxa in the stomach contents was 36 in the morphological observation and 80 in the DNA-based analyses. Nineteen identified taxa were common to both the methods used. The DNA-based analysis was able to identify more prey taxa than morphological observations, as found in previous studies. Two individual examples of the typical results are shown in Figure 1. In a juvenile chum salmon (Fig. 1a), the copepod *Eucalanus bungii* dominated as a prey item in the stomach contents when identified by morphological observation. However, in the DNA-based analysis, the sequence derived from a prey organism which could not be identified even at phylum level was dominant, and few sequences of *E. bungii* were detected. This prey species identification failure is probably due to the absence of COI sequence data from the phylum to which the unknown species belongs to in public gene databases. The shape of *E. bungii*'s exoskeleton in the stomach contents remained, although the internal tissues were more digested

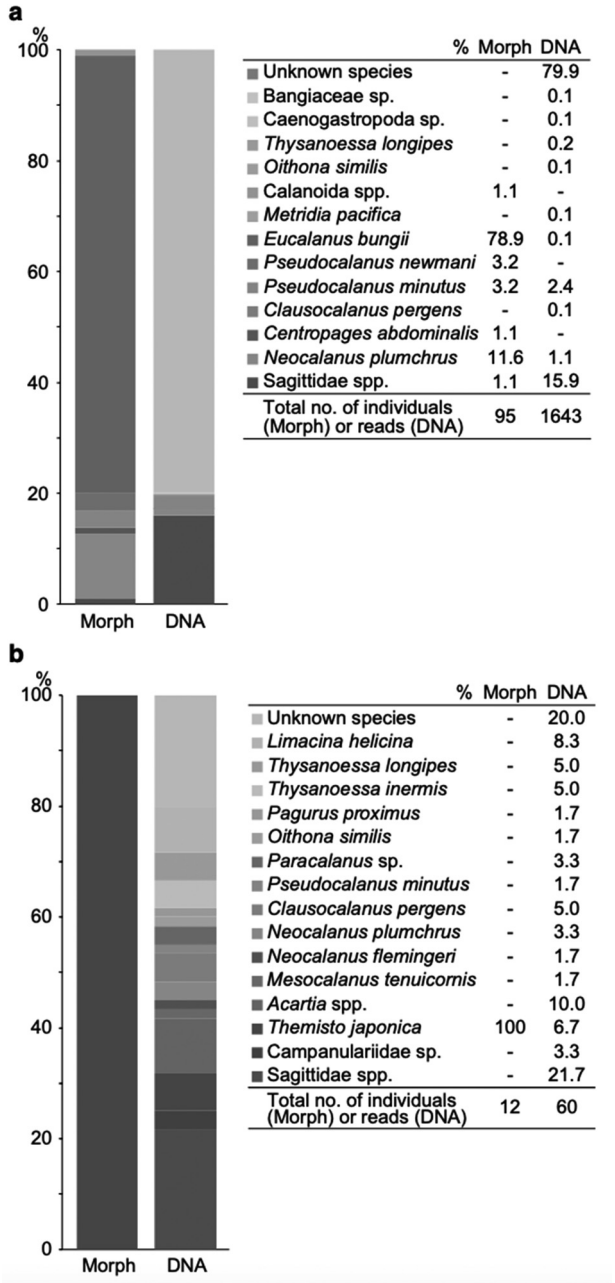

Fig. 1. Relative abundance and fauna of the stomach contents of juvenile chum salmon obtained by morphological observations and DNA-based analyses. (a) Stomach contents of an individual sample from Miyako Bay, Iwate Prefecture, northern Japan. (b) Stomach contents of an individual sample from Kamaishi Bay, Iwate Prefecture. Sources of the figures are Sakaguchi et al. (2017a), figure 4.

than the copepod *Neocalanus plumchrus* of the same body length. In another juvenile chum salmon (Fig. 1b), the amphipod *Themisto japonica* was identified as the only prey item in the stomach contents by morphological observation, whereas 16 prey taxa including the sequence of the above unknown prey organism were detected by DNA-based analysis.

This study shows that different prey items are identified between the morphological observation and the DNA-based analysis. The reasons for the differences are as follows: 1) Prey organisms of very small size or broken shape may not be identified using a microscope, but in the DNA-based analysis prey organisms can be identified regardless of the size or the shape. 2) The DNA may be hard to detect when the internal tissues are digested, even if the exoskeletons remain. 3) The DNA-based analysis may overestimate primary prey organisms by detecting secondary prey. For example, prey items from the stomach contents of amphipods which are prey organisms of juvenile chum salmon were detected and then recorded as prey of the juvenile chum salmon. 4) The DNA-based analysis may not precisely identify prey items to taxonomic level from the obtained DNA sequences due to lack of reference material in the public gene databases. Considering these results, we suggest that while prey identification studies using DNA-based analysis can make up for some of the shortcomings of only using morphological observation, it is clear that it also has some shortcomings. It is therefore considered that the feeding habits (diversity of prey organisms) can be more accurately understood by using both morphological observation and DNA-based analysis methods together.

DISCUSSION

DNA-based analysis is becoming commonplace in the dietary study of various aquatic and terrestrial organisms (de Sousa et al. 2019). However, some studies suggest cautionary points in using DNA-based analysis, such as the need to use suitable DNA markers and primer sets; and in correctly converting the outputs to quantitative data (Tournayre et al. 2019, Deagle et al. 2019). Sakaguchi et al. (2017a) also mentions DNA-based identification issues caused by either no, or few sequence data in public gene databases as mentioned in previous studies (Deagle et al. 2007, Hargrove et al. 2012, Berry et al. 2015). Some prey organisms could only be identified at the order, family, and genus levels in DNA-based analyses due to their species sequence data not being present in the reference databases. Furthermore, the COI sequence derived from an organism which could not be assigned even at the phylum level was frequently detected. To identify this unknown prey organism, Sakaguchi et al. (2017b) analyzed the stomach contents using *in situ* hybridization and another DNA marker (18S rRNA), identifying the organism as the appendicularian *Oikopleura longicauda. Oikopleura* is a common zooplankton in the sea. Some COI sequences from "*Oikopleura*" have been registered in public gene databases. However, these sequences are actually derived from completely different taxa (bacteria and cnidarians). This suggests that there is no available the COI sequence data of "true" *Oikopleura* in the public databases, despite the fact that *Oikopleura* is a well-known zooplankton group. The COI region is frequently used as a DNA marker in the DNA barcoding of metazoans, and the COI sequence data have been broadly accumulated in the public gene databases (Bucklin et al. 2011). However, if there is either no, or a poor, COI sequence of a taxon in the database, any newly obtained COI sequence derived from an organism belonging to the taxon should be carefully checked and registered in the databases. Furthermore, when performing diet analysis using the COI region, it is important to note the lack of sequence data in the database.

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