Ancient DNA Analysis of Pacific Cod (*Gadus macrocephalus*) Remains from Hokkaido, Japan

Yuka Shichiza[1], Katsunori Takase[2], Hiroshi Ushiro[3], Christine Conlan[1], Hua Zhang[1]
Thomas C.A. Royle[1], Dongya Y. Yang[1]

[1].Department of Archaeology, Simon Fraser University, Burnaby, BC, Canada
[2].Graduate School of Humanities and Human Sciences, Hokkaido University, Sapporo, Hokkaido, Japan
[3].Hokkaido Museum, Sapporo, Hokkaido, Japan

Introduction

Despite its cultural and economic importance, the archaeology of Pacific Cod fisheries at ancestral Ainu sites in Hokkaido, Japan, has not been the focus of extensive investigations[1,2]. Similarly, ethnographic records concerning Pacific Cod are limited relative to those available for salmon.
To investigate long-term Ainu-Pacific Cod interactions, our study will apply ancient DNA (aDNA) analysis to archaeological Pacific Cod remains.

Ainu People and Pacific Cod

Pacific Cod was of considerable subsistence importance to the Ainu people, especially among groups living in coastal regions[3,4]. Liver oil from the species, for instance, is one the essential seasonings in Ainu cuisine. Dried fish was also a prominent trade item that was exported to mainland Japan.

However, *Wajin*, the ethnic majority Japanese, introduced unfair trading systems and took over the fishery in Hokkaido[5]. As a result, many Ainu people were unable to fish freely after the 18th century, limiting their access to Pacific Cod.

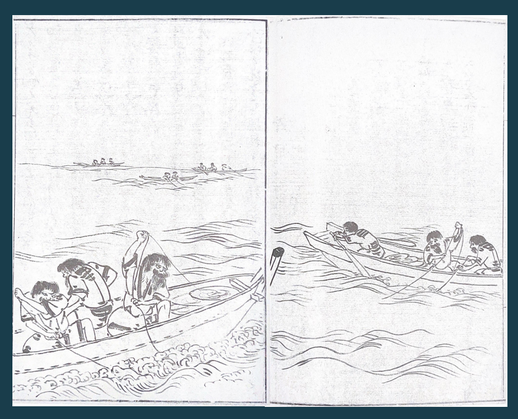
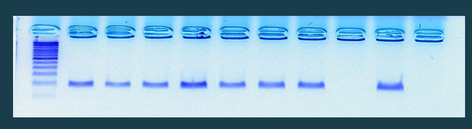


Figure1. Ainu People Fishing Pacific Cod
(Taken from Takeshiro Matsuura (1997), *Ezo Kunmou Zui*（蝦夷訓蒙図彙))[4]

Preliminary Results

- DNA was successfully amplified from 29 out of 30 samples (success rate of 97%).
- DNA is well preserved in fish remains from Hokkaido, Japan.
- The observed DNA sequence variation can potentially be used to reconstruct genetic diversity change of Pacific Cod over time.



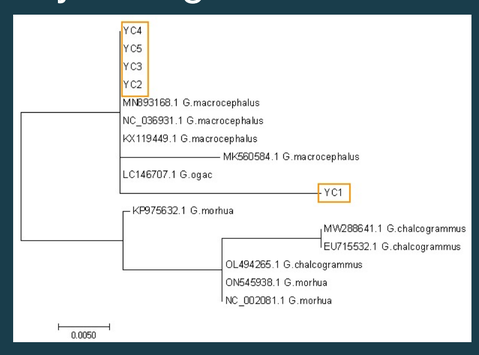


Figure4. (Left). Ancinet DNA was successufully amplified in most of samples as shown in gel electrophoresis results. Figure5. (Right) Phylogenetic analysis confirmed the species identity to be Pacific Cod. (Subset of samples (N=5) and some reference sequences are included here.)

Materials and Methods

- To date, we have applied aDNA analysis to 30 Pacific Cod remains from three ancestral Ainu sites (ca. 2400 to 1200 cal BP). Decontamination, DNA extraction, and PCR setup were conducted in a dedicated ancient DNA laboratory in the Department of Archaeology at Simon Fraser University (British Columbia, Canada). DNA was extracted following the modified silica-spin column method[6].
- To assess DNA preservation and confirm the morphology-species identity of the remains, we amplified and sequence a 168 bp fragment of the mitochondrial cytochrome c oxidase I (COI) gene using universal primers for fish[7].

Figure2. Archaeological Pacific Cod Remain

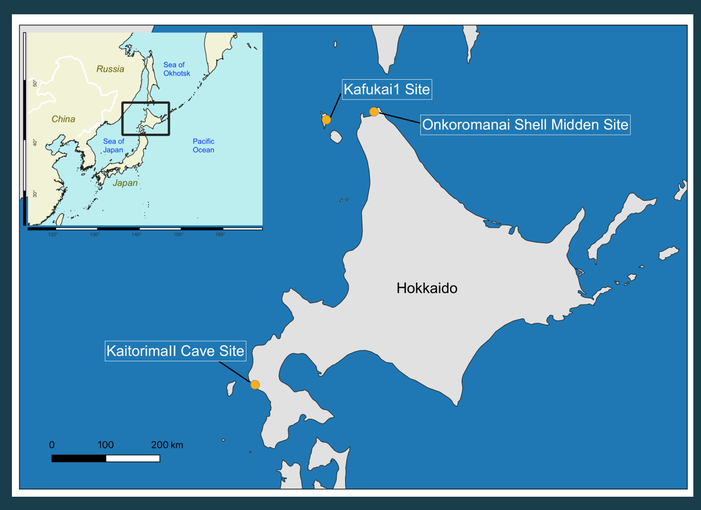


Figure3. Pacific Cod Remains were collected from three Archaeological Sites in Hokkaido, Japan

What's Next

- We seek to expand our sample size by collecting and analyzing remains from other sites located all around Hokkaido.
- We will explore the historical population structure and genetic diversity of Pacific Cod by sequencing regions of the mitochondrial genome that exhibit intra-specific variation. These data will allow us to conduct regional and chronological comparative analyses.
- Stable carbon and nitrogen isotope analyses will be conducted and integrated with the genetic data.
- The quantity of Pacific Cod remains recovered from archaeological sites varies across time and space. Applying aDNA analysis to archaeological remains will shed new light on how the Ainu-Pacific Cod interactions developed throughout history and examine the effects of various environmental and anthropogenic changes on Pacific Cod populations.

References
[1] Tsutaya, T., Takahashi, T., Omori, T., Yamazaki, K., Sato, T., Yoneda, M., Schulting, R. J., Kato, H., & Weber, A. W. (2021). Reconstruction of diachronic changes in human fishing activity and marine ecosystems from carbon and nitrogen stable isotope ratios of archaeological fish remains. *Quaternary International*, S1040618221005759. |[2] Uzawa, K. (1992). Fish Otoliths as Indicator of Cod Fishing Seasonality in a Jomon Shell Midden (Usu-10 Site). *Journal of the Anthropological Society of N*ippon, 100(3), 331–339.| [3]Haginaka, M. (1992). *Kikigaki Ainu no shokuji*. Tōkyō : Nō-san-gyoson Bunka Kyōkai.|[4]Matsuura, T., (1997). *Ezo kunmo zui: Ezo sankai meisan zue*. (Akiba, M., Trans) Sapporo-shi: Hokkaido Shuppan Kikaku Centre. |[5]Shirayama, T. (1961). *Matsumae ezochi basho ukeoi seido seiritsu katei no kenkyu*. Hakodate: Hokkaido Keizaishi Kenkyujo. |[6] Yang, D. Y., Eng, B., Waye, J. S., Dudar, J. C., & Saunders, S. R. (1998). Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology*, 105(4), 539–543.| [7] Royle, T. C. (2021). Ancient DNA analysis of archaeological fish remains: Methods and applications [Doctoral dissertation, Simon Fraser University]

SFU SIMON FRASER UNIVERSITY
北海道大学 HOKKAIDO UNIVERSITY
北海道博物館 HOKKAIDO MUSEUM