



Current status of Pinniped conservation research in KOREA

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1. Introduction

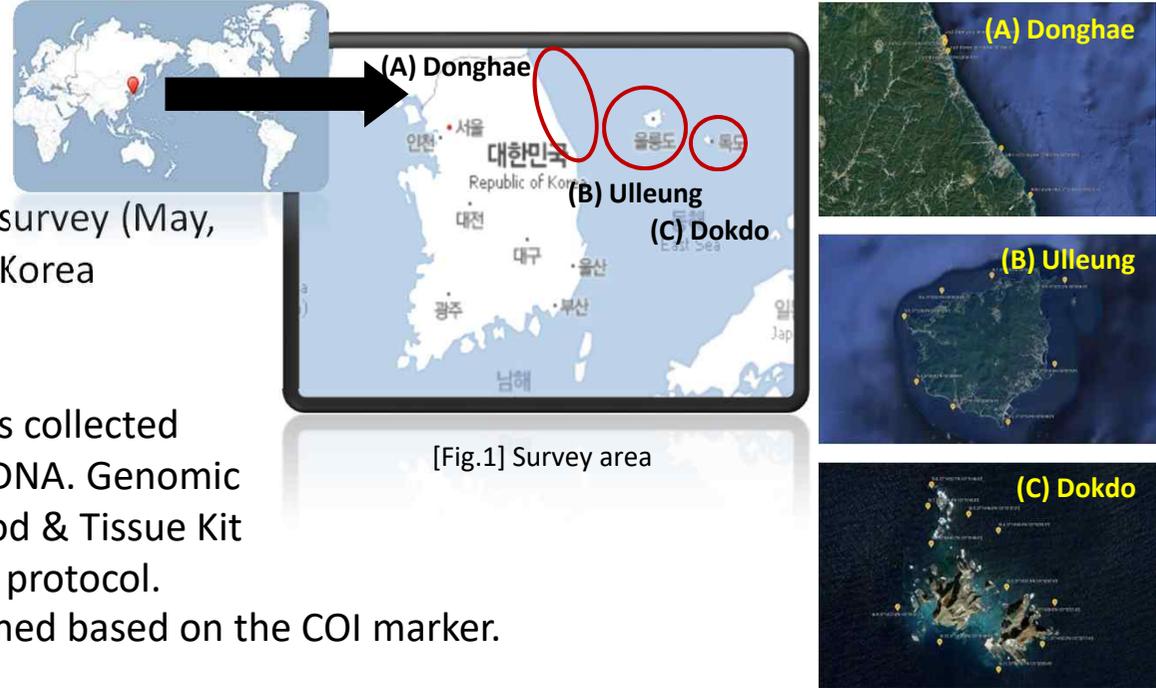
Six pinniped species are **currently** designated as **Marine protected species (MPS)** in KOREA. Their main breeding grounds are known to be China and Russia, and the waters along the Korean peninsula are considered as the marginal region for those species with extremely low individuals. Among them, Researches have been limited to the spotted seals (*Phoca largha*), the most abundant pinniped species in the Yellow Sea, Korea. Most of their studies have been mainly dependent on the traditional monitoring methods from visual surveys to count individuals of air-breathing vertebrates, which required a high-degree of labors and costs. As one of promising novel alternative tools for the traditional surveys, molecular biological analyses using **environmental DNA (eDNA)** has been paid attention for marine ecological studies due to its low cost and labors and high sensitivity in taxon recovery.

We here introduce current status of **research for pinniped conservation in Republic of Korea** using both traditional and novel molecular tools. In addition, satellite monitoring results and environmental conditions around breeding grounds are compared and analyzed.



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2. Materials & Methods



① **Visual surveys** 3 times of field survey (May, August, October, 2020) in the East Sea of Korea (Donghae, Ulleung Island, and Dokdo).

② **eDNA analysis** Seawater (1ℓ) was collected from each visual survey site to analyze eDNA. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacture’s protocol. The pinniped-specific primers were designed based on the COI marker.

③ **Satellite Image Analysis** Satellite images with spatial resolution of 50cm were provided from the “Skymap50” of SOAR platform.

④ **DNA identification of *Zalophus japonicus*** In Ulleung island, over 40 bones were collected in the coastal cave where *Z. japonicus* inhabited in historical literature. Primers for mitochondrial control region (D-loop) were used for its detection from the eDNA samples.



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3. Results & Discussion

① Visual surveys

- In the traditional ship surveys and UAV, any fur seals and Steller sea lion have **not identified** in the East Sea.

➔ **This means that access to new investigation methods is needed, moving forward with existing investigations.**

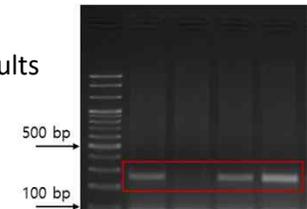
② eDNA analysis – Preliminary results

- **DNA primers were designed for six different pinniped species.**

(*Phoca largha*, *Histiophoca fasciata*, *Pusa hispida*, *Callorhinus ursinus*, *Zalophus* sp. *Eumetopias jubatus*)

- PCR analysis using eDNA samples showed reliability of the designed primers without cross-amplifications
- All the amplified signals were identified as **spotted seals, *Phoca largha***.
- The highest concentrations of eDNA (3461.76 ± 1229.14 copies/L) were identified in the Yellow Sea (Baeng-nyeong Island stations), where approximately 200~300 individuals of *P. largha* were witnessed from spring to autumn.
- Only spotted seal was identified by eDNA metabarcoding analysis with taxon-specific primers, which was correspondent to qPCR analysis results.

[Fig.2] PCR Results





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3. Results & Discussion (continued)

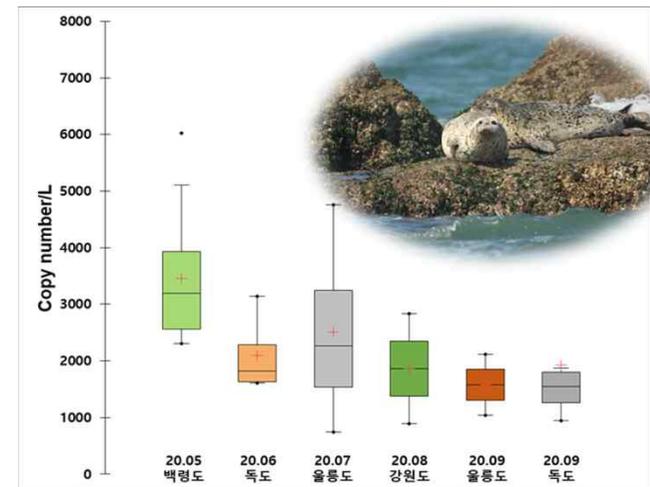
② eDNA analysis - Regional comparison

- In **spring and summer**, eDNA concentration of the spotted seals were highest at **Baeng-nyeong Islands (Yellow sea)**.
- It has been reported that 200 to 300 spotted seals inhabit in Baeng-nyeong Island (KOEM, 2020).

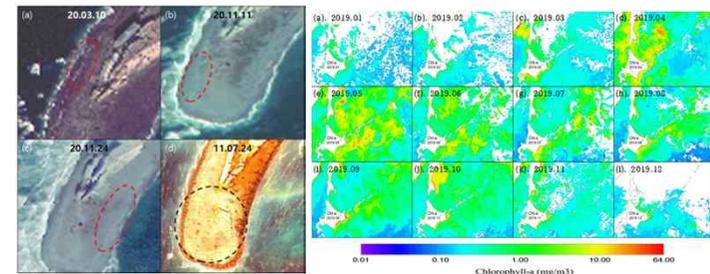
- ➔ **This result indicated that eDNA analysis is one of the reliable tools for the quantitative analysis of spotted seals.**
- ➔ **Further study should be conducted to know the reliability of eDNA analysis for seals and Steller sea lions during their migration in winter season in Korea.**

③ Satellite Image Analysis

- Many seals were distributed in May when chlorophyll-*a* concentrations were high.
- ➔ **Satellite monitoring could be a potential approach for monitoring pinnipeds related with environmental conditions around their breeding grounds.**



[Fig.3] DNA concentration by region



[Fig.4] Satellite Image and Chlorophyll-a variation in Tyuleniy Island



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3. Results & Discussion (continued)

④ DNA identification of *Zalophus japonicus*

- Around **40 bone samples** of pinnipeds were collected in Ulleung Island in the East Sea.
- From the BLAST analysis, Korean *Zalophus* D-loop sequences showed **high sequence similarity** with Japanese *Z. japonicus* (99.39%) with AB262362.
- * Clearly distinct from two other sea lions, *Z. californianus* and *Z. wollebaeki* (91~92%)

➔ Further study should be conducted to detect any trait of *Zalophus japonicus* in Korean waters using eDNA analysis.



[Fig.5] Sea lion bones

4. Research Cooperation Proposal

- ❖ Possibility of collaboration in environmental genetic research in Sakhalin, Kamchatka, Kuril, Alaska, etc.
- ❖ Suggestion to attend the international (online) workshop held by KOEM in September 2021.

* (Acknowledgment) This project was funded by the Korea Marine Habitat Restoration program in Ministry of Oceans and Fisheries

Description	Common Name	Max Score	Total Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> <i>Zalophus japonicus</i> JAL018.134133.1.1.D-loop...mitochondrial	<i>Zalophus japonicus</i>	339	339	9e-89	96.87%	204	NC020011.1
<input checked="" type="checkbox"/> <i>Zalophus japonicus</i> subantarctic (see also accession G_20141219) The 16S rRNA and D-loop...mitochondrial	<i>Zalophus japonicus</i>	316	316	2e-83	92.32%	493	MG230212.2
<input checked="" type="checkbox"/> <i>Zalophus wollebaeki</i> mitochondrial partial D-loop...mitochondrial	<i>Zalophus wollebaeki</i>	303	303	1e-78	92.45%	423	AF514322.1
<input checked="" type="checkbox"/> <i>Zalophus californianus</i> JAL018.134133.1.1.D-loop...mitochondrial	<i>Zalophus californianus</i>	298	298	1e-77	91.90%	397	MG230212.1
<input checked="" type="checkbox"/> <i>Zalophus wollebaeki</i> mitochondrial partial D-loop...mitochondrial	<i>Zalophus wollebaeki</i>	298	298	1e-76	91.96%	397	MG230212.1
<input checked="" type="checkbox"/> <i>Zalophus wollebaeki</i> mitochondrial partial D-loop...mitochondrial	<i>Zalophus wollebaeki</i>	298	298	1e-76	91.96%	422	AF514322.1
<input checked="" type="checkbox"/> <i>Zalophus wollebaeki</i> mitochondrial partial D-loop...mitochondrial	<i>Zalophus wollebaeki</i>	298	298	1e-76	91.96%	422	AF514322.1
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[Fig.6] Results of BLAST analysis based on mitochondrial D-loop sequence