
CLIMATE CHANGE: A VIEW THROUGH THE PRISM OF STELLER'S SEA COW EXTINCTION.

Phase 1:

- *Collection of Steller's sea cow bones on the Commander Islands.*
- *Pilot study: ancient DNA analysis of Steller's sea cow bones.*

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КФ ТИГ
ДВО РАН



Abstract

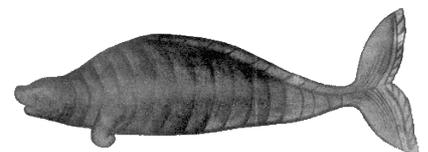
When discovered in 1741, Steller's sea cow had already been reduced from a wide North Pacific range to a relict population around the Commander Islands (Savinetsky et al. 2004; Domning et al. 2007). By 1768 the last sea cow was killed on Bering Island. The extinction of this unusual animal seemed to follow an all-too-familiar pattern of overhunting. Although that seems to have been the ultimate cause of the species' demise, we hypothesize that this process was started long before 1741.

We believe that climate fluctuation, changes in the near shore communities, and possibly some genetic mechanisms may have combined to make the remnant population of the sea cow particularly vulnerable to effects of overhunting and to extinction. In other words, we would like to test the hypothesis that factors other than 27 years of direct harvest had compromised the population viability and evolutionary potential of the species. For example, analysis of the age of the bones on the island could test the hypotheses that the only human hunting occurred between 1741 and 1768, following discovery of the Commander Islands and the sea cow by Vitus Bering, and that genetic diversity was already in decline prior to Russian contact which may reveal that the population was already on the wane.

Our main task in this project is to collect and archive as many Steller's sea cow remains as possible and to extract bone powder samples (via drilling) for ancient-DNA (aDNA) and radiocarbon dating analyses. The second objective is to conduct a pilot aDNA study on a sample of the osteological material.

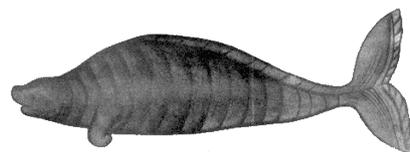
We report here on our findings from the pilot genetic study. Utilizing a Level III clean room dedicated to aDNA research, total DNA was extracted from 11 Steller's sea cow bone powder samples via silica-based aDNA methods. Using multiple primer sets, over 700bp of mtDNA control region and adjacent tRNA genes were successfully amplified from 9 samples. A total of 581bp of the threonine tRNA gene and control region were sequenced from 7 individuals. Six variable sites characterizing 3 unique haplotypes were documented. Re-extractions, PCRs and sequencing of this sample set are currently being conducted to confirm these findings and hopefully increase the number of individuals sequenced successfully.

This pilot study has successfully established an efficient lab method for studying aDNA from Steller's sea cow, and has provided the first data on genetic diversity within this recently extinct sirenian. We are confident that we can expand the scope of this study to include more samples across a longer time frame, and more genetic markers, including genes associated with individual fitness and population viability. Understanding the genetic aspects of the demise of the Steller's sea cow will ideally assist in formulating research and recovery priorities for extant sirenians.



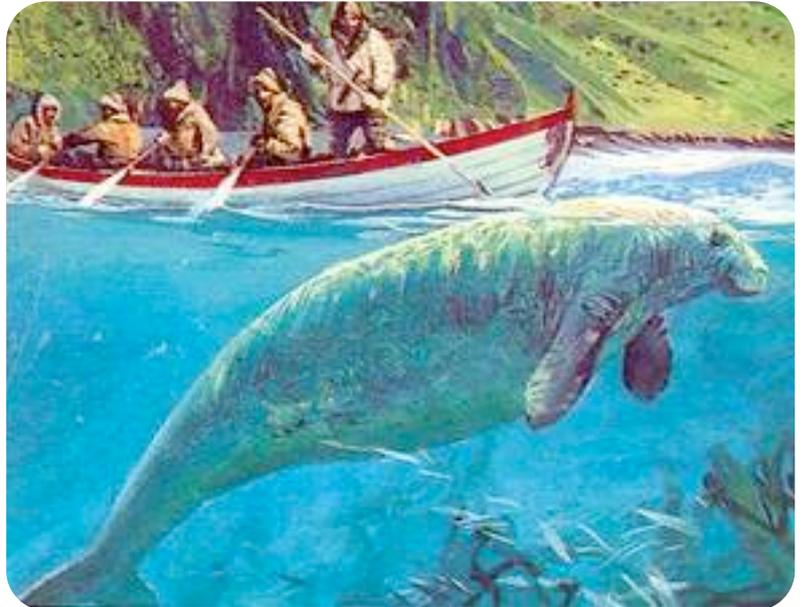
Contents

Introduction	4-6
Goals	7
Study area	7-9
Field work	9
Logistic	9-10
Results	10
Samples collection	10-12
Laboratory analysis	12-13
Discussion	14
Conclusion	14-15
References	16-17



Introduction

The underlying explanations for the sudden extinction of more than half of the New World's megafauna at the Pleistocene/Holocene border is a topic of long-standing interest and debate. This debate initially focused on the relative importance of two potential drivers-- climate/environmental change associated with late



Pleistocene glacial recessions, and the impacts of early humans as they crossed Beringia and spread into the New World. The absence of extinctions in the New World during earlier interglacial periods together with evidence that similar losses of large animals followed the peopling of various other continents and islands, but at widely differing times, has cast doubt on the primacy of climate change as the principal reason for the extinctions. This reasoning together with a growing weight of other evidence has led, instead, to the now widely held belief that human impacts figured prominently in megafauna extinctions worldwide (Barnowsky et al. 2004).

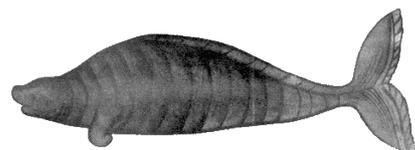
The more intriguing question is just how people did this? Was the sudden disappearance of so many large animals a consequence of hunting and overexploitation, a pandemic from diseases introduced by early humans or the domesticated animals they may have brought with them, human-induced environmental changes (as, for example, might have accompanied deforestation or burning), or some combination of these processes? Although the rapid and concurrent loss of so many

species implies a common cause, the exact details may well have varied considerably across species. The science of ecology has grown to realize that the dynamics of nature is controlled through complex interaction webs, and that certain species, variously referred to as keystones, foundation species, and ecosystem engineers, often have disproportionately strong influences on population and ecosystem dynamics. Many of these strong interactors are large bodied vertebrates (Terborgh and Estes, 2010). The commensalisms that result from this deeply interconnected web of life mean that a loss of one species may have led to the loss of others.

The case of Steller's sea cow (*Hydromalis gigas*) is an intriguing and potentially informative example of megafaunal extinction dynamics. This is because various aspects of the rise and fall of this species are well known; the final step in the sea cows' demise occurred just several hundred years ago, in the presence of modern human observers; and the dynamics of the coastal kelp forest interaction web, to which the sea cow was closely linked, have been extensively studied and are reasonably well understood. Although aboriginal people (and the early Russian fur traders in the Commander Islands) exploited sea cows and may have reduced or even exterminated local populations, humans also overexploited sea otter populations to such low levels that their keystone role in maintaining the kelp forest ecosystem was undoubtedly lost (Kenyon 1969, Simenstad et al. 1978, Estes and Duggins 1995), thus raising the question of the relative importance of direct human exploitation vs. the indirect effect of reduced food or habitat through the ecological extinction of sea otters to the sea cow's demise.

Risely and Turvey (2006) concluded that direct hunting losses from the Russian fur hunters were sufficient to account for the extinction of sea cows in the Commander Islands. Their conclusion is based on what we believe to be a poorly supported assumption—that sea cow mortality from human exploitation can be inferred from the number of Russian fur hunters overwintering on the Commander Islands.

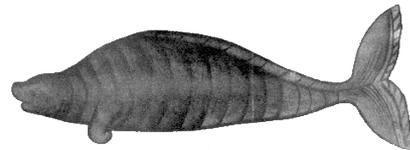
We believe that climate fluctuation, changes in the near shore communities, and possibly some genetic mechanisms may have combined to make the remnant population of the sea cow particularly vulnerable to effects of overhunting and to extinction. In other words, we would like to test the hypothesis that factors other than 27 years of direct harvest had compromised the population viability and evolutionary potential of the species. For example, analysis of the age of the bones on the island could test the hypotheses that the only human hunting occurred between 1741 and 1768, following discovery of the Commander Islands and the sea cow by Vitus Bering, and that genetic diversity was already in decline prior to Russian contact which may reveal that the population was already on the wane.



Goals

The main goals of our pilot project was:

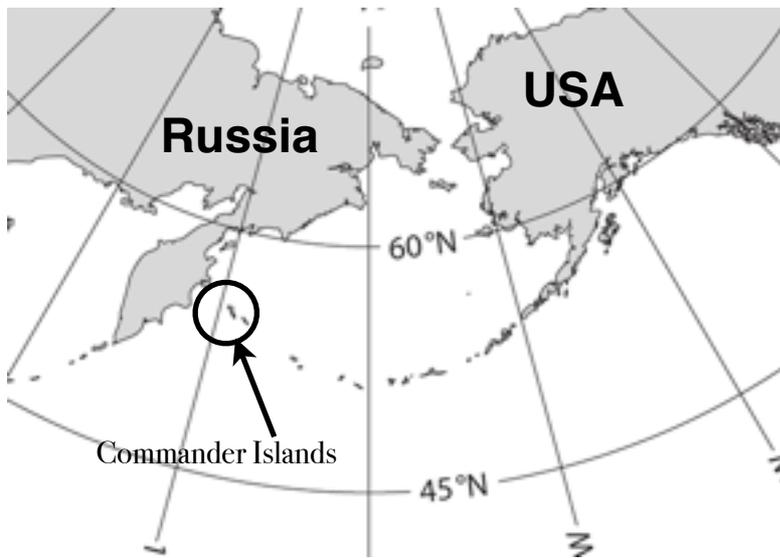
- To conduct field work on the Commander Islands, collect and archive any Steller's sea cow bones on the beaches of Bering and Medny Island,
- Take photos and video and work with the Nikolskoe village community, explaining the importance of collecting the valuable Steller's sea cow remains.
- To extract bone powder samples (via drilling) for ancient-DNA (aDNA) and radiocarbon dating analyses.
- To conduct a pilot ancient-DNA study on a sample of the osteological material



Study area

The **Commander Islands** or **Командорские острова** in Russian, located 175 km (109 mi) east of the Kamchatka Peninsula (closest point - Africa Cape) in the Western Part of Bering Sea. The Commanders Archipelago consist of two big Islands: Bering Island - 95 km (59 mi) by 15 km (9.3 mi)), and Medny Island (55 km (34 mi) by 5 km (3.1 mi)), and fifteen smaller ones, the largest of which are Toporkov Island (*Puffin Island*, 15 hectares (37 acres)) and Ariy Kamen Island, which are between 3 kilometres (1.9 mi) and 13 kilometres (8.1 mi) west of the only settlement, Nikolskoe village. Nikolskoe is an administrative center of national Aleutsky District compose of Kamchatsky Krai.

The Commander Islands (Russia, Kamchatsky Krai) are the westernmost extension of the Aleutian Islands (Alaska, USA), though



they are separated from the rest of the chain by 207 miles (333 km) strait between Medny Island and Attu Island. The Commander Islands have a lot of similarity with the Western Aleutians, and the main landscapes are

presented

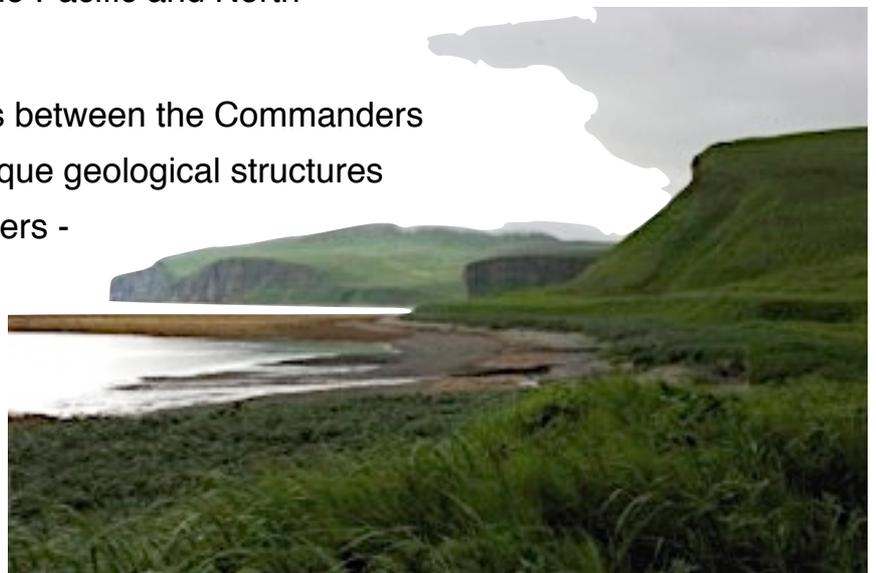
Fig. 1 Bering Sea and Northern Pacific

by mountain tundra. These oceanic islands are treeless, and have many small lakes, streams, rivers. The relief is somewhat diverse, encompassing folded-block mountains, volcanic plateaus, terraced plains, and low mountains. The geologic origins are long-extinct volcanoes on the edge of the Pacific and North American Plates.

One of the main differences between the Commanders and Aleutians is are the unique geological structures presented of the Commanders -

so called by geologists "bench". This structure increases the area of the shallow sublittoral zone and provides the optimal conditions for development of brown

algae - the main food of extinct Steller sea cow - the only Sirenian adapted to utilize the brown algae.



Compared with the Aleutians, the variety of animal species in the Commanders is higher: four northern fur seals rookeries, with almost 200,000 seals located on both Bering and Medny islands. More than 7,000 sea otters, about 2,000 harbor seal, one reproductive rookery of Steller sea lion, numerous marine colonial birds, etc., inhabit the Commander Islands. Thirty nautical miles of a marine protected zone surrounding the Commander Islands support abundant fish, squid and other invertebrates communities, and provide ample food resources for birds, pinnipeds and cetaceans.

Field work 2010-2011

Logistics

To conduct summer field research in 2010-2011 we used a 4-wheeler

“Yamaha Grizzly” (rented in Petropavlovsk-Kamchatsky), shipped by cargo boat with gas and other supplies to Bering Island as the main transport for collection of



the bones. A new boat and outboard motor were purchased in Petropavlovsk-Kamchatsky, and also shipped to Nikolskoe. We used this boat to visit distant locations on Bering Island that we could not reach by the 4-wheeler.

Poor communication between Kamchatka and the Commanders, and especially between Bering and Medny Islands, hindered our project, and required more funding and efforts that had been planned.

For logistic and safety reasons we postponed our trip to Medny Island, located across the 25 nm strait from Bering Island, but we did get a few samples from the bones collected on Medny Island for our research



needs. Because Steller's sea cows inhabited both Bering and Medny Islands, we were expecting that exchange between these two populations was very limited; thus, it is extremely important to compare samples from both islands.

Students from Russian universities (Moscow State University, Saratov State University, South Federal

University) and local Nikolskoe high school were also involved in the bone collection process and some people from Nikolskoe donated a few Steller's sea cow bones. High school student had a hiking trip on the East coast of Bering Island from Buyan Bay to Commander Bay and some portion of West coast from Gladkovskaya Bay to Podutesnaya Bay.

This hiking journey and Steller sea cow bone collection expedition, including camping in the tents and small cabins was an excellent experience for young students, to learn about history of exploration, unique nature, animals, birds, fish and modern problems of nature conservation in the Commander Islands.

Results

Samples collection

In the 2010 - 2011 field seasons, 93 Steller's sea cow ribs, one scapula and three vertebrae were collected on our trips along the Bering Island beaches, mainly on the east coast, and those also discovered at Nikolskoe village in the Commander Islands Nature Reserve storage that had been acquired from local citizens. These bones were collected for different goals; for example a scientist from Commander Islands Nature Reserve made an excavation of

an almost full Steller's sea cow skeleton, and all the bones belonged to a single animal. But mostly people collected bones as a potential source for carving and souvenir production. In spite of illegal trade of Steller's sea cow bones, it is possible to find carvings in the souvenir stores in Kamchatka peninsula and even in other Russian Far East towns. We suggest it was very important to sample these bones, for the following reasons:



1. Usually collectors select bones of good quality: dense, without damage, and with a good upper layer.
2. It seems to us, that ribs and bones in such good condition are probably the most recent, and present the last generations of Steller's sea cows prior to human contact and soon after that. The more "recent" bones we can analyze, the more accurate we can estimate genetic variation of this species.

In early 2011 we provided 44 samples collected in 2010, which were drilled from Steller sea cow bones used to test possibilities of DNA extraction from the sub-fossilized bones. These powder samples were sent for genetic analysis to Dr. O'Corry-Crowe. In 2011, 40 more Steller's sea cow bone samples were provided, and the development of samples collected in 2010 are now in progress.

This laboratory analysis was initiated because the National Park Service agreed to use some of the grant funds to buy supplies for genetic research.

Bones collected on the field trips were moved to Nikolskoe and sampled inside the office. We used standard 6 mm bits sterilized in 96% alcohol. For each bone different sterile bits were used. When drilling, to avoid the alien DNA in the samples, the powder from the upper bone layer (about 10 mm deep) was collected to the separate vials for age determination.

The powder from the interior bone layer was collected to the 2 ml sterile vials as genetic sample.

Both vials (for age and for genetic study) were labeled as a same sample but marked as for Genetic and Age and archived.

In 2011 we cut bigger pieces of Steller sea cow rib (about 500 g), in case it will be necessary for age estimation. The rest of the bones were stored in Komandorsky Nature Preserve for future use.

Laboratory analysis

All samples collected on the coast of Bering and Medny Islands were forwarded to ancient DNA genetic Lab in Harbor Branch Oceanographic Institute, Florida Atlantic University, Fort Pierce, Florida.

Total DNA was extracted from 11 bone powder samples of Steller's sea cow collected from bones at six sites on Bering Island (Figure 1) between 1994 and 2009. A roughly 700bp fragment of mtDNA was successfully amplified from nine samples. Subsequent sequence analysis yielded high quality light and heavy strand sequence for 581bp of this fragment in seven individuals (Figure 2). An eighth sample yielded good quality sequence from a somewhat shorter read length (Table 1).

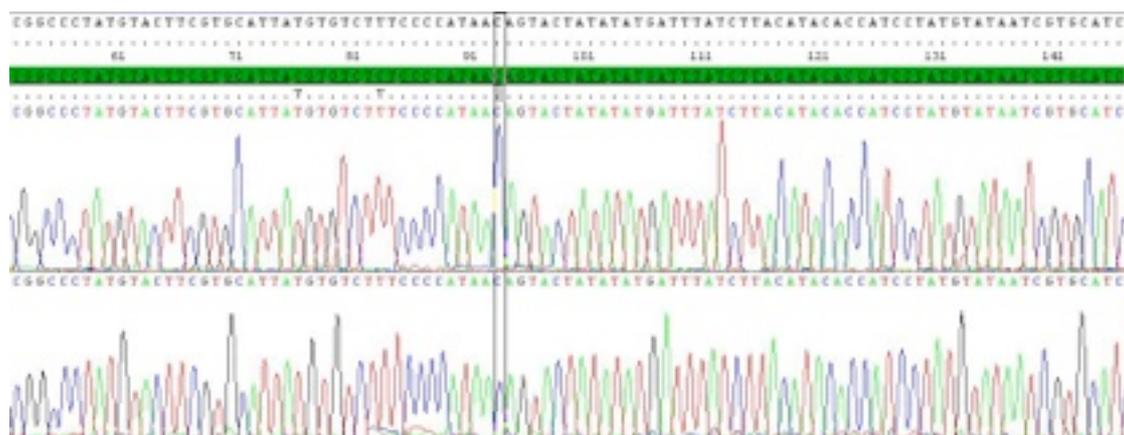


Figure 2. An electropherogram of Steller sea cow (*Hydrodamalis gigas*) mtDNA control region sequence from DNA extracted from sea cow bones collected on Bering Island, the larger of the two main Commander Islands, Russia.

Six variable sites defining three unique mtDNA haplotypes were documented among the seven samples successfully sequenced for the entire 581bp (Figure 3). Two of the haplotypes were found in a single sample, the third was documented in the remaining five samples. While there is a possibility that some of the bone samples with the same haplotype may have originated from the same individual, the sample and haplotype distribution indicate a minimum of x samples.

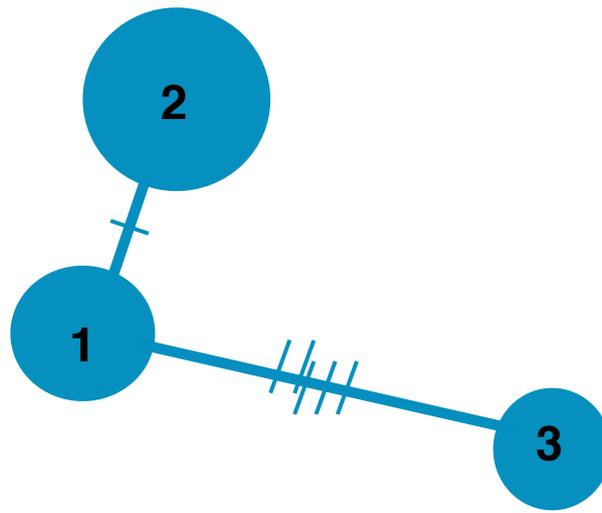


Figure 3. Minimum spanning network of Steller sea cow mtDNA control region haplotypes.

HBOI ID	Field ID	Bone Type	Collectin location	Date Collected	DNA Successfully Extracted	DNA Amplified by PCR	DNA Successfully Sequenced	Haplotype
HA36	1	Rib	Bering Island - Drovenskaya bay	07/25/94	x	x	Y	1
HA37	2	Rib	Bering Island - Drovenskaya bay	07/26/94	x	x	Y	na
HA38	3	Rib	Bering Island - Peredovaya bay	07/27/94	x	x	Y	2
HA39	4	Rib	Bering Island - NW cape	03/03/95	x	x	Y	3
HA40	5	Rib	Bering Island - Manatiape	05/30/95	x	x	N	na
HA41	6	Rib	Bering Island - Bobrovaya bay	07/24/95	x	x	Y	could not call, seq too short
HA42	7	Rib	Bering Island - Poludennaya bay	08/30/09	x	x	Y	2
HA43	8	Rib	Bering Island - Poludennaya bay	08/31/09	x	x	Y	2
HA44	9	Rib	Bering Island - ND	09/04/09	x	x	N	na
HA45	10	Rib	Bering Island - ND	09/04/09	x	x	Y	2
HA46	11	Rib	Bering Island - ND	09/04/09	x	x	Y	2

Table 1. Summary of Steller sea cow bone samples collected on Bering Island and analyzed as part of this pilot aDNA study. Success in each process is denoted by an 'x'.

Discussion

This pilot study successfully established an efficient lab method for studying aDNA from Steller's sea cow, and has provided the first data on genetic diversity within this extinct sirenian. A concern with such aDNA studies is that the quality and quantity of DNA extracted from the material will be low, thus affecting PCR success and sequencing quality. Few aDNA studies of large sample sets have been conducted for these reasons, limiting the application of this technology in intra-specific studies where sample sizes and read lengths have to be quite large. The quality and length of DNA sequence reads for Steller sea cow mtDNA in the current study are exceptional, even for contemporary tissue samples, and augur well for high throughput analyses of bone samples necessary for quantitative genetic analysis. We are confident that we can expand the scope of this study to include more samples across a longer time frame, and more genetic markers, including genes associated with individual fitness and population viability. Understanding the genetic aspects of the demise of the Steller's sea cow will ideally assist in formulating research and recovery priorities for extant species of dugong and manatee where little emphasis has been placed on the genetic component of population viability and recovery.

Conclusion

After two years of this project we believe we achieved our main goals. First of all we collected and archived about 100 samples of sub-fossilized Steller sea cow bones, and according to the available funding initiated a pilot project for extracting ancient DNA from the bones. This

pilot study demonstrates that DNA extraction from Steller sea cow bones is possible.

We realize that increasing our efforts for bone collection after a short period of time has limitations, because all noticeable bones are usually collected in a one trip. It is best to wait for further collection until a storm will move sand or gravel, or to try to visit previously unchecked locations. Collecting Steller sea cow remains in a distant or hard to reach locations are problematic, because of problem to transport heavy bones.

Future development of our project will include dating of collected bones. Without genetic analysis of each collected samples to date age of bones, the investigation of climate effects related to Steller sea cow extinction is impossible. We realize, that estimation and analyzing the geological age of bones without knowing genetic information will produce significant errors.

Because the number of collected samples is increasing, bones collected in a different time, but at the same or close-by locations can be from the same animal. Following big time scale analysis of bones age will increase number of animals of the same geological age. This will distort real temporal aspect of Steller sea cow number fluctuations.

But without known geological age of genetic samples we will be unable to investigate the temporal aspect of Steller sea cow genetic diversity, and possible terms of population bottleneck if it happened.

After all, we believe this project can be extremely important for future research not only for study serenian evolution, mechanisms of climate change effect to extinction of megafauna species, but also help to understand the modern problems of ecosystem stability in the Northern Pacific.

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