Duke of Edinburgh Prize to:

Naohiko Ohkouchi
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for "Development and Application of a Method for Analyzing Changes in the Biological World Using Advanced Analysis of Compound-Specific Isotope Ratio"

Outline of the work:

Dr. Naohiko Ohkouchi received his Ph.D. from the Graduate School of Science, the University of Tokyo, where his research focused on reconstructing past climate changes by ultra-trace analysis of lipid molecules in the oceanic sediments. During that time, he also developed advanced chemical analysis techniques for the purpose. After graduation, he became a postdoctoral fellow at the Center for Ecological Research, Kyoto University, where he studied ecology. Later, as an assistant professor at the Institute of Low Temperature Science, Hokkaido University, he participated in a project in the Sea of Okhotsk and returned to the study of marine sediments. In 1999, he relocated to the U. S. to work at the Woods Hole Oceanographic Institution, where he developed a method for radiocarbon dating of specific organic compounds isolated from oceanic sediments using various chromatographic and wet chemical techniques.

He was hired as a research scientist in 2002 when the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) established the Institute for Frontier Research on Earth Evolution. He has since improved the method for isolating various compounds from natural products and developed a technique for precisely measuring the isotopic compositions of bio-elements (primarily carbon and nitrogen) in them.

He has used compound-specific isotope ratios to uncover the details of natural processes in the environment by analyzing changes in the biological world. As Director of the Biogeochemistry Center at JAMSTEC, he is currently leading the ecosystems and environmental studies by using the above methodology. His research results have been published in over 250 papers, including four in *Science*, four in *PNAS*, and three in sister journals of *Nature*, with over 8,700 citations (Google Scholar, as of July 2021). Moreover, he has written three books for the general public, one of which was highly acclaimed and won the Kodansha Science Publishing Award.

The major achievements of Dr. Ohkouchi are summarized as the following.

1. Study of the dynamics of natural organic matter in the ocean and their paleoenvironment using fossil molecules (organic molecules produced in old times)

Dr. Ohkouchi isolated and radiocarbon dated a wide variety of molecules distributed on the seafloor, including chlorophyll and fatty acids, revealing for the first time that they have a wide range of ages. He also discovered that such molecules were collected from both land and sea and that the dynamics of these molecules changed significantly even during small-scale climatic changes such as the Little Ice Age and the Medieval Warm Period (Ohkouchi *et al*. 2002). The results of this study, which were published in *Science*, cautioned about the shortcomings of the sedimentary organic matter method, which is commonly used in paleoenvironmental research (i.e., assumption that the age at which sediments accumulated equals the age at which the organic matter in the sediments was produced). Simultaneously, it demonstrated for the first time that climate change affects the dynamics of particles drifting in the deep sea.

To precisely determine the stable carbon and nitrogen isotope ratios of individual trace organic compounds, Dr. Ohkouchi's group also successfully improved an isotope-ratio mass spectrometer system, which is currently an instrument with the highest signal sensitivity in the world (Ogawa *et al*. 2010). It precisely determines the nitrogen isotope ratio at 5 nmol and carbon isotope ratio at 15 nmol, making them possible to target a wide variety of compounds distributed in the ocean, such as chlorophyll, heme, membrane lipids, fatty acids, amino acids, and hydrocarbons.

Dr. Ohkouchi's team also discovered that the hydrocarbon (i.e., isoprenoid) parts of the membrane lipids of archaea living on the seafloor are not synthesized by the archaea themselves, but are reused by incorporating them into the cells.

In the sea, the chlorophyll side-chain phytol exhibits the same behavior. In other words, before using their ability to synthesize compounds required for life, microorganisms in nature look for "reusable" compounds in the environment. These findings demonstrated the critical nature of organisms living in the ocean, which is severely limited in terms of energy and nutrition (Takano *et al*. 2010).

2. Dynamics of cyanobacterial bloom emergence and accumulation of atmospheric oxygen in deep time

The tetrapyrrole structure (porphyrin), which forms the central ring of chlorophylls, is preserved in sediments for a long time and can be used to reconstruct primary producers and biogeochemical processes in the past.

Dr. Ohkouchi was able to successfully purify a series of porphyrins from 94-million-year-old (Cretaceous), organic-enriched black shales, the source rock of petroleum whose origin remains unknown. He discovered that the organic matter in black shale was formed primarily by a diatom-cyanobacteria symbiotic system. He also discovered that its bloom was related to geological events that occurred when deep mantle materials were supplied to the Earth's surface (Kuroda *et al*. 2007). This study contributed to the picture of a cyanobacterial world in which the repetition of such blooms produced petroleum source rock and pulsed accumulation of atmospheric oxygen. From the black shale, he also discovered porphyrins originating from bacteriochlorophylls, which are synthesized only by strictly anaerobic photosynthetic bacteria. It proved the existence of anoxic water masses within the photic layer of the ocean surface at that time. The same methodology was used to discover the world's oldest chlorophyll fossils in 1.1-billion-year-old strata, and nitrogen isotope ratios revealed that nitrogen-fixing cyanobacteria were the major producers in the ocean at the time (Ohkouchi *et al*. 2006, 2015; Gueneli *et al*. 2018).

The investigation of these organic-rich shales revealed the significance of cyanobacterial blooms in the evolution of the marine ecosystem over time.

3. Structural analysis of the food chain: Determination of precise trophic level by nitrogen isotope analysis of amino acids and their applications

Dr. Ohkouchi's team was the first to demonstrate that the nitrogen isotope ratios of two amino acids, glutamate and phenylalanine, record the food web in natural ecosystems. They discovered that a constant ^{15}N-enrichment with increasing trophic level occurs in the deamination of amino acids, and obtained the following empirical equation that accurately determines the trophic level of an organism (Chikaraishi *et al*. 2009; Ohkouchi *et al*. 2017).

$$[\text{Trophic level}] = (\delta^{15}N_{\text{Glutamate}} - \delta^{15}N_{\text{Phenylalanine}} + \beta) / 7.6 + 1.$$
$$\beta = -3.4‰ \text{ (aquatic organisms)}, +8.4‰ \text{ (terrestrial organisms)}$$

Knowing an organism's trophic level means understanding its place in the food web, which is one of the most fundamental pieces of information about living things in their natural environment.

Dr. Ohkouchi has been leading the ecosystem analysis using this method and has made contributions to a variety of fields such as ecology, fisheries science, anthropology, and so on. He also demonstrated that this method can be applied to fossil samples such as bones found in archaeological sites, as well as archaeological and anthropological problems. They were successful in analyzing the dietary habits of Jomon and Neanderthal people.

The following is a list of the various outcomes revealed by this methodology.

(1) Using eutrophic Lake Biwa as an example, the environmental changes in the aquatic environment caused by eutrophication in the 20th century have not changed the trophic niches of various fishes (Ogawa *et al*. 2013).

(2) Using Lake Baikal as an example, the trophic level of the top predator, the Baikal seal, is up to 5.1, which is the highest trophic level in nature known today (Ohkouchi *et al*. 2015).

(3) Many fish in the reef area had trophic levels ranging from 2.9 to 3.6 (Chikaraishi *et al*. 2014).

(4) Marine snow is the primary food source for juvenile eels in the wild. The results of this study were later used to develop a diet for cultured eels (Miller *et al*. 2012).

(5) According to bone records, salmon have a migratory pathway in which they grow and mature on the continental shelf of the eastern Bering Sea before returning to Japan (Matsubayashi *et al*. 2020).

(6) The trophic level of the people from the inland Middle Jomon period was 2.6, and at least 60% of their protein was derived from terrestrial animals (Naito *et al*. 2010).

(7) The Neanderthals who once lived in the Belgium interior had an average trophic level of 2.9 and were heavily carnivorous (Naito *et al*. 2016).

In conclusion, Dr. Naohiko Ohkouchi has paved the way for the application of compound-specific isotope ratio to existing fields such as oceanography, ecology, anthropology, and fisheries science, and has achieved results that develop him as a pioneer in the field.

This method has evolved into a powerful tool for analyzing changes in the biological world during the Anthropocene, a period since the 20th century during which the Earth's environment has been disrupted by human activity. In the Anthropocene, there is an urgent need to "protect ecosystems and conserve species" from a comprehensive and integrated perspective, from global to the local level, and from living to non-living things. In this regard, Dr. Naohiko Ohkouchi's establishment of "advanced analysis of compound-specific isotope ratio" is expected to reveal more and more important knowledge in the future.

Major Publication List

(1) Study of the dynamics of natural organic matter in the ocean and their paleoenvironment using fossil molecules (organic molecules produced in old times)

Ohkouchi, N., Eglinton, T. I., Keigwin, L. D., and Hayes, J. M. (2002) Spatial and temporal offsets between proxy records in a sediment drift. *Science*, **298**, 1224–1227.

Kashiyama, Y., Miyashita, H., Ogawa, N.O., Chikaraishi, Y., Takano, Y., Suga, H., Toyofuku, T., Nomaki, H., Kitazato, H., Nagata, T., and Ohkouchi, N. (2008) Evidence of global chlorophyll *d*. *Science*, **321**, 658.

Takano, Y., Chikaraishi, Y., Ogawa, N. O., Nomaki, H., Morono, Y., Inagaki, F., Kitazato, H., Hinrichs, K.-U., and Ohkouchi, N. (2010) Sedimentary membrane lipids recycled by deep-sea benthic archaea. *Nature Geosciences*, **3**, 858–861.

Ogawa, N.O., Nagata, T., Kitazato, H., and Ohkouchi, N. (2010) Ultra-sensitive elemental analyzer/isotope ratio mass spectrometer for stable nitrogen and carcon isotopic analyses. In *Earth, Life, and Isotopes* (Eds. Ohkouchi, N., Tayasu, I. and Koba, K.), Kyoto University Press, Kyoto, pp. 339–353.

Inagaki, F. *et al*. (Dr. Ohkouchi is the 29th author out of 44 authors) (2015) Exploring deep microbial life in coal-bearing sediment down to ~2.5 km below the ocean floor. *Science*, **369**, 420–424.

Yokoyama, Y., Yamazaki, T., Miyairi, Y., Anderson, J. B., Koizumi, M., Suga, H., Kusahara, K., Hasumi, H., Southon, J. R., and Ohkouchi, N. (2016) Widespread collapse of the Ross Ice Shelf during the late Holocene. *Proceedings of the National Academy of Sciences, USA*, **113**, 2354–2359.

Takano, Y., Chikaraishi, Y., Imachi, H., Miyairi, Y., Ogawa, N. O., Kaneko, M., Yokoyama, Y., Kruger, M., and Ohkouchi, N. (2018) Insight into anaerobic methanotrophy from $^{13}C/^{12}C$- amino acids and ^{14}C-ANME cells in seafloor microbial ecology. *Scientific Reports*, **8**, 14070.

Ishikawa, N. F., Itahashi, Y., Blattmann, T., Takano, Y., Ogawa, N. O., Yamane, M., Yokoyama, Y., Nagata, T., Yoneda, M., Eglinton, T. I., and Ohkouchi, N. (2018) An improved method for isolation and purification of underivatized amino acids for radiocarbon analysis. *Analytical Chemistry*, **90**, 12035–12041.

(2) Dynamics of cyanobacterial bloom emergence and accumulation of atmospheric oxygen in deep time

Ohkouchi, N., Nakajima, Y., Okada, H., Ogawa, N. O., Suga, H., Oguri, K., and Kitazato, H. (2005)

Biogeochemical processes in the meromictic lake Kaiike: Implications from carbon and nitrogen isotopic compositions of photosynthetic pigments. *Environmental Microbiology*, **7**, 1009–1016.

Ohkouchi, N., Kashiyama, Y., Kuroda, J., Ogawa, N. O., and Kitazato, H. (2006) The importance of diazotrophic cyanobacteria as primary producers during Cretaceous Oceanic Anoxic Event 2. *Biogeosciences*, **3**, 467–478.

Kuroda, J., Ogawa, N. O., Tanimizu, M., Coffin, M. F., Tokuyama, H., Kitazato, H., and Ohkouchi, N. (2007) Contemporaneous massive subaerial volcanism and late Cretaceous Oceanic Anoxic Event 2. *Earth and Planetary Science Letters*, **256**, 211–223.

Ohkouchi, N., Nakajima, Y., Okada, H., Ogawa, N. O., Chikaraishi, Y., Suga, H., Sakai, S., and Kitazato, H. (2008) Carbon isotopic composition of tetrapyrrole nucleus in chloropigments from a saline meromictic lake: A mechanistic view for interpreting the isotopic signature of alkyl porphyrins in geological samples. *Organic Geochemistry*, **39**, 521–531.

Sekine, Y., Suzuki, K., Senda, R., Tajika, E., Tada, R., Goto, K., Yamamoto, S., Ohkouchi, N., and Ogawa, N. O. (2011) Osmium evidence for a correlation of the rise of oxygen and Paleoproterozoic glaciations. *Nature Communications*, **2**, 502.

Nakayama, T., Kamikawa, R., Tanifuji, G., Kashiyama, Y., Ohkouchi, N., Archibald, J.M., and Inagaki, Y. (2014) Complete genome sequence of a non-photosynthetic cyanobacterium: Insight into bacterium-eukaryote endosymbiosis from the spheroid bodies of rhopalodiacean diatoms. *Proceedings of the National Academy of Sciences, USA*, **111**, 11407–11412.

Ohkouchi, N., Kuroda, J., and Taira, A. (2015) The origin of Cretaceous black shales: a change in the surface ocean ecosystem and its triggers. *Proceedings of the Japan Academy, Series B*, **91**, 273–291.

Nunoura, T., Chikaraishi, Y., Harada, T., Mori, K., Kato, Y., Sato, T., Yanagawa, K., Miyazaki, M., Shuto, A., Ohkouchi, N., Fujita, N., Atomi, H., Takaki, Y., and Takai, K. (2018) A primordial reversible TCA cycle in a facultatively chemolithoautotrophic thermophile. *Science*, **359**, 559–563.

Gueneli, N., McKenna, A. M., Ohkouchi, N., Boreham, C., Beghin, J., Javaux, E. J., and Brocks, J. J. (2018) 1.1 billion years old porphyrins and their isotopic composition establish a marine ecosystem dominated by bacterial primary producers. *Proceedings of the National Academy of Sciences, USA*, **115**, E6978–E6986.

(3) Structural analysis of the food chain: Determination of precise trophic level by nitrogen isotope analysis of amino acids and their applications

Chikaraishi, Y., Ogawa, N. O., Kashiyama, Y., Takano, Y., Suga, H., Tomitani, A., Miyashita, H., Kitazato, H., and Ohkouchi, N. (2009) Determination of aquatic food-web structure based on compound-specific nitrogen isotopic composition of amino acids. *Limnology and Oceanography: Methods*, **7**, 740–750.

Naito, Y., Honch, N. V., Chikaraishi, Y., Ohkouchi, N., and Yoneda, M. (2010) Quantitative evaluation of marine protein contribution in ancient diets based on nitrogen isotope ratios of individual amino acids in bone collagen: An investigation at the Kitakogane Jomon site. *American Journal of Physical Anthropology*, **143**, 31–40.

Miller, M., Chikaraishi, Y., Ogawa, N. O., Yamada, Y., Tsukamoto, K., and Ohkouchi, N. (2012) A low trophic position of Japanese eel larvae. *Biology Letters*, **9**, 20120826.

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Ogawa, N. O., Chikaraishi, Y., and Ohkouchi, N. (2013) Trophic position estimates of formalin-fixed samples with nitrogen isotopic compositions of amino acids: an application to gobiid fish (Isaza) in Lake Biwa, Japan. *Ecological Research*, **28**, 697–702.

Chikaraishi, Y., Steffan, S. A., Ogawa, N. O., Ishikawa, N. F., Sasaki, Y., Tsuchiya, M., and Ohkouchi, N. (2014) High-resolution food webs based on nitrogen isotopic composition of amino acids. *Ecology and Evolution*, **4**, 2423–2449.

Ohkouchi, N. and Takano, Y. (2014) Organic nitrogen: Sources, fates, and chemistry. In *Treatise on Geochemistry, 2nd Ed.* (Eds. P. Falkowski and K. H. Freeman), Elsevier, London, pp. 251–289.

Steffan, S. A., Chikaraishi, Y., Currie, C., Gaines Day, H. R., Pauli, J. N., Zalapa, J. E., and Ohkouchi, N. (2015) Microbes are trophic analogs of animals: Unification of the micro- and macrobiome in food web ecology. *Proceedings of the National Academy of Sciences, USA*, **112**, 15119–15124.

Ohkouchi, N., Ogawa, N. O., Chikaraishi, Y., Tanaka, H., and Wada, E. (2015) Biochemical and physiological bases for the application of carbon and nitrogen isotopes to the environmental and ecological studies. *Progress in Earth and Planetary Science*, **2**, 1.

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(4) Books and others

Ohkouchi, N. (2008) *Changing Blue: Approaching the Mystery of Climate Change*. Iwanami-shoten, Tokyo. (in Japanese; Kodansha Science Publishing Award; Korean translation in 2013)

Ohkouchi, N., Tayasu, I., and Koba, K. (eds.) (2010) *Earth, Life, and Isotopes*. Kyoto University Press, Kyoto.

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Ohkouchi, N. and Yamafuku, A. (2017) *A Story of Oil*. Takusan-no-Fushigi (A lot of Wonders), Fukuinkan-shoten, Tokyo. (in Japanese; Chinese translation in 2019)