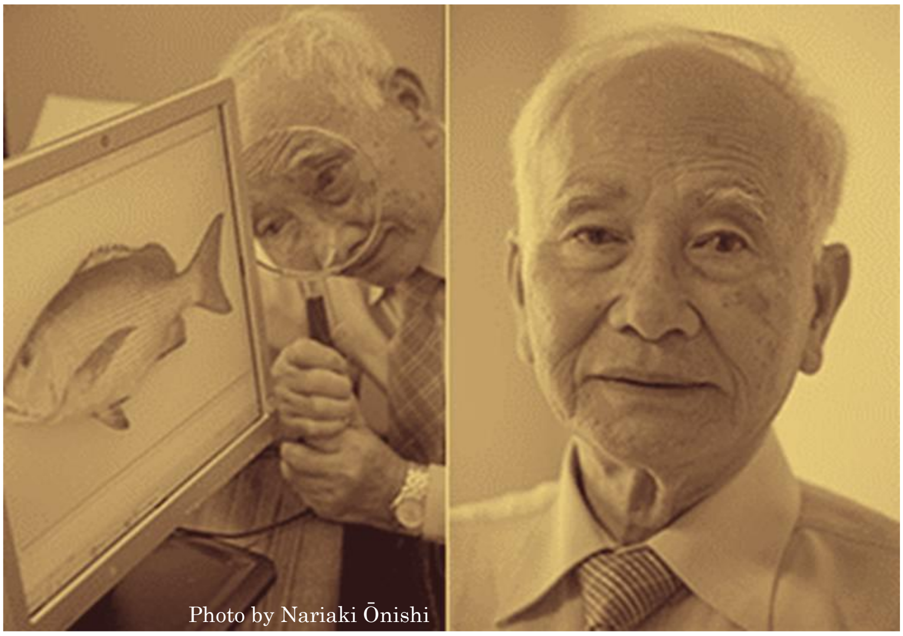
Toxins in the Sea as Seen Through the Eyes of Chemistry

Takeshi Yasumoto



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(https://brh.co.jp/s_library/interview/86/).

Biography

1935 Born in Okinawa, Japan

1958 Graduated from the Department of Fisheries, Faculty of Agriculture, the University of Tokyo

1960 Assistant Professor, Laboratory of Marine Biochemistry, Faculty of Agriculture, the University of Tokyo

1966 Postdoctoral Fellow, the Department of Chemistry, the University of Hawaii

1969 Associate Professor, Faculty of Agriculture, Tohoku University

1976 WHO Short Term Consultant, Louis Malardé Institute

1977 Professor, Faculty of Agriculture, Tohoku University

1998 Advisor, Japan Food Research Laboratories

1998 Professor Emeritus, Faculty of Agriculture, Tohoku University

Major Awards

1989 Toxic Plankton International Society Award

1992 JSBBA Award (Japanese Society for Bioscience, Biotechnology, and Agrochemistry)

1994 P. J. Scheuer Award for Marine Natural Products Chemistry

1994 The 26th Naito Foundation Merit Award

1997 Japan Prize of Agricultural Science, The Yomiuri Prize of Agricultural Science

1999 The Medal with Purple Ribbon

2004 Imperial Prize, Japan Academy Prize

2006 Nakanishi Prize

2010 The Order of the Sacred Treasure, Gold Rays with Neck Ribbon*

2017 Elected as a member of the Japan Academy*

*Added on the occasion of translation to English.

01　A Mother Who Raised Eight Children

I was born in Naha City, Okinawa Prefecture, the fourth of eight children. The Pacific War broke out the same year I entered Tenpyo National School, and in the summer of my junior year, Saipan fell and the residents of the Southwestern Islands were ordered to evacuate the area, which led to my family's evacuation to Kagoshima. My father, an employee of Kangyo Bank, stayed behind in Okinawa to protect the books of the Naha branch, but he was caught up in the ground war and died at the young age of 38. My mother always said with bitterness, "He should have run away as soon as possible." We were frequently hit by air raids in Kyushu, where we were evacuated, and every time a town burned down, we had to flee. I escaped from Sendai (Satsumasendai City), where I was first stationed, just before the air raid and evacuated to the countryside, and then moved to Waifu-machi (Kikuchi City, Kumamoto Pref.). When the war ended and I was forced to paint my textbooks black with black ink, I thought to myself as a child that I would not blindly trust the established authorities.

It was one year after the end of the war that I was able to return to Okinawa. As anyone who has experienced life with nothing after the war will tell you, it instilled in me a spirit of mutual support and a sense of self-reliance to take care of myself. Even though I lost my father, my mother raised eight children and sent them all off to college. Perhaps my mother had leadership qualities, because after the war she gathered together a group of women and acted as a coordinator, negotiating with outside parties and taking on work. Even so, it must have been difficult for her to make ends meet. In those days, no matter what time of night I woke up, I would always find my mother stepping on the sewing machine or doing the laundry. None of my siblings and I can ever be proud of her. She will always be a proud mother to us.

A picture of my family with my aunt and cousins when I was a child. My mother (far right) protected her eight children from air raids with her own hands and raised them in the poverty of postwar life (I am on the far left).

I was doing the big wheel on the bars at high school. I was an all-around sportsman. Perhaps it was my muscularity and heaviness that made me suited to diving in the sea.

02 A fisherman Told me about "Drunken Fish"

I like to be physically active, and in high school I was passionate about playing softball tennis. When I finished my last match in July of my senior year of high school, I decided to go to Iheya Island, north of the main island of Okinawa, with my friends to have some fun before I started studying for the entrance examinations. Looking back, this summer experience was my first encounter with marine toxins, the subject of my life-long research.

Iheya Island is a small island surrounded by blue sea and coral reefs. I enjoyed my last summer vacation in high school by diving into the sea every day to see creatures and fish. The coral reef sea has countless small and large waterways, and the tides are complex. The island's fishermen know the tides well, so I asked them every day where the tide was going today, and we became good friends and learned many things about sea creatures. One day, he told me, "If you eat fish caught in that area of the coral reef, be careful because you can get drunk." I wondered why he said "get drunk" instead of "get sick" by the fish, and asked him about it. He told me it was because when you eat fish caught in that area, about one in roughly 100 fish you eat makes you feel sluggish like a hangover. Oddly enough, during that time, any contact with cold water causes shock and severe pain, as if one were electrocuted. What is even more interesting is that the place where it is caught shifts in cycles of several years, just like a living creature, and any kind of fish in that place can cause the intoxication. The fisherman told us that perhaps it is not the fish themselves that are making the toxin, but the seaweed they eat or something else. I remember that my curiosity about the true nature of the toxin lurking in the sea was aroused at that time.

03 Tackling a Mystery Since Columbus

Because of my love of the sea and the stories of fishermen that had stayed with me, I chose the Department of Fisheries in the Faculty of Agriculture at the University of Tokyo. When I researched at the university, I found out that the symptom of "fish intoxication" was "ciguatera" poisoning, which was found in regions around the world where coral reefs have developed, and was a well-known seafood poisoning that has been recorded since Columbus' time. Moreover, what kind of substance was the causative toxin and what kind of organism produces it was still a mystery. I thought this was interesting, and was also stimulated by a book titled "Report on Survey of Toxic Fish in the Southern Ocean (see below)," compiled by Dr. Yoshio Hiyama, also a professor in the Department of Fisheries. It was a report on the research on Ciguatera conducted by Dr. Hiyama and his colleagues in Saipan just before the Pacific War, in which they investigated the toxicity of more than 70 species of fish by interviewing islanders and testing them on mice. When I opened the report, I found many familiar fish listed and was surprised to learn that many of the fish I had seen and caught in Okinawa could be poisonous. I became more and more interested in Ciguatera, and whenever I returned to Okinawa for vacation, I began visiting the fishing cooperative to interview them about the types of poisonous fish and the waters where they were caught. When I went to talk to Professor Hiyama about my research, he encouraged me to do my best, saying, "Research on Ciguatera is interesting."

I came across the famous book "Report on Survey of Poisonous Fishes of the South Seas" when I was in college. Along with original color illustrations of South Seas fish, it described in detail the types of poisonous fish, symptoms of poisoning, and even how to remove the poisonous internal organs.

With my mentor, Professor Yoshiro Hashimoto (left), with whom I began my research on Ciguatera. He played matchmaker at my wedding (right).

I wanted to go on to graduate school and continue my research on Ciguatera, and at first, I thought about going on to the biology laboratory where Professor Hiyama was working. However, biology at that time focused on studies that described the characteristics of organisms, such as taxonomy and morphology, and not the study of trying to clarify things in an analytical manner. I wanted to find out the mechanism by which fish become poisonous, so I thought that an approach from chemistry might be interesting, and chose the Fisheries Chemistry Laboratory. It was an unexpected opportunity that led me to begin serious research on Ciguatera here. At the time, Okinawa Prefecture was still under U.S. administration, and passports were required to enter and leave the prefecture. During the first spring break of my master's program, I was about half a month late for the new semester because my passport was issued late. Prof. Yoshiro Hashimoto got angry, saying "Where have you been playing around?" I returned to my seat with a bloated look on my face, saying that it was not my fault that I was late, but I handed him my notes on the ciguatera research that I had compiled during my delay. When the professor saw the notes, he said, "This is interesting!" He was overjoyed. Prof. Hashimoto, a Navy veteran, had been to the South Seas during the war and was familiar with Ciguatera, so he was thrilled to learn that a ciguatera survey could be conducted in Okinawa. At the time, Tokyo was experiencing an outbreak of Ciguatera in fish imported from the south, and the unfamiliar addiction was attracting attention. Also, as soon as Prof. Hashimoto found out that the cause of the delay was the passport, he said, "I'm sorry. It was my fault for getting angry." I was surprised that a professor of Tokyo University bowed down to a student who had just started graduate school, and at the same time, I was impressed by his grace. Together with Prof. Hashimoto, who was eager to "go to Okinawa right away," we embarked on a full-scale investigation of Ciguatera.

04 Meeting the Pioneers of Ciguatera Research

It was good that Prof. Hashimoto and I began our research in Okinawa, but we soon realized that we would not be able to study Ciguatera chemically in time to collect even a small number of samples. Since only about one fish in every 100 develops Ciguatera, and the toxic components in each fish are extremely small, it was a daunting task to collect enough samples for detailed chemical analysis. At that time, Prof. Scheuer of the University of Hawaii visited the University of Tokyo with the same problem. At the time, the University of Hawaii was leading the way in ciguatera research, and Scheuer's group was the first in the world to successfully isolate a ciguatera toxin and name it "ciguatoxin. However, due to a lack of samples and the limitations of analytical technology at the time, the detailed chemical properties and source of the toxin had yet to be elucidated. In 1976, in recognition of my work in joint research, I was dispatched as a short-term consultant of the World Health Organization (WHO) to the French territory of Tahiti, where Ciguatera is common. Dr. Hokama, my collaborator at the University of Hawaii, who had initially requested me, said, "I don't want to go to Tahiti because I don't speak French," and he introduced me to the WHO instead. I could manage to read French, and I also had a yearning for the beautiful islands that fascinated Gauguin, so I said, "I can manage if I go there," and I decided to go. By the way, this was a big turning point for me.

The collaboration between Japan, the U.S., and French Polynesia brought together some of the pioneers of ciguatera research. The leader, Prof. Banner of the University of Hawaii (Albert H. Banner: center), Prof. Paul J. Scheuer (far right), the namesake of ciguatoxin, Prof. Hashimoto (second from left), and Dr. Raymond Bagnis (second from right) of the Louis Malardé Institute in Tahiti.

05　The Origin of Ciguatera Gripped on Gauguin's Island

The Louis Malardé Medical Institute in Tahiti was home to experts in various fields and had collected many species on a large scale, but had found no organisms that could be the origin of Ciguatera. I changed my perspective a bit and started by taking a closer look at the same species of fish and comparing what makes them different, toxic or not. Using mice, I looked at the toxicity of herbivorous fish and turban shells, sent from different regions of Tahiti, and found that individuals from a region called the Gambier Islands were particularly toxic. When we opened its digestive tract, we noticed that it contained a lot of disc-shaped algae, about 0.1 mm in diameter, which we had never seen before. I immediately flew to the Gambier Islands to check the situation there.

The surgeonfish is a herbivorous species that lives on coral reefs, characterized by a ripple-like pattern on its body. The algae (Gambierdiscus toxicus), which is thought to be the source of ciguatoxin, was discovered for the first time in the digestive tract of this fish.

The Gambier Islands are a group of isolated islands located near Moruroa Atoll on the eastern edge of French Polynesia, blessed with beautiful coral reefs and abundant seafood. When we visited, however, Ciguatera was so frequent that none of seafood would be edible, and the islanders were living on canned food. When we examined the seafloor, as we expected, we found the small disk-shaped algae. These were dinoflagellates, a new genus and species, and we discovered that they have the unprecedented property of growing by attaching themselves to seaweed. It was named *Gambierdiscus toxicus* because of its Gambier Islands (Gambier), its disc-like shape (disc), and the fact that it would be poisonous (tox). In fact, it was the construction of coral reef development that triggered the large proliferation of *G. toxicus*. An observatory to monitor hydrogen bomb testing was built on the summit of Mangareva, the largest island of the Gambier Islands, and the coral in the area was killed off when the seabed was dug up to build a harbor on the island. The surface of the calcareous algae was covered with lime algae, which in turn were covered with *G. toxicus*.

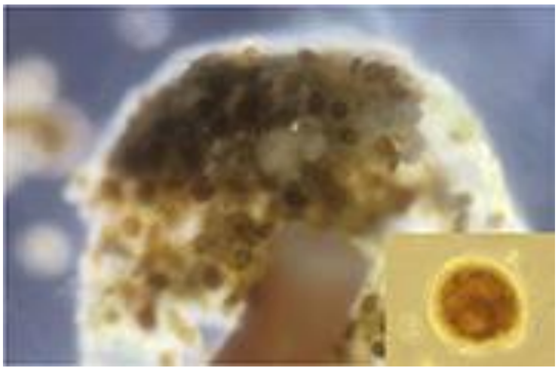
Mangareva Island, the largest of the Gambier Islands. At the top of the island was a monitoring center for hydrogen bomb tests.

The seabed of the Gambier Islands. White calcareous algae covered the entire area. The algae were growing on the surface of the dead coral, creating conditions conducive to the propagation of G. toxicus.

We immediately set to work collecting *G. toxicus* to see if it carried ciguatoxin. We dived into the ocean, scraped off the surface of the dead coral with a brush, and sifted it through a sieve we had brought from Japan to obtain *G. toxicus*. I continued this diligent work with Dr. Raymond Bagnis, a leading researcher on Ciguatera in Tahiti, and Dr. Akio Inoue, a professor at Kagoshima University (now professor emeritus).

Jack Bennett, the best diver and fisherman in Tahiti, was a great help in collecting dead coral from the ocean floor. He became my best friend and we even became brothers. In fact, he was the one who saved me from a dangerous situation. As the days passed in the Gambier Islands, Inoue-san and I began to lose patience with the same corned-beef lifestyle we had been living every day. I really wanted to eat fish, so I ate it, thinking that a little bit would be okay. At that moment, Jack stopped me by grabbing my arm and saying, Stop! I don't know what would have happened if he hadn't stopped me.

From the 400 grams of *G. toxicus* we collected, we were able to extract 0.75 mg of ciguatoxin, just as we had hoped. If we cultivated and purified *G. toxicus* in large quantities, we would have enough ciguatoxin for analysis. I brought the sample home and began culturing it with great anticipation. However, no ciguatoxin was detected from the cultured *G. toxicus*. Even after various changes in culture conditions and trials by research groups around the world, *G. toxicus* did not produce ciguatoxin. Speculations and criticisms were rife that other organisms were making the toxin, let alone producing large quantities of it, and the search for the cause of the problem seemed to be getting away from us once again.

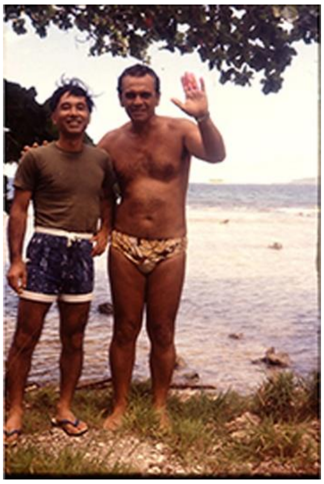


Gambierdiscus toxicus covering the surface of the calcareous algae and its enlargement (bottom right). The dinoflagellate G. toxicus grows attached to large seaweeds, but it can also swim free from the seaweeds. Prof. Yasuo Fukuyo, who wrote the paper described this species, initially wanted to name it G. yasumotoi after me, but I told him that I did not want my name attached to a toxic organism and asked him to change it to G. toxicus.

The reason that immediately came to mind as to why the ciguatoxin was undetectable was that all of the *G. toxicus* used in the culture were clonal strains, starting from a single cell. In order to save time and effort in management, we kept only one of the strains that we brought back that was growing well and disposed of the others. Because we had no experience with microorganisms, we were unaware that different strains might produce different substances depending on their genotype. Since large-scale collection and cultivation requires a certain amount of money and manpower, we could not immediately embark on another investigation. Eventually, after 10 years, we were ready again and found a strain that produced ciguatoxins, finally proving that *G. toxicus* was the origin of Ciguatera.

Our first failure was a bitter lesson, but we were now convinced that dinoflagellates on the seafloor were a cause of poisoning that could not be ignored. So, for the next 10 years, until we conducted another survey, we broadened our view to various types of benthic dinoflagellates and investigated their toxicity, and unexpectedly, we found a series of other poisoning-causing substances and new substances that had not yet been discovered. So I don't think 10 years was a waste of time.

Prof. Akio Inoue (left) and Dr. Raymond Bagnis (right) sift G. toxicus from an algae mixture scraped off coral using several different sieves.



With Jack Bennett (right), Tahiti's best diver and close friend. We always worked together as diving partners during oceanographic expeditions (I am on the left).

A report on the results of research supported by the Toyota Foundation, which gave us our first funding grant. The most difficult part of the ciguatera research was fundraising, and we went directly to various international organizations to ask for support. The report was well received around the world and was reprinted three times.

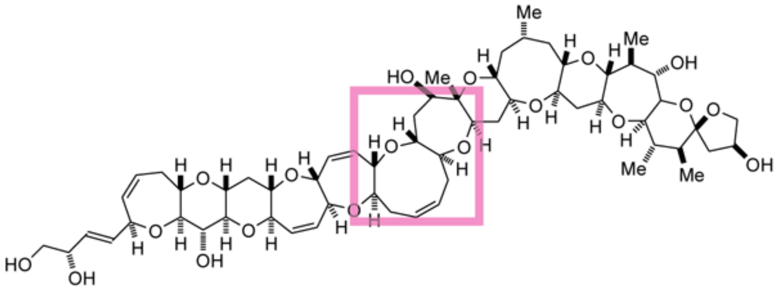
The benthic dinoflagellate Ostreopsis siamensis (left), closely related to G. toxicus, and Prorocentrum lima (center) were also found to produce substances that cause seafood poisoning. Amphidinium klebsii (right), which causes red tide, was found to have potent anti-fungal components.

06 Ciguatoxin Finally Revealed

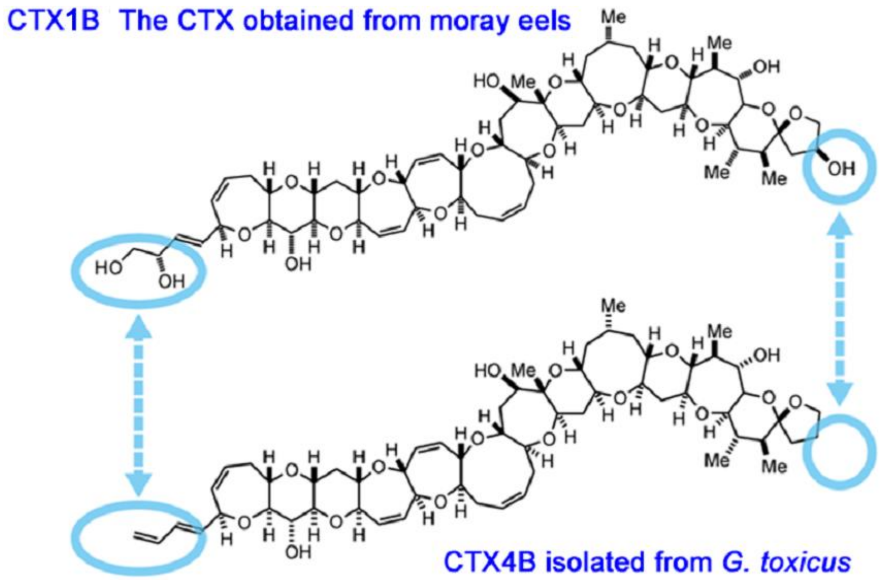
Since we could not immediately obtain the toxin from *G. toxicus*, we decided to use a carnivorous fish, the moray eel, which accumulates high concentrations of the toxin, as material for the analysis of the chemical structure of ciguatoxin. Once again, Jack Bennett and I dived into the sea to capture moray eels, and over a period of 10 years, we collected 830 fish (approximately 4 tons worth) and extracted 0.4 mg of ciguatoxin from 125 kg of their guts. I had considerable experience in chemical structure analysis from my research on benthic dinoflagellates, but I knew that ciguatoxin was a huge molecule that no one had ever handled before, and the sample was only 0.4 mg. Structural analysis required both careful planning and bold decisions. Together with my colleague Michio Murata (currently professor at Osaka University), we first carefully estimated the elemental composition and confirmed that the molecular formula was $C_{60}H_{86}O_{19}$, and then estimated the chemical structure by making full use of NMR and computer simulations, which were the most advanced at the time. However, the proposed structure did not match the molecular formula, and there were eight carbons missing. We thought that there might be signals that were not visible in NMR spectra due to the flexible three-dimensional structure of the molecule, so we cooled the NMR to suppress the movement of the molecule. When the NMR sample was cooled to –20°C, a large ring consisting of one oxygen and eight carbon atoms emerged in the center of the molecule. The toxin was transferred from *G. toxicus* to herbivorous fish, then to carnivorous fish such as moray eels, and so on through the food chain.

Even more surprising was the diversity of ciguatoxin types. There were as many as 23 homologues of ciguatoxin with similar basic skeletons. Thanks to new technologies, such as the development of effective reagents for analysis by Prof. Hiroshi Ohrui (currently Professor Emeritus at Tohoku University) and others in a neighboring laboratory, and the development of a state-of-the-art mass spectrometer (FAB-MS) by JEOL, we were able to clarify the structures of all 23 analogs, including their stereochemical structures, in a sample of less than 5 µg. For example, the toxicity of ciguatoxin in the moray eel was about 10 times higher than that of its ciguatoxin homologue in *G. toxicus* due to oxidation of the toxin in the moray eel body. It seems that the moray eel does not seem to be intoxicated even though it has accumulated a strong toxin that is normally excreted immediately from the body, indicating that ciguatoxin, which originated in *G. toxicus*, has been transformed into various forms through the food chain and is circulating in the ecosystem. After 40 years since our first encounter with Ciguatera, the whole picture has finally become clear.

Moray eel collected for the extraction of ciguatoxin.



Chemical structure of ciguatoxin (CTX 1B). The shape of many fused rings is called a "ladder-shaped polyether" structure, which is unique to marine biological compounds. The 9- and 7-membered rings in the center of the molecule (in the frame) change 3D structure by conformational exchange, bending the molecule like a hinge. In recent years, it has become clear that this flexible structure is closely related to the development of toxicity.



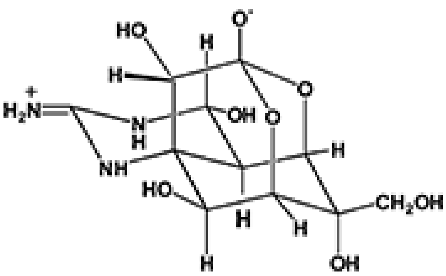
Comparing the structures of ciguatoxins extracted from G. toxicus and moray eels, the moray eel ciguatoxin (CTX1B) has both ends of the molecule oxidized (circles) from that of G. toxicus (CTX4B). The reason why moray eels store more toxic ciguatoxin is still being elucidated, but there is no indication that they actively use the toxin for defense or other purposes, as is the case with puffer fish.

07 The Mystery of Pufferfish Toxin was Solved in the Course of the Ciguatera Research

Let me go back for a moment and tell you how the mystery of pufferfish toxin was solved in the course of my research on Ciguatera. Shortly before I was sent to Tahiti, I was introduced by Prof. Hashimoto to the laboratory of Prof. Hisashi Kaneda of Tohoku University, where I was engaged in the separation and structural analysis of fish oils. Although ciguatera research was a different theme at Tohoku University, I was grateful that Prof. Kaneda allowed me to continue without saying a word. By the way, it is known that pufferfish toxicity varies from region to region, and when I did a trial study in the Tohoku region, I found that pufferfish from the Sanriku coast were particularly toxic. Pufferfish poisoning has been familiar to the Japanese people since ancient times, as in Buson's poem, "Fugu-jiru no ware ikiteiru nezame kana (Japanese)," or "After eating fugu soup, I am still alive with sleepyhead." The reason why there are still cases of poisoning every year is because there is a large variation in toxicity among regions and individuals, and the rule of thumb that "it is safe to eat this much" does not apply. In fact, while testing the toxicity of various seafood species in Okinawa and Tahiti for the purpose of studying ciguatera toxicity, I had noticed that some organisms produced trace amounts of tetrodotoxin (TTX), the poisonous component of pufferfish toxin. The fact that the toxins were produced by all species of organisms and that the toxicity varied among regions and individuals was similar to the occurrence of Ciguatera, which led us to believe that the pufferfish toxin TTX was not produced by the pufferfish itself, but rather accumulated through the food chain.

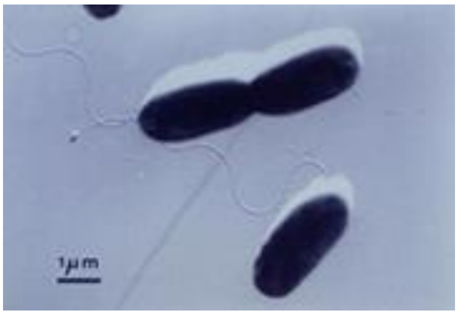
However, since pufferfish are high on the food chain, it is difficult to find organisms that produce TTX. The key to solving the mystery, then, came to me in the form of a crab that lives in the Southern Ocean. We had long observed that the sea squirt crab, which can accumulate TTX like pufferfish, feeds primarily on calcareous algae. As expected, TTX was detected in the calcareous algae *Jania* sp. which is eaten by the sea crab, so at first I thought that this calcareous algae might be the origin of TTX. However, since the concentration of TTX in the seaweed increased and decreased significantly after the summer season, we reconsidered the possibility that invisible bacteria attached to the seaweed were the real culprits. We discovered the TTX-producing bacterium *Shewanella* *alga*. In fact, a student had the good fortune to find TTX in a culture of this bacterium that had been left to grow, and later discovered that it produces a lot of toxin under anaerobic, or tormented, conditions. We did not expect to find bacteria rather than dinoflagellates as the source of toxins in fish and shellfish. It made me realize the diversity of poisonous organisms.

With Prof. Kaneda, my mentor at Tohoku University, who helped me analyze fish oils and fats.



Stereochemical structure of tetrodotoxin (TTX), the pufferfish toxin. Despite its small molecule size, the toxin has a very complex structure.

The sea urchin crab, which was the material to solve the mystery of pufferfish poisoning, and the calcareous algae Himemosazuki, which the crabs feed on. When examined, TTX was detected in both.



The TTX-producing bacterium Shewanella alga was discovered from the Himemosazuki, and this discovery led to the focus of attention on bacteria as a cause of pufferfish poisoning and the discovery of several other species of TTX-producing bacteria.

08　Running Around the Seven Seas

Perhaps it was these achievements in research on Ciguatera and pufferfish poisoning that earned me a reputation as a person who could handle anything you asked Yasumoto to do. From around the 1990s, whenever a case of seafood poisoning occurred anywhere in the world, the WHO, universities, and other organizations began requesting me to conduct research. I received so many reports of overseas business trips to remote islands such as Tahiti and Fiji that I was even questioned by the faculty committee as to whether I was really there to conduct research. I think I have been to almost every island in the world.

I will never forget the time I went to Madagascar after receiving word of a mass food poisoning of sea turtles. The site of the poisoning was in the middle of nowhere, so I flew from Antananarivo, the capital of Madagascar, to the countryside, and then drove across the desert in a four-wheel drive vehicle that I procured locally to the site. We arrived at a secluded seaside village, and were surprised to see curious children running out of the door as soon as we set foot inside. Two months had already passed since the outbreak of poisoning, but we managed to dig up the leftovers from the sea turtles buried deep underground and carefully brought them back to the university. Extraction and purification were performed in the laboratory while enduring a foul odor, and the poisonous substance lyngbiatoxin A was detected. We were able to identify this substance thanks to the testimony of local children who told us, "When I recently entered the sea, I got a rash on my body." Lyngbiatoxin A causes irritation when it comes into contact with the skin, and poisoning when ingested orally. This poisonous substance was produced by cyanobacteria attached to eelgrass, a favorite food of sea turtles.

Where a four-wheel drive vehicle crosses the desert of Madagascar to the site of a sea turtle poisoning.

A seaside village on the island of Madagascar that was the site of a sea turtle food poisoning outbreak.

A large number of children popped out of what appeared to be a secluded village. The children's testimonies also provided clues to the identification of the toxic substance.

Sardine poisoning is common in the Philippines and Fiji. The poisoning is so intense that it is said that the symptoms already appear while the tail is still outside the mouth, without finishing eating a single sardine. I have interviewed people in various villages, and there are stories of families eating sardines that their children had caught and the father who ate the largest one died, and chickens and cats that had been kept died soon after eating leftover sardines. The cause of the poisoning was unknown for a long time, but it was only when I witnessed a case of sardine poisoning in one village and crab poisoning in a neighboring village at about the same time that the sardine poisoning occurred that I realized what was happening. A toxic substance called palytoxin, which accumulates in crabs, can cause poisoning symptoms similar to those of sardine poisoning. I had confirmed during my research on Ciguatera that a type of benthic dinoflagellate produces palytoxin, so sardines may also have taken in palytoxin from dinoflagellates on the sea floor. I was happy when I obtained a single sardine head left uneaten by a poisoned patient at the Madagascar Pasteur Institute, prayed for it, analyzed it, and was able to detect palytoxin as expected.

Information from the local people is essential to elucidate the cause of poisoning. Fishermen, in particular, know everything about the sea. When I asked a Filipino fisherman about sardine poisoning, he replied, "Open the gills of the sardines you catch; the kind with mud in them is dangerous because they eat algae on the ocean floor. Isn't that obvious?" I said. I was surprised that he knew that much. I remember the first fisherman I met on Iheya Island when I was in high school was also very knowledgeable. In fact, I think now that I got a clue to solving both pufferfish poisoning and sea turtle poisoning from that fisherman.

A house in the Philippines where sardine poisoning was interviewed. Three days before the sardine poisoning occurred in this house, a crab poisoning occurred in a neighboring village, and the relationship between the two was first noticed.

The highly toxic Cobra crabs. The genus name, Demania, is derived from "demon," and is named for its highly poisonous nature.

Sardines obtained in Madagascar, leftovers from a poisoned patient. One of the two pieces was found to contain palytoxin, the same poisonous substance that causes crab poisoning.

Filipino fishermen who told me more about sardine poisoning.

Most seafood poisoning cases occur on islands far from cities. People around me say that it is a hard job because you rush to the scene without knowing whether or not there is a guarantee of food, clothing, and shelter at the destination. However, I myself have never thought of it as hard work. It is a unique and wonderful experience to live with the local people and go into the sea. I want to make it possible for them to predict outbreaks of poisoning.

Speaking of which, there is one poisoning that I discovered firsthand. In the Kanada Laboratory, it was a regular event for everyone to go to the beach together during summer vacation. While playing mahjong at night at a local hotel, we all got diarrhea after eating boiled mussels that the hotel owner brought over for us. Ordinary people would have just gotten it from food poisoning, but since the poisoning occurred even though the mussels were well boiled, I realized that it was a shellfish poison, which had never been reported in the past, and did some research. We had discovered that a type of benthic dinoflagellate that we had seen during my research on Ciguatera produced a substance called okadaic acid, which can cause diarrheal poisoning. This experience notified us the cause. *D. fortii* is a nuisance that can toxify shellfish when present in small numbers in seawater. We were surprised to learn that it was a type of algae that does not photosynthesize itself but preys on other flagellates. The ecology of small dinoflagellates is unique.

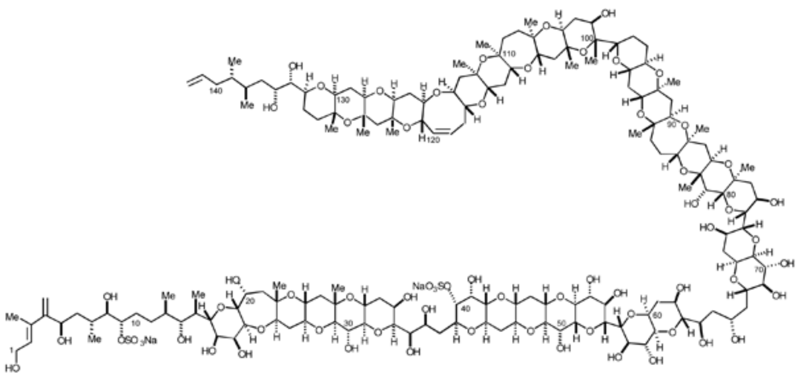
With Professor Jose Antonio Rodoriguez Vazquez (Vigo University, Spain). I taught him the know-how of shellfish toxin analysis because the same shellfish poisoning found in a guest house in Tohoku occurred in Spain.

09 The Challenge of the Largest and Strongest Toxin

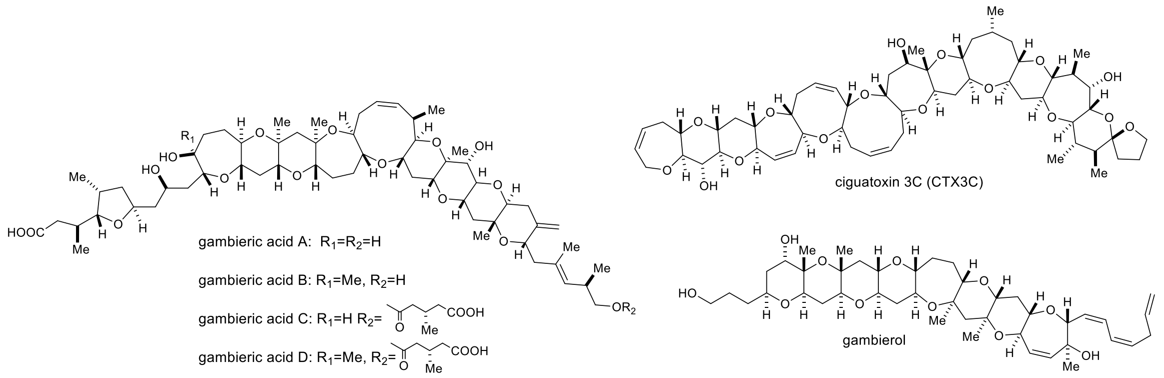
In fact, the symptoms of Ciguatera are so diverse that many mysteries still remain. One such example is maitotoxin, which I discovered when I first studied the surgeonfish in Tahiti and named it after the fish's local name, "maito."

Maitotoxin can be found in very small amounts in seafood and causes symptoms similar to those of Ciguatera. It is the strongest non-protein natural toxin, and if injected, just 1 mg is powerful enough to kill a million mice. The physiological mechanism that causes poisoning is not fully understood, but at very low concentrations it manipulates ion channels in cell membranes, causing an influx of calcium ions into the cells. In addition, maitotoxin is not only highly toxic, but also has a molecular weight of 3422, which is much larger than that of ciguatoxin. Although it was not a simple matter to elucidate the chemical structure, we were able to do so with the cooperation of many researchers.

First, in order to reduce the size of the molecule to a size suitable for analysis, the macromolecule was divided into three fragments, and the chemical structure of each fragment was estimated using NMR and mass spectrometry. Michio Murata, my colleague, played a central role in this process. For the final confirmation of the total structure, we cut the NMR 3D spectrum along the Z-axis, extracted the signals, and stitched the cross sections together to reproduce the structure. In other words, this is the same principle as a medical CT scan, which reconstructs the three-dimensional human body by connecting cross sections. For this analysis, we rented a state-of-the-art NMR system and ran it continuously for a week. We were able to maintain a stable magnetic field for a long period of time thanks to the technical capabilities of JEOL, which cooperated with us in the analysis. Furthermore, the steric structure was found by Professor Kazuo Tachibana of School of Science at the University of Tokyo, who reproduced it by partial synthesis. After 10 years of research, maitotoxin was finally revealed as a molecule with a chain-like skeleton consisting of 32 rings. *G. toxicus* produces ciguatoxin, maitotoxin, and many other complex compounds. The biosynthetic mechanism of dinoflagellates is astonishing.



Chemical structure of maitotoxin. It is the largest compound in the natural product family, and it took 10 years to determine its chemical structure.



Various substances produced by G. toxicus. Some cause Ciguatera and others have anti-fungal properties.

Taking a break from tennis. Tohoku University collaborators were also tennis buddies. Profs. Hiroshi Ohrui (second from left), Akio Miyazawa (second from right), and Yasukatsu Oshima (Professor Emeritus of Graduate School of Life Sciences, Tohoku University: far right). (myself, far left).

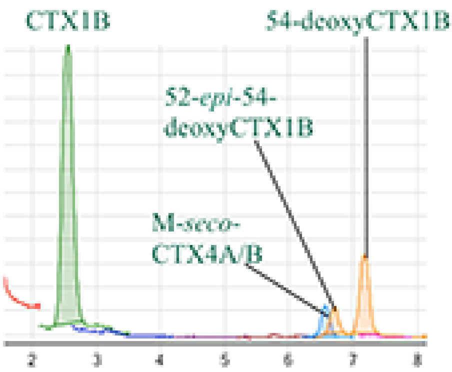
10　Continuing to Face the Toxins of the Sea

I believe that our research on Ciguatera has finally reached the stage of fitting the last piece of the jigsaw puzzle. For a long time, humans had no way to identify the fish that cause Ciguatera, nor could we predict when or where outbreaks would occur. Now we are developing practical methods to make them possible.

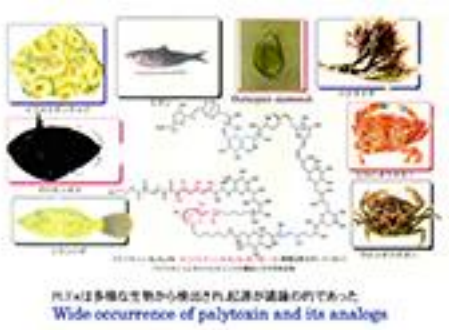
We are working with Osaka Prefecture University (Osaka Metropolitan University) and the U.S. Food and Drug Administration (FDA) to create a universal index for evaluating toxicity, so that fish and shellfish that develop Ciguatera can be determined through chemical analysis. To this end, we have refined a standard for ciguatoxin and started providing it to 12 institutions in Japan and overseas. The standard is the equivalent of a metric standard, so to speak, and will serve as the basis for all analyses. This is my role as the first to clarify the structure of ciguatoxins, and I believe it is my way of returning the favor.

When I received the Japan Prize of Agricultural Science and the Yomiuri Prize of Agricultural Science. The daughter (far right) is holding a tennis racket gifted by alumni as a gift in celebration, which she still uses today.

When I received the Japan Academy Prize and the Imperial Prize. Explained to Their Majesties the Emperor and Empress about the toxins of marine organisms.



We have developed a method to detect all ciguatoxin homologues at once by LC-MS/MS (a type of mass spectrometer). Every single peak in the graph corresponds to a different ciguatoxin homologue.

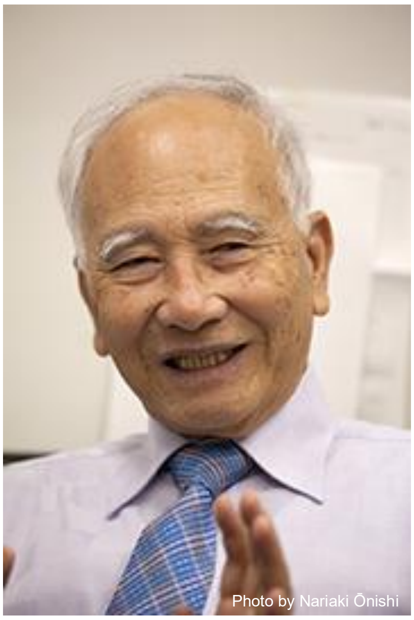


Some of the slides from a conference in 2014. I presented a method for determining the origin of palytoxin (center of the slide) and analyzing its homologues, which are present in large numbers in the ocean.

Also, since advanced analysis is not possible on remote islands or in fishing villages far from cities, a test kit that can simply detect ciguatoxin is needed. I am working with Profs. Masahiro Hirama at Tohoku University and Takeshi Tsumuraya at Osaka Metropolitan University to create an antibody that reacts with ciguatoxin to produce coloration. Since ciguatoxin has a wide variety of homologues, we need to adjust antibodies for each of them. We are now running around Okinawa, Amami, Hawaii, and Tahiti, analyzing the ciguatoxin composition of various fish species and trying to develop practical kits. I want to research as long as I have the strength and energy to do so and reduce poisoning as much as possible.

On the other hand, I am passionate about analyzing the spectra of palytoxin homologues in order to find the origin of palytoxin in the sea, which has not yet reached its conclusion. The substances produced by dinoflagellates, such as palytoxin and maitotoxin, have huge and complex structures and are extremely toxic. The most common question I receive after presentations at conferences is, "What do dinoflagellates make such complex substances for?" to which I cannot yet answer. What I am envisioning now is that these substances may be responsible for intracellular signaling in dinoflagellates. Since both palytoxin and ciguatoxin, like maitotoxin, exert their toxicity by acting on ion channels in the cells of ingested organisms, I think that these substances may be involved in ion permeation in marine organisms. The flexible three-dimensional structure and the hydrophilic and hydrophobic groups are similar to peptide hormones, which are responsible for signal transduction in our bodies. They may still retain inefficient mechanisms that life has discarded in the course of evolution, or conversely, the acquisition of this mechanism on their own may have been the dead end of their evolution. You may think this is an outlandish hypothesis, but it is a great mystery that I would like to try to solve next.

11 “I want to paint the sea on canvas.”



To find interesting phenomena from creatures in the field, it is important to have the ability to think outside the box. This may be a digression. Prof. Koji Nakanishi is so good at magic tricks that his nickname at academic conferences is "Magician Nakanishi.” I don't do magic tricks, but I am sometimes called "Magician Yasumoto" because it is like a magic trick to take organisms or chemical substances that no one has ever seen from the sea and elucidate their chemical structures with a small amount of samples. I chose a chemistry course when I was a student, but my love of living creatures has not changed since childhood. Observing and continually thinking about the feeding habits and life histories of various creatures may have led to ideas that others did not have.

When I received an honorary doctorate from the University of Vigo, Spain. I continue to collaborate with the University of Vigo, while traveling back and forth to Japan (myself: right).

When I received an honorary doctorate from Kalmar University in Sweden.

The 2002 Yasumoto Lifetime Achievement Award recipient, Prof. Smayda (left), has elucidated the causes of algal blooms, such as the red tide, and their impact on the ecosystem.

I have had my feet planted in both chemistry and biology, and I have been blessed with good colleagues in both fields. Analyzing huge and complex compounds has been a series of unprecedented challenges, so I cannot express my gratitude enough to my fellow chemists who have worked hard and developed their skills together with me. I was also happy that my fellow biologists appreciated my chemical achievements. I am very proud of the "Yasumoto lifetime achievement award" of the International Society for the Study of Harmful Algae. The award recognizes researchers who have made significant achievements in the field of toxic algae. The first recipient was Prof. Max Taylor of the University of British Columbia, who wrote a major book covering the taxonomy, ecology, and evolution of dinoflagellates, and subsequent recipients have included Prof. Theodore J. Smayda of the University of Rhode Island and Prof. Yasuo Fukuyo of the University of Tokyo, and others, all of whom I admire very much. I am always filled with gratitude and pride at the award ceremony.

Since I was the first to start ciguatera research in Japan, I myself have never belonged to any academic clique. I have never taken care of my students' job placement. I always tell my students, "If you are doing the same thing as everyone else, you will always be compared with each other, and that will be boring. Do something different and do a good job. As a result, there are many graduates who have become professors or associate professors at universities, but they are all opening up new fields on their own. I guess my role is like a courier who brings interesting materials from the field and passes them on to students.

When I received the Okinawa Times Award, I asked my 96-year-old mother to attend the award ceremony on my behalf. Until just before she passed away at the age of 100, my mother was out and about in good health, enjoying traveling abroad and collecting ceramics.

Eight grown-up siblings with their mother (front row, center). One of my siblings became an architect, one became a wife of a politician, and one became a dyeing and weaving researcher. All of them share the same love of travel and the arts, which I believe is also inherited from my mother (myself, second from the right in the back row).

I want to know the whole picture of natural phenomena in the sea through the process of toxification of marine organisms. All of my siblings except me paint, and just as a painter stands in front of a canvas to conceptualize the whole picture, I stand in front of a natural phenomenon to think about what kind of investigation and analysis is necessary. This naturally brings in chemistry and biology, so I am often told that my research is natural history. The canvas gradually fills in with various matters, such as the chemical structure of toxins, their ecological origins, physiological effects, and the life histories of organisms, until it becomes a single picture. I would like to do research in such a way that other researchers can look at it and know, "This is Yasumoto's picture." Of course, I cannot do all the research by myself, so I go to specialists in various fields and say, "We have interesting materials. Why don't we do research together?" I have found some interesting materials. The things I found had unique chemical structures and physiological effects, so I was grateful that so many people were interested in them. I was confident that we were the most advanced in this field, so I provided them with valuable samples and taught them how to conduct experiments. We have been working together with researchers from all over the world, regardless of nationality, and this has now expanded to a large extent. Even so, there are still many things we don't know and would like to know. The bigger the picture gets, the more fun it is, because the more mysterious things keep coming up one after another.


Photo by Nariaki Ōnishi