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Guam Seaweed Poisoning: Seaweed Testing on Guam

VALERIE J. PAUL, PH.D.

Director, Marine Laboratory
University of Guam
UOG Station
Mangilao, Guam 96923

Abstract—*In vitro* and *in vivo* studies of aqueous and organic extracts of *Gracilaria* seaweed harvested from several sites on Guam have shown them to contain biotoxins which are believed to serve as natural deterrents to grazing by herbivorous fish. The highest levels of these toxins were observed in *Gracilaria* collected from the Tanguisson Beach area soon after an incident of human seaweed poisoning involving *Gracilaria* harvested from the same site.

My training at Scripps Institution of Oceanography included studies in marine natural products chemistry so I was very interested in the Guam seaweed-associated poisoning incident from the beginning. The early suspicion that this incident was due to ciguatoxin or palytoxin, toxins which have been extensively studied by Professor Yasumoto, prompted us to seek his assistance in verifying the cause. Now very early on Professor Yasumoto indicated to me that he was quite convinced that the toxin involved was not palytoxin. His testing of samples from the original collections led him to suspect that we were dealing with a previously undescribed toxin, possibly unique to *Gracilaria*.

My research here at the University of Guam Marine Laboratory has involved primarily chemical ecology. This is the study of marine natural products, including marine toxins, and their function in the marine environment. We believe that in many cases these compounds serve as feeding deterrents against natural predators (Duffy & Paul 1992, Paul & Pennings, 1991). We have found that variability in levels of these natural products in seaweeds, as well as other marine organisms, is very common (Alstyne et al. 1992, Meyer & Paul 1992). So what we are hearing today about the variability of toxicity between populations of plants is not surprising, although we certainly do not understand what is contributing to or controlling this variation.

I would like to describe some of the work we have been doing with marine toxins. Rather than studying the structural chemistry of these compounds we have been investigating their biological effects towards potential natural predators. I would like to illustrate variability of these natural products by using a seaweed that we have worked quite a bit with. *Caulerpa*, or sea grapes, is another seaweed that is commonly collected and eaten on Guam. So far as we know the natural products that this seaweed produces are not highly toxic to humans. We believe, however, that because grazing levels on coral reefs are very high due to the presence of many different types of herbivorous fishes, *Caulerpa* and other seaweeds produce these toxins to survive.

In our studies we employ assays that actually test the feeding deterrent effects of compounds toward natural predators in the marine environment. In one such assay we take seaweed that fish would normally eat and coat it with seaweed extracts or metabolites that we suspect may serve as feeding deterrents. By placing samples treated in this manner in the ocean and then observing the reaction of herbivorous fish we can actually test whether or not specific compounds function for this purpose. We also conduct similar assays utilizing artificial diets by incorporating extracts or pure compounds into gelatin cubes. Using these methods we have found a great deal of chemical variability and even the presence of different kinds of compounds when we have studied different populations of *Caulerpa*. We can also find very different biological activities in some of these compounds. Some are quite deterrent towards fish while others are not deterrent at all as far as we can tell.

After we first heard about the seaweed poisoning cases we decided to employ these assays in testing *Gracilaria* extracts. We have commonly been making two types of extracts. One is what I'll call an organic extract, which is basically a dichloromethane methanol extraction. This extract contains lipophilic substances that are in the seaweed. The second is an aqueous extract which is usually an ethanol/water extract. This contains compounds that are more polar or water soluble.

In the *Gracilaria* collected at Tanguisson soon after the poisonings, both aqueous and organic extracts were highly deterrent towards fishes in our assays. Fish did not like to eat these extracts although some people apparently did. The extracts were also highly cytotoxic to cells in culture, so there appears to be some correlation between cytotoxicity (which is an *in vitro* assay) and our feeding deterrent tests in the natural environment.

This preliminary work on the cytotoxicity and feeding deterrent activity of extracts from *Gracilaria* collected in the Tanguisson area suggested that there were at least two groups or two types of toxins present, the more organic soluble toxin as well as a more aqueous soluble toxin. In the fall of 1991 Professor Bob Richmond and some of the students in my laboratory were interested in seeing whether the *Gracilaria* collected from sites on Guam other than Tanguisson was also toxic. Bob and Mel Borja collected specimens from Agat and my students hiked down to remote Sella Bay in the Southern end of Guam and also made quite a large collection. All collections proved to be toxic so it is clear that we are not dealing with a problem unique to the Tanguisson site but something apparently common to *Gracilaria* on Guam. However, all of the subsequent collections have not been as toxic as the original collection made around the time of the poisonings last year. In testing the Sella Bay extracts for feeding deterrent activities we also found a slightly different pattern in that the organic extract was very deterrent but the aqueous extract was not. We further fractionated the organic extract by column chromatography and found some particular fractions of rather intermediate polarity that were very active. These may actually correspond well with what we have been calling in our laboratory the "Yasumoto toxin."

Prof. Richmond also performed some manipulation of these *Gracilaria* samples in an attempt to determine what might have caused the apparent high levels of toxicity at the time of the seaweed poisoning incident. He did a variety of different things including heat treating the algae, desiccating it, and letting it sit in fresh water. We were unable to observe that this made any difference as all samples were cytotoxic at relatively low levels but none were as toxic as the original collections last year.

As has been pointed out, we do not know what may be making the seaweed toxic at certain times of the year. After speaking with Dr. Yasumoto today a couple of ideas have been developed that we would like to follow. One concerns the reproductive condition of the algae. The red seaweeds produce tetrasporophytes at certain times of their life cycle and perhaps that could have some influence on toxicity levels. My major area of interest will continue to be the examination of environmental or biological factors that may have contributed to this poisoning incident. Perhaps after today we will have more ideas as to which directions we should pursue in continuing this research.

References

Alstyne, K. L., C. R. Wylie, V. J. Paul & K. Meyer. 1992. Antipredator defenses in tropical Pacific soft corals (Coelenterata: Alcyonacea) I. Sclerites as defenses against generalist carnivorous fishes. Biol. Bull. 182: 231–240.

Duffy, J. E. & V. J. Paul. 1992. Prey nutritional quality and the effectiveness of chemical defenses against tropical reef fishes. Oecologia 90: 333–339.

Meyer, K. D. & V. J. Paul. 1992. Intraplant variation in secondary metabolite concentration in three species of *Caulerpa* (Chlorophyta: Caulerpales) and its effects on herbivorous fishes. Mar. Ecol. Prog. Ser. 82: 249–257.

Paul, V. J. & S. C. Pennings. 1991. Diet-derived chemical defenses in the sea hare *Stylocheilus longicauda* (Quoy et Gaimard 1824). J. Exp. Mar. Biol. Ecol. 151: 227–243.