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## CIGUATERA IN THE NORTHEASTERN CARIBBEAN

An estimated 20,000-30,000 cases of ciguatera are reported annually in Puerto Rico and the U.S. Virgin Islands. Besides a host of gastrointestinal problems, ciguatera causes severe itching, tingling in the extremities, temperature sensory reversal and various other neurological symptoms, which range widely among ciguatera victims, and may persist for months. Worst of all, the symptoms, once cleared up, may recur with alcohol consumption, ingestion of non-toxic fish, and stress. Little is known about the cause of ciguatera, and medical treatment is symptomatic; no antidote is known.

Ciguatera adversely affects the fisheries industry of the Caribbean Basin. A recent Puerto Rican Supreme Court ruling clears restaurants of liability for serving ciguatoxic fish, since there is no way of detecting the presence of the toxin. The general population of Puerto Rico has been alerted by the Department of Natural Resources as to the risks involved in eating certain reef dwelling fishes, frequently reported to be poisonous.

Thomas R. Tosteson, Ph.D., physiologist of the Department of Marine Sciences at the University of Puerto Rico, has received Sea Grant funds to work with and coordinate an international group of scientists who are investigating the origin, chemical nature and detection of toxins responsible for ciguatera fish poisoning. "So far," he explains, "based on relative toxicity and both immunological and pharmacological assays, there appear to be four distinct toxins in the ciguatoxic barracuda extracts."

Tosteson is working toward the development of a desperately-needed dockside test, which would determine the presence of ciguatera in recently caught fish.

**D**r. T.R. Tosteson, physiologist at the Department of Marine Sciences of the University of Puerto Rico-Mayagüez, is the coordinating member of a group of scientists in Puerto Rico and the continental United States who are investigating the origin, chemical nature and detection of toxins responsible for ciguatera fish poisoning in

the northeastern Caribbean. The investigators are also studying the toxicity of marine microbial populations and the physiological activity of their metabolic products. These efforts are supported by grants from the Sea Grant College Program of the University of Puerto Rico, the National Science Foundation, the National

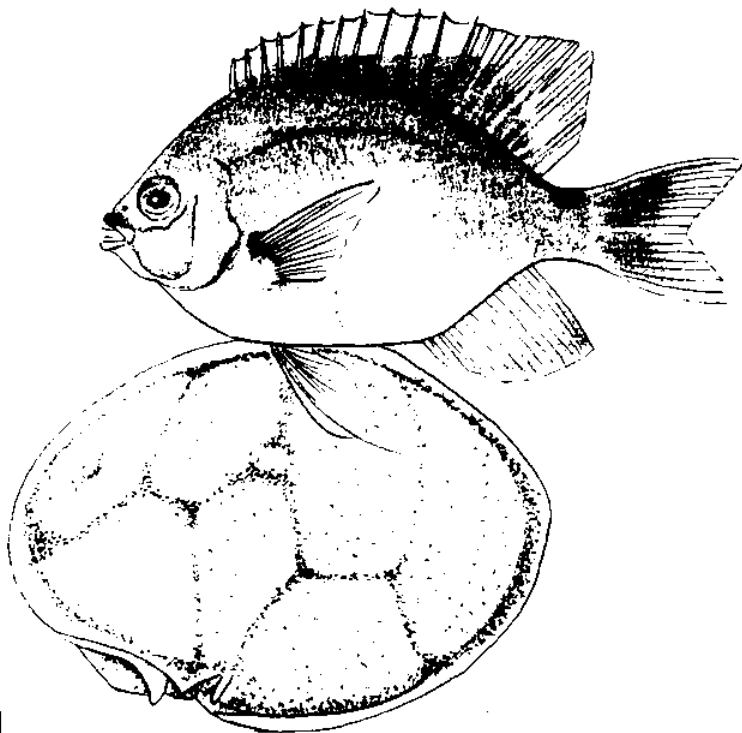
Institutes of Health and the Office of Naval Research.

Ciguatera fish poisoning is a human health problem that affects the people of Puerto Rico and all persons living near tropical seas, for whom marine fish represent a significant source of food.

This type of poisoning has been recognized as a public health problem in the Caribbean. Symptoms of ciguatera include gastrointestinal inflammation, nausea and diarrhea, however, distinctive characteristics of this poisoning are severe itching and tingling of the extremities and temperature sensory reversal. Neurological symptoms may persist for months, and recur with stress, alcohol consumption and ingestion of non-toxic fish. Treatment of ciguatera is symptomatic, with no antidote presently known. In Puerto Rico and the U.S. Virgin Islands there are an estimated 20,000 to 30,000 cases of ciguatera per year. Outbreaks of this poisoning cause significant financial losses to fishing industries in the northern Caribbean-southern Florida area.

Ciguatera traditionally has been limited to tropical regions; however, refrigeration and air transport have made the marketing of tropical reef fish world-wide. Thus, the frequency of this type of fish poisoning in temperate regions of the Earth has increased.

Cooking, smoking, drying, salting or freezing do not destroy the toxin(s) responsible for ciguatera fish poisoning. Whether this poisoning is caused by one or several toxins is not known. The precise origin of toxin(s) responsible for ciguatera fish poisoning is not known. Human activities in marine environments, pollution, dredging, and construc-



*Stegastes planifrons*

A coral reef fish being utilized in the development of a dock-side assay for ciguatera.

*Gambierdiscus toxicus* (x 1,000)

A benthic dinoflagellate implicated as one of the vectors of ciguatera fish poisoning.

tion involving the destruction of coral reefs, have been suggested as causes of the increased occurrence of ciguateric fish. Benthic bacterial and/or algal micro-organisms have also been suggested as possible vectors of ciguatera toxin(s).

Toxins associated with these microbes eaten by herbivorous fish would be passed through the food chain when these animals are consumed by larger carnivorous fish. Chemical and pharmacological similarities among "ciguatoxins" and those toxins isolated from presumed benthic microalgal vectors have not been conclusively demonstrated.

Barracuda is a frequently implicated species in ciguatera fish poisoning in the Caribbean. Ciguatoxicity of barracuda (*Sphyraena barracuda*) was

determined in 219 specimens caught along the southwest coast of Puerto Rico from 1985 to 1987. Twenty nine percent of these fish were toxic. Monthly frequencies of ciguatoxic barracuda showed seasonal variability, with peak values (60-70% of captured fish were poisonous) in the fall (October-November) and late winter-early spring (February-March). Minimal frequencies (0-10% captured fish toxic) were observed during summer and early winter. Seasonal variation in frequency of ciguatoxicity suggests that ciguatera toxins, at least in their active form, are not accumulated in barracuda tissues for extended periods of time.

Ciguatoxic barracuda tissues weighing 180.4 kg were extracted in aqueous methanol (MeOH), followed by back-extraction of residual water with ethyl acetate (EtAc). One thousand eighty (1080)  $\mu\text{g}$  toxic extract/g fish tissue extracted were recovered, for a total of 195 gm. Toxic residues were chromatographically purified using silicic acid (Bio-Sil A) and Sephadex LH-20, and subsequently reverse phase high performance liquid chromatography (HPLC). Results indicated that the barracuda ciguatoxic fractions were significantly more hydrophobic than dinoflagellate toxins. Based on relative toxicity, immunological and pharmacological assays, there appear to be four distinct toxins in the ciguatoxic barracuda extracts. These data and the

observed seasonality in ciguatoxic barracuda suggest that diverse, multiple toxins cause ciguatera fish poisoning.

Variability in barracuda ciguatoxicity may reflect seasonal fluctuations in toxic vectors, benthic dinoflagellates and smaller reef fishes, or changes in the ability of the barracuda to detoxify ingested poisons. Population dynamics and toxicities of the benthic dinoflagellate *Ostreopsis lenticularis* were studied in the coastal area of southwestern Puerto Rico from 1983 to 1986. Population densities were not strongly correlated with temperature or rainfall, however, *Ostreopsis* showed seasonal fluctuations, with peak toxicities in the fall (October) of 1985 and 1986. Thus, while the fall peak in ciguatoxic fish frequency may correspond with peak dinoflagellate (*Ostreopsis*) toxicities, the February-March peak in ciguatoxic fish showed no apparent correlation with benthic dinoflagellate population density or toxicity.

Studies of the toxicity and associated bacterial flora of *O. lenticularis* grown in laboratory culture have indicated the involvement of bacteria in dinoflagellate toxicity. Microscopic examination of the cells in laboratory grown cultures of this dinoflagellate revealed the presence of bacterial exo- and endosymbionts, and viral particles.



The number of bacteria directly associated with dinoflagellate cell surfaces increases dramatically during the static phase of their growth in laboratory culture, a period during which the dinoflagellates are most toxic. Thus, toxic bacteria may also play a role in dinoflagellate toxicity and ciguatera fish poisoning.

HPLC purified ciguatoxins are being compared to toxins extracted from *Ostreopsis lenticularis* in order to establish the relationship between toxic benthic

dinoflagellates and ciguatera fish poisoning. Fish ciguatoxins and toxic dinoflagellate extracts are being used to experimentally poison captured reef fishes maintained in large aquaria. Tests of fish color adaptations to dark and light backgrounds are being examined to assess the effects of both toxins on the chromatic responses of these fishes. The action of these toxins on fish coloration responses may be the basis for the design of a dockside test for ciguatoxic fish.

### Literature:

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