Rotenone: An Essential but Demonized Tool for Assessing Marine Fish Diversity

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Coral reefs, one of the most biologically diverse and important ecosystems on Earth, are experiencing unprecedented and increasing ecological decline, yet the fish faunas of such reefs and other tropical shoreline habitats remain poorly known in many areas. Rotenone, a natural substance traditionally used by subsistence fishers, is a uniquely efficient tool for sampling reef and other shore fishes for marine research. Unfortunately, such sampling is perceived as being highly destructive, and increasing prohibitions against using rotenone in many countries will soon cripple essential research on reef-fish biodiversity worldwide. In this article we dispel common misconceptions about the environmental effects of small-scale rotenone sampling in marine research.

Keywords: coral reef fishes, biodiversity research, rotenone sampling

Rotenone, a natural chemical produced by leguminous plants native to Southeast Asia and South America, has traditionally been used by indigenous subsistence fishers in the fresh and marine waters of those areas (Bearez 1998, Lockett 1998, Ling 2003). Rotenone kills fishes and other organisms by blocking the cellular uptake of oxygen (Singer and Ramsay 1994). Freshwater fishery managers routinely employ it in quantities of up to hundreds of metric tons to eliminate alien species to help conserve native fishes, and to eliminate unsuitable fishes before seeding water bodies with fishes that support recreational fisheries (McClay 2000, Ling 2003).

Rotenone is the active ingredient of organic insecticides commonly used on household pets and gardens, and in agriculture and animal husbandry. Information about rotenone's use as a pesticide, drawn from government reports and a wide range of studies in the peer-reviewed literature, and about the implications of its use for human health and the environment, is available from the following sources: the World Health Organization (WHO; *www.who.int/ipcs/publications/pesticides_hazard_rev_3.pdf*), the Extension Toxicology Network (*http://extoxnet.orst.edu/pips/rotenone.htm*), and the American Fisheries Society (AFS; *www.fisheries.org/units/rotenone/index.htm;* relevant reports from the US Environmental Protection Agency are also available at the AFS site); also see reviews by Lockett (1998) and Ling (2003). In summary, these sources regard rotenone as a relatively safe pesticide for use in agriculture and animal husbandry. There is no clear evidence that it is carcinogenic or teratogenic in rats. The WHO classifies rotenone, along with pyrethrin, another commonly used organic household insecticide, as moderately hazardous: it is categorized as a level 3 on a scale of 1 (most toxic) to 4 (least toxic). When ingested in large amounts, rotenone has low toxicity for birds, but it is moderately toxic to rats. The main human health hazard associated with rotenone usage in fisheries management (and research) arises from inhalation of powder or spray, which can be prevented through the use of respirators. Fishes killed with rotenone retain very small amounts of it, mostly in inedible body parts. There are no reported effects on human consumers of fish collected using rotenone, and both authors have consumed fish collected in this way without noticeable, immediate effects. Moreover, because rotenone is thermally liable, cooking reduces the potential risk to humans who consume fish exposed to the compound. Rigorously designed, large-scale studies for determining any long-term effects on humans produced by consumption of rotenone-exposed fishes are unlikely to occur, as they are expensive and typically restricted to pesticides used in developed countries, where rotenone use is highly regulated and its use by fishers typically is illegal. The AFS maintains that an experimental

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study that found brain damage and Parkinson's disease-like symptoms in rats following chronic intravenous injection of rotenone (Betarbet et al. 2000) has dubious relevance to rotenone usage in fisheries management. The AFS assessment of that study (see *www.fisheries.org/units/rotenone/index.htm;* also see Ling [2003]) noted that (a) rotenone is not absorbed by the mammalian digestive system and is rapidly degraded by the liver; (b) in the study by Betarbet and colleagues (2000), delivery of rotenone to the rat brain required intravenous injection; (c) no Parkinson's-like symptoms or anatomical changes were produced in rats that were fed rotenone over extended periods in previous studies; and (d) there is no evidence of any link between use of rotenone in fisheries management over many decades and Parkinson's disease in humans.

Restrictions on rotenone use in marine research are increasingly being imposed worldwide. Many countries in the global centers of diversity for tropical and subtropical shore fishes either prohibit or strongly restrict researchers from using rotenone to collect reef and shore fishes. A partial list includes the United States (mainland, Virgin Islands, and Hawaii), where rotenone is legally used in large-scale freshwater fisheries management and in the restoration of native freshwater fish populations; Mexico; Belize; Honduras; Colombia; Ecuador; Venezuela; the Cayman Islands; Brazil, where rotenone has historically been used by indigenous subsistence fishers; French Polynesia; Japan; Palau; Australia; New Zealand; India; and South Africa. We believe, as we show here, that such prohibitions or undue restrictions result from managers' lack of information about the necessity for small-scale rotenone sampling, and unawareness of the temporary and trivial nature of the environmental side effects of such sampling.

Rotenone sampling produces essential data on marine biodiversity

Taxonomy is fundamental to understanding biodiversity and evolutionary processes, and it has an essential role in conservation biology (Dubois 2003), facts that managers often do not appreciate but need to take into consideration. All biological research requires accurate identification of species, which depends on museum systematists having sufficient specimens collected over wide areas and through time (Cotterill 1995). Voucher specimens are essential for research on the biodiversity of shore fishes; on their taxonomy (morphological and genetic); and on their ecological, evolutionary, biogeographic, and population responses to climate change. Rotenone sampling has been crucial for the development of all comprehensive modern regional identification guides for tropical shore fishes, which necessarily include cryptic fishes in their coverage. These guides, including that by Randall (2007) and his nine earlier regional identification guides on Indo-Pacific and Atlantic reef fishes (see also Carpenter [2002] and three earlier regional guides to tropical Indo-Pacific fishes, issued by the Food and Agriculture Organization), demonstrate the importance of such sampling for understanding of tropical shore fish biodiversity worldwide. However, large areas of the tropics have not been surveyed, and it is far from clear how many species of tropical shore fishes exist even in areas that are relatively well sampled (see Zapata and Robertson [2006]). Further, comprehensive information from rotenone surveys on the habitat usage, ecology, and geographic distributions of shore fishes is vital for the conservation and management of those fishes—the organisms most vulnerable to local or global extinction due to adverse conditions are the ones with small geographic ranges and special habitat and other ecological requirements.

According to Gerald R. Allen of the Western Australian Museum in Perth (personal communication, 13 June 2007), rotenone sampling proved essential for his recent biodiversity surveys in support of conservation activities at key locations in the Philippines, Indonesia, Papua New Guinea, and the Solomon Islands. Combined with visual surveys, such sampling represented a powerful tool for obtaining a complete "snapshot" of the fauna for any given site he surveyed. He has used rotenone sampling regularly over the past 10 years as part of "rapid biological assessments" in those areas.

These assessments are a key element of conservation strategies employed by organizations such as Conservation International (CI), The Nature Conservancy (TNC), and the World Wildlife Fund. Such sampling played a vital part in surveys conducted by CI and TNC in the Raja Ampat Islands, off the western coast of New Guinea. The combination of visual surveys and small rotenone stations in those islands allowed Allen's team to produce an impressive list of more than 1100 fish species from that area, undeniable evidence that this area is among the richest in the world for tropical reef fishes. As a direct result of this documentation, a network of six marine protected areas is being established in those islands—an outcome that would not have been possible without rotenone sampling.

Rotenone usage in marine research

Historically, three different formulations of rotenone have been used by researchers collecting fishes: (1) weak aqueous preparations, consisting of powdered root (5 to 8 percent strength) mixed to a slurry with water, often with the addition of biodegradable household dish soap to enhance the emulsion of the powder; (2) diluted commercial petroleum-based liquid preparations composed of rotenone resin (up to 50 percent strength) in a mixture of petroleum solvents and emulsifiers that allow the liquid preparation to mix with water; and (3) 97 percent crystalline rotenone dissolved in alcohol or acetone (Gilmore et al. 1981, McClay 2000). Commercial petroleum-based preparations, which are commonly used in freshwater fisheries management (McClay 2000), are expensive, unreliable after prolonged storage, and, because of their flammability, hazardous to transport. Crystalline rotenone is extremely expensive. For these reasons, marine researchers typically use aqueous preparations. When sampling reef and shore fishes, collectors manually spread a small amount of aqueous rotenone slurry (approximately 1 kilogram of powder mixed in several liters of water) across the bottom. In open reef waters. this quantity samples fishes from an area approximately 10 meters in diameter for less than an hour, after which the rotenone has dispersed to the point that it is ineffective against fishes entering the treated area. Such sampling is typically limited to areas with low water flow, because many fish species are resistant to rotenone (some require approximately 20 minutes' exposure to become collectible), and rotenone disperses too rapidly when current flow is moderate to high.

Rotenone sampling is one of a set of complementary—not alternative—research tools. Visual surveys provide accurate information on visible species in the clear waters of coral reefs, but not on species in turbid environments such as estuaries and mangroves, nor on cryptic fishes that live hidden in crevices or burrows in reefs, mangroves, sand, or mud. Rotenone surveys do provide comprehensive data about cryptic and turbid-habitat fishes; they also greatly enhance the collecting of deep-reef fishes. Richard Pyle, of the Bishop Museum in Hawaii (personal communication, 15 June 2007), is one of the handful of scientific divers worldwide who use rebreathers to sample reef fishes at depths between 45 and 150 meters. In his experience, rotenone surveys at those depths triple the rate of discovery of new species of cryptic deep-reef fishes (e.g., see Smith-Vaniz [2005] for a description of one such species and information on rebreather collecting). John E. McCosker, of the California Academy of Sciences in San Francisco (personal communication, 12 June 2007), maintains that rotenone surveys are essential for effective sampling of very deep habitats (> 150 meters) using research submarines, which use a flexible arm to deliver rotenone from an onboard reservoir to a small cave or area of the bottom.

Cryptic shore fishes belong to a broad range of fish families that make up almost half the species in Neotropical shore fish faunas (see Carpenter [2002], Robertson and Allen [2006]), and are of similar importance in shore-fish faunas throughout the tropics. Rotenone sampling reveals cryptic species usually undetected in visual surveys of an area, typically almost doubling the number of species known to occur in an area (Harmelin-Vivien et al. 1985, Dibble 1991, Lockett 1998, Ackerman and Bellwood 2000, Collette et al. 2003, Dennis et al. 2005, Smith-Vaniz et al. 2006), although Willis (2001) found six times as many species using rotenone. Visual surveys not only fail to document the occurrence of many cryptic reef fishes but also grossly underestimate population densities of such fishes. For example, Ackerman and Bellwood (2000) visually censused small areas before sampling with rotenone; they collected 50 to 75 percent more individual fish using rotenone; Willis (2001) and Dibble (1991) collected 4 times and 16 times as many, respectively (see also Brock [1982] and Kulbiki [1990]).

Collecting with anesthetics, such as quinaldine and clove oil, is effective when small quantities are squirted from a plastic squeeze-bottle at individual fishes that can be approached closely. Overall, however, anesthetics are much less effective than rotenone for collecting hidden fishes, which leave their hiding places (and become collectible) when they are exposed to rotenone, but usually fail to do so when anesthetized. Furthermore, rotenone produces more persistent disorientation in fishes than do anesthetics, making the fish easier to collect (Ackerman and Bellwood 2002). Also, most anesthetics require the use of solvents such as alcohol or acetone. Although anesthetics can be used to sample small enclosed areas such as intertidal rock pools (Griffiths 2000), use of anesthetics in the large amounts necessary in such sampling entails the release of solvents in quantities that are likely to adversely affect organisms other than fishes.

Other collecting techniques all have substantial limitations for sampling marine shore fishes: explosives are generally destructive; traps and nets are highly selective in the types of fishes captured, and can be destructive of some habitats; and electrofishing does not work in salt water. Like rotenone, antimycin has been used for collecting fishes; however, this antibiotic is toxic to a wider range of organisms than is rotenone, is substantially more toxic to mammals than rotenone, is highly variable in its action against different species of fishes, and is ineffective at high pH conditions such as occur in mangroves and some other marine habitats (Marking 1992).

From our experiences and those of other colleagues who collect shore fishes, rotenone sampling is by far the most effective method for collecting a broad range of cryptic shore fishes found in turbid water and in deep water during biodiversity surveys.

Rotenone is strongly selective for fishes. Rotenone has low toxicity to birds, and marine birds show no adverse affects after consuming fish affected by rotenone during research collection (John E. McCosker, personal communication, 15 June 2007); in any case, the fish retain very little rotenone in their tissues. Besides fishes, rotenone principally affects small, planktonic crustaceans, and a typical rotenone collecting station yields limited numbers of brittle-starfish, small benthic shrimp, and octopuses, which themselves are useful for research (Bussing 1972, Lockett 1998, Ling 2003; Gerald R. Allen, personal communication, 12 June 2007; John E. Randall, Bishop Museum, Honolulu, personal communication, 13 June 2007).

The few relevant experiments that have been conducted found no deleterious effects on corals exposed to the diluted rotenone used by research collectors. Jaap and Wheaton (1975) found that undiluted (full-strength) petroleum-based liquid rotenone, instead of dispersing properly, formed droplets that collected on and remained on horizontal surfaces of hard corals. This caused "partial mortality" of coral colonies, in which patches of tissue die but the entire colony does not. The coral can later overgrow the area of dead skeleton. There were no such effects of full-strength rotenone on soft corals, which, unlike the hard corals, have a vertical growth form and do not retain rotenone-solution droplets. Those authors found no effects on hard and soft corals from diluted petroleum-based rotenone that dispersed normally. Because researchers collecting fishes dilute rotenone before delivering it underwater, and because it quickly disperses from collecting stations, corals do not experience prolonged contact with concentrated rotenone during research collecting.

In various other field studies of reef fishes that employed typical small rotenone stations, no adverse effects were observed on corals. For example, Bright and colleagues (1974) found no effects after using petroleum-based rotenone under a plastic "tent" that was weighted down around its edges to enclose an area of the bottom, thus limiting the dispersion of rotenone for about an hour before removal to allow collectors to work in the area. Smith (1973) used petroleum-based rotenone to repeatedly sample coral patch reefs up to three times over periods of up to three years, and noted no adverse effects on corals (C. Lavett Smith, American Museum of Natural History, New York, personal communication, 13 June 2007).

Other than fishes, the only macroscopic organisms killed by application of petroleum-based rotenone to a large tide pool on a central Pacific reef were shrimps and octopuses (but not crabs; Bussing 1972). National Park management personnel who made a follow-up assessment of side effects of aqueous-rotenone sampling by Smith-Vaniz and coworkers (2006) in the US Virgin Islands observed no adverse effects on corals (Z. Hillis-Starr, Buck Island Reef National Monument, St. Croix, personal communication, 18 June 2007).

Lack of any observed effects on corals subjected to brief exposure to diluted rotenone in typical collecting stations is consistent with our own observations over many years of collecting activity. We and other researchers who have used both aqueous and petroleum-based rotenone (e.g., Gilmore et al. 1981) have observed less mortality of invertebrates with aqueous preparations, indicating that the petroleum products used in commercial preparations of rotenone are toxic to invertebrates as well as to fishes. Restricting scientific collecting in shallow water to the use of aqueous rotenone would not hinder research activity. Petroleum-based rotenone is most useful for deep-water collecting because the research divers who collect there (only a handful worldwide) have little time to complete their work, and lower temperatures dramatically reduce the effectiveness of aqueous rotenone (Richard L. Pyle, personal communication, 15 June 2007; John E. McCosker, personal communication, 12 June 2007).

Rotenone sampling does not remove all fishes or destroy fish habitats. Sampling from small rotenone stations in open water does not kill all fishes in the area. Fish must remain in the treated area for some time to become collectible, and large, mobile fish that move briefly through a treated area are not affected. Many mobile fishes avoid rotenone and leave the treated area temporarily until the rotenone has dispersed, although small groupers are attracted to collecting sites to prey on disoriented fishes. Visual surveys can provide suitable data for fishes underrepresented in rotenone samples on reefs because of such avoidance reactions. In rotenone surveys aimed primarily at assessing the presence of cryptic fish species, the escape of mobile, visible species actually is desirable. If there is a need to retain mobile fishes (for example, when estimating the abundance composition of a community), barrier nets can be set up around the sampling area before administration of the rotenone (e.g., Ackerman and Bellwood 2000). In addition, fishes do recover from mild rotenone intoxication (Schultz 1948, Bussing 1972, Smith 1973, Ling 2003). In fact, Galzin (1979) successfully used rotenone as an "anesthetic" on coral reef fishes, squirting tiny amounts of highly diluted material from a squeeze-bottle at individual fishes until they were sufficiently disoriented to be captured with an aquarium net, after which they were tagged and released. He observed 95 percent of the tagged fish in the field one month after their initial capture with rotenone.

Finally, in contrast to mechanical collecting methods such as trawl sampling, which can destroy or disturb large areas of sessile benthic communities that provide fish habitat, rotenone sampling by divers does not involve physical destruction of marine habitats.

Rotenone disperses quickly and degrades rapidly. Bubbles produced during scuba diving, combined with the swimming motions of the divers, soon carry water and rotenone away from where it was applied. An hour's activity by a pair of scuba-diving collectors at an open-water collecting site with no current flow is sufficient to disperse the rotenone to the extent that it no longer affects fishes that enter the previously treated area. Water currents greatly speed up the dispersion process.

Information on the degradation of rotenone in aquatic and terrestrial environments is available from the same sources that provide information about rotenone's use as a pesticide (see above). It is widely recognized that rotenone is chemically unstable and degrades rapidly in the environment through abiotic mechanisms (photolysis and hydrolysis). The rate of rotenone's degradation in water is determined mostly by temperature, but also by turbidity and by the levels of light, pH, and oxygen. Degradation is fastest in warm, well-illuminated, well-oxygenated waters, where it can be completed in less than a week. Although previous studies have not specifically examined rotenone degradation on coral reefs, study results do indicate that under conditions like those typically associated with coral reefs (warm, clear, well-lighted, well-oxygenated), complete degradation of rotenone occurs within a few days. The potential for rotenone to bioaccumulate through food chains is low—rotenone has a half-life of about a day in fish.

Shore-fish populations are resilient. Shore-fish populations recover rapidly from short-term natural disasters and from the much more localized effects of rotenone sampling. Large-scale use of rotenone by fisheries managers can produce desired "permanent" changes in the freshwater fish communities of lakes and reservoirs, because unwanted fishes cannot naturally repopulate isolated water bodies. In contrast, local shore-fish communities are strongly interconnected by dispersal of the pelagic larvae that most marine fishes produce.

Communities of reef fishes in small areas naturally fluctuate in species composition and in the abundance of individual species, in large part because of interspecific variation in the tempo and levels of pelagic larval recruitment to shore habitats. The absolute abundance of a species varies in relation to recruitment fluctuations, and the relative abundances of different fishes (both prey and predators) change because recruitment patterns are not the same for all species in all years (e.g., Sale 1988)—in short, shore-fish communities are naturally dynamic entities.

Communities of marine organisms along the world's shorelines do suffer from global-scale stresses, such as global warming, and from strong, long-term stresses, such as those caused by intensive, continuous, large-scale human activities. However, shore environments are highly dynamic, and are repeatedly subjected to natural disturbances (e.g., floods, storms, and hurricanes) that reduce populations of shore fishes over large areas and both destroy and construct fish habitats. Larval dispersal enables populations of such fishes to recover rapidly from the natural depopulation resulting from such relatively brief, occasional events, and it also enables local shore-fish communities to repopulate within months from the much more limited effects of small-scale rotenone sampling. Various studies have documented recovery by shore fishes from defaunation resulting from (a) natural events along the Gulf of California (Thomson and Lehner 1976); (b) atomic tests in French Polynesia (Planes et al. 2005); (c) defaunation of entire tide pools with anesthetics in California (Grossman 1982); and (d) rotenone sampling in the US Pacific Northwest (Polivka and Chotkowski 1998), Caribbean reefs (Smith 1973, Mahon and Mahon 1994), the Gulf of Mexico (Ross and Doherty 1994), the eastern United States (Collette 1986), South Africa (Beckley 1985), Brazil (Rosa et al. 1997), Australia (Lardner et al. 1993), and New Zealand (Willis and Roberts 1996, Roberts and Stewart 2006). In the Gulf of Aqaba, a major accidental spill of chemicals that included pesticides much more potent than rotenone produced the total elimination of coral reef fishes throughout an area several hundred times the size of the area sampled in a typical small rotenone station. Gundermann and Popper (1975) monitored fish populations on coral heads in that area before and after that event and found that recovery occurred within 10 to 12 months of the spill.

Rotenone surveys are few, far between, and very small scale. A typical two-week biodiversity survey by a team of several collectors will make two to three rotenone collections per day, and scatter that sampling across a range of habitats and locations. In contrast, the net of a small coastal shrimp trawler would take approximately two minutes to drag through an area equivalent to that sampled during an entire two-week biodiversity survey, with the trawl catching many organisms other than fishes and disrupting bottom habitats in the process.

Conclusions

Small-scale rotenone sampling is one of the best tools available for discovering vital information about the biodiversity of tropical shore fishes. Such sampling combines strong selectivity and efficiency with minimal, highly transient environmental effects. Shoreline communities of fishes are resilient and have a well-demonstrated capacity to recover from temporary medium-scale stresses, such as those caused by hurricanes, as well as from briefer and much smaller-scale stresses like those produced by small rotenone stations. The effects of small-scale rotenone sampling are vanishingly small at the scale of a reef system, estuary, or country, relative to the destructive effects of natural events or compared with the effects of a broad range of human activities on shore fishes (widespread overfishing, pollution, and habitat disruption).

Because research use of rotenone lacks a social or economic base of political influence, it provides a soft and convenient target for prohibitions. However, to be effective, science and management decisions must made on the basis of the best data available, and the best available methods for obtaining that information on marine biodiversity involve small-scale rotenone sampling. We therefore urge marine resource managers to responsibly and objectively consider requests from marine scientists for the use of rotenone in research programs that have clearly stated scientific and management goals.

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