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Terrestriality in the Cyprinodontid Fish *Rivulus Marmoratus:* Potential Utility in H_2S and Other Bioassays[1]

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Guidelines for the selection of fishes as test organisms in systems monitoring water quality have been published or reviewed by several authors (Rosenberger et al. 1978; U.S. EPA 1975; Buikema et al. 1982). Criteria vary with the specific purpose of each system. However, in all cases it is of practical importance that the species selected be readily available as well as easy and inexpensive to culture. For applications in biological early warning systems and general toxicity testing, it is desirable that the species be refractory to nonharmful variation in water quality, e.g., salinity fluctuations (Cairns and van der Schalie 1980). Other factors being equal, the utility of a species for biological testing increases with the width of its tolerance range to innocuous variation. Currently, several members of the family Cyprinodontidae are employed in biological monitoring, most notably Jordanella floridae (Smith 1973), Cyprinodon variegatus (Schimmel et al. 1974), and Fundulus heteroclitus (LaRoche 1970). The objective of this paper is to propose an addition to that list, the euryhaline cyprinodontid Rivulus marmoratus, whose suitability as a bioassay fish compares favorably to and in some areas surpasses that of other species currently used in that capacity.

Rivulus marmoratus, which attains a maximum length of 50 mm and weight of 1.5 g, inhabits mangrove forests, swamps, and lagoons of Florida and several Caribbean islands (Rosen 1973). The species consists of synchronous, self-fertilizing hermaphrodites, which has resulted in homozygous individuals comprising isogenic lines (clones), three of which thus far have been described (Harrington 1963; Kallman and Harrington 1964; Harrington and Kallman 1968). The species is amphibious, emergence occurring as a nonspecific response to environmental stress (Abel 1981). The unique genetic composition of the species, the ability of R. marmoratus to leave the water and respire in air, plus other attributes suggest that R. marmoratus may be useful as a bioassay organism.

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Hydrogen sulfide was selected as a reference toxicant for this study because H_2S is an extremely potent metabolic poison found in industrial effluent into aquatic environments (Ellis 1937; Van Horn *et al.* 1949; Dorris *et al.* 1960) and is also a product of sewage decomposition. Additionally, a concurrent project was examining the role of H_2S produced from natural organic decomposition in eliciting terrestriality in the habitat of *Rivulus marmoratus*.

MATERIALS AND METHODS

Stocks of *Rivulus marmoratus* that have been maintained in laboratory culture for at least 20 generations were used in all studies. Because ongoing experiments required the use of most of the fish on hand, experiments for this project were conducted using a mixture of individuals from all three clones. Fish were maintained on a 14 hr photoperiod in synthetic seawater adjusted to 14 ± 1 parts per thousand (ppt) at a constant temperature of 25 ± 1°C (Koenig and McLean 1980). The assay was conducted in 400-ml glass beakers modified for flow-through capacity. An oyster shell attached inside each beaker slightly above the level of the overflow tube served as a platform onto which the fish could jump and lie. Five beakers, each connected to a different head tank, were used in each run. To three tanks selected by random number table, different concentrations of H_2S solution were added. Two other randomly chosen tanks served as controls, one normoxic, the other hypoxic. Except for the control containing air-equilibrated water, O_2 concentrations of 2.0 ± 0.05 parts per million were established in all tanks. The experiments were run under hypoxia to duplicate conditions frequently associated with the presence of H_2S. In all tanks pH was adjusted to 7.5 ± 0.05.

Five fish from 0.3 - 0.5 g, starved for three days, were randomly selected and each was added to a different beaker containing 25 ml normoxic water. The experimental solutions were then introduced at a rate of 2.0 ± 0.05 $l \cdot hr^{-1}$, which was maintained throughout each run. After the beakers filled (300 ml), the fish were allowed 1 hr of acclimation followed by 1 hr of observation. Because preliminary experiments suggested that the ability of a fish to flop onto the platform required learning, a positive response to the test solution was chosen to be not only (1) successfully leaping onto the platform, but also (2) jumping completely from the water regardless of whether the fish landed on the platform. For those fish satisfying criterion (1), total time emergent was recorded. Eleven runs utilizing 55 different fish (the results from two of which were discarded for various reasons) were performed. A single observer scored all runs from a distance of several feet without knowledge of the relative or absolute H_2S concentrations of the solutions.

H_2S and O_2 concentrations were measured in water samples taken from the beakers at the beginning of acclimation, at least once during an experiment, and at the conclusion. Additionally, blanks (beakers without fish) were run and sampled. O_2 concentrations did not vary significantly over the time span. However, H_2S, which oxidizes rapidly, decreased in concentration by 50% or more in some chambers over the course of an experiment. Because of this, H_2S concentrations measured at the beginning of acclimation were used in all calculations. Nominal solutions of H_2S were prepared by adding calculated amounts of sodium sulfide to deoxygenated water. H_2S was measured by the spectrophotometric method (American Public Health Association 1976). Water was deoxygenated by bubbling nitrogen through it while monitoring O_2 concentration with a YSI model 51-A O_2 meter, calibrated initially by the azide modification of the Winkler method (American Public Health Association 1976), and in air prior to each use. Because H_2S affects the function of the O_2 probe, O_2 determinations in samples from all test chambers were performed by titration.

Probit analysis (Finney 1971) was used to analyze the quantal response of jumping. Data were grouped into intervals of 100 parts per billion (ppb) H_2S and the midpoints used in the calculations. Spearman's Coefficient of Rank Correlation (Steel and Torrie 1980) was used to evaluate the relationship between H_2S concentration and duration emergent.

RESULTS AND DISCUSSION

Figure 1 depicts the results of this study. The concentration of H_2S that elicited a positive response in 50% of the fish (median effective concentration, EC50) was 123.6 ppb (3.633 μM) [95% confidence limits: 63.7 - 182.0 ppb (1.873 - 5.351 μM)]. Sixteen of the 20 fish that responded positively jumped onto the platform. No positive responses were recorded from either the normoxic or hypoxic control. There was a significant positive correlation between duration of emergence and H_2S concentration ($r = 0.52$, $p < 0.05$, Table 1). The high variability at the higher concentrations in Table 1 and the low correlation coefficient are apparently results of toxic effects of H_2S. Individuals in the more dilute solutions of H_2S frequently ventilated at the surface for the duration of a run. Fish in hypoxic controls exhibited the same behavior, although the duration was more variable. Fish lying on the platform generally remained motionless and were refractory to gentle prodding.

Thus, this experiment demonstrates the ability of *Rivulus marmoratus* to detect and avoid water contaminated with H_2S. It is true that sublethal responses reported for freshwater fishes are more sensitive [e.g., *Lepomis macrochirus* exhibited decreased swimming endurance at 1.5 ppb H_2S (Oseid and Smith 1972)]. However, because there is a dearth of euryhaline species suitable for such assays, considered with other advantages of *R. marmoratus*, more comprehensive testing of the leaping response of this species is warranted, specifically relating to H_2S pollution, and in biological monitoring systems in general. In addition, quantification of other reactions, such as surface alignment or activity patterns, could result in a more sensitive response to H_2S.

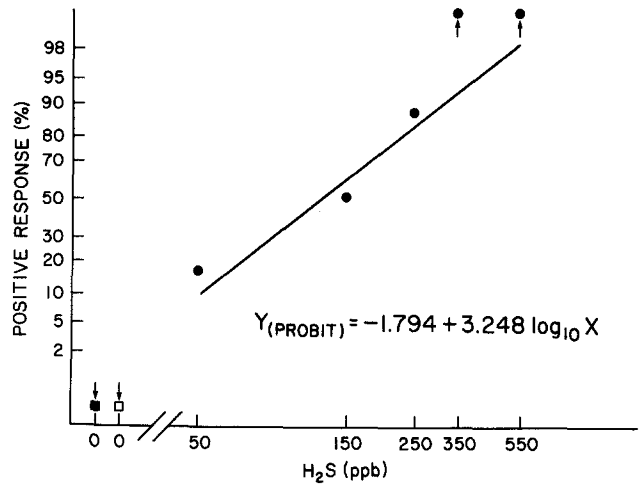

Figure 1: Log-probit plot of % positive response vs H_2S concentration. Solid dots represent fish exposed to H_2S solutions. Solid block denotes normoxic controls. Open block represents hypoxic controls. Downward and upward arrows are directed at points representing 0 and 100% response, respectively. H_2S concentrations are midpoints of 100 ppb intervals.

Table 1: Duration emergent for each of the 20 fish responding positively. * represents response judged positive by criterion (2) (see text).

H_2S Concentration (ppb)	Total Time Emergent (min)	H_2S Concentration	Total Time Emergent
25	0*	233	51
108	15	252	60
147	14	270	39
172	17	304	31
172	0*	339	27
195	0*	399	31
200	47	525	54
209	0*	564	28
211	38	565	15
232	45	579	52

Koenig and McLean (1980) discussed the manifold advantages of using the *Rivulus marmoratus* bioassay system relative to chronic marine bioassays. Here, the characteristics of the system are presented as they pertain to biological warning systems, using criteria given in Cairns and van der Schalie (1980).

1. The behavior used in this bioassay is easily quantified through electronic devices, such as the one described by Cripe *et al*. (1975).
2. Avoidance reactions such as leaping remove the fish from physical contact with the toxicant, thereby reducing stress.
3. Although rapidity of response was not measured here, I have observed it to be very fast (seconds) to such contaminants as H_2S, ammonia, formalin, and rotenone, none in high concentrations.
4. Although the response of *Rivulus marmoratus* to nonharmful variation is not well-known, I have maintained this species at a variety of temperatures in salinities from 0 (distilled water) to over 50 ppt without observing avoidance behavior or ill effects. Thus, *R. marmoratus* is suitable for fresh water and marine bioassays. Aquatic hypoxia alone does not induce jumping, which is consistent with the interpretation of the functional morphology of the head (dorsoventral flattening, upturned mouth), which facilitates respiratory use of the surface microlayer (Lewis 1970; Kramer 1983).
5. An important characteristic of the system (although not taken advantage of here) is genetic uniformity. Genetic variability should therefore not enter as a source of experimental error, although behavioral responses generally show a high degree of variability.
6. Because *Rivulus marmoratus* inhabits still waters, it adapts well and quickly to living in culture dishes in the laboratory. Therefore, large numbers of fish can be maintained in small areas at low cost. In addition, fish older than four months produce a constant supply of eggs throughout the year.

Clearly, *Rivulus marmoratus* possesses attractive qualities for use in a wide variety of applications in monitoring water quality. There are also, of course, potential disadvantages in the use of any single test animal. For example, homozygosity and isogenicity may be disadvantageous to experiments purporting to extrapolate to responses of heterozygous populations. Also, *R. marmoratus* is quite aggressive, requiring that adults be held singly or be given refuges when held communally. Still, although more comprehensive testing is required and appropriate interfacing techniques applied to complete the system, the use of *R. marmoratus*, including the novel application here, has potential promise in water quality management.

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