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Scale Morphologic Response to Aromatic Hydrocarbons

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Numerous fish kills have been reported by commercial news media. Some kills have been attributed to water quality and others to contamination. Fish kills appear to be recurring events (KUSHLAN, 1974). Recently in Louisiana, there has been concern about the primary contributing agent to fish kills. Methods are available to sample fish populations and analyze for contaminants (BRUNN, 1982; ZAZBIK et al., 1982). These methods require a sacrifice of animals as well as expensive and sophisticated equipment. A simple and less expensive laboratory method (DRUMMOND et al., 1974; DRUMMOND et al., 1973) utilized the cough response in the brook trout (Salvelinus fontinalis) as an indicator for mercuric compounds. This procedure may not be a comfortable one for the untrained person. The purpose of this study was to design a simple and inexpensive procedure utilizing scales without sacrificing the fish in an effort to assess the environment.

METHODS AND MATERIALS

Mosquito fish (Gambusia affinis) for this study were collected from a private pond in Grambling, Louisiana. The pond has never been exposed to any type of known contaminant. The fish were transported to Wright-Patterson AFB, OH and maintained under ambient laboratory conditions before they were used in experimental tests. Twenty fish were used for each of the control and the experimental groups. Ten fish were placed in a 2-liter glass container. A water-soluble fraction of shale-derived JP-4 fuel was prepared and used to make the test water. The experimental fish were exposed to 3.45 mg/liter hydrocarbons whose principal components were benzene, toluene, xylene, and naphthalene. After a 48 h exposure scales were removed from 10 fish and placed in Karnovsky's fixative (1965). A few scales were unfixed and mounted in tap water for immediate examination by light microscopy. Scales fixed in Karnovsky's solution were used for light microscopy and scanning electron microscopy (SEM). Scales for SEM were washed with sodium cacodylate (pH 7.6) and post-fixed in 2% osmium tetroxide. Post-fixed scales were dehydrated in a graded series of acetone solutions, critical-point dried, coated lightly with gold, and examined by SEM.

RESULTS AND DISCUSSION

Mosquito fish exposed to a sublethal concentration did not display any behavior responses different from those of the control. The experimental fish did appear to be slightly darker than controls. Results of examination of scales by light microscopy are recorded in Table 1. Melanophores observed fell into the following categories: 1) contracted (Fig. 1), 2) reticulate, and 3) stellate-reticulate (Fig. 2).

TABLE 1. Melanophore Response to Hydrocarbons

Total Hydrocarbon conc. (mg/L)	Melanophores Present in Anal fin (N=20)	Average Melanophores per scale (N=100)		
		Contracted	Reticulate	Stellate-Reticulate
0.0 (control)	0	6.84	2.37	1.43
3.45	20	1.16	0.64	12.29
	0.001*	0.001*	0.001*	0.001*

* Level of significance

The following ranges were noted for control: 1) contracted, 0-38; 2) reticulate, 0-13; and 3) stellate-reticulate, 0-20. In control scales, 16% were without contracted melanophores, 30% of which represented scales without any melanophores. The reticulate melanophores were not observed in 38% of the scales, 12% were without any melanophores. The stellate-reticulate melanophores were absent in 57% of the scales examined, and 10% were without any melanophores. The experimental fish had the following ranges: 1) contracted, 0-10; 2) reticulate, 0-5; and 3) stellate-reticulate, 0-41. In experimental scales 86% were without contracted melanophores and 5% of these had no melanophores. The reticulate melanophores were not observed in 55%, 9% of these had no melanophores. The stellate-reticulate melanophores were absent in 9%, with 50% of these with no melanophores.

Lipophores were observed in fresh wet-mounts. The lipophores in the treated fish were observed 3 times more frequently than in the controls. Prolonged periods in Karnovsky's fixative led to bleaching of this light yellow pigment.

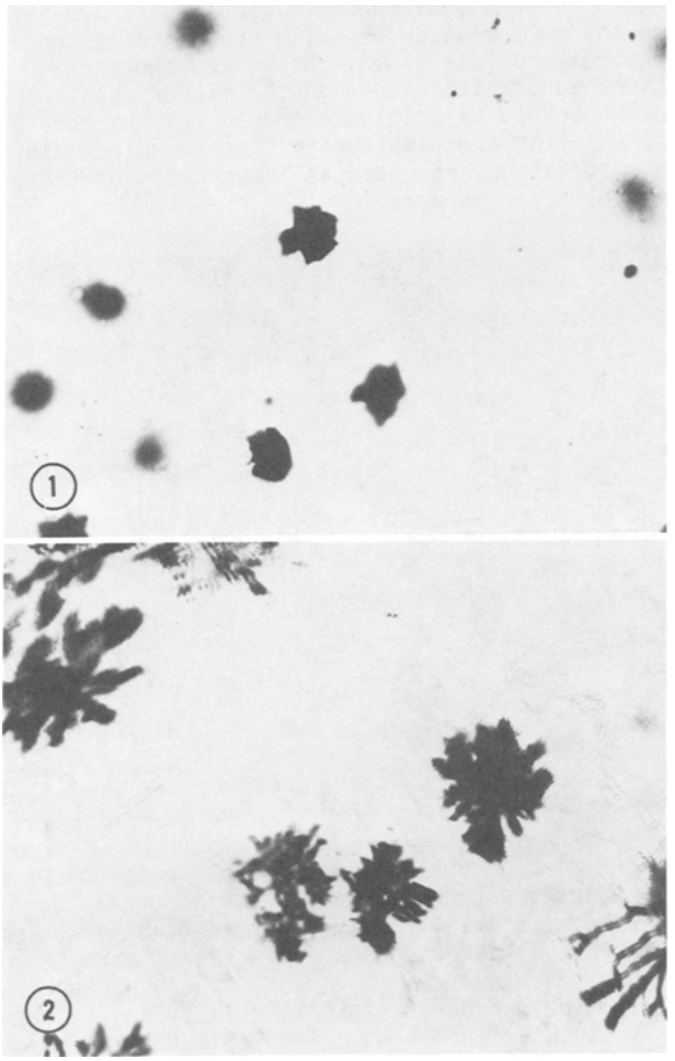

Figure 1. A light micrograph showing the contracted melanophore as the dominant type. Scale from control fish. X 120

Figure 2. A light micrograph showing the stellate-reticulate melanophore as the dominant type. Scale from treated fish. X 120

SEM Analysis

Control. The surface epidermis (Fig. 3) of scale pocket of control fish was characterized by extensive concentric arranged microridges. Long, uninterrupted microridges, usually in pairs, delineated the cell boundaries while shorter ones covered the rest of the cell surface. Small blebs and amorphous accumulations were associated with the microridges. These microridges appeared to originate from a central area of the cell which was flat and displayed a fine granular material. The amorphous material was extruded at the corners of these epithelial cells in one area.

Figure 3. SEM micrograph of control fish. Note concentrically arranged mircroridges (arrows), the blebs on microridges (arrow heads), amorphous material (A) and flat, central areas of the cell (C) from which microridges arise. Bar = 10 μ. X 2,000

Experimental. The surface epithelial cells (Fig. 4) ranged from defined units to undefined ones. The defined units have demonstrable boundaries. The regular surface microridges paralleled the boundary at the cell where some were in concentric whorls, others circular and obtuse. The microridges were found over the entire cell surface; there was an absence of a flat central microridge-free area on the cell. There was a corresponding absence of both fine granular material and a point of origin for the microridges. The point from which the normal extrusion of amorphous material occurred formed a shallow cavity

surrounded by fusion of microridges. The undefined units possessed the same features just described, but in addition the microridges were shorter and not arranged in a circular or an obtuse pattern. Instead, they were less regularly arranged than ones observed in the control.

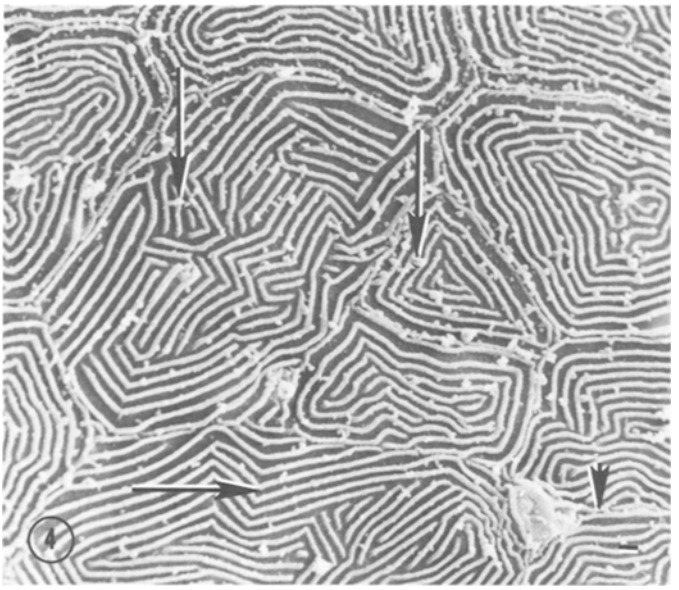

Figure 4. SEM micrograph of experimental fish. Note the difference in the orientation of the microridges (arrows), absence of blebs, flat central area of the cell, and the fusion of microridges to form a pocket (arrow head). Bar - 1.0 μ. X 2,000

The response of melanophores to 3.45 mg/liter hydrocarbons is morphologic and appears to be a reliable indicator of contamination. Observations by light microscopy alone would allow one to accurately assess an aquatic environment based on scale morphology. An earlier report (HENDERSON et al., 1981) described reductions in eye and body pigment in the fathead minnow (Pimephales promelas). Other studies revealed that hydrazine (HENDERSON et al., 1983) and fuel hydrocarbons (HENDERSON, Unpublished data) induce similar morphological changes. The changes observed in the present study manifested themselves in an arborization and an increase in total number of melanophores leading to preponderance of stellate-reticulate melanophores. The increase in pigment was a function of the type and number of melanophores present. The treated fish were

darker because they had expanded melanophores as well as a greater number of this cell type. The converse was true of control. The amount of pigment in the sea lamprey (MANION, 1972) has been correlated with the dominant melanophore type and the number of cells; this appears to be also true in the mosquito fish. The explanation for the melanophore response seems to reside in the stimulation of the autonomic nervous system. Hydrocarbons of shale-derived JP-4 cause damage to the myelin sheath of nerves (HENDERSON, unpublished) and therefore render ineffective impulse transmission via melanin-aggregating nerve fibers. This condition has all of the features of the Parker effect (PARKER, 1942). This effect leads to an increase in pigmentation due to a release of a melanin-dispersing transmitter. The color change described in this report is morphologic since the time required for it is greater than 10-20 minutes (FUJI and NOVALES, 1965).

SEM analysis can be utilized in addition to light microscopy. Surface cell morphology provides another parameter for assessing damage to cells. Since treated cells show less amorphous material, one could assume that these cells are no longer capable of producing adequate quantities of this glycoprotein which was cytochemically determined in another study (HENDERSON unpublished).

The work described in this study is useful as a quick assessment procedure for determining the toxic effects of hydrocarbons on the mosquito fish. Scales can be removed and examined in a wet-mount preparation immediately or prepared for SEM analysis and either procedure can drastically reduce the evaluation time required to assess pollutants.

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REFERENCES

BRUNN, H. and D. MANZ, Bull. Environm. Contam. & Toxicol. 28, 599 (1982).

DRUMMOND, R., W. SPOOR, and G. OLSON, J. Fish. Res. Bd. of Can. 29, 1035 (1973)

DRUMMOND, R., G. OLSON, and A. BATTERMAN, Trans. Amer. Fish. Soc. 103, 244 (1974).

FUJI, R. and R. NOVALES, Amer. Zoo. 9, 453 (1969).
HENDERSON, V., J. FISHER, R. D'ALLESANDRIS, Environm. Contam. & Toxicol. 26, 807 (1981).
HENDERSON, V., J. FISHER, R. D'ALLESSANDRIS, J. LIVINGSTON, Trans. Amer. Fish. Soc. 112, 100 (1983).
KARNOVSKY, M. J., J. Cell Biol. 27, 137a (1965).
KUSHLAN, J. A., Trans. Amer. Fish. Soc. 103, 235 (1974).
MANION, P. J., Trans. Amer. Fish. Soc. 101, 662 (1972).
PARKER, G., Proc. Natl. Acad. Sci. US. 28, 164 (1942).
ZABIK, M. E., C. MERRILL, and M. ZABIK, Bull. Environm. Contam. & Toxicol. 28, 592 (1982).

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