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Abstract

Cadmium is a heavy metal ion that can cause deleterious effects on aquatic animals. This study uses both electrophysiological recordings from lateral line nerves and videotaping of schooling behavior to investigate the effects of cadmium exposure on fish. The fathead minnows were exposed to cadmium at a concentration of 450 μg/l over a 24-hr period. Extracellular recording with a silver hook electrode was used to record compound action potentials from the lateral lines of control and experimental fish. After a short time exposure (24 hr) to cadmium ions, all of the electrophysiological activities of the lateral line nerves were suppressed. However, after a 10-day recovery in clean water, the function of the lateral line nerves was regained. Schooling behavior observed under lighted conditions showed no significant difference (in terms of percentage of time forming a school) between control and experimental fish. Schooling behavior, however, was completely lost for experimental fish when observed under complete darkness but it was regained after a 10-day recovery in clean water. The current study shows that both electrophysiological recording from the lateral line nerve and observation of schooling behavior can be used as effective assay methods for cadmium toxicity studies.

Focus Categories: MET, TS, WQL

Keywords: Biomonitoring, Heavy Metals, Lateral Line, Schooling Behavior, Mining, Pollutants, Toxic Substances

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The fish used in this study were provided by Frankfort State Hatchery. Dr. Li Guan of University of Maryland College Park provided technical assistance in electrophysiological recording.

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Chapter I - Introduction

Objectives

Water is one of the most valuable resources for the survival of all living creatures. Man's dependence on water resources has prompted considerable research on pollution levels hazardous to aquatic life. Fish or aquatic invertebrates have been utilized as model animals for water pollution study. Several research procedures have been established to assess the effects of water pollution on fish physiology. These procedures include respiratory and cardiovascular responses, hematology, uptake and excretion of xenobiotics, osmotic regulation, physiological energetics, cellular enzyme activity, behavior and nervous system function, reproduction and growth (see reviews in Adams, 1990; Heath, 1987). Among all these assessment methods, nervous system function change is the most sensitive assay but traditionally has received the least attention. Few aquatic biologists have training in neuroscience and subsequently view nervous system function change assessment procedures as difficult or impossible. As a result, little attention has been paid to neurophysiological changes, one of the most sensitive biological assays, for water quality assessment. The purpose of this study is to use electrophysiological recording and neurobehavioral procedures from lateral line nerves of fish to develop an easy and accurate assay method for biological assessment of water quality.

It has been well documented (see Kelly 1988 for general review) that mining activities could have severe impacts on water quality in watershed areas surrounding mines. The results of mining can include acid drainage water, sediment, soil erosion and heavy metals pollution. These types of large scale deleterious impacts have been reported in Kentucky where strip mining is widely used (Branson and Batch, 1972; Dyer, 1983). Traditional water quality monitoring programs in mining areas have merit for gauging long term changes in water quality caused by strip mining. However, physical and chemical evaluations of water quality are not sufficient to understand the deleterious impacts on biological systems. Therefore, an efficient and sensitive biological assessment procedure is needed for Kentucky mining areas. Heavy metals pollution is one of the major concerns from mining operations and among heavy metals, cadmium (Cd) requires special attention due to bioaccumulation in food chains (Nriagu and Sprague, 1987). Because of its constant contact with water, the lateral line system (a vibration detection system) of fish is one of the first organ systems to be affected by cadmium pollution. Changes in neurophysiological and neurobehavioral activities related to the lateral line system are simple and sensitive methods for monitoring cadmium pollution, even at sublethal concentrations. The laboratory study described here establishes baseline data to relate various cadmium concentrations to functional changes of the lateral line.

The lateral line is a low frequency vibration detection system and is mainly used as a non-visual predator-prey detection system in fish. An intact non-visual predator-prey detection system is crucial for the survival of any fish species, in particular for piscivore species, such as striped bass. Exposure to cadmium, a known heavy metal contaminant in the freshwater and brackishwater of the United States, even at sublethal concentrations might have deleterious effects on the lateral line systems of many fish species including striped bass. The effect of chronic exposure to sublethal concentrations of cadmium on lateral line system of fathead minnows were assessed. Two major objectives of this study were: 1) To evaluate how cadmium would affect the electrophysiological response properties of sensory hair cells of the lateral line system of the fathead minnow; and 2) To investigate changes of schooling behavior under both light and dark conditions after exposure to cadmium. Multi-unit electrophysiological recordings from the lateral line were taken before and after cadmium exposure. The study attempts to develop an integrated neurophysiological and neurobehavioral assay method to assess the effect of cadmium on the non-visual predatory-prey detection system of fathead minnow. Such an integrated assay can provide a very sensitive and effective methodology of evaluating heavy metal toxicity on lateral line systems of fish at sublethal concentration levels.

Background

Fish use a variety of sensory modalities to learn about their environment (Atema et al., 1989). Damage to one or more of these modalities, such as by toxic materials in the water, can severely impair the ability of a fish to survive. There is considerable evidence that aquatic contaminants affect behavior in fish species. Since the behavior of an animal represents an integrated response of all of the cellular processes, behavior may be the most sensitive measure of animal health and the effects of a toxicant. Therefore, observations of behavior provide a unique toxicological perspective that links the physiological, biochemical and ecological consequences of environmental contamination (Marcucella and Abramson, 1978; Little and Finger, 1990). In fish, behavioral changes in foraging (Cairns and Loos, 1967), predator-prey interaction (Hatfield and Anderson, 1972), ventilatory frequency (Cairns and Garton, 1982), swimming (Howard, 1975) and avoidance reaction (Hartwell et al., 1987) have been used as indicators for assessing effects of sublethal toxicity from contaminants. However, the underlying neurophysiological mechanisms leading to these behavioral changes have never been addressed.

The lateral line system of fish is a major non-visual predator defense and prey searching system for the detection of moving objects (Kalmijn, 1989; Platt et al., 1989). It is known that fish use their lateral lines to detect low frequency vibration (up to 200 Hz), such as beating tails of other swimming fish or movement of aquatic invertebrates (Sand, 1984). Except during daylight in very low turbidity aquatic environments, the lateral line system comprises the major system for providing fish with information about their environment.

The fathead minnow (*Pimephales promelas*) is a cyprinid fish and has very wide geographical distribution ranging from central Canada to Gulf of Mexico coastal areas. The fathead minnow is considered as one of the major prey species to many predators such as bass, frogs, reptiles (water snakes) and birds. Therefore, an effective and intact non-visual predator-prey detection system in fathead minnow is crucial to the survival of the species in its natural habitats.

It is known that the uptake of cadmium in aquatic animals is through cellular or organelle of epithelial tissue (Coombs, 1979). The lateral line receptors in most fish lie in canals on the head, and in a canal extending along the body (Webb, 1989). As a result, lateral line receptors have direct contact with water containing contaminants. Previous in vitro studies on the lateral line system of catfish show extensive damage by cadmium at a very low sublethal concentration (Zwart and Herwing, 1989). Furthermore, Blaxter and Fuiman (1989) have shown that physical and chemical ablation of the lateral line system in herring (Clupea harengus) result in loss of ability to evade predatory fishes. Damage of the lateral line cripples the non-visual, predator-prey detection system, and subsequently leads to the fish being either preyed upon or unable to catch prey. Such destruction of the defense and foraging capabilities of fathead minnows could potentially result in more damage to the fathead minnow population than gradual accumulation of cadmium in internal organs. However, it is not known how chronic toxicity of cadmium in sublethal concentration would affect the lateral line system of fathead minnows.

The purpose of this study was to develop an integrated and sensitive neurophysiological and neurobehavioral assay method to assess the effect of a sublethal level of cadmium on the lateral line system of the fathead minnow. By studying sensory behavior under controlled conditions we were able to begin to determine those aspects of behavior that were affected by the toxicant. The neurophysiological and neurobehavioral aspects of the work provide a direct evaluation of the effects of cadmium on the lateral line system and help evaluate the role of changes in such a system on overall behavior of fathead minnows. It should be emphasized that although only cadmium and fathead minnows were addressed in this study, the protocol developed can be easily applied to other aquatic species and water pollutants of concern in Kentucky. Most importantly, the integrated novel monitoring system developed in this study allows us to address many water pollution related questions from a perspective that has never been considered before.

Chapter II - Research Procedures

(I) Experimental animals.

The fathead minnows (*Pimephales promelas)* used in this study were obtained from the Frankfort Hatchery. The fish were housed in glass-wall aquaria (from 40-l to 600-l) with constant aeration. Water temperature was maintained around 25 °C. Partial water changes of the aquaria were conducted every week. Fish were fed with Tetramin flake food on daily basis at about 5% of body weight of fish.

(II) Cadmium exposure.

A stock solution of cadmium chloride was prepared by dissolving $CdCl_2$ into distilled water at a concentration of 4500 μg/cc. Tested fish were then transferred into a 10-l holding tank and 1 ml of stock solution was added to achieve a final concentration of 450 μg/l concentration. The exposure time was 24 hours. During the cadmium exposure, vigorous aeration was provided to the tank water but a filter system was not used to prevent the potential removal of cadmium ions by the filter system.

(III) Electrophysiological recording of lateral line activity.

A silver hook electrode (**Ø** 1.25 mm) was used to record electrophysiological activities of the posterior lateral line nerve (PLLN). Stimuli were given through a B&K 4810 minishaker positioned above the caudal peduncle region of the experimental animals, fathead minnows (see Figure 1 for details of setup). Frequency testing ranged from 10 Hz to 200 Hz. Spikes of compound action potentials generated from the stimuli given were used to measure the effect of

Cd exposure on the lateral line. At least ten fish from each treatment were used in the evaluations.

(IV) Schooling behavior change.

Schooling behavior of control and experimental fish were recorded under light and dark (with Infrared light and IR-camera) conditions to evaluate how intact and damaged (through exposure to Cd) lateral lines play a role in schooling formation especially under dark condition. The recording tank was a black-colored plastic tank of 1.2m diameter with a water depth of 30 cm. Four largemouth bass (as predators) were housed with 30 fathead minnows (either control or experimental animals) under light or dark conditions and videotaped for 2 hours (see Figure 5 for details). For data analysis, the tapes were viewed in a VCR and for every 15 min. interval, a 60-sec. period was used to count how many seconds fathead minnows stayed together within three body lengths of each other. The time (in seconds) that the fish stayed as a school was then converted into a percentage. For each 120-min tape, seven sets of percentage data (at 15, 30, 45, 60, 75. 90, 115-min.) were obtained. These data were averaged and presented as percentage that the fish exhibited schooling behavior.

Chapter III-Data and Results

I. Electrophysiological Recordings

Preliminary data showed that at 80 Hz, the lateral line nerves of fathead minnow had the lowest threshold to vibrational stimuli. Therefore the most sensitive frequency (80 Hz) was used to assess the effect of cadmium exposure on fathead minnow. For the control fish, before and after the onset of stimulus, the firing rate of spontaneous spikes was 431/ sec (threshold value set at 0.16 mV) (Figure 2, upper panel). With the onset of stimuli (as indicated in the lower panel of Figure 1 with sinusoidal wave), multiple high amplitude spikes were generated at a rate of 1020/sec (Figure 2).

Table 1. Mean (number/sec) compound action potential firing rate of lateral line nerves.

	Stimulus Off	Stimulus On
Control Fish	431	1020
Cd-exposed Fish	0	0
10-Day Recovery Fish	59	107

For experimental fish, (24hr exposure to cadmium at a concentration of 450 μg/l), the lateral line nerves showed significant changes in the spike rate. First, the amplitudes of the responses were reduced to only about 1/10 of those control fish (notice the scale in Figure 3 is

After 10 days of recovery in clean water, there was no significant difference between the schooling behavior under lighted conditions of the control fish and the experimental fish (Figure 6). When observed under complete darkness, it was obvious that after recovery the schooling behavior of the experimental fish regained as compared with 24 hr cadmium-exposed fish (Figure 6).

Table 2. Mean percentage of time (over a 2 hr period) of schooling formation.

	Light Condition	Dark Condition
Control Fish	88.9	39.4
Cd-exposed Fish	82.5	1.2
10-Day Recovery Fish	95.5	57.2

Figure 1. Experimental set-up for lateral line nerve activity recording. P15: Grass preamplifier. Minishaker (B&K 4810) is used to generate vibration signals to tested fish.

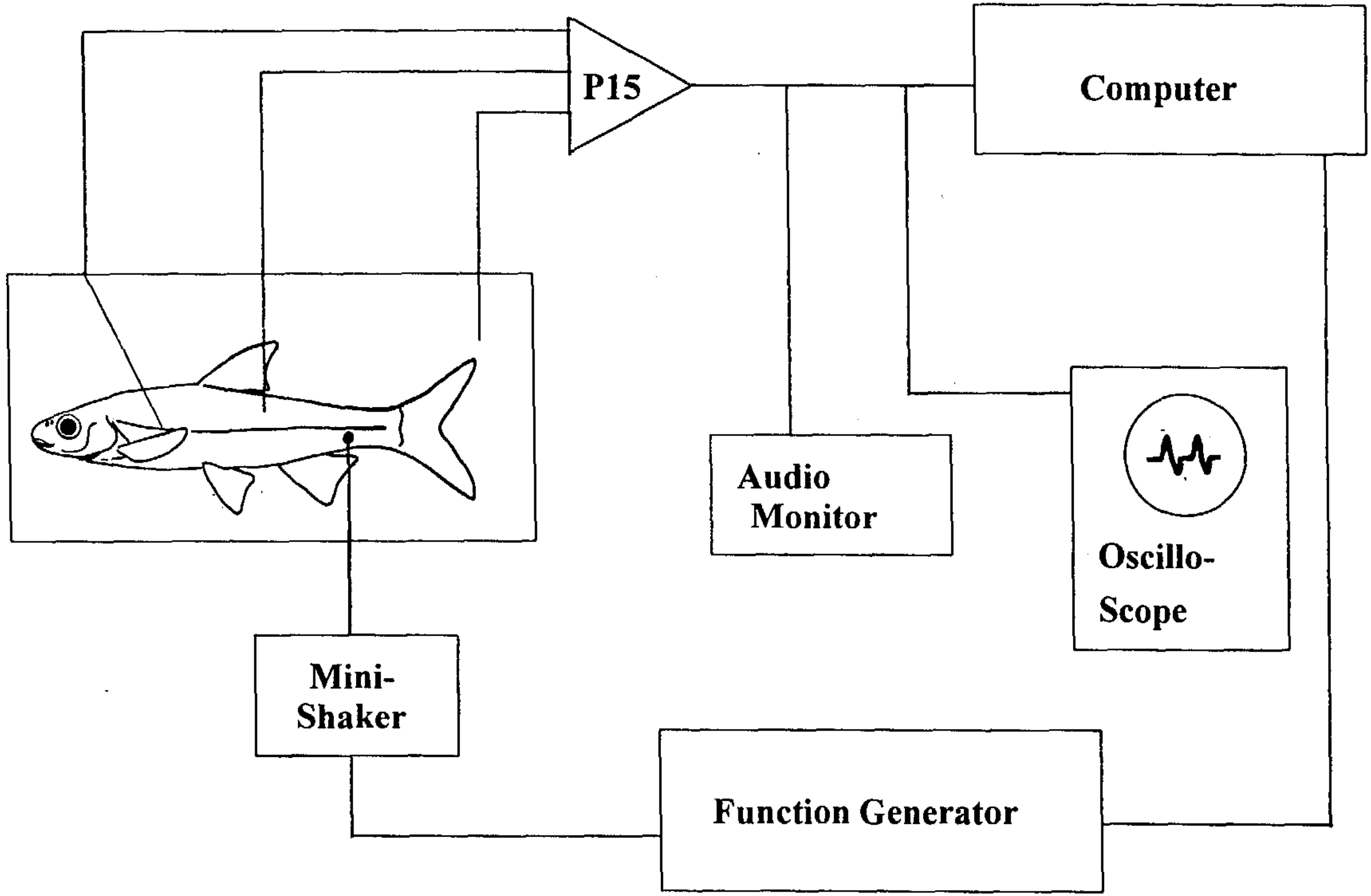


Figure 2. A sample compound action potential recording from a control fish when an 80 Hz (400 ms duration, 17 m/s^2 acceleration) vibration stimulus is given to the fish. Spike amplitude bar = 0.625 mv.

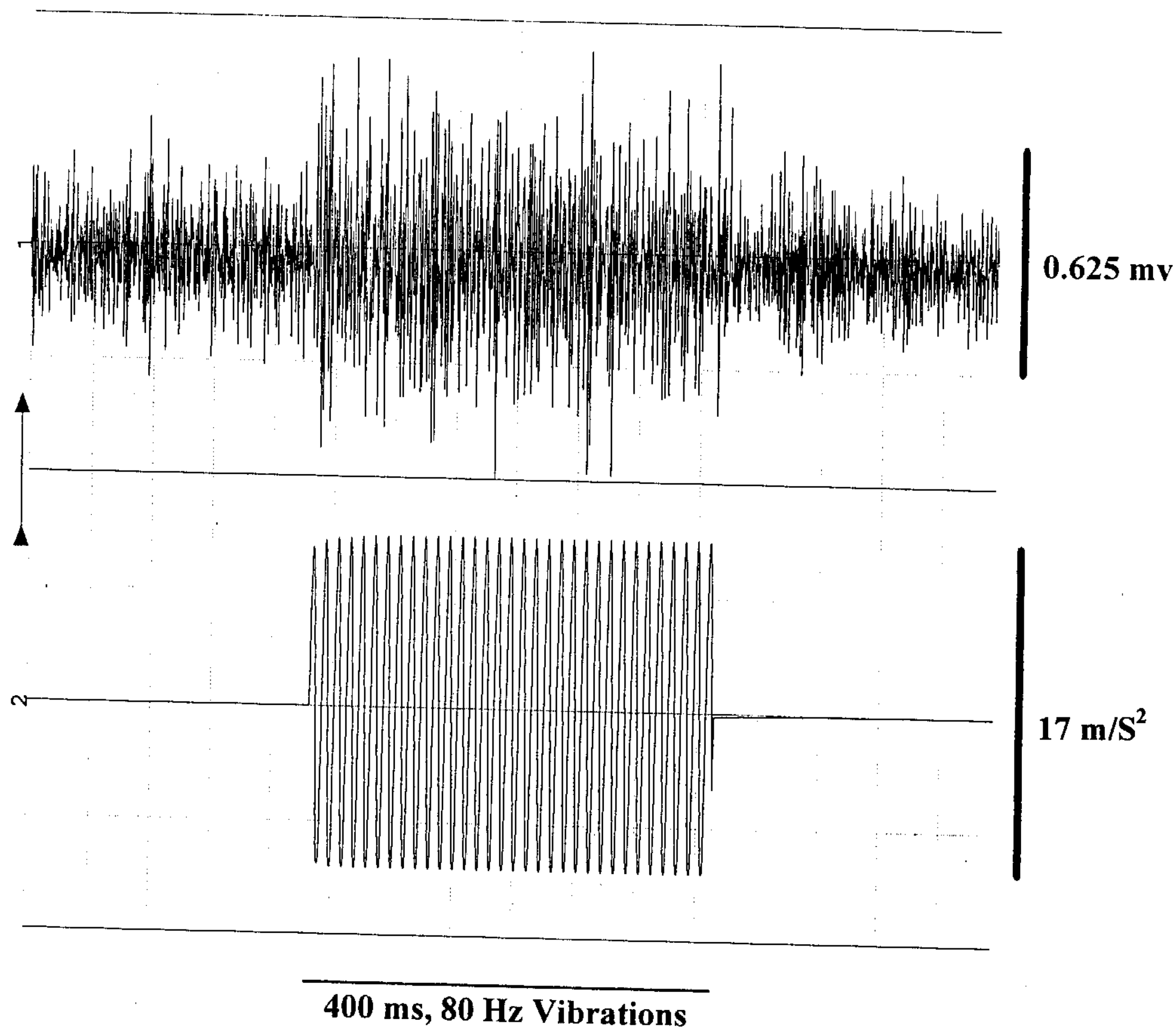


Figure 3. A sample compound action potential recording from a Cd-exposed fish (450 μg/l, 24 hrs) when an 80 Hz (400 ms duration, 17 m/s² acceleration) vibration stimulus is given to the fish. Spike amplitude bar = 0.0625 mv.

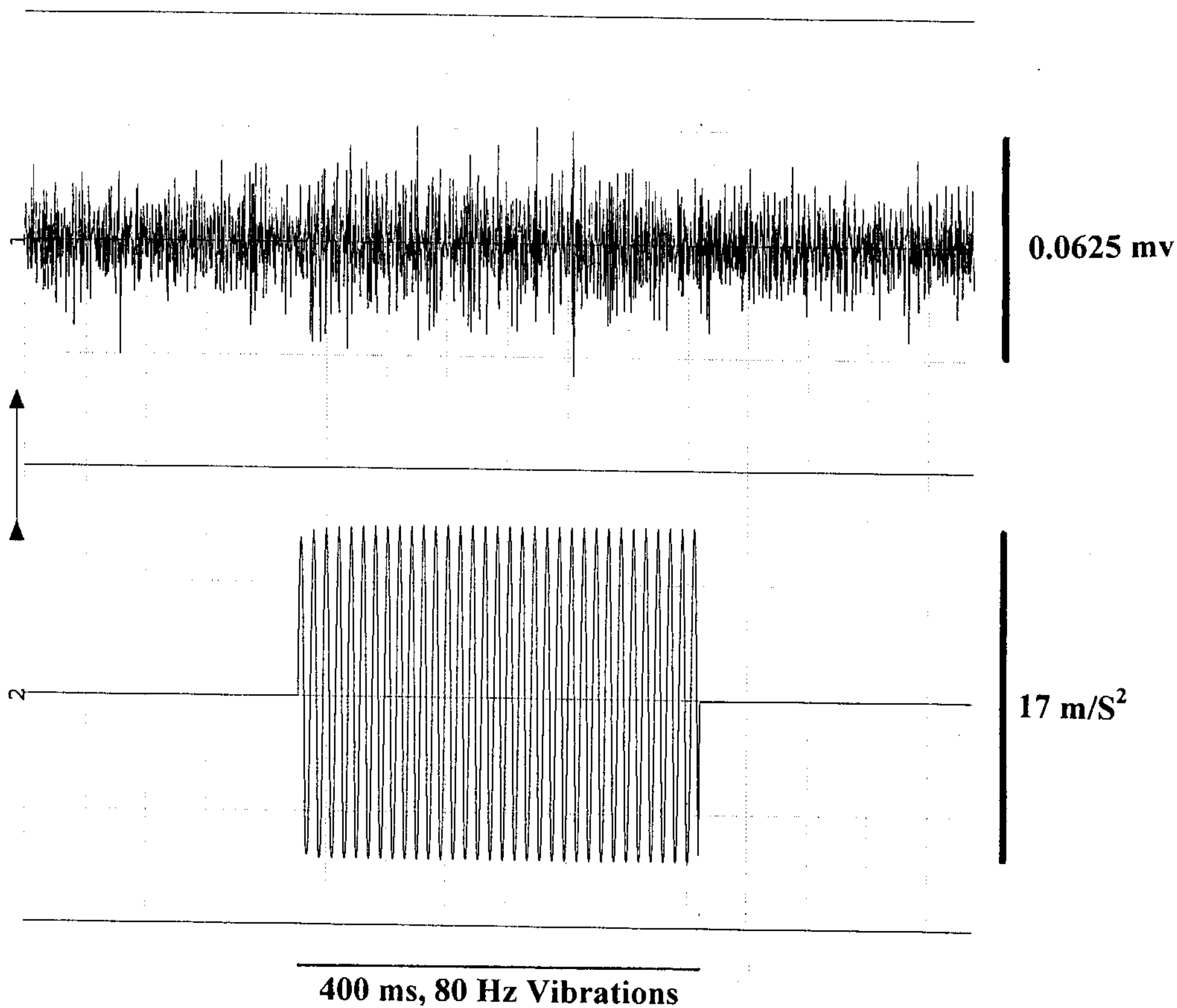


Figure 4. A sample compound action potential recording from a recovering fish (first exposed to Cd 450 µg/l, 24 hrs then 10 days in clean water) when an 80 Hz (400 ms duration, 17 m/s^2 acceleration) vibration stimulus is given to the fish. Spike amplitude bar = 0.20 mv.

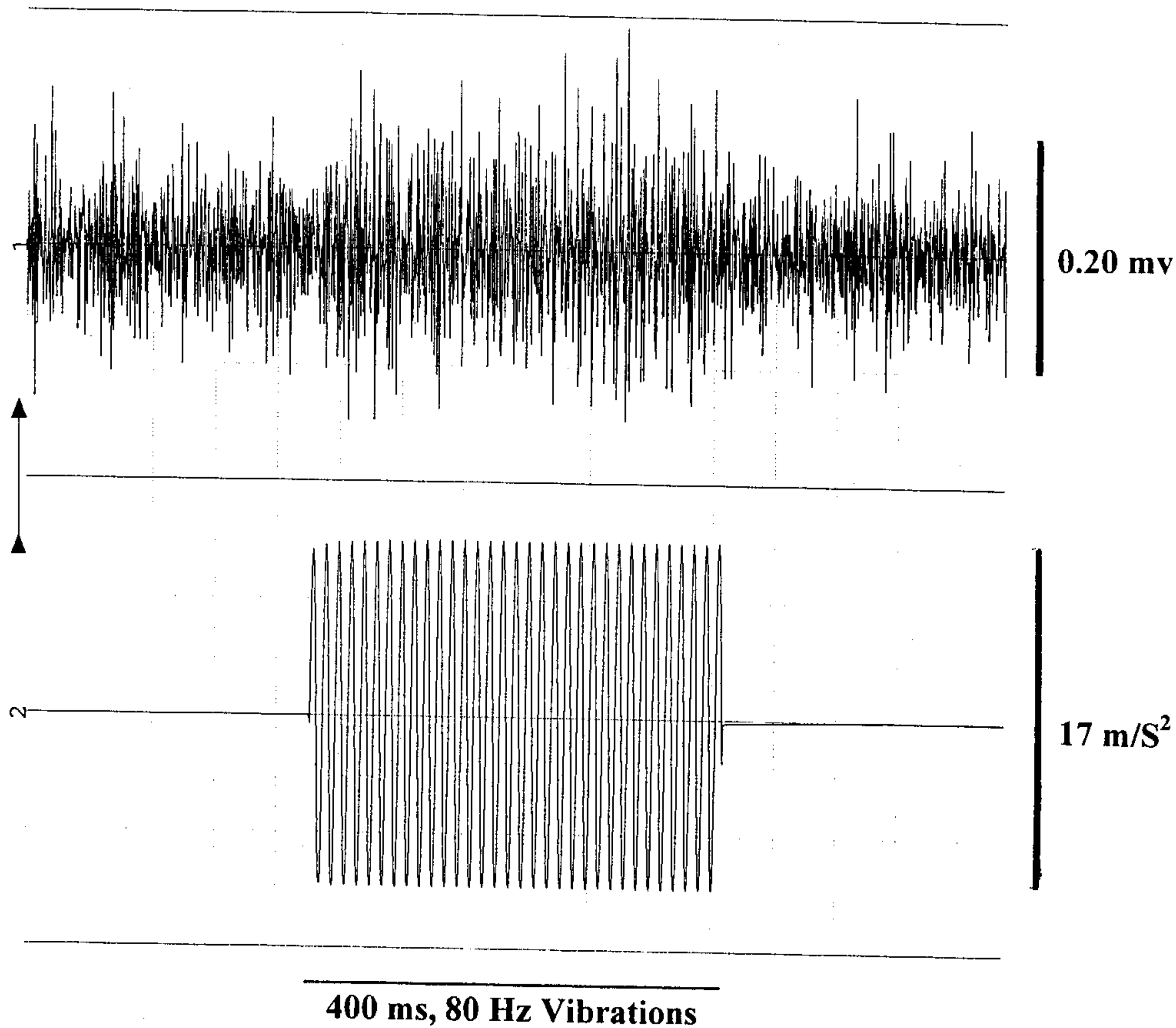


Figure 5. Recording set-up for schooling behavior formation observation. IR: infra-red light. Four largemouth bass and 30 fathead minnows are used.

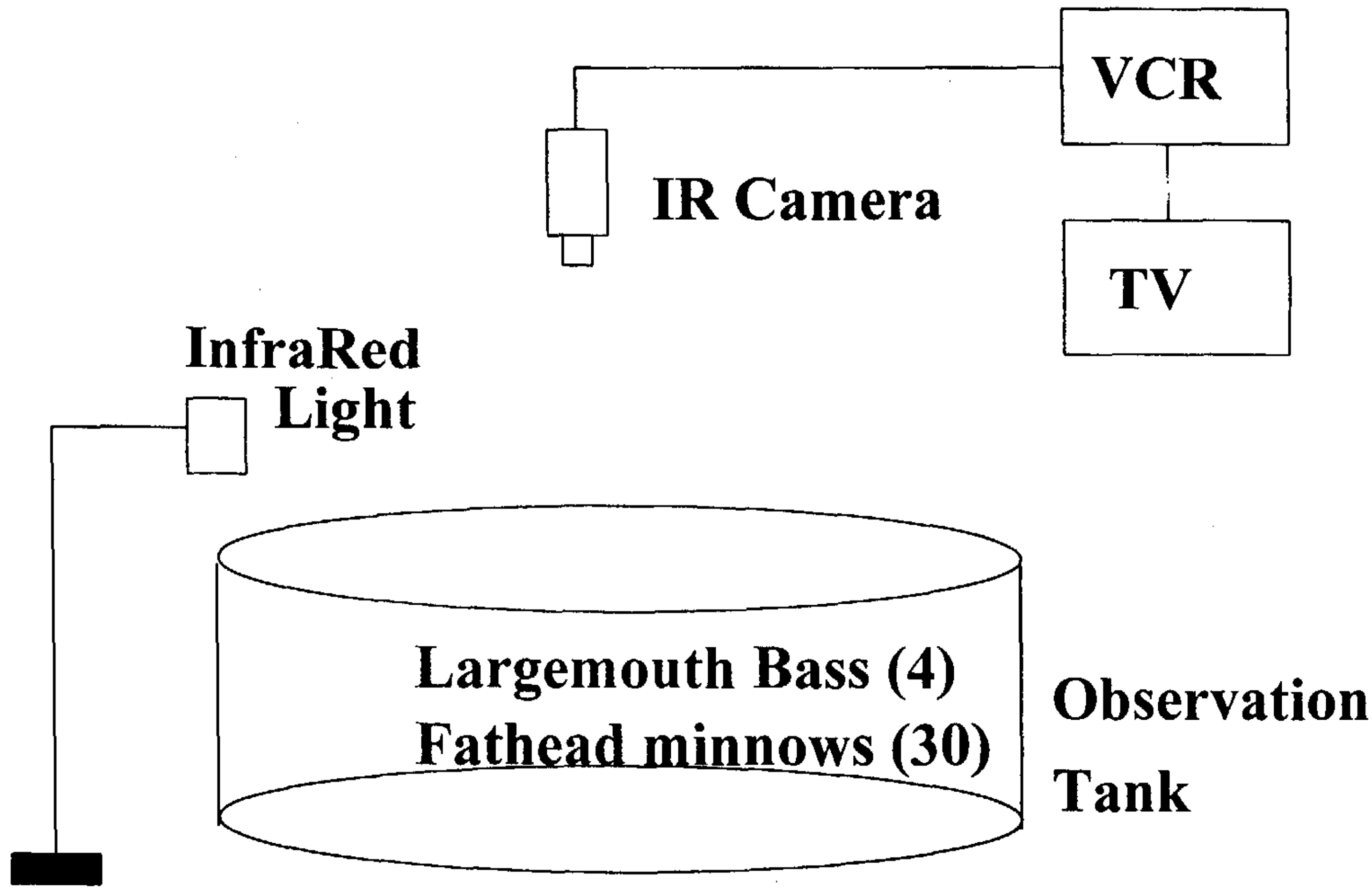
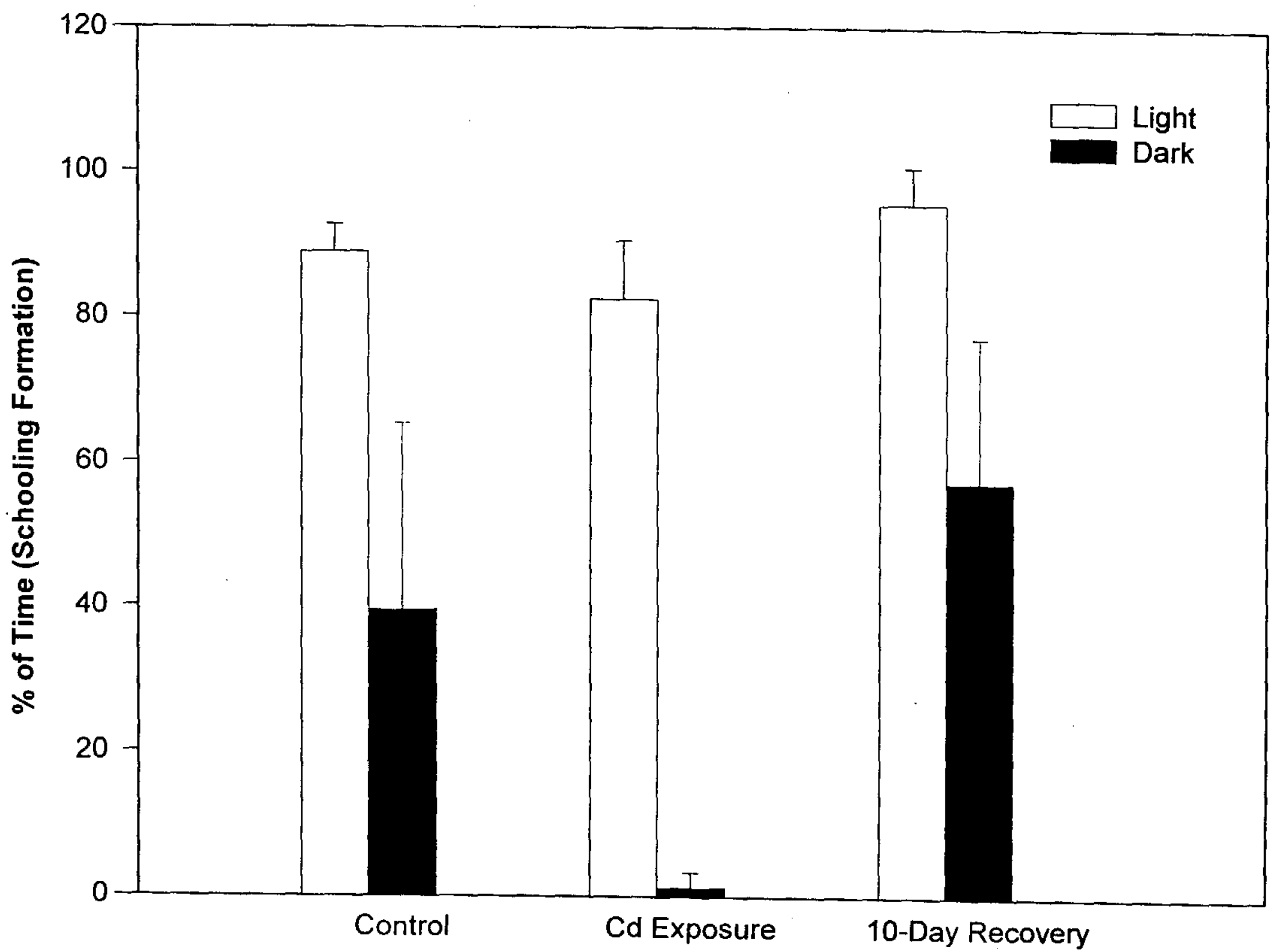


Figure 6. Percentage of schooling formation time (over a 2-hr period) of control, Cd-exposed (450 µg/l, 24 hrs) and 10 days in clean water recovery fish. Open bar: observed under lighted condition. Solid bar: observed under total darkness with the aid of infrared light.



Chapter IV-Summary and Conclusions

Both electrophysiological recordings from the lateral line nerves and schooling behavior observations clearly indicate that exposure to cadmium can temporarily disrupt the normal physiological function of the lateral line nerves. When the lateral line nerves cease to generate any compound action potential, it is a clear sign that normal physiological activities are compromised. The absence of schooling behavior under darkness with the Cd-treated group further corroborates the finding from the physiological recording. It is obvious that exposure to cadmium ion led to temporary suppression of the lateral line function and also reduced the schooling behavior mediated by the system. It is known from patch clamping studies that the presence of cadmium ions, even at a very low concentration of 10 μM to 20 μM, can block calcium ions (Byerly and Hagiwara, 1988) . Without the proper presence of calcium ions inside the neuromast cells (the basic functional units of the lateral line nerves) both fast high voltage activated (HVA) and slow HVA calcium channels cease to function. The end result is that no compound action potentials are generated from the lateral line nerves and schooling ability under darkness (with the presence of predators, e.g. largemouth bass) is lost.

The results of this study clearly show that after just 24 hours of exposure to cadmium at a concentration of 450 μg/l, the lateral line function is eliminated. The consequence of this effect is that an anti-predation function (i.e., schooling behavior) is also suppressed. Therefore, even if the cadmium exposure itself does not kill the fish, the loss of lateral line function could reduce the ability of fish to detect the presence of predators or prey. Eventually this could lead to the death of affected fish.

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