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SWIMMING BEHAVIOR AS AN INDICATOR OF SUBLETHAL TOXICITY IN FISH

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Abstract – Swimming behavior of fish is impaired by exposure to a diversity of contaminants. Gross aberrations in swimming can be qualitatively assessed while subtle changes in swimming behavior arising from sublethal exposures can be detected through a more detailed analysis of this response. Compared to other swimming behavior variables, the physical capacity to swim against water flow tends to be affected at relatively high toxicant concentrations and often presages mortality. Orientation to water flow, however, is altered at sublethal concentrations. Frequency of activity is a more sensitive measure in detecting contamination than measurements of survival alone. Alterations in swimming behavior have been detected during exposures to various contaminants at concentrations as low as 0.7 to 5% of their LC50 values and at concentrations that subsequently inhibited growth after longer periods of exposure. Analysis of swimming patterns provides even higher resolution for analysis of swimming behavior, and increased availability of the instrumentation necessary for such measurements should facilitate use of this approach. Fish swimming activity can easily be incorporated in test protocols to expand the sensitivity of standard toxicity tests.

Keywords – Behavioral toxicology Swimming activity Swimming capacity Aquatic organisms

INTRODUCTION

Changes in swimming behavior caused by sublethal exposure to contaminants may impair the ability of fish to feed, avoid predation or reproduce [1]. Such behavioral modifications provide an index of sublethal toxicity and also indicate the potential for subsequent mortality. In the present study, we evaluated swimming behavior as an indicator of sublethal toxicosis in fish by reviewing pertinent toxicological literature. We compared behavioral measurements with the standard evaluations of mortality and growth in both laboratory and field studies, and discussed the use of behavior as a routine measure in toxicity assessment.

SWIMMING VARIABLES IN TOXICITY TESTS

Swimming behavior is the most frequently assessed behavioral response during toxicity investigations of fish. Swimming capacity and swimming activity, two general measures of swimming behavior, are commonly used to assess contaminant-related changes in locomotion. Swimming capacity is a measure of orientation to water flow [2], as well as the physical capacity to swim against it [3]. Swimming activity includes such variables as frequency and duration of movements [4], speed and distance traveled during movement [5], frequency and angle of turns [6], position in the water column and form and pattern of swimming.

Toxicant-related modifications in swimming behavior generally occur appreciably earlier than mortality, regardless of the type of behavior evaluated. Table 1 lists the concentrations and durations of exposure at which significant changes in both locomotory behavior and survival were reported in the literature. Based on this information, the average toxicant concentration that induced changes in swimming behavior was less than 16% of the concentration that caused mortality. Although most of these studies did not establish threshold concentrations for behavioral effects, values ranging from 0.1 to 5.0% of the LC50 were commonly reported (Table 1). In studies where multiple observations were made [e.g., 7,8], behavioral changes commonly occurred 75% earlier than the onset of mortality.

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Table 1. Contaminant concentrations and exposure durations that cause significant changes in swimming behavior compared with exposures that induce mortality in aquatic organisms

Species, contaminant and reference	Parameter measured[a]	Behavior exposure		Lethal exposure	
		μg/L	Duration	μg/L	Duration
Oncorhynchus kisutch					
Fenitrothion [8]	FM,SC	480	2 h	1,300	96 h
Kraft mill effluent [3]	SC	3.7–9.0% v/v	18 h	18–45% v/v	96 h
Oncorhynchus mykiss					
Carbaryl [17,37[b]]	SC	1,000	96 h	1,950	96 h
	A	1,000	96 h	1,950	96 h
Chlordane [17,37[b]]	SC	>20	96 h	42	96 h
	A	2	96 h	42	96 h
DEF [17,37[b]]	SC	5	96 h	660	96 h
	A	50	96 h	660	96 h
Pentachlorophenol [17,37[b]]	SC	>20	96 h	52	96 h
	A	2	96 h	52	96 h
Diquat [2]	R	500	24 h	90,000	24 h
Simazine [2]	R	1,000	24 h	200,000	24 h
Copper pH 6 [16]	SC	10	96 h	40	96 h
TCDD [9]	FM	0.000038	27 d	0.00176	21 d
	A	0.000038	19 d	0.00176	21 d
Phenol [10]	FM	8,000	7 min	8,900	96 h
Salvelinus fontinalis					
Fenitrothion [15]	SC	500	24 h	1,500	24 h
Malathion [14]	SC	40	10 d	120	5 d
Copper sulfate [7]	A	6–9	2 h	9.5	>161 d
Aluminum pH 5.6 [32]	A,FM	142	30 d	142	30 d
	SC	68	60 d	142	60 d
Carassius auratus					
Parathion [6,37[b]]	FM	330	24 h	1,830	96 h
DDT [38]	FM	1	3 d	30–100	96 h
Cyprinus carpio					
Distillery effluent [39]	A	20% v/v	24 h	17.6% v/v	48 h
DDT [13]	R	50	3–4 h	57	48 h
Pimephales promelas					
TNT [40]	FM,A	460	96 h	2,580	96 h
Ptychocheilus lucius					
Shale oil [35]	SC	1,700	96 h	4,200	96 h
Arius felis					
Copper [27]	FM	50	72 h	>200	72 h
Lepomis macrochirus					
Methyl parathion [28]	FM	3	10–14 h	4,380	96 h
Cadmium [41]	A	100	3 d	500	14 d
Chromium [41]	A	50	3 d	>24,000	14 d
Zinc [41]	A	100	3 d	>5,000	14 d
DDT [19]	A	0.008	8 d	0.2–1.0	96 h
Fluorene [18]	SC	1,000	30 d	1,000	30 d
	A	1,000	30 d	1,000	30 d
Micropterus salmoides					
Copper [42,43[b]]	A	100	6 h	960	48 h
Cadmium [42,43[b]]	A	100	16 h	1,500	48 h
Phenol [42,43[b]]	A	1,000	5 h	20,400	48 h
Ammonia [42,43[b]]	A	500	16 h	16,700	48 h
Cyanide [42,43[b]]	A	50	5 h	100	48 h
Pseudopleuronectes americanus					
Polyoxyethylene Monolaurate [44]	A	1,000	24 h	>10,000	24 h

[a]A, activity (frequency and duration); FM, form (posture and pattern); SC, swimming capacity; R, rheotaxis.
[b]Reference source for toxicity information.

Because these swimming variables are interrelated, several aspects of swimming behavior may be simultaneously affected by a toxicant. For example, a 28-d exposure of rainbow trout (*Oncorhynchus mykiss*) to dioxin (29 pg/L) reduced frequency of activity, changed the posture of swimming and affected positioning in the water column [9]. Another study documented simultaneous declines in the distance traveled, frequency and duration of activity and swimming capacity of brook trout (*Salvelinus fontinalis*) after exposure to aluminum (300 μg/L) in water with an acidity of pH 5.5 [4].

The response of each locomotory variable to a toxicant may change during exposure. Because fish may be irritated by a toxicant or attempt to escape exposure, changes in orientation to water flow, spatial selection and speed of movement may occur initially [10]. As these sensory reactions subside, more severe aberrations in locomotory behavior may occur as toxicants saturate detoxifying enzymes or alter neurochemical or metabolic processes [11,12].

Swimming capacity

Swimming capacity reflects the ability of fish to swim against sustained or incrementally increasing velocities of water flow. Besch et al. [13] found that measures of swimming capacity provided a rapid indication of toxicity; swimming failure occurred within 2 h of exposure to a toxicant such as DDT, which was lethal after 48 h. Changes in swimming capacity have also been documented at toxicant concentrations where no appreciable mortality occurred (Table 1). For example, swimming capacity of brook trout was altered by malathion and fenitrothion at concentrations of about 33% of the LC50 [14,15], and swimming capacity of rainbow trout was reduced after exposure to copper at 12% of the LC50 [16]. However, swimming capacity is not consistently affected by sublethal contaminant exposure. In a study that assessed the toxicity of six agricultural chemicals to rainbow trout [17], exposure to chlordane, methyl parathion or pentachlorophenol at concentrations up to 50% of the LC50 failed to influence the response, although similar concentrations of carbaryl, 2,4-DMA and DEF caused significant declines in swimming capacity. Behavioral responses may not be directly related to toxicant concentrations. For example, Little et al. [17] observed heightened swimming capacity among fish exposed to DEF or 2,4-DMA at 0.5% of LC50, while greater concentrations (5–50% of LC50) significantly reduced swimming capacity. A 30-d sublethal exposure of bluegill to low concentrations of fluorene (0.12–0.25 mg/L) significantly increased swimming capacity, whereas exposure to an increased concentration (1.0 mg/L) significantly decreased swimming capacity [18].

Orientation to water flow, or rheotaxis, is an important variable in swimming capacity tests and is also influenced by contaminant exposure. For example, chinook salmon (*Oncorhynchus kisutch*) exposed to fenitrothion ceased to orient against water flow, and moved with the current instead [8]. Contaminant effects on rheotaxis may be independent of the effects on swimming capacity and may reflect an attempt by the fish to escape the contaminant gradient. Dodson and Mayfield [2] showed that rainbow trout failed to orient toward movement of a striped background after exposure to diquat and simazine at concentrations of 0.5% of the LC50.

Swimming activity

Grossly altered swimming movements such as changes in water column position (surfacing, resting on bottom), swimming posture (head-up swimming), body movements (increased or decreased waveform of body movement) or swimming patterns (frequent turns or spiralling) often occur during toxicant exposure. In extreme cases, a loss of coordination, convulsive movements or loss of equilibrium may occur. The all-or-none occurrence of these responses has been successfully used to qualitatively assess behavioral toxicity syndromes [11] and to describe behavioral effects of dioxin exposure [9].

Accurate, consistent descriptions of subtle changes in swimming activity such as analysis of swimming patterns or observations of frequencies of movements require more extensive measures of the response. Hypoactivity and hyperactivity have been measured as the frequency of entries and exits through a dividing partition [19], or number of grid lines crossed. Frequency of activity of larval striped bass (*Morone saxatilis*) was measured as the number of visually detectable body movements that resulted in forward movement of the larva [20], and also as rate of change of direction [10]. Duration of time in which fish are active [17] or inactive [18] has also been measured to determine frequency of activity. In a study with rainbow trout [17], duration of swimming activity was consistently reduced by each of six chemicals after 96-h sublethal exposures (Table 1). This response was sensitive at 0.5 to 50% of the LC50 values for chlordane, pentachlorophenol and an organophosphorus defoliant, DEF.

Measurements of activity markedly increased the sensitivity of tests conducted as static renewals to assess toxicity of riverwater to larval striped bass in South Carolina [20]. Reduced frequency of movement, measured as total number of movements over a 3-min period, were observed 48 h before the onset of mortality among fish exposed to 50 to 100% riverwater. At some sites, no significant mortality occurred yet significant hypoactivity was observed in 50 to 100% riverwater that was later determined to contain phenolic compounds.

Pattern analysis of swimming behavior involves quantification of the component variables such as the radius of turns and frequency of turning behavior, the distance of linear travel between turns and the speed of travel, as well as the frequency and duration of movements [21]. The assessment of these variables usually requires specialized equipment because measurements can be extensive. Motion is readily detected with high resolution in photodetection chambers [22,23] and computer-interfaced video systems [5,24,25]. For example, Rand used electronic techniques to determine that frequency of turning behavior and the angle of turns increased for goldfish (*Carassius auratus*) [6], bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) [26] during sublethal exposure to parathion. Sea catfish (*Arius felis*) also exhibited broader turning angles following 72-h sublethal exposures to copper (0.1 mg/L) [27]. Orientation angles, observed in turning behavior, reflect the integration of sensory information and locomotory control essential in the orientation to environmental stimuli [21]. Speed of movement and angular velocity have also been measured. Analysis of video processed images by Smith and Bailey [10] detected decreased speed of movement and angular velocity of steelhead trout (*Oncorhynchus mykiss*) within 7 min of exposure to phenol (8 mg/L).

Stereotypic body movements that do not result in locomotion also reflect toxicant exposure. Fin flickering, a grooming motion of bluegill, showed strong dose–response relations with increased concentrations of methyl parathion [28]. In addition, the frequency of stereotypic movements of the fathead minnow (*Pimephales promelas*) decreased during exposure to acidic water [29].

DISCUSSION

Swimming activity is a consistent indicator of sublethal toxicity for a wide variety of species exposed to different chemicals under various conditions. Based on Rand's [30] criteria for selecting behavioral endpoints for toxicity studies, we feel that swimming activity is possibly the most suitable measure of swimming behavior for routine use in toxicity studies. It is highly sensitive, appropriate for numerous aquatic species, relevant to survival and easy to measure. In a study comparing swimming activity, swimming capacity, feeding and predator avoidance, duration of swimming activity of rainbow trout was consistently reduced by sublethal exposure to each of six chemicals [17]. In contrast, swimming capacity was unaffected by three of the chemicals.

Swimming activity is not only influenced by chemicals before mortality occurs, but can also be influenced before reductions in growth are detected. For example, swimming activity was significantly reduced in rainbow trout after 96-h exposure to 5 μg/L DEF [17]; growth was reduced at similar concentrations after 30 d [31]. Also, frequency of movements and duration of activity were both significantly inhibited in brook trout at lower aluminum concentrations than affected survival or growth under acidic conditions [32]. Such sensitivity has regulatory implications. For example, chlordane reduced swimming activity of rainbow trout at concentrations below the U.S. Environmental Protection Agency (EPA) "not-to-be-exceeded" concentration for chlordane in freshwater [33] and copper inhibited swimming activity of brook trout at concentrations below the maximum allowable toxicant concentration for copper [7].

Assessment of swimming activity is useful in defining no-effect concentrations in the laboratory or under controlled field conditions. However, as with other toxicological endpoints, the extrapolation of behavioral responses to effects that may occur in natural populations is difficult to interpret. The ecological consequence of aberrant swimming behavior is implied mainly through the impairment of adaptive behaviors such as migration, predation or predator success. Hypoactivity and hyperactivity, as well as deviations in adaptive diurnal rhythmicity, can disrupt feeding and increase vulnerability to predation. Heightened activity may increase an organism's vulnerability to predation, whereas reductions in swimming activity lessen the chance of encountering prey by reducing search areas [34]. Deviations in swimming response can also interfere with the capture of prey or impair the efficiency of foraging behavior and decrease the amount of energy available for growth. Impairments in feeding behavior of fish associated with altered swimming behavior have also been observed after sublethal exposures to low pH and aluminum [4], metals [7], organophosphates [8], dioxins [9] and petroleum hydrocarbons [35].

Behavioral data are often criticized because they lack standardization. Indeed, no single standardized procedure exists for measuring any variable of swimming activity largely because swimming behavior varies among species as well as among lifestages within a species. More appropriately, test methods must be tailored to a particular life stage of a single species, such as the methods developed for tests with larval striped bass [20]. Standardized methods are not lacking so much as consensus on guidelines for the conduct and quality controls for such studies. The recent development of electronic instrumentation and appropriate software can facilitate both the standardization and the routine use of swimming activity in toxicity testing.

No numerical value or range of values have been defined as the norm for swimming activity for any aquatic species; therefore, detection of abnormal activity is based on comparisons of responses of exposed fish either with activity measured during a baseline, preexposure period or observations of fish under a control treatment. Swimming behavior is affected by maturity and physical condition of the test organism, the organism's social environment, degree of exposure to other stressors, acclimation and time of testing. Physical aspects of the testing situation such as temperature, water quality, illumination or size and shape of the test chamber also affect results [36]. Thus, tests involving behavioral responses require control treatments with rigorous uniformity of the test environment and handling regime of the test organisms. The influence of nonexperimental variables should be considered during the development of a behavioral method as a bioassay tool, with the goal of reducing variation and increasing precision of measurement.

Swimming behavior is a valid and consistent index of sublethal toxicity that can be easily incorporated in test protocols to expand the sensitivity of standard toxicity tests. Behavioral responses are sensitive to a number of contaminants across a wide range of species. In addition, measures of swimming behavior are nondestructive and can be made with minimal stress to the fish, thus allowing repeated measurements on the same individuals throughout an exposure. Activity measurements can be rigorously monitored in the laboratory with modern electronic equipment and can be easily adapted for use in field toxicity testing. Because of their consistency, sensitivity and utility, behavioral measurements should be included in standard protocols for aquatic toxicity assessment.

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