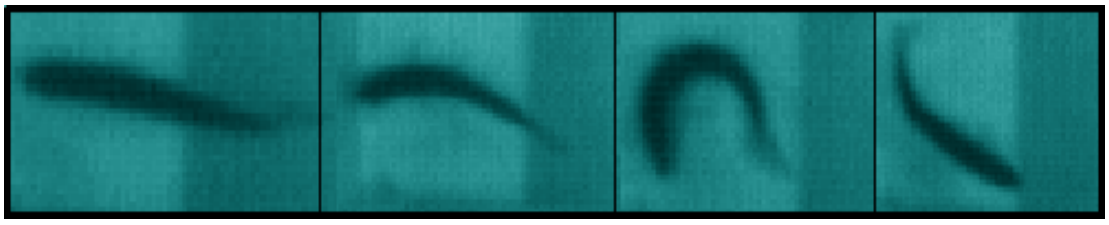
ORNL/TM-2003/195

Development of a New Technique to Assess Susceptibility to Predation Resulting from Sublethal Stresses (Indirect Mortality)

August 2003

Prepared by
Glenn F. Cada
Michael G. Ryon
Dennis A. Wolf
Brennan T. Smith



U.S. Department of Energy
Energy Efficiency and Renewable Energy
Wind and Hydropower Technologies

Bringing you a prosperous future where energy is clean, abundant, reliable, and affordable.

ORNL/TM-2003/195

Environmental Sciences Division

DEVELOPMENT OF A NEW TECHNIQUE TO ASSESS SUSCEPTIBILITY TO PREDATION RESULTING FROM SUBLETHAL STRESSES (INDIRECT MORTALITY)

Glenn F. Cada
Michael G. Ryon
Environmental Sciences Division
Oak Ridge National Laboratory

Dennis A. Wolf
Computing & Computational Sciences Division
Oak Ridge National Laboratory

Brennan T. Smith
Tennessee Valley Authority
Norris, Tennessee

Date Published: August 2003

Prepared for
U.S. Department of Energy
Office of Energy Efficiency and Renewable Energy
Wind and Hydropower Technologies

Prepared by
OAK RIDGE NATIONAL LABORATORY
P. O. Box 2008
Oak Ridge, Tennessee 37831-6036
managed by
UT-Battelle, LLC
for the

U.S. DEPARTMENT OF ENERGY
under contract DE-AC05-00OR22725

CONTENTS

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF TABLES

ACKNOWLEDGMENTS

We thank the staff of the ORNL Aquatic Laboratory for their assistance in designing and conducting these experiments. In particular, the work of Jessica Busey, Kitty McCracken and Gail Morris to run the fish exposures and help analyze videos was very helpful. Contributions by George Houser, Kenneth Lowe, and Kelly Roy were instrumental in providing prey and predator fish for the tests. Instrumentation assistance was provided by Jeff Riggs, Danny Sluss, Randy Smith, and Ed Sanford. Photographic assistance was provided by Jim Richmond of ORNL Photography. Samantha Childers, Tennessee Valley Authority, assisted in the turbulence measurements. Valuable suggestions on the manuscript were provided by Chuck Coutant and Mark Bevelhimer of Oak Ridge National Laboratory and by the Technical Committee of the U.S. Department of Energy's Advanced Hydropower Turbine Systems Program. This report is a product of the Wind and Hydropower Technologies Program, U. S. Department of Energy.

SUMMARY

Fish that pass through a hydroelectric turbine may not be killed directly, but may nonetheless experience sublethal stresses that will increase their susceptibility to predators (indirect mortality). There is a need to develop reliable tests for indirect mortality so that the full consequences of passage through turbines (and other routes around a hydroelectric dam) can be assessed. We evaluated a new technique for assessing indirect mortality, based on a behavioral response to a startling stimulus (akin to perceiving an approaching predator). We compare this technique to the standard predator preference test.

The behavioral response is a rapid movement commonly referred to as a startle response, escape response, or C-shape, based on the characteristic body position assumed by the fish. When viewed from above, a startled fish bends into a C-shape, then springs back and swims away in a direction different from its original orientation. This predator avoidance (escape) behavior can be compromised by sublethal stresses that temporarily stun or disorient the fish.

We subjected striped shiners and fathead minnows to varying intensities of either turbulence (10-, 20- or 30-min) or 2-min exposures to a fish anesthetic (100 or 200 mg/L of tricaine methanesulfonate), and evaluated their subsequent behavior. Individual fish were given a startle stimulus and filmed with a high-speed video camera. Each fish was startled and filmed twice before being stressed, and then at 1-, 5-, 15-, and 30-min post-exposure.

The resulting image files were analyzed for a variety of behavioral measures including: presence of a response, time to first reaction, duration of reaction, time to formation of maximum C-shape, time to completion of C-shape, and completeness of C-shape.

The most immediate measure of potential changes in fish behavior was whether stressed fish exhibited a startle response. For striped shiners, the number of fish not responding to the stimulus was significantly different from controls at 1-min post-exposure and for fathead minnows at 1- and 5-min post-exposure. The greatest effects occurred with exposure to the fish anesthetic; in fathead minnows all of the recorded measures were significantly different from controls at 1-min and 5-min post-exposure at the 100 mg/L dose. For striped shiners all recorded behavioral measures were significantly different from controls at 1-min at the 200 and 100 mg/L doses and for selected behavioral measures at 5-min. Turbulence also had significant effects on striped shiner startle responses following 20- and 30-min exposures for all behavioral measures at 1-min. The patterns suggest that any effects on startle response due to turbulence or low doses of anesthetic are short-lived, but can be evaluated using the escape behavior technique.

The most useful indication of changes in escape behavior in these tests was the simple reaction/no reaction to the startle stimulus. The startle response occurred reliably among unstressed fish, and was frequently reduced or eliminated in fish exposed to turbulence or anesthesia. The other behavioral parameters observed were often altered by the sublethal stresses as well.

A standard predator preference test was also conducted with largemouth bass as the predators and fathead minnows as prey. In this test design, groups of 10 unstressed fish (controls) and 10 stressed fish were put in a tank with a predator. The stressed fathead minnows were exposed to turbulence or fish anesthetic. The predator was allowed to eat half of the prey, and the data were evaluated to determine whether predators consumed greater proportions of stressed minnows than control minnows. The predation test indicated that exposure to MS-222 resulted in significant predation in fathead minnows, but exposure to turbulence did not. This pattern was the same as seen in fathead minnows using the startle response (escape behavior) test.

For the sublethal stresses we applied, evaluation of changes in fish escape behavior yielded results comparable to traditional predator preference tests. Because this fish behavior test is simpler and quicker to conduct than predator preference tests, it shows promise as a useful technique for assessing indirect mortality resulting from sublethal stresses.

1. INTRODUCTION

Many studies of downstream fish passage at hydroelectric power plants have focused on *direct mortality*, i.e., death directly due to injuries or physiological stresses received during passage through the turbine, spillways, or turbine bypasses. However, it is also recognized that turbine-passed fish may not be killed directly, and may appear to be uninjured, but nonetheless could experience sublethal stresses that could result in *indirect mortality*. For example, the immune response of stressed fish may be compromised such that they succumb to disease days or weeks after the passage stress occurred. On a shorter time scale, the swimming behavior of stressed fish may be altered in a way that makes them more susceptible to predators in the tailwaters. There is a need to develop reliable tests for indirect mortality so that the full consequences of passage through conventional and advanced turbines (and other routes around the hydroelectric dam) can be assessed. This report describes a new technique to test the potential for increased susceptibility to predation following exposure to sublethal stresses.

The most commonly used laboratory technique for assessing susceptibility to predation involves mixing batches of sublethally stressed and unstressed (control) fish and exposing the mixed batch to predators in a tank (Coutant 1973; Cada et al. 1981; Neitzel et al. 2000). After about half of the prey are eaten, the remainder are retrieved and the relative proportions of stressed and control fish are determined. If a significantly higher proportion of stressed fish is eaten, one concludes that the population of fish exposed to these sublethal stresses had been impaired sufficiently to be more susceptible (than controls) to mortality due to predation.

These predator preference tests have been used to study the effects of a variety of sublethal stresses, from heated discharges to chemicals to hydraulic stresses. Although the tests have demonstrated increased susceptibility to predation in the laboratory, they are difficult to conduct, require a certain amount of "training" of the predators to feed reliably in a laboratory tank (and therefore have a degree of artificiality), often have low precision (i.e., high variability in response), require the sacrifice of prey species (which may limit their application for rare species), and have rarely been validated in the field.

There is a large body of literature describing high-energy swimming bursts (called "fast-start" behavior or startle responses) that fish use to capture prey or avoid predators (see Eaton and DiDomenico 1986; Webb 1986; Domenici and Blake 1997; and Hale 1996). One form of fast-start swimming is the "C-start." When viewed from above, a startled fish bends into a C-shape, then springs back and swims away in a direction different from its original orientation (Figure 1.1). Some literature demonstrates that this predator avoidance (escape) behavior can be compromised by sublethal stresses that temporarily stun or disorient the fish (Mesa et al. 1994). Tests that examine changes in escape behavior may be preferable to existing predator preference tests because (1) they focus on a particularly relevant aspect of overall swimming performance, i.e., escape behavior; (2) they eliminate the need for a predator-prey test system, including predator training, and thus are less complicated to conduct; and (3) they could be made sufficiently compact and portable for use at field sites.

The purpose of this study was to look for changes in escape behavior in sublethally stressed fish that could be used as predictors of changes in susceptibility to predation. We subjected prey fish to stresses in the laboratory and looked for quantifiable, reproducible changes in escape behavior, compared to unstressed controls. We used two types of sublethal stresses: disorienting levels of turbulence (such as might occur in the draft tube and tailrace of a hydropower plant) and brief exposure to the common chemical fish anaesthetic MS-222 (tricaine methanesulfonate). Potentially this technique could be used to assess the effects on fish of a wide variety of physical stresses (sublethal shear or strike), temperature stresses, chemical stresses (e.g., low dissolved oxygen or gas supersaturation) or cumulative impacts. In addition, we conducted a series of conventional predator preference tests in order to determine which technique (escape behavior or predator preference) was a more sensitive indicator of effects of sublethal stresses on fish in the laboratory.

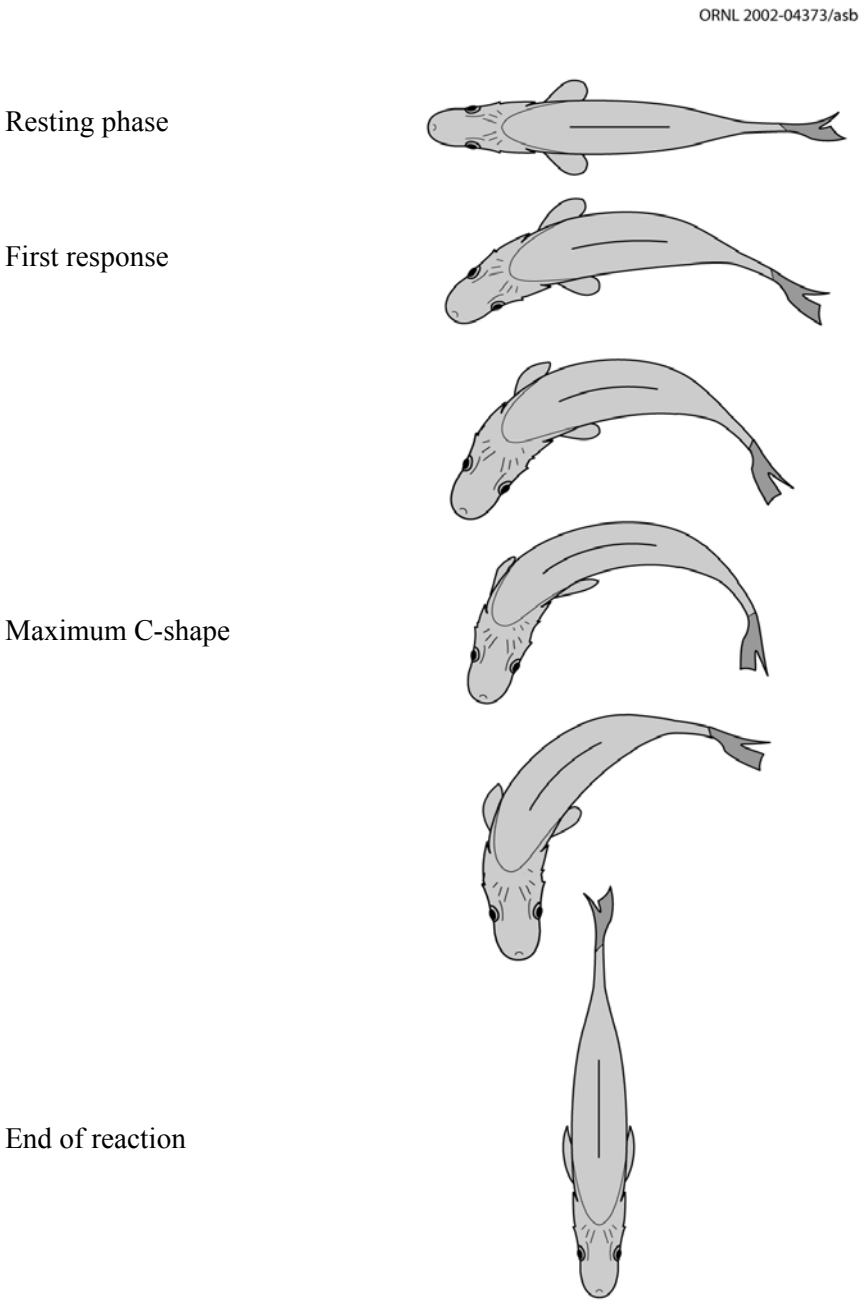


Fig. 1.1. Schematic diagram of the C-shape behavior in fish.

2. MATERIALS AND METHODS

Two types of laboratory experiments were performed to evaluate potential effects of non-lethal stresses on predation susceptibility. The first set of experiments focused on image analysis of escape behaviors in two test species. The behavior was elicited from the fish by initiating a threat or startle action which resulted in an innate, reflex response. The second set of experiments used a more traditional predator preference test. This evaluation was based on actual consumption of a mixed batch of stressed and non-stressed prey by a predator. The proportion of stressed to non-stressed prey eaten reflected the impact of the stress on the survivability of the test fish.

2.1 ESCAPE BEHAVIOR TESTS

The startle response test was conducted in the laboratory using two fish species and two primary stressors. Briefly, individual test fish were transferred from the holding tank to the observation tank, their initial response (swimming behavior) to a startling stimulus was filmed with an overhead camera, they were given a sublethal stress, and their startle response was filmed again to try to detect changes. The species holding tanks, test and observation tank, camera, analysis and test procedures were the same or very similar for all the experiments.

2.1.1 Test Species

The two test species used were the striped shiner (*Luxilus chrysocephalus*) and the fathead minnow (*Pimephales promelas*). The startle response we measured in these experiments is a common behavior among fish, and has been demonstrated in both minnow and trout families (Webb 1978, Hale 1996). The majority of behavior tests used the striped shiner as procedures were fine-tuned, and additional tests were performed using fathead minnows because they were used for both behavioral and predator preference tests. The striped shiners were collected by seining or electrofishing local streams with primarily rural land use watersheds and the fathead minnows were purchased from local bait shops. The striped shiners averaged 6 to 12 cm in total length, while the fathead minnows were generally smaller at 4 to 6 cm. Both species were fed rations of frozen brine shrimp, daily.

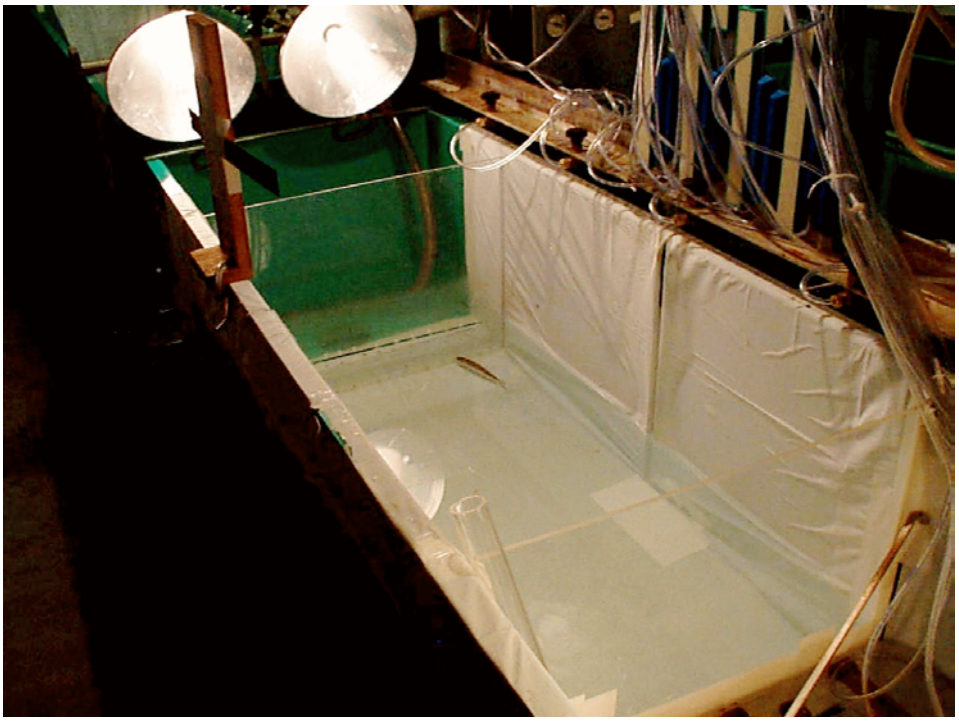
2.1.2 Holding Tank and Water Conditions

The fish were kept in isolated holding tanks before testing and then transferred to a separate holding tank afterwards. The tanks were fiberglass Living Streams®, 2.1 m long x 55 cm wide x 30 cm deep. Dechlorinated water was supplied continuously at 350 L/h at ambient temperatures of 18-22 EC. The same water source supplied both holding tanks and observation tanks. To limit fungal infections, the holding tanks were treated weekly with a mild solution (0.1 mg/L) of malachite green. Ambient light was supplemented by two commercial fluorescent lights on an 8-hr daylight cycle.

2.1.3 Observation - Test Tank

The observation tank was a separate Living Stream® tank with plexiglass dividers and lined with white plastic fabric (Fig. 2.1). The dividers created an observation area of 100 cm x 55 cm (Fig. 2.2) This arena was similar in size to those used in other studies of startle response (Webb 1978, Eaton and Emberly 1991, Wakeling et al. 1999), sufficient to allow for escape behavior, and still be within the field of view of the camera. Water depth was restricted to 19 cm to limit vertical movements of test fish. The observation tank was subdivided in half to create a small area for stressing the fish (Fig. 2.1).

(a)



(b)

Fig. 2.1. Photo of observation tank showing (a) observation area lined by white plastic and (b) stress area with turbulence generated by aerators and water pumps.

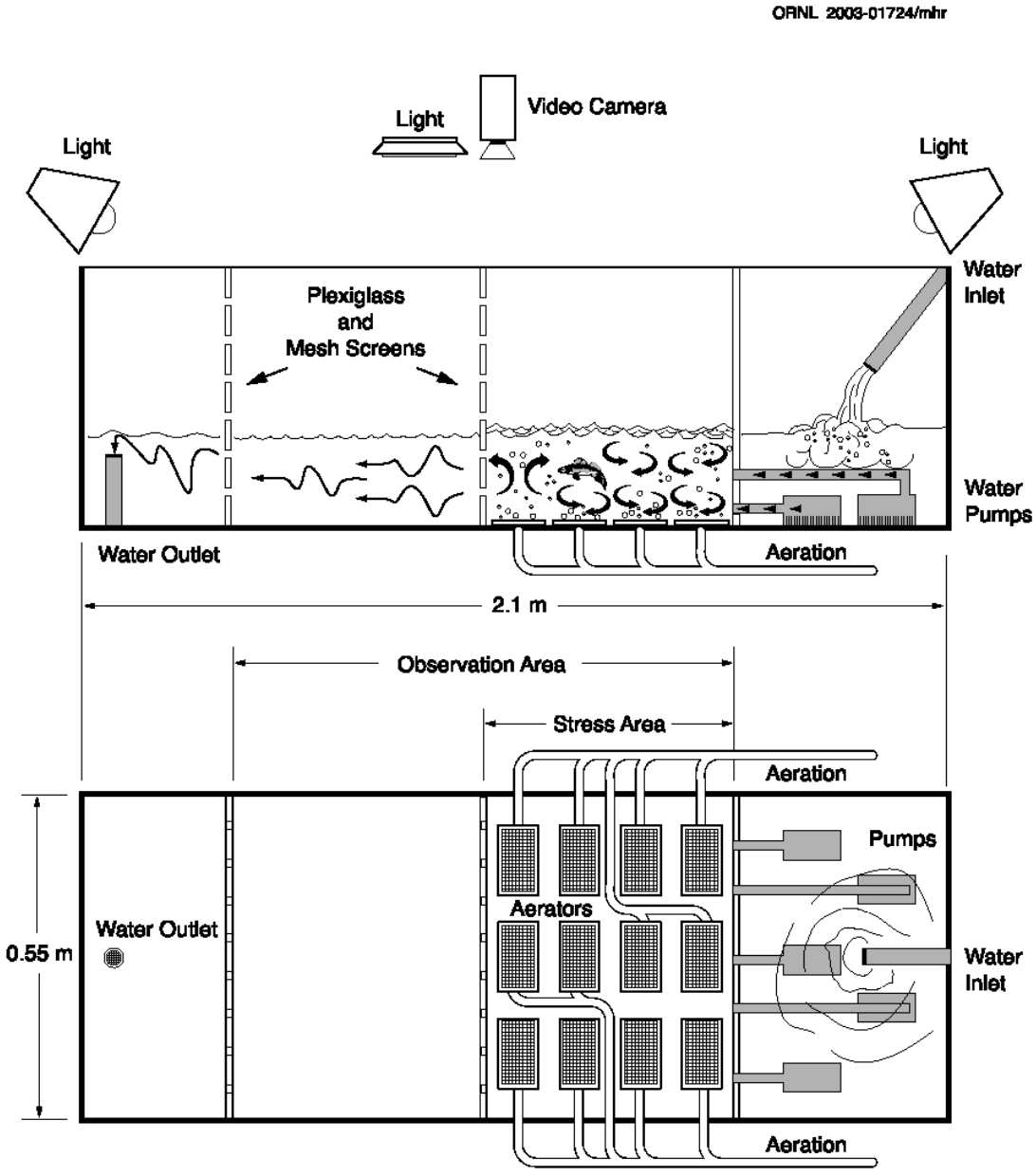


Fig. 2.2. Schematic diagram of observation tank showing turbulence stress area and observation area as seen from side view (top) and overhead (bottom).

2.1.4 High-Speed Camera and Light Conditions

Behavioral tests were recorded using a Photron Fastcam PCI®, black and white, high-speed camera fitted with a 12.5- to 75-mm zoom lens. Observations were digitally recorded at 500 fps and a shutter speed of 1/3000 s. Images were saved as .AVI files on a Dell PC with a maximum recording duration of 4.3 s. The observation arena was lit using a combination of four angled incandescent (100 W) bulbs in reflectors with an overhead tungsten light (1000 W). When fully lit, the observation arena had light levels at the water surface of 204 lux and underwater at the bottom of the tank of 111 lux. These levels are similar to natural sunlight levels, e.g. 350 to 600 lux measured at mid-day.

2.1.5 Stressors

The tests were conducted with two stressors, one chemical and one physical. A fish anesthetic tricaine methanesulfonate (MS-222) was used because of its known effects on fish swimming. MS-222 has a narcotic effect on fish that suppresses swimming and reaction abilities. High levels of turbulence were generated in the test tank to simulate real life stressors. The MS-222 was applied as an aqueous bath for 2 min at concentrations of 100 mg/L to fathead minnows and striped shiners and also at 200 mg/L to the striped shiners. The exposure rapidly produced a temporary loss of equilibrium in both species. For the turbulence test, a combination of submerged pumps and aeration was used to create chaotic water movements in the observation tank (see Fig. 2.1). On one side of the observation tank, five Little Giant®1/5 HP submerged pumps (500 gal/h capacity) were set up in an array, so that jets of water were directed across the arena. On the bottom of the arena 26 large flat air stones were supplied with up to 75 psi air pressure to create an upwelling in the test arena. Fish were exposed to turbulence created by the pumps and air stones at durations of 30, 20, or 10 min. Controls were fish placed in the observation tank but not exposed to either MS-222 or turbulence.

Velocity data for the observation tank were obtained with a 16 MHz acoustic-Doppler velocimeter (ADV). Although the response of the ADV to intense turbulence is not yet well understood, the ADV appears to have potential as an indexing instrument for turbulent flows. The data presented herein are a first attempt to characterize a complicated velocity field with an imperfect instrument—improvements to the sensor and the analysis techniques are anticipated.

The ADV infers fluid velocity by transmitting pulses of acoustic energy into the water and relying on small particles dispersed throughout the water to alter the phase of the reflected acoustic energy via the Doppler effect. Details of acoustic-Doppler physics and signal processing in turbulent flows may be found in several sources (see for example, Lohrmann et al., 1994 and McClelland and Nicholas, 2000). Velocity time series were collected at eight locations in the observation tank (Fig. 2.3). The measuring volume of the ADV was positioned at the mid-depth of 9 centimeters. Data were collected at a rate of 50 Hertz for 340 seconds, yielding a three-component velocity time series of 17,000 samples at each location. Results for only one of the tank quadrants (positions 3 and 7) are presented herein, as they are representative of the data obtained from the remaining three quadrants.

Air-water mixtures are challenging environments for acoustic-Doppler sensors because the measurement physics depend upon the acoustic wave speed, which is sensitive to the void fraction of the mixture. Measurements were made in the tank with and without the aerators in service to determine better the effect of air on the measurements. The aerated and non-aerated flows are different because of the extra energy imparted to the flow by the buoyancy and kinetic energy of the bubbles. However, some insight into the aerated flow may be gained by examining the more robust velocity time series obtained from the non-aerated flow.

The proximity of the well boundaries to the water jets produces a flow that is turbulent and three-dimensional, in terms of the mean flow and the turbulent velocity fluctuations. Excepting the flow in areas near the jet nozzles, there is no significant persistent direction for the time-averaged flow in the

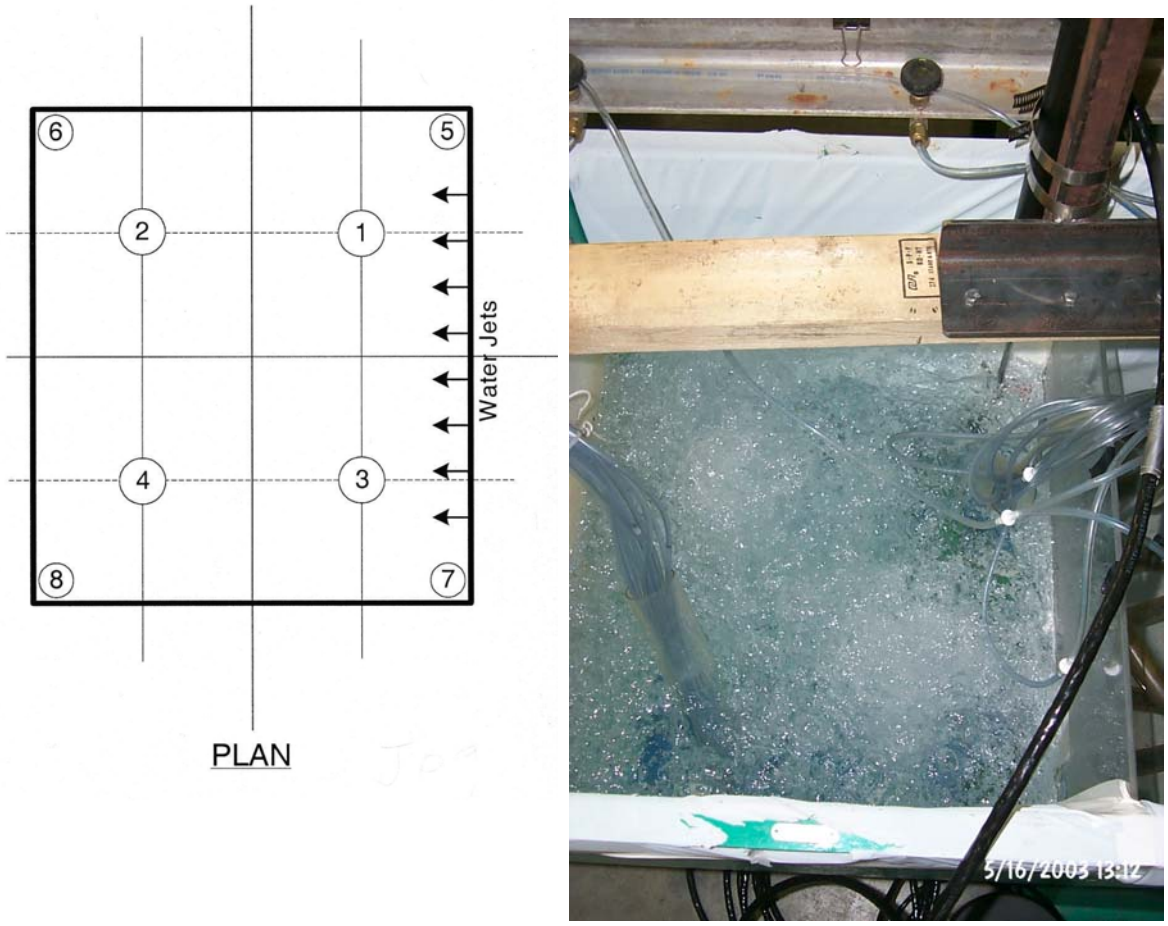


Fig. 2.3. Locations of velocity measurements (photograph shows ADV positioned at location 5).

tank. It is convenient, in the absence such direction, to analyze components of velocity aligned with the non-orthogonal bisecting angles of the ADV beams. The benefit of this coordinate system is that one can examine the turbulent fluctuations recorded at each acoustic receiver of the ADV. In this way, noise spikes and biases arising from the instrument physics are not obscured by transformation to a Cartesian coordinate system.

In Figs. 2.4 through 2.7, turbulent velocity data are analyzed and plotted through three mechanisms:

1. a histogram of velocities, in which the 17,000 samples are grouped into 0.5 cm/sec bins, that approximates the probability distribution function of the time series;
2. a power spectral density transformation of the time series, showing the energy present in the flow signal for a range of temporal frequencies; and
3. time series plots.

The data include erroneous spikes which, although most evident in the time series plots, affect all three analyses. The data presented in Figs. 2.4 through 2.7 have been filtered to eliminate velocity samples outside the range of $-60 < v < 60$ cm/sec. Samples thus rejected are almost exclusively single-sample spikes arising from non-physical causes. However, numerous spikes of smaller magnitude remain in the record. These spikes are symptomatic of the measurement challenge presented by the intense

small-scale turbulence in the tank. In the case of the two-phase bubbly flow created by the admission of air to the well, the occurrence of spikes threatens to obscure the fluid and bubble velocity signal completely. Although a discussion of the causes and effects of these subtle noise spikes in ADV data is beyond the scope of this report, sophisticated techniques for detecting and correcting noise spikes are evolving (see Goring and Nikora, 2002). The presence of the spikes affects quantitative measures of turbulence and will need to be addressed if flows are to be compared based on their turbulent velocity statistics.

Despite the difficulties that noise spikes present, qualitative conclusions can be made from the data. First, the data confirm the intuitive result that the corners of the well are less turbulent than the interior portions. Histograms from the corner location (Fig. 2.6) are narrow than those from the interior (Fig. 2.4). The decreased energy of the turbulent fluctuations at the corner location is evident in the power spectral density and the time series plots in Figs. 2.4 and 2.6. Second, data collected in the non-aerated flow exhibit the -5/3 power-lay decay of energy (note the dashed lines in the power spectral density pots) with increasing temporal frequency indicative of *inertial subrange*, a characteristic of equilibrium turbulent flows. This suggests that the ADV is capturing much of the turbulent flow dynamics, at least in the non-aerated flow. The power spectral densities computed from the ADV data begin to flatten at approximately 20 Hertz due to Doppler noise—related to the unavoidable broadening of the acoustic signal as it travels through the water and is reflected from numerous scatterers in the finite measuring volume.

2.1.6 Experimental Protocol

The experimental protocol used a range of stress levels and controls to evaluate the behavioral responses, but the basic design was the same for each test. Ten test fish were used at each exposure level, and tested individually. A test fish was transferred to the tank, allowed to acclimate for 30 min and testing was initiated. To initiate the startle reaction, a 46-cm length of metal rebar was struck against the upper edge of the tank, to create both a visual and a pressure-wave stimulus. The rod was positioned so that the camera would record its movement. For each fish, two pre-stress tests were conducted to serve as an individual control of the fish's reference behavior. After the pre-tests, fish were exposed to a stress (turbulence or anesthesia), and returned for post-stress testing. Startle reactions were filmed at 1, 5, 15, and 30-min post stress to determine whether fish startle reaction was affected, and whether recovery occurred with time. After each test, the video image was saved to an .AVI file; the duration of the image was often reduced to 1 or 2 s, if the fish showed limited reaction to the stimulus. Fish were used for only one test of stress exposure and then transferred to a post-experiment holding tank. With the acclimation, pre-stress video recording, exposure and post-stress recording steps, about 1.5 to 2 hr were required for each test.

2.1.7 Motion Analysis Software

The movements of individual test fish were analyzed both by a frame-by-frame visual review (0.002 secs per frame) by an observer and an automated review using the commercial Visual Fusion® software program (vers. 3.2) designed by Sanders-Reed of Boeing-SVS, Inc. The program manually or automatically track targets in images created using high speed cameras. For our preliminary experiments, target fish were manually tracked by identifying the most anterior point of the head and the most posterior extension of the tail of their darkened silhouette and measuring the changes in distance between these points as the escape behavior progressed.

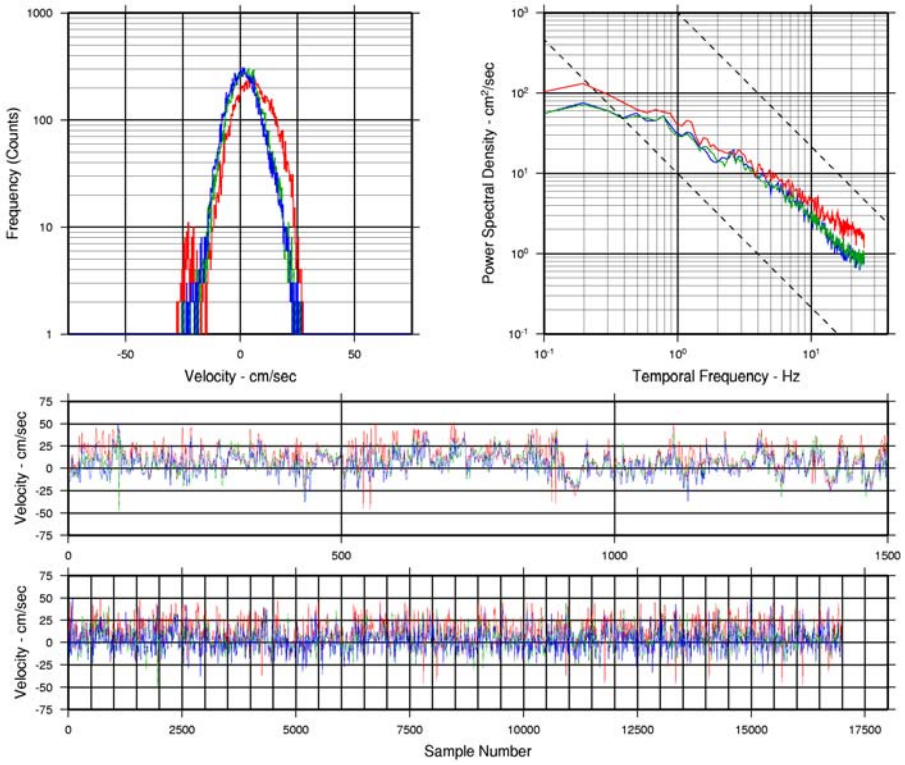


Fig. 2.4. Velocity data collected at location 4 without air admission.

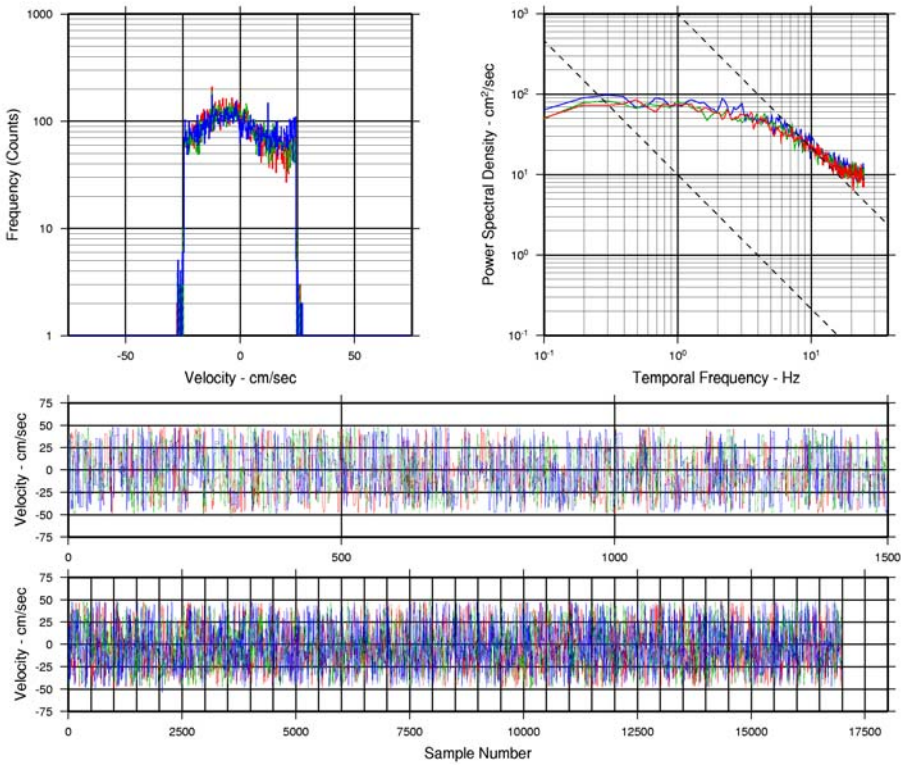


Fig. 2.5 Velocity data collected at location 4 with air admission.

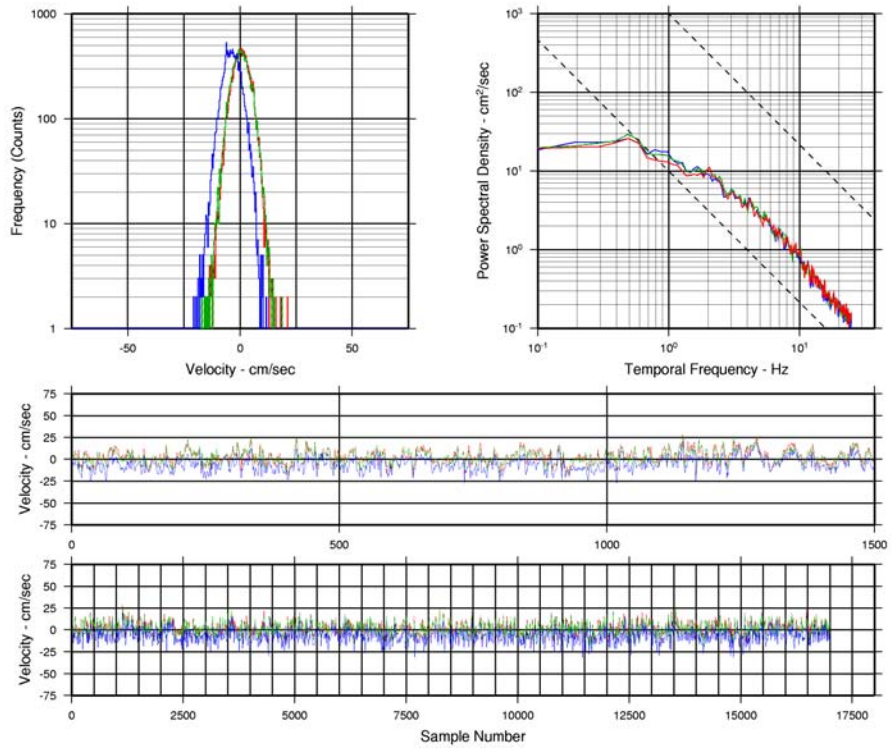


Fig. 2.6. Velocity data collected at location 7 without air admission.

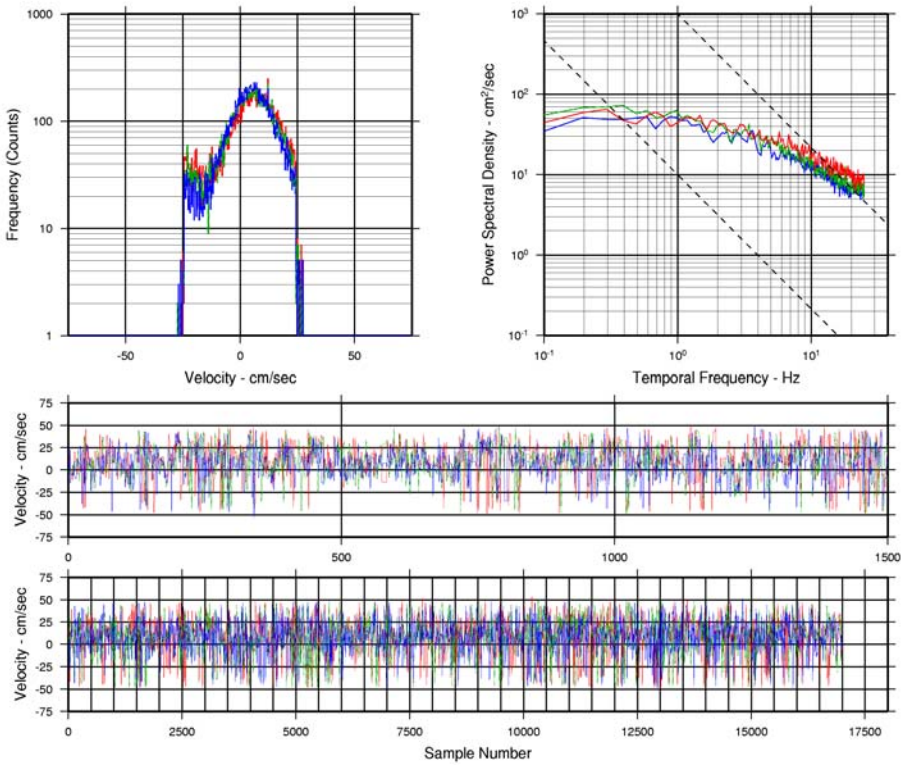


Fig. 2.7. Velocity data collected at location 7 with air admission.

2.1.8 Behavioral Measures Analyzed

A suite of response indicators was evaluated for each test. Although the overall test was based on the startle or escape behavior, there are many phases to this process and it is not necessary that the full complement of the startle response be demonstrated to differentiate between treatments. Table 2.1 displays the potential behavioral response indicators that were captured on video; not all of them are evaluated in this report.

Table 2.1 Behavioral response indicators evaluated for use in tests

Measurement parameter	How measured	Used in test
Presence/absence of response	Fish reacted suddenly and demonstrably to trigger or failed to register a reaction	Y
Time to first reaction	Difference between time when response occurred and trigger stimulus occurred	Y
Duration of reaction	Length of time fish displayed a continuous non-stop movement after trigger stimulus	Y
Acceleration of response	Rate of change in distance fish swam per second	N
Velocity of response	Distance fish swam per second for the first response	N
Angle of change from resting position	Degrees (0-360) that fish moved from resting position to first springing from C-shape	N
Distance moved in first response	Distance fish traveled after springing from C-shape until a pause in motion	N
Presence/absence of C-shape	Fish moved both head and tail to form some type of C-shape or failed to do so	N
Time to form maximum C-shape	Time from initial movement of head toward tail until the moment when the head and tail are closest to each other	Y
Time to end of C-shape behavior from the maximum C-shape	Time from point where head and tail are closest to each other until the moment when the head and tail are straightened out again	Y
Completeness of C-shape: ratio	Closest distance between head and tail divided by the total body length of the fish at rest	Y

The statistical methods used to analyze the data were chosen to answer the following questions:

A. Is there a significant effect of treatment (turbulence stress and anesthetic) on fish response as compared to control?
B. Which proposed measures of response are sensitive to treatment effects?
C. Do the fish become desensitized to the shock stimulus over time during the course of repeated tests?
D. How quickly do the fish recover from stress?
E. What portion of a fish's overall response is due to differences among fish and to variation within an individual fish?

2.1.9 Statistical Analysis

Not all fish responded to the trigger stimulus during testing. Non-response might have been the result of the severe effect of the treatment, the result of desensitization to the stimulus over time (habituation), or just because the fish chose not to respond. For some behavioral measurements, a zero for non-response would be appropriate, in that it indicates a lack of reaction. However, in some cases a zero would indicate a positive response to the stress, for example a zero for time to first response suggests the fish reacted very quickly to the startle trigger. Therefore, the data were transformed in some of the analyses to accommodate non-responding fish. Where appropriate the non-responding values were replaced by a value slightly larger than the maximum observed value, for example when we created graphical displays showing treatment effects. This allowed us to do approximate analyses using standard statistical techniques. A more direct approach to the problem of missing or non-responding data is to transform the data to ranks. For example, the time-to-first response data were ordered from smallest to largest, treating the no- response values as larger than all of the others. The ordered values then received their rank in the ordered sequence, ties receiving the average rank. The ranks then were analyzed using nonparametric statistical methods. Another transformation of each observation to "response" or "no response" permitted us to use other nonparametric techniques.

Graphical displays of the data were created to show the effects of stress on the various treatment groups. Box and whisker plots (boxplots for short) representing each of the different treatment groups provided a comparison of the overall range and the 25th, 50th (median), and 75th percentiles of the data. A boxplot is a rectangle (the "box"), the lower edge at the 25th percentile, the upper edge at the 75th percentile and a line cutting the rectangle at the median. Extending from either end are lines (the "whiskers") reaching to the extreme values (range) of the data for the treatment group.

The primary response/no response analysis used to assess treatment effects was a test for homogeneity of binomial samples (Snedecor and Cochran 1980). The data for treatment group i with x_i responders out of N_i total were displayed in a 2 by I table, where I is the number of treatments. If the treatments were all equally effective then the true proportion p_i of responders for the treatments would be the same. The Pearson's Chi-squared test was used to assess treatment effects at the various individual post-stress time intervals.

The Kruskal-Wallis test was the primary rank test used for testing treatment effects. This test can be thought of as a one way analysis of variance applied to data ranks. Large differences among the treatment mean rank values suggest significant treatment effects. Follow-up multiple comparisons of individual treatments to controls were investigated when the overall test of treatment effect was significant (Conover 1980).

To assess the effect of increased turbulence stress, we fit a linear model of fish behavioral response versus amount of time exposed to turbulence. A significant slope coefficient indicated a "dose" effect and reinforced the conclusion of significant treatment effect. This analysis was carried out at the 1-min post-stress period, where treatment effects were most evident.

Two other nonparametric analyses of ranks were performed to assess desensitization of the control, no treatment fish. Both tests take into account that the observations are correlated across post-stress times and can be thought of as nonparametric extensions of standard paired analyses like the paired t-test. The Quade test is an extension of the Wilcox on signed-rank test and the Friedman test is an extension of the nonparametric sign test [see Conover (1980), section 5.8]. One disadvantage of these two tests is that they require complete data, a response for each time category for each fish. Those with incomplete data are dropped from the analysis and if much of the data are discarded there may be some question as to the representativeness of the remaining data.

A generalization of the response/no response analysis called Cochran's Q was also used to test for fish desensitization. This generalization of the test for homogeneity of binomial proportions takes into account the fact that the responses within a fish are correlated. Large values of Cochran's Q are associated with situations where the proportions responding vary greatly among the time categories [see Conover (1980), section 4.6 or Fliess (1981), pp 126-128].

Similar analyses were performed to assess fish recovery after treatment by testing for differences among the time categories 3 through 6 for specific treatments. Provided that the effects of the treatment had been realized by the time the measurements are taken, significant differences among the time categories indicated recovery.

The first two time categories for all fish, for each fish species, provided estimates of the components of variance for the different response variables. We expressed the within-fish variance as a percentage of the total combined variance, within and among fish variance.

2.2 PREDATOR PREFERENCE TESTS

It has often been demonstrated in the laboratory that stressed fish that appear to be uninjured are nonetheless more susceptible to predators (Coutant 1973; Neitzel et al. 2000; review by Mesa et al. 1994). Such tests provide important information about the possible consequences of sublethal stresses. Whereas predator preference tests provide direct information about an impact of interest (increased loss of stressed fish to predation), they are difficult to conduct and have a high degree of artificiality.

It is useful to determine whether changes in escape behavior detected in the high-speed video camera might also be reflected in changes in predation in laboratory test tanks. Conversely, it is possible that subtle changes in behavior of sublethally stressed fish were not among the suite of escape behaviors we measured, but can nonetheless be detected by predators in a predation test. We conducted a series of conventional predator preference tests with turbulence-stressed or anaesthetized prey in order to determine whether susceptibility to predation was altered. Conducting both types of tests allowed a comparison of the relative sensitivities of the two techniques.

2.2.1 Test Fish and Holding Facilities

Prey fish were the same species used in the escape behavior tests, fathead minnows. They were obtained from the same sources and held in the laboratory under the same conditions. Adult largemouth bass (*Micropterus salmoides*) caught from local waterways were used as predators. After capture, the bass were observed daily for health conditions and conditioned to feed on live prey.

2.2.2 Experimental Protocol

Holding tanks were set up in the laboratory for the two largemouth bass predators (one per tank) and prey fish (Fig. 2.8). Predator tanks were circular, 1.2-m diameter Living Stream® tanks with approximately 500-600 L capacity, depending on the height of the standpipe. All tanks were supplied with flow-through dechlorinated water at a temperature of 18-22EC and were kept on an 8-hr daylight cycle with overhead flourescent lights. The tanks were isolated from unintended disturbances by a 7-ft privacy curtain.

Fathead minnows were fin-clipped to differentiate between test and control groups. All fish offered to predators had either the left or right pelvic fin clipped; location of the fin clips were randomly assigned between groups. Before testing began, the largemouth bass predators were allowed to acclimate to the laboratory for 5 days, and the fin-clipped fathead minnows for at least 3 days. At least 1 day elapsed between feeding tests.

A total of 20 prey fish (10 test and 10 controls) were offered to a largemouth bass predator. The 10 test fish were placed in the turbulence tank for 30 min to acclimate, and 10 control fish were placed in the nearby holding tank (identical except lacking turbulent water movements) for the same time. At the end of the acclimation period, the pumps and aeration were turned on to initiate the turbulence exposure (either 10-, 20-, or 30-min exposure). At the end of the turbulence exposure, the test fish were netted out and put in a transfer bucket. The control fish were also netted out of their tank and combined with the test fish in the transfer bucket.

Fig. 2.8. Photo of predator preference test tank with largemouth bass (*Micropterus salmoides*) predator and fathead minnow (*Pimephales promelas*) prey.

The combined test and control fish were poured into the large circular holding tank containing the largemouth bass. No cover was provided in the tank. The prey fish were allowed to stay in the predator tank for a maximum of 30 min, or until about ½ (10) had been eaten. At that point, the remaining minnows were netted out of the predator tank and examined for fin clips. Surviving prey were not used for additional tests.

An additional test using a fish anaesthetic (MS-222) as a source of sublethal stress was performed. Procedures were the same as for the turbulence tests, except that test fish were placed in a 5-gallon bucket containing a 100 mg/L solution of MS-222 for 2 min, then removed to a transfer bucket. Control fish were placed in a 5-gallon bucket containing plain water for 2 min before being placed in the same transfer bucket with the test fish. Procedures for determining predator preference between anaesthetized and control fish were the same as for turbulence-exposed fish.

2.2.3 Statistical Analyses

Comparisons of the number of stressed and control fish remaining at the end of the feeding period were made using a chi-square test. Our null hypothesis (H_o) was that predators would consume equal proportions of stressed and control prey. Five replicates were conducted for each of the 4 test conditions (10-, 20-, and 30-min exposure to turbulence; and 2-min exposure to anaesthetic). The chi-square test was applied to each replicate; with 1 degree of freedom, the null hypothesis was rejected at a chi-square value greater than 3.84 (α=0.05).

In addition, data from the 5 replicates of each test condition were combined for a heterogeneity chi-square analysis (Zar 1999). In this analysis, data were totaled (pooled) on the assumption that all five replicates came from the same population. The significance of the heterogeneity chi-square was tested to justify pooling the data; if the heterogeneity chi-square term is not significant, the replicates are considered to come from the same population and the data can be pooled. Yates correction for continuity (Lowry 2002) was applied to the pooled data.

3. RESULTS

3.1 ESCAPE BEHAVIOR TESTS

In general, the escape behavior tests were conducted smoothly. The overall test protocol and the experimental tank design allowed us to conduct many tests in a short time frame. Similarly, the camera and processing software worked virtually without fail. The few problems with the tests occurred partially as a result of this being the developmental phase of the overall test design. All of these were corrected and no systematic errors in the escape behavior tests were detected.

A total of 65 striped shiners and 50 fathead minnows were tested and evaluated for the six standard behavioral responses identified in Table 2.1. The summary data for these tests are presented in Appendix A. Tables A-1 and A-2 present data for all test fish and Tables A-3 and A-4 present data for only those fish that responded. One difficulty with interpreting the data was the wide variation in individual fish behavior over time. For most tests, approximately 77% of the variation in shiners and 100% for fathead minnows was due to within-fish variation. There was generally as much variation within an individual as there was variation among individuals in such measures as response / no response and time to first response.

3.1.1 Presence of a Response to Trigger

Table 3.1 displays the numbers of striped shiners that responded to the startle stimulus at different times and under different stress conditions. Most shiners were startled by the two pre-test stimuli (i.e., before any stresses were applied): a total of 54 out of 58 shiners exhibited escape behavior in the first pre-test series, and 52 out of 59 in the second pre-test series. In addition, nearly all control fish that were not exposed to turbulence or anaesthesia (0 min exposure to stress) were startled by the stimulus in the subsequent 1-min post-test series. However, some of the unstressed shiners appeared to become habituated to the startle stimulus: 4 of 10, 4 of 10, and 3 of 10 unstressed shiners ceased to react to the startle stimulus in the 5-min, 15-min, and 30-min post-test series, respectively (Table 3.1). Habituation is the relatively permanent reduction or elimination of a response in the absence of any overt punishment. In this case, the shiners may have learned that the periodic rapping on the side of the tank did not harm them and as a result stopped responding (or reduced the strength of their response).

Application of turbulence or anaesthesia generally decreased the numbers of fish that reacted to the stimulus (Table 3.1). For example, more than half of the test fish exposed to 10 min of turbulence did not exhibit escape behavior at 5, 15, and 30 min after the test. Similarly, most striped shiners given a high dose of MS-222 did not react to the stimulus, although there was an indication that fish had begun to recover by 30 min after the exposure. As with unstressed control fish, there is a possibility that some of the stressed fish may not have exhibited escape behavior in the post-test series simply because they had become habituated to the stimulus. However, many anaesthetized or turbulence-stressed fish appeared lethargic. It appears that the anaesthetic had a greater effect than turbulence in reducing the number of shiners that exhibited escape behavior.

Pearson's Chi-squared tests were applied to the counts in Table 3.1 in order to test the independence of treatment and reaction. Only the 1-min post-test series showed a statistically significant difference among treatments; nearly all of the anaesthetized striped shiners failed to react to the stimulus, compared to smaller proportions of turbulence-stressed fish that showed no reaction. These statistical tests indicate that for most time periods, turbulence and anaesthesia had similar effects on the ability of a startling stimulus to elicit a reaction among striped shiners.

Table 3.1. Pearson's Chi-squared tests of the independence of treatment and reaction for striped shiners exposed to turbulence or MS-222 fish anaesthetic

Values are the number of fish that responded to the startle stimulus

Response	0 min exposure to stress	10 min turbulence exposure	20 min turbulence exposure	30 min turbulence exposure	200 mg/L MS-222 exposure	100 mg/L MS-222 exposure	Row total	Statistical values
1st Pre-test Startle Stimulus								
No reaction	1	0	1	0	2	0	4	χ^2=7.13 df=5
Reaction	10	9	6	14	6	9	54	
Column total	11	9	7	14	8	9	58	p=0.211
2nd Pre-test Startle Stimulus								
No reaction	0	2	0	1	1	1	5	χ^2=4.24 df=5
Reaction	8	6	8	11	8	11	52	
Column total	8	8	8	12	9	12	57	p=0.5.16
1-Min Post-Test Startle Stimulus								
No reaction	1	0	2	7	11	11	32	χ^2=36.7 df=5
Reaction	10	8	6	6	0	1	31	
Column total	11	8	8	13	11	12	63	p=<0.001
5-Min Post-Test Startle Stimulus								
No reaction	4	5	3	7	9	10	38	χ^2=8.56 df=5
Reaction	6	4	5	6	2	2	25	
Column total	10	9	8	13	11	12	63	p=0.128
15-Min Post-Test Startle Stimulus								
No reaction	4	5	6	5	9	4	33	χ^2=10.03 df=5
Reaction	6	4	2	8	1	7	28	
Column Total	10	9	8	13	10	11	61	p=0.073
30-Min Post-Test Startle Stimulus								
No reaction	3	5	3	7	2	4	24	χ^2=3.81 df=5
Reaction	7	3	4	6	6	7	33	
Column Total	10	8	7	13	8	11	57	p=0.577

The fathead minnows responded very well to the trigger before the stressor was applied (Table 3.2), with more than 95% responding. After exposure to the stressor, the percentage of responders dropped by ten-fold, particularly for the 1- and 5- min tests. Most of the non-responders were in the medium MS-222 exposure.

For the turbulence tests, the number of non-responding fathead minnows was about the same for each duration of exposure. Overall the number of non-responding stressed fish was much lower in the fathead minnows than in the striped shiners.

Table 3.2 Pearson's Chi-squared tests of the independence of treatment and reaction for fathead minnows exposed to turbulence or MS-222 fish anaesthetic

Values are the number of fish that responded to the startle stimulus

Response	0 min exposure to stress	10 min turbulence exposure	20 min turbulence exposure	30 min turbulence exposure	100 mg/L MS-222 exposure	Row total	Statistical values
1st Pre-test Startle Stimulus							
No reaction	0	0	1	0	0	1	χ^2=3.64 df=4
Reaction	10	9	8	5	8	41	
Column total	10	9	9	5	8	42	p=0.456
2nd Pre-test Startle Stimulus							
No reaction	0	1	0	0	1	2	χ^2=3.32 df=4
Reaction	10	9	10	8	7	44	
Column total	10	10	10	8	8	46	p=0.506
1-Min Post-Test Startle Stimulus							
No reaction	3	1	2	2	9	17	χ^2=20.8 df=4
Reaction	8	8	7	8	0	31	
Column total	11	9	9	10	9	48	p=<0.001
5-Min Post-Test Startle Stimulus							
No reaction	1	2	0	2	7	12	χ^2=21.9 df=4
Reaction	9	7	7	6	0	29	
Column total	10	9	7	8	7	41	p=<0.001
15-Min Post-Test Startle Stimulus							
No reaction	0	2	1	3	1	7	χ^2=3.89 df=4
Reaction	10	8	8	7	7	40	
Column Total	10	10	9	10	8	42	p=0.421
30-Min Post-Test Startle Stimulus							
No reaction	4	1	1	2	2	10	χ^2=4.08 df=4
Reaction	5	9	8	6	6	34	
Column Total	9	10	9	8	8	44	p=0.396

Pearson's Chi-squared tests were applied to the counts in Table 3.2 in order to test the independence of treatment and reaction. At the 1-min and 5-min post-test series, a statistically significant difference was seen among treatments; all of the anaesthetized fathead minnows failed to react to the stimulus, compared to smaller proportions of turbulence-stressed fish that showed no reaction.

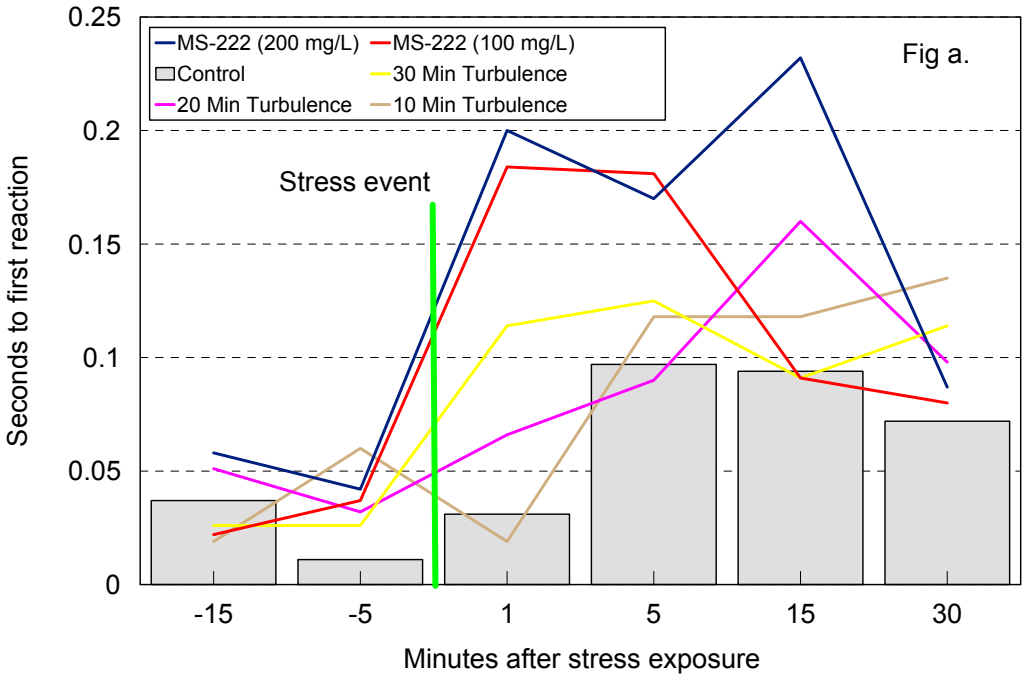
3.1.2 Time to First Reaction

If a stressed fish is slow to react to a stimulus, even if it does react, it may not be able to escape an attack by a predator. Sublethal stresses may increase the time to first reaction following a startling stimulus. In our study, this parameter was easy to measure and it occurred consistently in control fish. For most fish, the first response was triggered by the sound - pressure wave associated with the bar hitting the tank, but some fish picked up the motion cue of the bars movement toward the tank, and reacted prior to the strike. This suggests that the first response is a flight-based behavior, as it was triggered by more than one type of cue. Also, only in rare cases did the first response not progress rapidly into a full C-shape behavior. When fish did not respond at all to the stimuli, then a standard value of 0.2 s was used as a maximum, even though a longer period may have been filmed without any movement. This period of time was judged to be sufficiently long enough to indicate a delay in response and in a real predation situation allow a predator to capture the prey.

The pattern seen for this measure in striped shiners was a rapid response by all treatment groups, including controls, in the two pre-stress periods (Table A-1). After stress the response was generally much slower for the MS-222 and turbulence groups, with an indication of a dose-response pattern (Fig. 3.1a). The greatest delay in response occurred at 1-min post-stress, but some effect was indicated for the 5- and 15-min periods, as well. The controls continued to respond
quickly (<0.1 s), although their times gradually increased over the test cycle. Because of the wide variation between individual fish, the 1-min period was statistically significant ($p<0.05$) only for the MS-222 treatments and the 30-min turbulence exposure. After 5-min, only the MS-222 exposures were significantly different than controls (Table A.1).

To account for the individual variation, these data are corrected for self controls, i.e., the times to first reaction for each fish in the two pre-test periods are subtracted from the time to first reaction of that same fish after the stress had been applied (Fig. 3.2a). In Fig. 3.2, the x-axis shows controls (00), 10 min-turbulence (10), 20 min-turbulence (20), 30 min-turbulence (30), and MS-222 dose(s) (M = 100 mg/L; H = 200 mg/L) for the time periods after stress (3 = 1 min post-exposure, 4 = 5 min-post exposure, 5 = 15 min-post exposure, and 6 = 30 min-post exposure). If the time to first reaction does not change as a result of the stress, then the corrected value displayed in Fig. 3.2 is zero. On the other hand, if the stress causes a delay in the escape response, the corrected time to first reaction will have a positive value. Most striped shiners did not show a change in time to first reaction following the stress. Values for fish that received a high dose of MS-222 anaesthetic were above the 0.0 line in the 5- and 15-min post-test time periods, but these numbers represent only 2 and 1 fish, respectively, that reacted to the stimulus. All the other fish in this test condition did not display an escape behavior.

The pattern of time to first reaction for the fathead minnow was less consistent. Once again, all treatment and control groups responded quickly in pre-stress tests (Fig. 3.1b). After the stress exposure all groups had a delay in response, but so did the control group. The MS-222 test group was the most delayed and was statistically significantly different than controls ($p<0.01$) at the 1-min and 5-min post-exposure periods. The turbulence-exposed fish did not exhibit a statistically significant dose response. The patterns for fathead minnows remained the same when corrected for individual variations; essentially the turbulence had minimal effects on this parameter for this species (Fig. 3.2b).



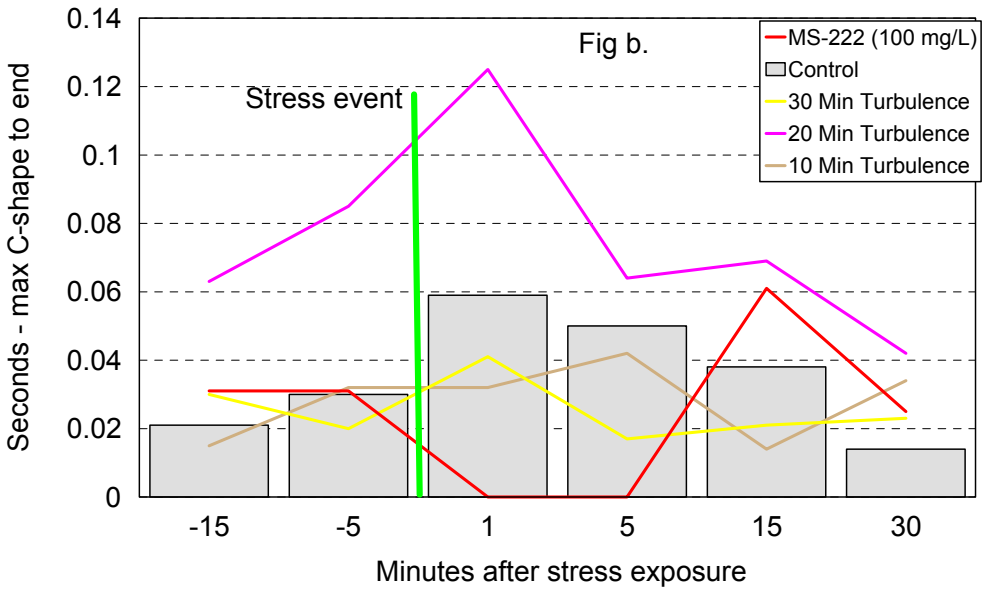
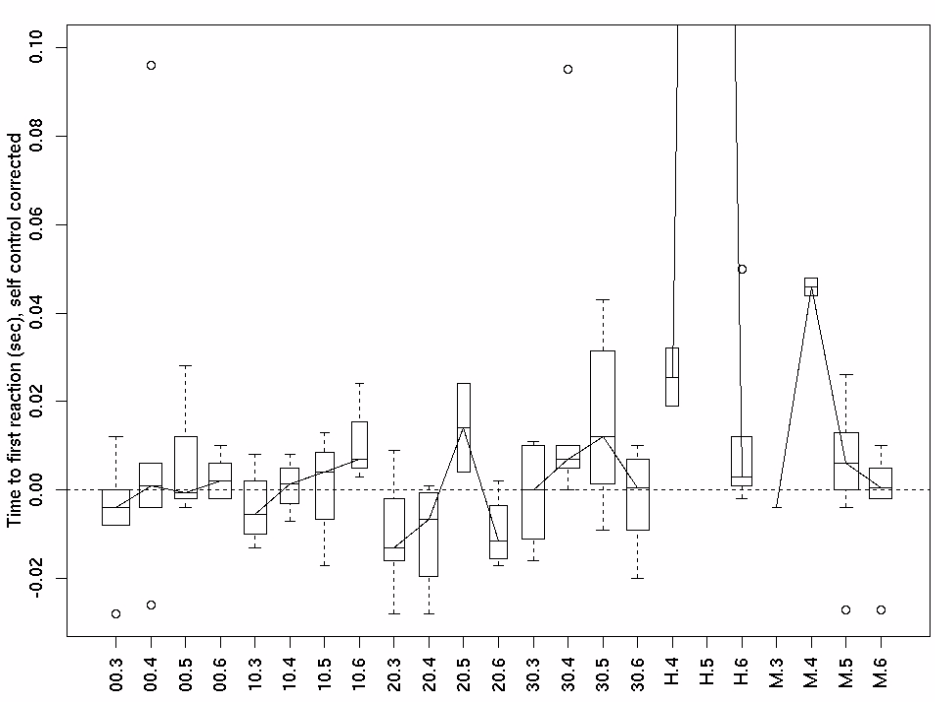


Fig. 3.1. Mean time to first reaction as affected by exposure to turbulence or tricaine methanesulfonate (MS-222), using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*) .

(a)



(b)

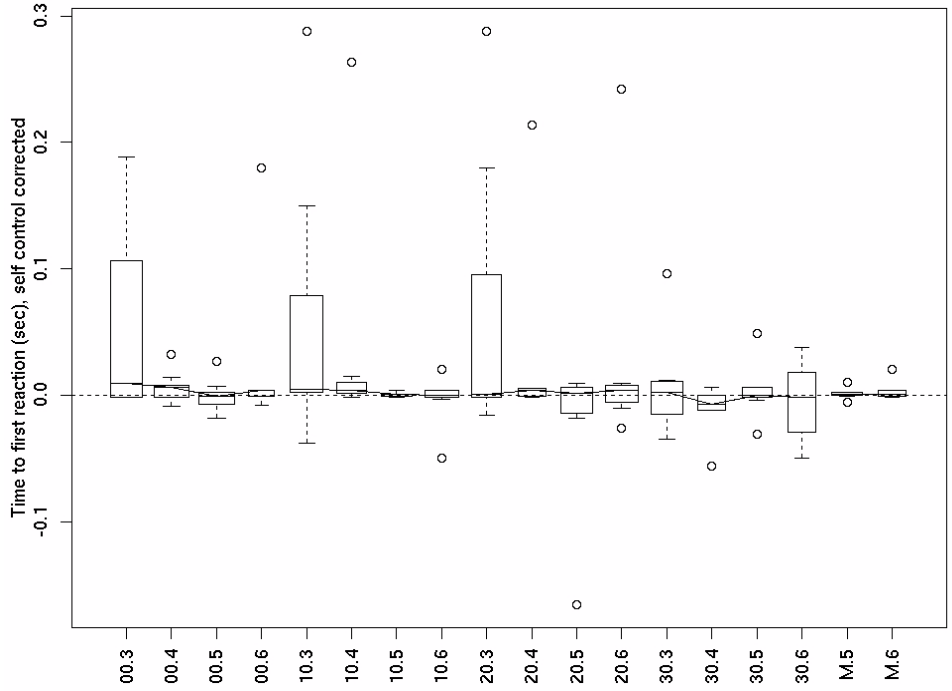


Fig. 3.2. Time to first reaction, adjusted for individual variation, as affected by exposure to turbulence (00, 10, 20, 30 min) or tricaine methanesulfonate (M, 100 mg/L) using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*). For each treatment, time post-exposure (1, 5, 15, or 30 min) is represented by a decimal (.3, .4, .5, or .6, respectively). The box represents the 25th to 75th percentiles, the line is the median, and the whiskers/dots represent the extreme data values.

3.1.3 Duration of Reaction

A stressed fish that stops its escape behavior too soon may still be captured by an attacking predator. A sublethal stress should not decrease the duration of reaction and perhaps, an increased duration of reaction may be protective. For our study, the duration response was another easily measured parameter that seemed fairly uniform in magnitude. The most difficult aspect of the parameter was delineating when the movement was complete. We applied a standard that the duration of the first response incorporated all movements, including a C-shape, until the fish began to coast, without power swimming. For fish that failed to respond at all to the stimuli, a default duration of zero was used.

Striped shiners showed a dose-related pattern of response to turbulence and MS-222, with a shorter duration of reaction for treatment groups compared to controls (Fig. 3.3a). The pre-stress tests all had extended motion periods, lasting from 0.1 to 0.3 s. After stress exposure, duration of reaction declined for the 1-min post-stress in all exposed groups, but increased for the controls. In the later test periods (i.e., $\geq$ 5 min), all groups were much more similar. For the 1-min post-stress test, there were statistically significant ($p < 0.05$) responses for the MS-222, 30-min turbulence, and 20-min turbulence groups (Table A-1). The response patterns were not significant at later post-exposure time periods.

When these data were corrected for Individual variation in response (self controls) by subtracting the durations of reaction for each fish in the two pre-test periods from the duration of the reaction of that same fish after the stress had been applied, there appeared to be little influence of turbulence on the durations of reaction (Fig. 3.4a). Most values were near the 0.0 line, indicating little change from pre-test values. Some of the striped shiners that were exposed to 20 or 30 min of turbulence or to a high dose of anaesthetic appeared to have shorter reaction durations, i.e., they stopped darting around the tank sooner in the post-test than in the pre-test series. There was no indication of habituation in this parameter. Fish that reacted tended to continue the escape behavior for the same amount of time, even at the sixth test series.

There was no apparent pattern in duration of response for fathead minnows (Fig. 3.3b). The duration was reduced significantly for the MS-222 exposed fish at 1-min and 5-min post-exposure (Table A-2), but mainly because most fish failed to respond. There was a dose-response pattern for turbulence exposures at the 1-min post-stress period, but the control fish also demonstrated a reduced duration at this time as well, so the pattern was not statistically significant. The lack of a consistent dose-response for turbulence was evident in the boxplots for self-adjusted data as well (Fig. 3.4b).

3.1.4 Time to Maximum C-Shape Formation

A key element of escape behavior is the speed at which a startled prey can form into a C-shape and spring out again. If a sublethal stress increases the amount of time a fish takes to reach the maximum C-shape, the fish may not be able to escape the attacking predator. In analyzing the video, it was easy to delineate the start and maximum C-shape positions in both control and treatment fish. The C-shape formation behavior was also very similar between the striped shiner and the fathead minnow.

Figure 3.5a displays the time (seconds) to formation of the maximum C-shape for striped shiners under each of the test conditions and post-test time periods. The most notable difference was the large variation in time to maximum C-shape formation associated with the 200 mg/L dose of MS-222. This variation was greatly influenced by the lack of a response of many anaesthetized fish, which resulted in wide changes in mean values calculated from small numbers of reacting fish. At the 1-min post-exposure period, the changes were statistically significant ($p<0.05$) for both MS-222 exposure levels and for the 20-min and 30-min turbulence exposures. The effects were short-lived, so that by the 5-min post-exposure test no changes were significant.

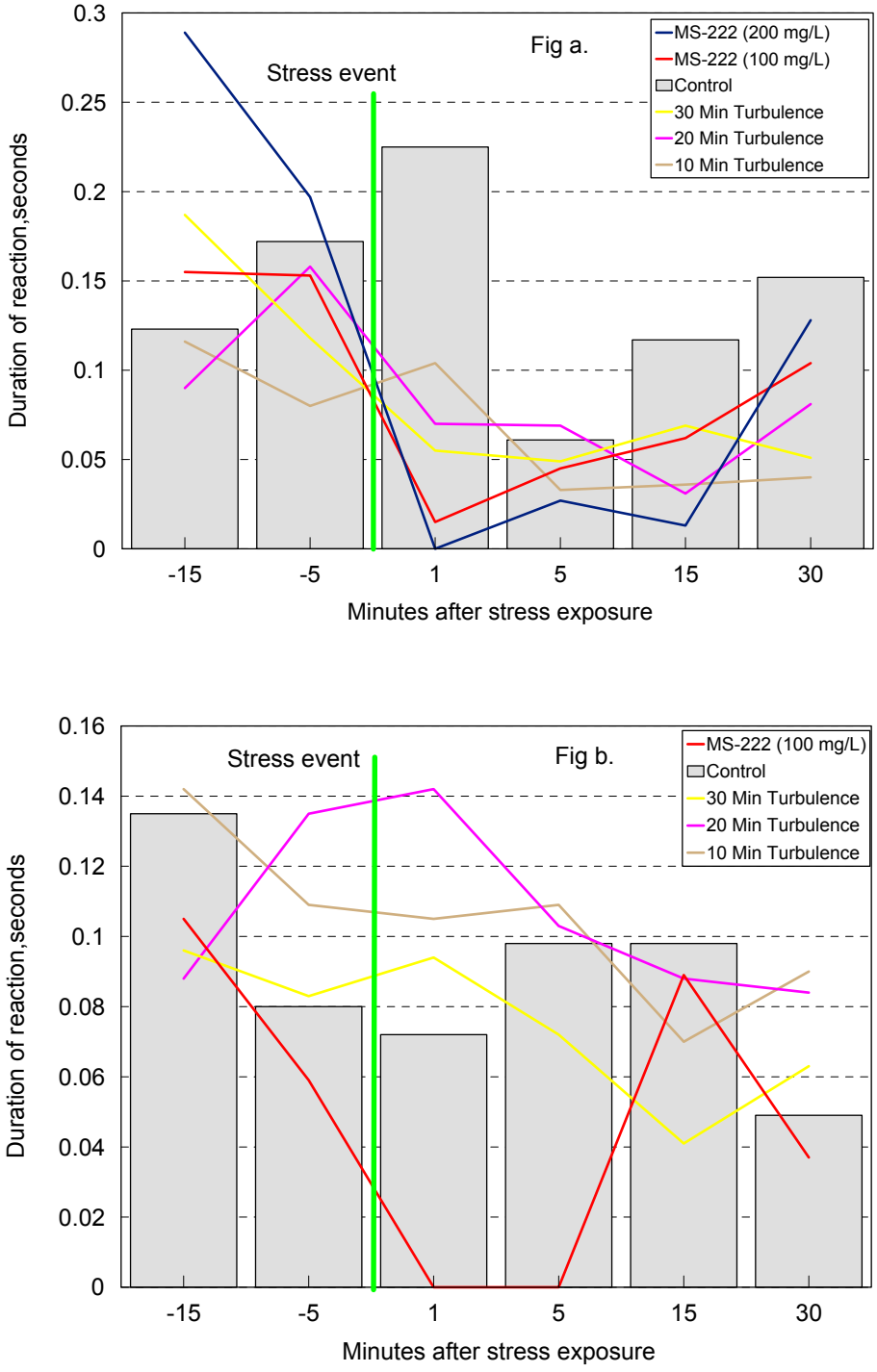
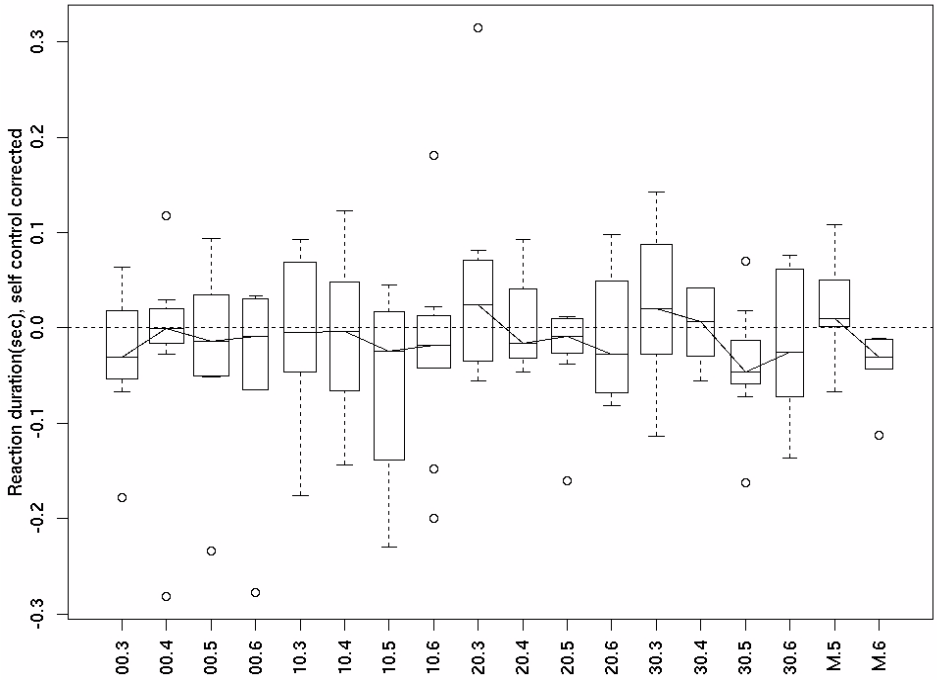


Fig. 3.3. Mean duration of reaction as affected by exposure to turbulence or tricaine methanesulfonate (MS-222) using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*).

(a)



(b)

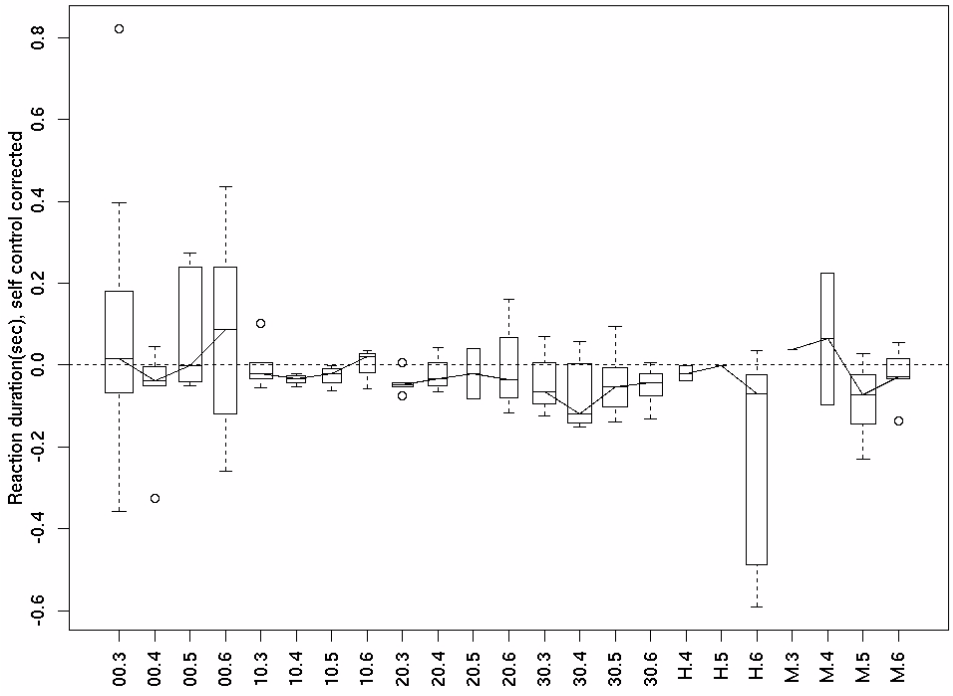


Fig. 3.4. Duration of reaction, adjusted for individual variation, as affected by exposure to turbulence (00, 10, 20, 30 min) or tricaine methanesulfonate (M, 100 mg/L) using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*). For each treatment, time post-exposure (1, 5, 15, or 30 min) is represented by a decimal (.3, .4, .5, or .6, respectively). The box represents the 25th to 75th percentiles, the line is the median, and the whiskers/dots represent the extreme data values.

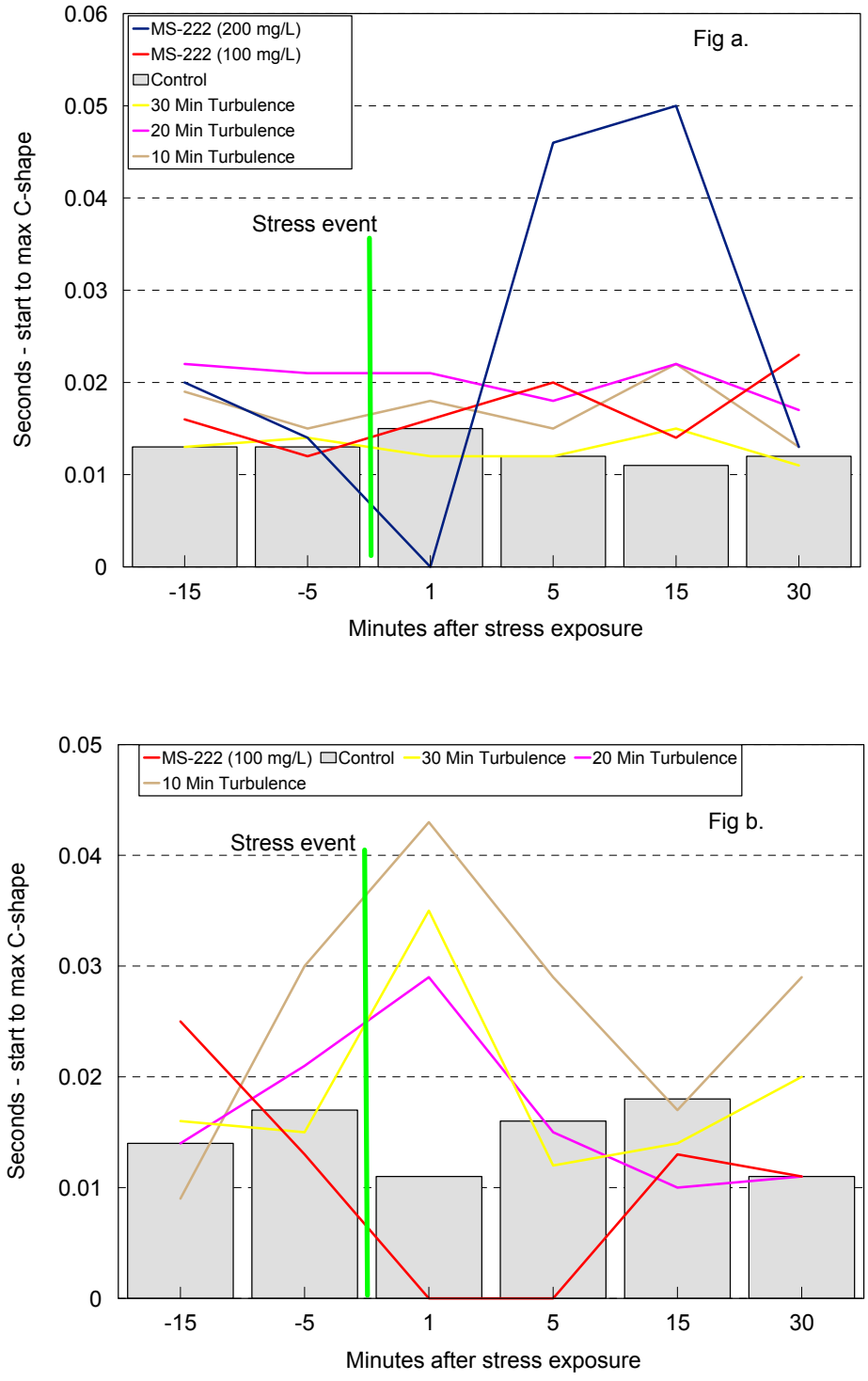


Fig. 3.5. Mean time to form maximum C-shape as affected by exposure to turbulence or tricaine methanesulfonate (MS-222) using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*).

This minimal response pattern was also seen for the data adjusted for self control (Fig. 3.6a). There appeared to be little influence of turbulence on the times to maximum C-shape formation. Most values were near the 0.0 line, indicating little change from pre-test values. Anaesthetized shiners may have taken longer to reach the maximum C-shape in the first 1- and 5-min post-tests, but observations are based on small numbers of fish because most anaesthetized shiners did not react at all. There was no indication of habituation in this parameter, i.e., the times did not increase or decrease in response to repeated startling stimuli.

The patterns for fathead minnow suggested that the turbulence-exposed fish took longer to reach the maximum C-shape than did the controls (Fig. 3.5b). However, this was not a dose-response pattern, only appeared at the 1-min post-exposure period, and was not statistically significant. The impact of the MS-222 exposure was significant ($p<0.05$) for both the 1-min and 5-min post-exposure periods (Table A-2). When the data were adjusted for individual variations (Fig. 3.6b), a similar response pattern was seen. There was no indication of habituation in this parameter, i.e., the times did not increase or decrease in response to repeated startling stimuli.

The patterns for fathead minnow suggested that the turbulence-exposed fish took longer to reach the maximum C-shape than did the controls (Fig. 3.5b). However, this was not a dose-response pattern, only appeared at the 1-min post-exposure period, and was not statistically significant. The impact of the MS-222 exposure was significant ($p<0.05$) for both the 1-min and 5-min post-exposure periods (Table A-2). When the data were adjusted for individual variations (Fig. 3.6b), a similar response pattern was seen. There was no indication of habituation in this parameter, i.e., the times did not increase or decrease in response to repeated startling stimuli.

3.1.5 Time from Maximum C-Shape to End of Reaction

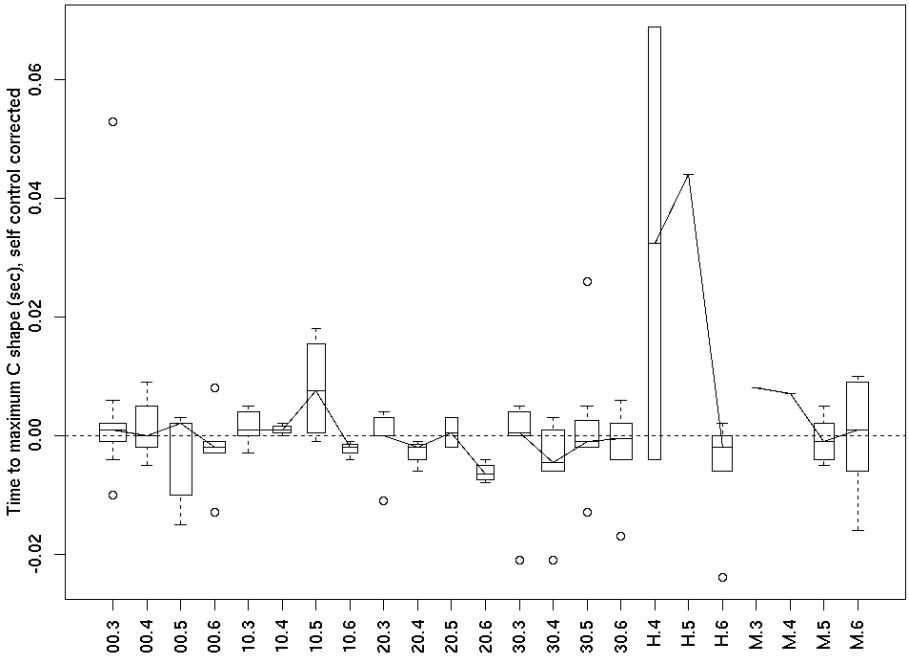
A key element of escape behavior is the speed at which a startled prey can form into a C-shape and spring out again. Once a fish had reached the maximum C-shape, it can spring out and even change direction of escape. If a sublethal stress increases the amount of time a fish takes to react out of the maximum C-shape, the fish may not be able to escape the attacking predator. As with other measures of the initial C-shape, this measure is fairly discrete and easy to determine. It appears to be uniform in both control and treatment groups.

Figure 3.7a displays the time (seconds) it took a striped shiner to straighten out its body from the maximum C-shape under each of the test conditions and post-test time periods. The pattern for this second phase of the C-shape process is very similar to that seen for the start to maximum phase. At 1-min post-exposure, the MS-222 exposures, the 20-min, and the 30-min exposures were all statistically significantly different than control (Table A-1). However the impact was limited; at the 5-min post-exposure none of the treatments was significant.

This minimal response pattern was also seen for the data adjusted for self control (Fig. 3.8a). There appeared to be little influence of turbulence on the times from maximum C-shape formation. Most values were near the 0.0 line, indicating little change from pre-test values. Anaesthetized shiners may have taken longer to uncoil from the maximum C-shape in the first 1- and 5-min post-tests, but observations are based on small numbers of fish because most anaesthetized shiners did not react at all. There was no indication of habituation in this parameter, i.e., the times did not increase or decrease in response to repeated startling stimuli.

The pattern (Fig. 3.7b) of response for fathead minnows was generally the same pattern found for the "start of reaction to maximum C-shape formation" paramater. The effects of the MS-222 dose were statistically significant ($p<0.05$) at both the 1-min and 5-min post-exposure periods, but were not in later periods (Table A-2). When adjusted for self-controls (Fig. 3.8b), the patterns did not differ much from zero, indicating the impacts were not significant.

(a)



(b)

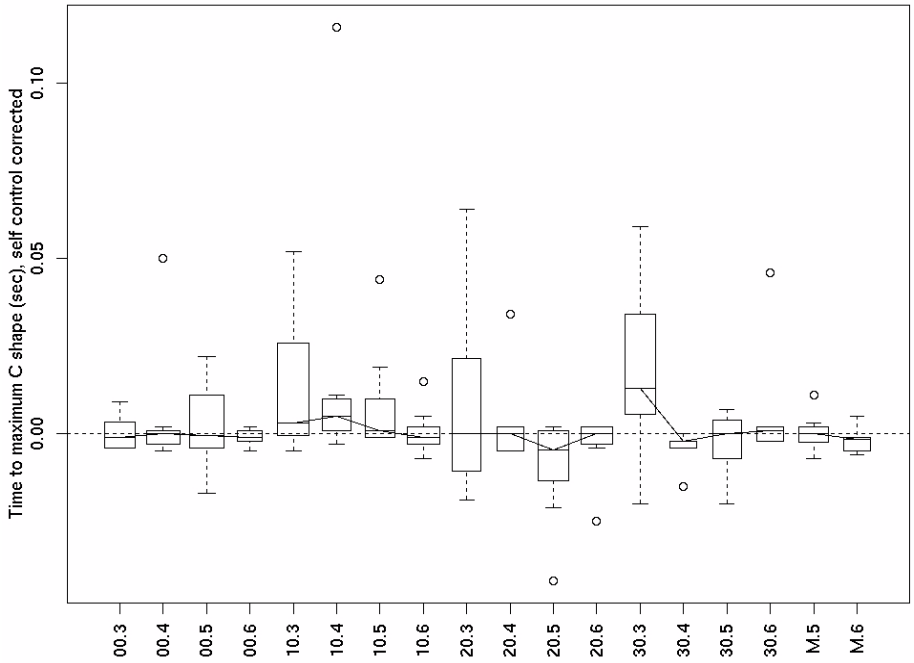
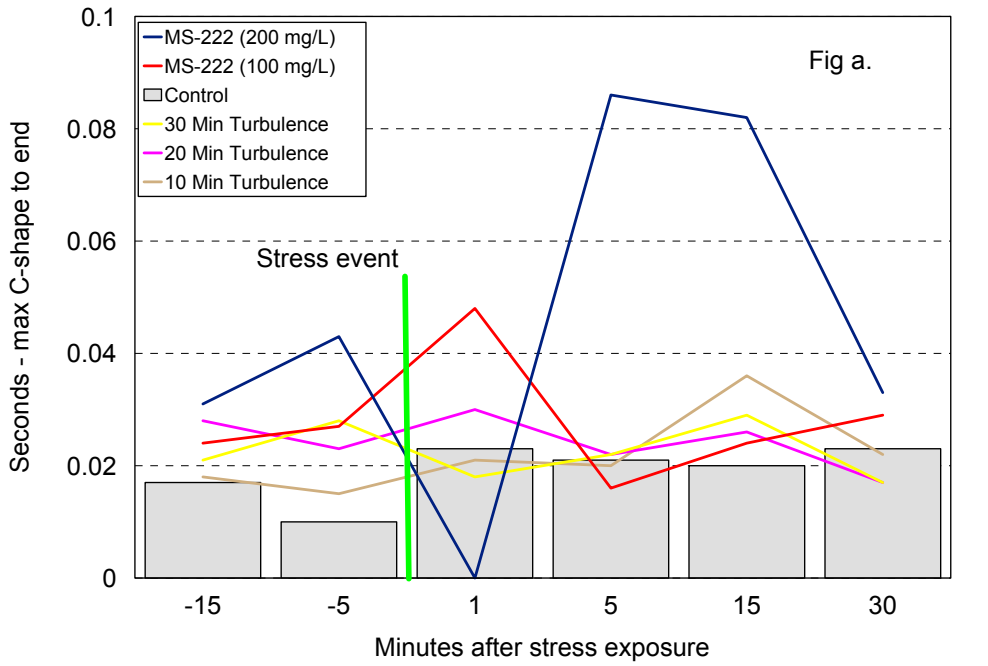


Fig. 3.6. Time to form maximum C-shape, adjusted for individual variation, as affected by exposure to turbulence (00, 10, 20, 30 min) or tricaine methanesulfonate (M, 100 mg/L) using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*). For each treatment, time post-exposure (1, 5, 15, or 30 min) is represented by a decimal (.3, .4, .5, or .6, respectively). The box represents the 25th to 75th percentiles, the line is the median, and the whiskers/dots represent the extreme data values.



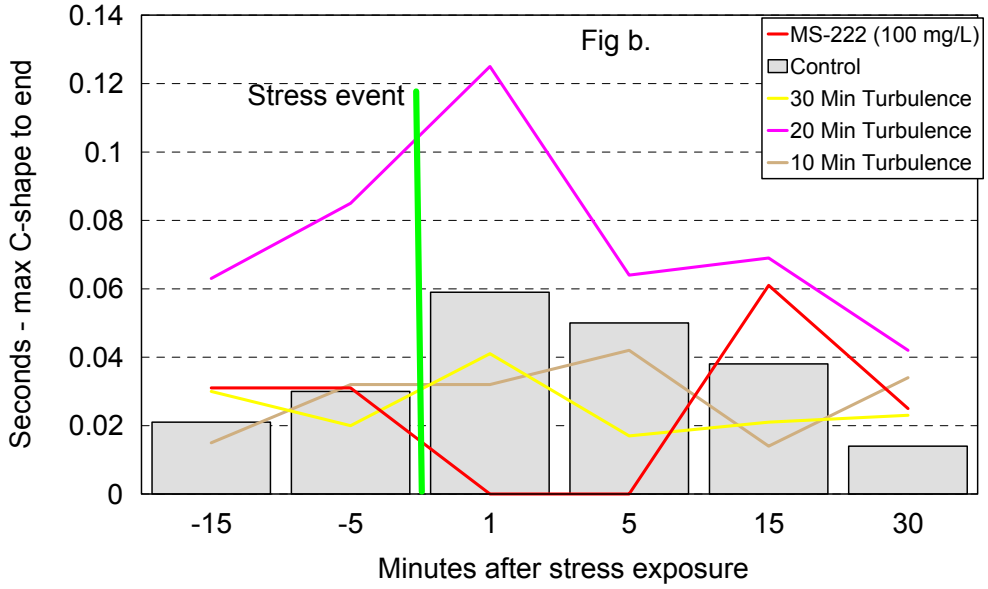
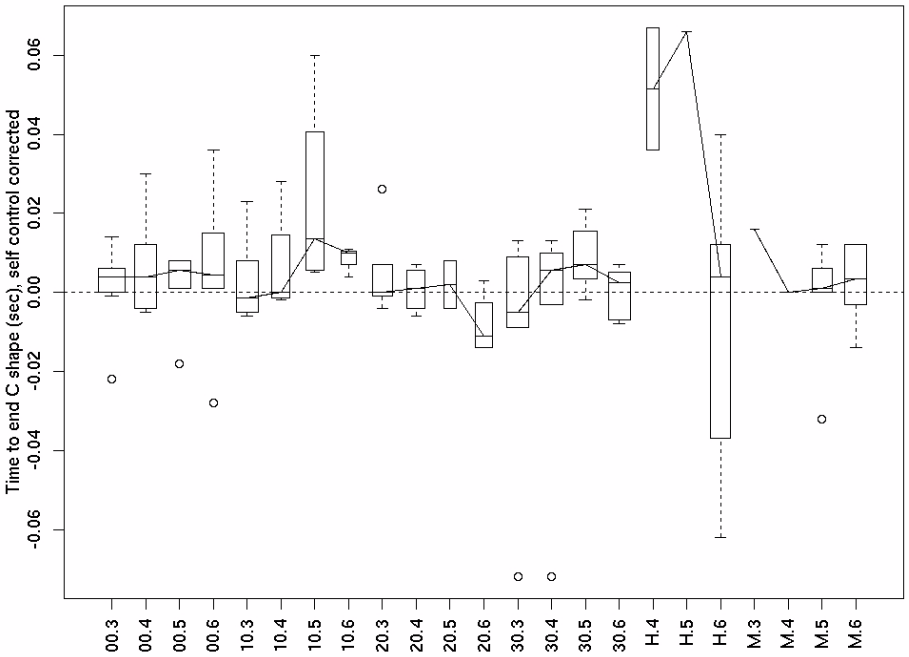


Fig. 3.7. Mean time from maximum C-shape to end of reaction as affected by exposure to turbulence or tricaine methanesulfonate (MS-222) using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*).

(a)



(b)

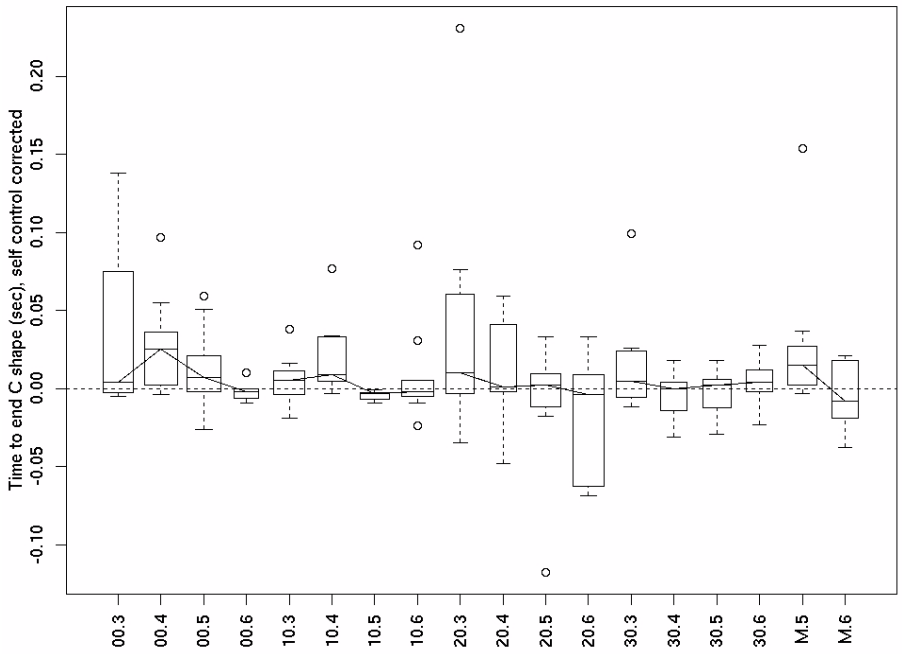


Fig. 3.8. Time from maximum C-shape to end of reaction, adjusted for individual variation, as affected by exposure to turbulence (00, 10, 20, 30 min) or tricaine methanesulfonate (M, 100mg/L) using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*). For each treatment, time post-exposure (1, 5, 15, or 30 min) is represented by a decimal (.3, .4, .5, or .6, respectively). The box represents the 25th to 75th percentiles, the line is the median, and the whiskers/dots represent the extreme data values.

3.1.6 Completeness of C-Shape Formation

The completeness of the C-shape has an influence on the speed of the prey's escape behavior. If a fish forms a strong C-shape, it will have more power and velocity when it springs out again than a fish that only flexes a small amount before swimming away. That is, if a sublethal stress reduces the completeness of the C-shape, the startled prey fish may not have enough propulsive power (speed) to escape an attack. Also, a more complete C-shape will allow the prey to choose several directions in which to escape. As the fish springs out it can alter the path of escape so it may change its heading by 90E. We expressed completeness of C-shape as the ratio of the head-to-tail distance at the point of maximum C-shape formation to the total length of the fish. The ratio could range from 1.0 (fish does not bend at all, so the head-to-tail distance is the same as the total length) to 0.0 (the head and tail touch at the peak of the C-shape formation, so that the head-to-tail distance is zero). We assume that the smaller the ratio, the more power and speed the fish can exert when escaping a startling stimulus. This was an easily measured parameter, only slightly more difficult to determine on small fish, or fish where the tail was hard to detect in the image.

Figure 3.9a displays the completeness ratio of the C-shape for striped shiners under each of the test conditions and post-test time periods. The ratio was significantly ($p<0.05$) different from controls at 1-min post-exposure for the MS-222 exposures and the 30-min turbulence exposure (Table A-1). The ratio remained significantly different than controls for the MS-222 exposures at the 5-min post-exposure.

The data were also corrected for self controls, i.e., the ratios that reflect completeness of the C-shape for each fish in the two pre-test periods are subtracted from the ratios for that same fish after the stress had been applied (Fig. 3.10a). If the ratio does not change as a result of the stress, then the corrected value displayed in Figure 3.10a is zero. On the other hand, if the stress causes a change in the completeness of the C-shape, the self-corrected value will have a positive or negative value. A positive value for the ratio indicates that as a result of the stress, the fish formed a less complete C-shape compared to pre-test controls. The figure only shows data for fish that reacted; many of the fish did not react at all to the startling stimulus after a stress had been applied, so there was no measurement of completeness of C-shape.

Many of the median values for control-corrected C-shape ratios were greater than 0.0 (Fig. 3.10a), indicating that both turbulence and anaesthesia reduced the amount of bending in shiners that reacted to a startling stimulus. There was no indication of habituation in this parameter; the median ratios for unstressed fish were near zero at the fifth and sixth test series.

The impact of the stressors on the C-shape ratio in fathead minnows was limited to the MS-222 dose treatment (Fig. 3.9b). The change was only statistically significant ($p<0.05$) for this group at the 1-min and 5-min post-exposures (Table A-2). As would be expected the pattern for data adjusted for self controls (Fig. 3.10b) also indicated little significant change. For these data, the 30-min turbulence indicated a less complete C-shape, but the wide variation per test made this a statistically non-significant change.

3.2 PREDATOR PREFERENCE TESTS

The two largemouth bass predators readily ate both stressed and control fathead minnow prey. In most tests, about one half of the prey were consumed in less than 30 min, and often the test was terminated in 1-2 min. Although turbulence-stressed or anaesthetized fish had no apparent injuries, they appeared to be lethargic. Compared to controls, stressed fish often sank to the bottom of the predator tank and remained motionless.

Predator preference data for the 10-min exposure to turbulence are shown in Table 3.3. Chi-square tests indicated that none of the 5 individual tests showed a statistically significant selection by predators for either stressed or control fish, despite the fact that a greater proportion of stressed fish were eaten in each of the 5 tests. This is because of the small numbers of prey fish (20) in each test. However, when the data were pooled (a total of 98 prey fish), there was an overall trend toward a greater consumption of turbulence-stressed prey. That is, there was a statistically significant tendency for largemouth bass predators to select stressed fish over control fish.

In contrast, predators did not show a preference for prey exposed to 20 min of turbulence (Table 3.4) or 30 min of turbulence (Table 3.5). None of the individual tests was statistically significant, nor were the pooled data. Fathead minnows exposed to the anesthetic MS-222 were preferred over unstressed controls in 2 of the 5 tests (Table 3.6); this preference was also statistically significant for the pooled data.

At the time of the testing, there appeared to be considerable differences in feeding behavior between the two bass. Predator 1 was a voracious feeder, rapidly consuming half of the prey in a few min. Feeding tests using Predator 1 could be conducted every day; there was no need to allow days off for the fish to recover its appetite. Conversely, Predator 2 was a slow and sometimes reluctant eater. In two of the 7 tests using Predator 2, the entire 30-min period was needed for it to eat one half (or less) of the prey. As much as 5 days were allowed to elapse between feeding tests with Predator 2 to allow its appetite to recover.

The data were pooled by predator in order to determine whether there were differences between the two predators in preference for stressed and unstressed fish. Predator 1 ate more stressed fish than control fish in 8 of 13 tests. Out of a total of 120 prey consumed by Predator 1, 73 were stressed and 47 were unstressed (control) fish. The chi-square value of 5.64 indicated that Predator 1 selected for stressed fish. Similarly, Predator 2 ate more stressed prey than control prey in 6 of 7 tests. Out of 84 total minnows consumed by Predator 2, 56 were stressed and 28 were controls. The chi-square value of 9.33 was significant at the 0.01 level, which indicated that Predator 2 also selected for stressed fish when offered equal numbers of stressed and control fish.

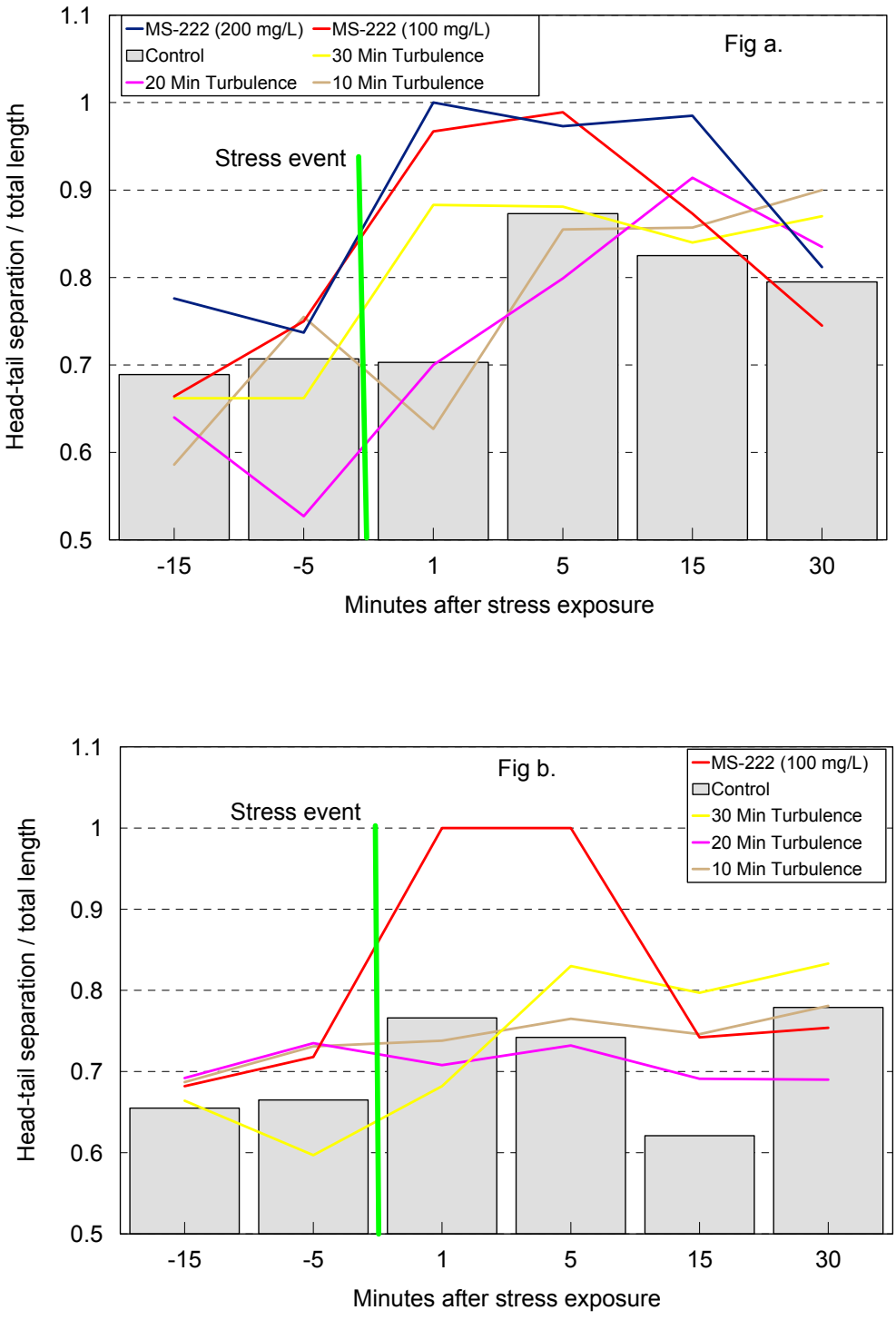
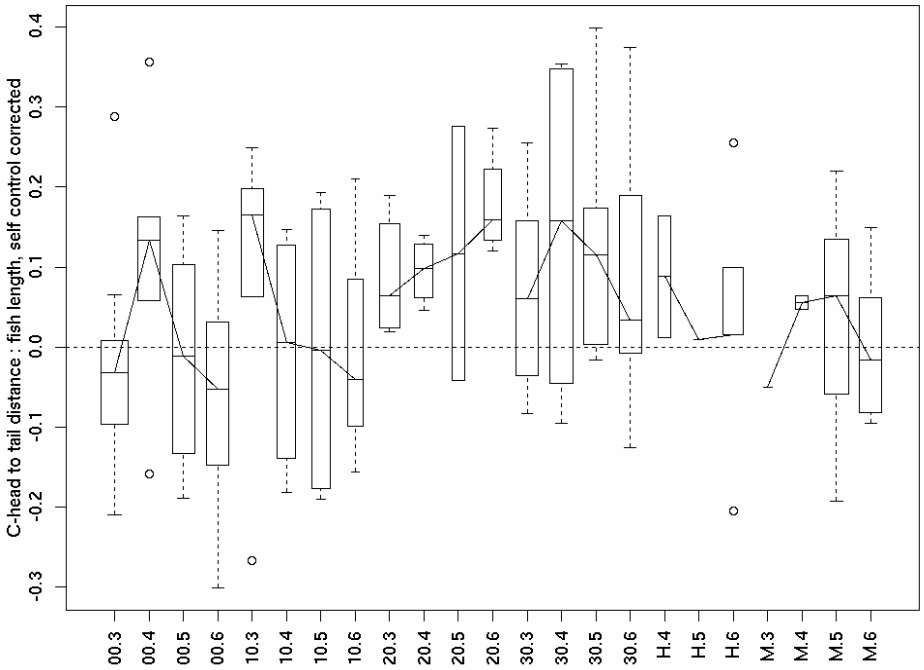


Fig. 3.9. Mean ratio of head-tail separation and body length as affected by exposure to turbulence or tricaine methanesulfonate (MS-222) using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*).

(a)



(b)

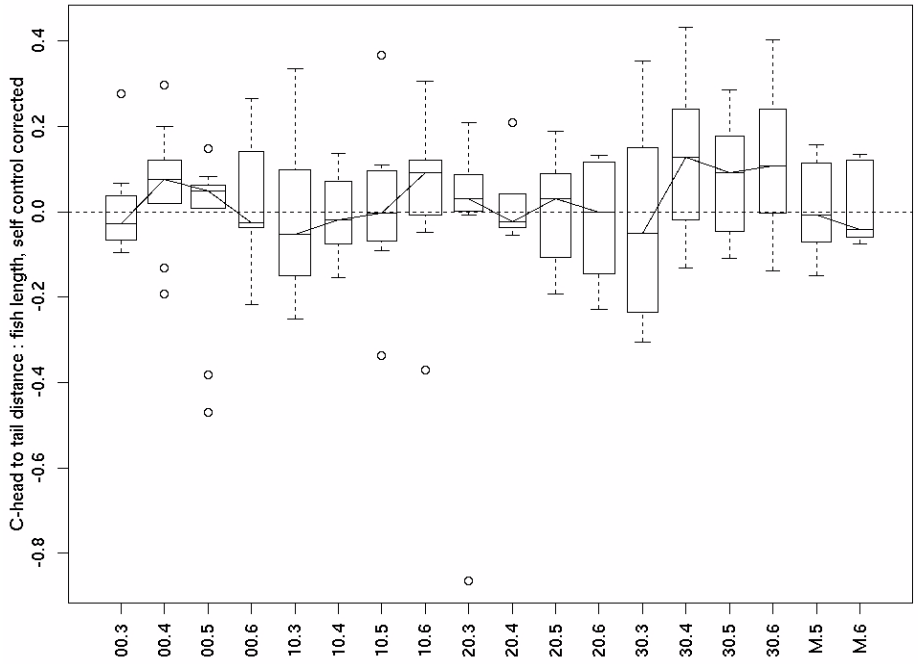


Fig. 3.10. Ratio of head-tail separation and body length, adjusted for individual variation, as affected by exposure to turbulence (00, 10, 20, 30 min) or tricaine methanesulfonate (M, 100 mg/L) using (a) striped shiners (*Luxilus chrysocephalus*) and (b) fathead minnows (*Pimephales promelas*). For each treatment, time post-exposure (1, 5, 15, or 30 min) is represented by a decimal (.3, .4, .5, or .6, respectively). The box represents the 25th to 75th percentiles, the line is the median, and the whiskers/dots represent the extreme data values.

Table 3.3. Predator preference experiment following exposure of fathead minnow prey to 10 min of turbulence

* indicates that predators did not select stressed and control fish in a 1:1 ratio ($\alpha = 0.05$)

Replicate	Number of prey at start of test	Number of prey at end of test	Predator	Number observed at end of test		Statistics	
				Control	Stressed	df	χ^2
1	20	10	1	7	3	1	1.60
2	20	10	2	6	4	1	0.40
3	20	10	1	6	4	1	0.40
4	20	10	2	7	3	1	1.60
5	18	9	1	7	2	1	2.60
Total χ^2						5	6.60
Pooled χ^2	98	49	-	33	16	1	5.90*
Heterogeneity χ^2						4	0.70

Table 3.4. Predator preference experiment following exposure of fathead minnow prey to 20 min of turbulence

* indicates that predators did not select stressed and control fish in a 1:1 ratio ($\alpha = 0.05$)

Replicate	Number of prey at start of test	Number of prey at end of test	Predator	Number observed at end of test		Statistics	
				Control	Stressed	df	χ^2
1	20	13	2	10	3	1	3.77
2	20	8	1	3	5	1	0.50
3	20	15	2	7	8	1	0.06
4	20	10	1	5	5	1	0.00
5	20	8	1	4	4	1	0.00
Total of χ^2s						5	4.33
Pooled χ^2	100	54		29	25	1	0.31
Heterogeneity χ^2						4	4.02

Table 3.5. Predator preference experiment following exposure of fathead minnow prey to 30 min of turbulence.

* indicates that predators did not select stressed and control fish in a 1:1 ratio ($\alpha = 0.05$)

Replicate	Number of prey at start of test	Number of prey at end of test	Predator	Number Observed at end of test		Statistics	
				Control	Stressed	df	χ^2
1	20	9	1	4	5	1	0.11
2	20	10	1	6	4	1	0.40
3	20	14	2	9	5	1	1.14
4	20	9	1	7	2	1	2.78
5	20	12	2	8	4	1	1.33
Total of χ^2						5	5.76
Pooled χ^2	100	54		34	20	1	3.65
Heterogeneity χ^2						4	2.11

Table 3.6. Predator preference experiment following 2-min exposure of fathead minnow prey to anaesthetic (MS-222)

* indicates that predators did not select stressed and control fish in a 1:1 ratio ($\alpha = 0.05$)

Replicate	Number of prey at start of test	Number of prey at end of test	Predator	Number Observed at end of test		Statistics	
				Control	Stressed	df	χ^2
1	20	10	1	9	1	1	6.40*
2	20	9	1	3	6	1	1.00
3	20	9	1	7	2	1	2.78
4	20	10	2	9	1	1	6.40*
5	20	9	1	5	4	1	0.11
Total of χ^2						5	16.69*
Pooled χ^2	100	47		33	14	1	7.70*
Heterogeneity χ^2						4	8.99

4. DISCUSSION

Many of the components of escape behavior that we measured were altered by sublethal exposures to anaesthetic or turbulence. For example, the escape behavior tests using wild striped shiners indicated that exposure to turbulence affected all the aspects of startle response behavior that we studied, ranging from whether or not the prey responded to a startling stimulus to the duration and magnitude of the subsequent escape reaction. It is likely that these changes would also increase the susceptibility of turbulence-stressed fish to predators in a natural system.

With these particular stressors, the most straightforward indication of changes in escape behavior was the simple reaction/no reaction to the startle stimulus. This response occurred reliably among unstressed fish, and was frequently reduced or eliminated in fish exposed to turbulence or MS-222. In a best case scenario, the impact of a sublethal stressor could be determined just using the reaction/no reaction response pattern. However, if the stressor only caused more subtle changes in behavior, then analysis of the other behavioral parameters would be needed. The other parameters observed were often altered by the sublethal stresses as well. With different sublethal stresses or different exposures, some of these other measures might prove to be more important indicators of decreased escape ability than just the presence/absence of a startle response.

Consequently, the initial analysis of changes in the behavior of sublethally stressed fish should be a simple tally of the proportions of fish that react to a startling stimulus. This would provide a useful and rapid estimate of potential indirect mortality associated with downstream passage. If this analysis is not definitive, then analyses of the other relevant behavioral measures evaluated in this study could be added. A subtle change in the normal execution of these behaviors could be used to quantify the magnitude of the impact and help decide the relative contribution of the stress to overall indirect mortality. Owing to the substantial "within-fish" variability that we measured, more test fish should be included in the test design to result in more definitive statistical analyses.

The escape behavior and predator preference tests had similar results. Predators consumed a significantly higher proportion of fathead minnows that were given a 100 mg/L dose of MS-222 than control minnows. Similarly, fathead minnows given the same dose of MS-222 displayed significant changes in all the escape behavior variables that we quantified (time to first reaction, reaction duration, time to form the maximum C-shape, time to the end of the C-shape, and extent of C-shape). Exposure of fathead minnows to 20 min or 30 min of turbulence did not significantly affect either the predator preference or escape behavior in these tests. There was a weak indication that predators selected a higher proportion of fathead minnows exposed to 10 min of turbulence (compared to controls), but this sublethal stress did not significantly alter the escape behavior parameters that were measured. Based on these limited comparisons, the escape behavior tests provided an indication of the effects of sublethal stresses that was comparable to the traditional predator preference tests.

In designing these experiments, it was assumed that the impact of the stress was a temporary one and generally acute in nature. The fish would be exposed to the stress, their behavior would be disrupted, and then they would recover within a short time. This is likely the case for many passage events at hydropower plants, where a fish encounters a single facility and has limited, if any, physical damage. However, there are instances in which migrating fish must pass several dams and are exposed to repeated passage stress events. In these cases, the impact of the stressor may be cumulative or more chronic in nature. There may be actual physical damage, e.g., to the sensory systems, that results in a greater disruption of the escape behavior response. The physical damage may not be immediately
obvious without sacrificing the fish. Our tests have not examined this level of stress in the current experiments, but an evaluation of these chronic impacts would be a logical use for the escape behavior approach.

It would be desirable to conduct these tests at field sites, in order to determine whether significant changes in escape behavior occurs among fish that pass downstream through hydroelectric turbines or over the spillways. As with direct mortality studies, tagged fish could be released above the dam, collected in the

tailwaters, and transferred to streamside holding tanks for filming and behavior analysis. In fact, the same tagged fish that are used for direct mortality could be used; these additional behavioral tests could be performed on uninjured, turbine-passed fish during the normal post-test holding period. Probably the greatest difficulty in getting reliable results from field tests will be the amount of time required to recover fish and transfer them to observation tanks. Our laboratory studies indicate that the debilitating effects of turbulence on escape behavior wore off with time. Many of the aspects of swimming behavior that were significantly compromised 1 min after exposure to turbulence were no longer significantly different from controls after 5 min. We do not know if the levels of turbulence associated with turbine passage, spillway passage, or extended residence in the tailrace have effects comparable to those we generated in the laboratory tanks. Studies have shown that tagged salmon take from 35 to 230 min to travel 1.7 km down the tailrace below the Dalles Dam on the Columbia River (Allen et al. 2001), with the longer times associated with powerhouse passage. Thus the 30-min duration that did affect escape behavior is certainly comparable to some field conditions. Whether the levels of turbulence in this tailrace are similar to our laboratory studies are unknown. Further, we do not know if fish recover faster in the quiet water of a laboratory tank than they would in the turbulent tailwaters below a dam. If turbine- or spillway-passed fish respond the same way as the striped shiners in our laboratory tests, they would have increased susceptibility to predation for a few minutes after passage. Once in calmer water, they might recover from their initial disorientation in a similarly brief time. It would be important to recover fish quickly from the tailwaters in order to detect temporary alterations in escape behavior.

Compared to traditional predator preference tests, escape behavior tests have the advantages of being simpler to run, less subject to behavioral problems unrelated to the treatment (e.g., reluctance of predators to feed; the effects of size of the tank and amount of cover provided on the predator preference results), and are more easily conducted at field sites. Other applications of the escape behavior test would include evaluations where the target species can not easily be sacrificed through a predation test, e.g., if the species is a protected one. If the laboratory technique we have described in this report can be validated at field sites, it would be a useful indicator of a source of indirect fish passage mortality that is presently poorly understood, i.e., the potential increased susceptibility to predators of sublethally stressed fish.

5. REFERENCES

Allen, B., T. L. Liedtke, A. Daniel, J. Begala, M. Salway, and J. Beeman. 2001. Monitoring tailrace egress in the stilling basin, the ice-trash sluiceway, and the powerhouse of the Dalles Dam, 2000. U. S. Geological Survey, Western Fisheries Research Center, Columbia River Research Laboratory, Cook WS. 62 pp.

Cada, G.F., J.A. Solomon, and J.M. Loar. 1981. Effects of sublethal entrainment stresses on the vulnerability of juvenile bluegill sunfish to predation. ORNL/TM-7801. Oak Ridge National Laboratory, Oak Ridge, TN. 34 p.

Conover, W. J. 1980. Practical Nonparametric Statistics, John Wiley & Sons, Inc., New York.

Coutant, C.C. 1973. Effect of thermal shock on vulnerability of juvenile salmonids to predation. Journal of the Fisheries Research Board of Canada 30(7):965-973.

Domenici, P. and R.W. Blake. 1997. The kinematics and performance of fish fast-start swimming. The Journal of Experimental Biology 200:1165-1178.

Eaton, R.C. and R. DiDomenico. 1986. Role of the teleost escape response during development. Transactions of the American Fisheries Society 115:128-142.

Eaton, R.C. and D.S. Emberley. 1991. How stimulus direction determines the trajectory of the Mauthner-initiated escape response in a teleost fish. The Journal of Experimental Biology 161:469-487.

Goring, D. G. and V. I. Nikora. 2002. Despiking acoustic Doppler velocimeter data. Journal of Hydraulic Engineering, 128(1), 117-126.

Fliess, J. L. 1981. Statistical Methods for Rates and Proportions, John Wiley & Sons, Inc., New York.

Hale, M.E. 1996. The development of fast-start performance in fishes: Escape kinematics of the chinook salmon (*Oncorhynchus tshawytscha*). American Zoologist 36:695-709.

Lowry, R. 2002. Chi-square procedures for the analysis of categorical frequency data. Chapter 8, Parts 1-3. http://faculty.vassar.edu/lowry/ch8pt1.html

Lohrmann, A., R. Cabrera, and N. C. Krauss. 1994. Acoustic-Doppler velocimeter (ADV) for laboratory use. Proceedings of the Symposium on Fundamentals and Advancements in Hydraulic Measurement and Experimentation, Buffalo, New York, August 1-5, 1994. ASCE.

Mesa, M.G., T.P. Poe, D.M. Gadomski, and J.H. Petersen. 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. The Journal of Fish Biology 45 (Supplement A): 81-96.

McClelland, S. J. and A. P. Nicholas. 2000. A new method for evaluating errors in high frequency ADV measurements. Hydrological Processes, 14, 351-366.

Neitzel, D.A., D.D. Dauble, M. Richmond, and G.F. Cada. 2000. Laboratory studies of the effects of shear and turbulence on fish. Draft Progress Report to the Advanced Hydropower Turbine Systems Program, U.S. Department of Energy, Idaho Operations Office, Idaho Fall, ID.

Snedecor, G. W. and Cochran, W. G. 1980. Statistical Methods (seventh edition), Iowa State University Press, Ames, Iowa.

Wakeling, J.M., K.M. Kemp, and I.A. Johnston. 1999. The biomechanics of fast-starts during ontogeny in the common carp *Cyprinus carpio*. The Journal of Experimental Biology 202:3057-3067.

Webb, P.W. 1978. Fast-start performance and body form in seven species of teleost fish. The Journal of Experimental Biology 74:211-226.

Webb, P.W. 1986. Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Micropterus salmoides*). Canadian Journal of Fisheries and Aquatic Sciences 43:763-771.

Zar, J. H. 1999. Biostatistical Analysis (fourth edition), Prentice Hall, Upper Saddle River, New Jersey.

APPENDIX A

FISH BEHAVIOR DATA

Table A.1. Behavioral reaction measurements of all striped shiners (*Luxilus chrysocephalus*) after exposure to turbulence or tricaine methanesulfonate (MS-222)

Values are mean ± SD. Statistically significant ($p<0.05$) changes are indicated by *

Type of stressor	Obs. time	No. of test fish	Time to first reaction (s)	Duration of reaction (s)	Time to max.C-shape formation (s)	Time from max C-shape to end of reaction (s)	C-shape ratio (Head-tail distance: total body length)
Control	T1	11	0.037 ± 0.058	0.123 ± 0.078	0.013 ± 0.009	0.017 ± 0.013	0.689 ± 0.174
	T2	8	0.011 ± 0.004	0.172 ± 0.164	0.013 ± 0.007	0.010 ± 0.006	0.707 ± 0.132
	T3	11	0.031 ± 0.057	0.225 ± 0.285	0.015 ± 0.015	0.023 ± 0.015	0.703 ± 0.131
	T4	10	0.097 ± 0.093	0.061 ± 0.055	0.012 ± 0.005	0.021 ± 0.014	0.873 ± 0.146
	T5	10	0.094 ± 0.093	0.117 ± 0.139	0.011 ± 0.003	0.020 ± 0.007	0.825 ± 0.164
	T6	10	0.072 ± 0.089	0.152 ± 0.181	0.012 ± 0.006	0.023 ± 0.015	0.795 ± 0.166
Turbulence, 10 min	T1	9	0.019 ± 0.015	0.116 ± 0.023	0.019 ± 0.003	0.018 ± 0.008	0.586 ± 0.216
	T2	8	0.060 ± 0.086	0.080 ± 0.055	0.015 ± 0.005	0.015 ± 0.005	0.755 ± 0.199
	T3	8	0.019 ± 0.019	0.104 ± 0.059	0.018 ± 0.005	0.021 ± 0.009	0.627 ± 0.095
	T4	9	0.118 ± 0.097	0.033 ± 0.041	0.015 ± 0.003	0.020 ± 0.014	0.855 ± 0.188
	T5	9	0.118 ± 0.097	0.036 ± 0.044	0.022 ± 0.011	0.036 ± 0.025	0.857 ± 0.178
	T6	8	0.135 ± 0.090	0.040 ± 0.057	0.013 ± 0.004	0.022 ± 0.007	0.900 ± 0.141
Turbulence, 20 min	T1	7	0.051 ± 0.069	0.090 ± 0.051	0.022 ± 0.004	0.028 ± 0.013	0.640 ± 0.206
	T2	8	0.032 ± 0.017	0.158 ± 0.044	0.021 ± 0.006	0.023 ± 0.013	0.527 ± 0.109
	T3	8	0.066 ± 0.085	0.070 ± 0.055*	0.021 ± 0.004*	0.030 ± 0.012*	0.700 ± 0.190
	T4	8	0.090 ± 0.093	0.069 ± 0.069	0.018 ± 0.003	0.022 ± 0.006	0.779 ± 0.194
	T5	8	0.160 ± 0.075	0.031 ± 0.058	0.022 ± 0	0.026 ± 0.011	0.914 ± 0.171
	T6	7	0.098 ± 0.096	0.081 ± 0.108	0.017 ± 0.001	0.017 ± 0.003	0.835 ± 0.165

Table A. 1 (continued)

Type of stressor	Obs. time	No. of test fish	Time to first reaction (s)	Duration of reaction (s)	Time to max.C-shape formation (s)	Time from max C-shape to end of reaction (s)	C-shape ratio (Head-tail distance: total body length)
	T6	7	0.098 ± 0.096	0.081 ± 0.108	0.017 ± 0.001	0.017 ± 0.003	0.835 ± 0.165
Turbulence, 30 min	T1	14	0.026 ± 0.025	0.187 ± 0.025	0.013 ± 0.005	0.021 ± 0.018	0.662 ± 0.108
	T2	13	0.026 ± 0.055	0.118 ± 0.059	0.014 ± 0.009	0.028 ± 0.024	0.662 ± 0.173
	T3	13	0.114 ± 0.097*	0.055 ± 0.070*	0.012 ± 0.002*	0.018 ± 0.008*	0.883 ± 0.161*
	T4	13	0.125 ± 0.090	0.049 ± 0.061	0.012 ± 0.009	0.022 ± 0.012	0.881 ± 0.178
	T5	13	0.091 ± 0.090	0.069 ± 0.065	0.015 ± 0.009	0.029± 0.013	0.840 ± 0.147
	T6	13	0.114 ± 0.097	0.051 ± 0.060	0.011 ± 0.003	0.017 ± 0.006	0.870 ± 0.170
MS-222, 200 mg/L	T1	8	0.058 ± 0.088	0.289 ± 0.325	0.020 ± 0.011	0.031 ± 0.032	0.776 ± 0.162
	T2	9	0.042 ± 0.062	0.197 ± 0.181	0.014 ± 0.007	0.043 ± 0.030	0.737 ± 0.169
	T3	11	0.200 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	1.0 ± 0*
	T4	11	0.170 ± 0.066*	0.027 ± 0.061	0.046 ± 0.048	0.086 ± 0.023	0.973 ± 0.082*
	T5	10	0.232 ± 0.102	0.013 ± 0.042	0.050 ± 0.050	0.082 ± 0.050	0.985 ± 0.048
	T6	8	0.087 ± 0.090	0.128 ± 0.124	0.013 ± 0.003	0.033 ± 0.015	0.812 ± 0.153
MS-222, 100 mg/L	T1	9	0.022 ± 0.018	0.155 ± 0.114	0.016 ± 0.006	0.024 ± 0.010	0.664 ± 0.161
	T2	12	0.037 ± 0.055	0.153 ± 0.111	0.012 ± 0.006	0.027 ± 0.015	0.750 ± 0.168
	T3	12	0.184 ± 0.054*	0.015 ± 0.039*	0.016*	0.048*	0.967 ± 0.108*
	T4	12	0.181 ± 0.046*	0.045 ± 0.108	0.02	0.016	0.989 ± 0.039*
	T5	11	0.091 ± 0.088	0.062 ± 0.057	0.014 ± 0.003	0.024 ± 0.010	0.873 ± 0.187
	T6	12	0.080 ± 0.089	0.104 ± 0.139	0.023 ± 0.014	0.029 ± 0.014	0.745 ± 0.204

Table A.2. Behavioral reaction measurements of all fathead minnows (*Pimephales promelas*) after exposure to turbulence or tricaine methanesulfonate (MS-222)

Values are mean ±SD

Type of stressor	Obs. time	No. of test fish	Tine to first reaction (s)	Duration of reaction (s)	Time to max.C-shape formation (s)	Time from max C-shape to end of reaction (s)	C-shape ratio (Head-tail distance: total body length)
Control	T1	10	0.012 ± 0.004	0.135 ± 0.095	0.014 ± 0.006	0.021 ± 0.012	0.655 ± 0.181
	T2	10	0.013 ± 0.009	0.080 ± 0.026	0.017 ± 0.012	0.030 ± 0.028	0.665 ± 0.128
	T3	11	0.100 ± 0.096	0.072 ± 0.055	0.011 ± 0.007	0.059 ± 0.059	0.766 ± 0.201
	T4	10	0.036 ± 0.059	0.098 ± 0.059	0.016 ± 0.021	0.050 ± 0.044	0.742 ± 0.223
	T5	10	0.013 ± 0.011	0.098 ± 0.045	0.018 ± 0.011	0.038 ± 0.038	0.621 ± 0.219
	T6	9	0.115 ± 0.101	0.049 ± 0.049	0.011 ± 0.004	0.014 ± 0.005	0.779 ± 0.273
Turbulence, 10 min	T1	9	0.010 ± 0.003	0.142 ± 0.139	0.009 ± 0.003	0.015 ± 0.007	0.687 ± 0.152
	T2	10	0.034 ± 0.061	0.109 ± 0.100	0.030 ± 0.060	0.032 ± 0.027	0.731 ± 0.175
	T3	9	0.084 ± 0.107	0.105 ± 0.112	0.043 ± 0.062	0.032 ± 0.015	0.738 ± 0.219
	T4	9	0.084 ± 0.108	0.109 ± 0.087	0.029 ± 0.044	0.042 ± 0.039	0.765 ± 0.200
	T5	10	0.048 ± 0.080	0.070 ± 0.050	0.017 ± 0.017	0.014 ± 0.011	0.746 ± 0.199
	T6	10	0.032 ± 0.060	0.090 ± 0.064	0.029 ± 0.060	0.034 ± 0.035	0.781 ± 0.181
Turbulence, 20 min	T1	9	0.035 ± 0.062	0.088 ± 0.060	0.014 ± 0.004	0.063 ± 0.062	0.692 ± 0.212
	T2	10	0.049 ± 0.109	0.135 ± 0.108	0.021 ± 0.025	0.085 ± 0.102	0.735 ± 0.154
	T3	9	0.126 ± 0.158	0.142 ± 0.129	0.029 ± 0.024	0.125 ± 0.118	0.708 ± 0.302
	T4	7	0.048 ± 0.083	0.103 ± 0.036	0.015 ± 0.013	0.064 ± 0.053	0.732 ± 0.107
	T5	10	0.032 ± 0.059	0.088 ± 0.057	0.010 ± 0.004	0.069 ± 0.063	0.691 ± 0.172
	T6	10	0.055 ± 0.094	0.084 ± 0.066	0.011 ± 0.004	0.042 ± 0.038	0.690 ± 0.166

Table A. 2 (continued)

Type of stressor	Obs. time	No. of test fish	Tine to first reaction (s)	Duration of reaction (s)	Time to max.C-shape formation (s)	Time from max C-shape to end of reaction (s)	C-shape ratio (Head-tail distance: total body length)
	T6	10	0.055 ± 0.094	0.084 ± 0.066	0.011 ± 0.004	0.042 ± 0.038	0.690 ± 0.166
Turbulence, 30 min	T1	5	0.013 ± 0.003	0.096 ± 0.058	0.016 ± 0.006	0.030 ± 0.018	0.664 ± 0.216
	T2	7	0.027 ± 0.024	0.083 ± 0.027	0.015 ± 0.010	0.020 ± 0.011	0.597 ± 0.150
	T3	10	0.063 ± 0.078	0.094 ± 0.071	0.035 ± 0.022	0.041 ± 0.034	0.682 ± 0.253
	T4	9	0.057 ± 0.089	0.072 ± 0.068	0.012 ± 0.007	0.017 ± 0.010	0.830 ± 0.179
	T5	10	0.079 ± 0.086	0.041 ± 0.049	0.014 ± 0.009	0.021 ± 0.007	0.797 ± 0.201
	T6	7	0.068 ± 0.084	0.063 ± 0.071	0.020 ± 0.021	0.023 ± 0.010	0.833 ± 0.180
MS-222, 100 mg/L	T1	9	0.010 ± 0.005	0.105 ± 0.058	0.025 ± 0.037	0.031 ± 0.027	0.682 ± 0.131
	T2	9	0.036 ± 0.066	0.059 ± 0.034	0.013 ± 0.004	0.031 ± 0.022	0.718 ± 0.188
	T3	9	0.200 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	1.0 ± 0*
	T4	6	0.200 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	1.0 ± 0*
	T5	8	0.037 ± 0.066	0.089 ± 0.056	0.013 ± 0.006	0.061 ± 0.057	0.742 ± 0.186
	T6	9	0.061 ± 0.086	0.037 ± 0.031	0.011 ± 0.005	0.025 ± 0.012	0.754 ± 0.163

Table A.3. Behavioral reaction measurements of responding striped shiners (*Luxilus chrysocephalus*) after exposure to turbulence or tricaine methanesulfonate (MS-222)

Values are mean ±SD

Type of stressor	Obs. time	No. of test fish reacting	Time to first reaction (s)	Duration of reaction (s)	Time to max.C-shape formation (s)	Time from max C-shape to end of reaction (s)	C-shape ratio (Head-tail distance: total body length)
Control	T1	10	0.021 ± 0.022	0.135 ± 0.070	0.013 ± 0.009	0.017 ± 0.013	0.658 ± 0.147
	T2	8	0.011 ± 0.004	0.172 ± 0.164	0.013 ± 0.007	0.010 ± 0.006	0.707 ± 0.132
	T3	10	0.014 ± 0.009	0.247 ± 0.290	0.015 ± 0.015	0.023 ± 0.015	0.673 ± 0.090
	T4	6	0.029 ± 0.038	0.101 ± 0.023	0.012 ± 0.005	0.021 ± 0.014	0.789 ± 0.131
	T5	6	0.023 ± 0.027	0.195 ± 0.128	0.011 ± 0.003	0.020 ± 0.007	0.709 ± 0.087
	T6	7	0.017± 0.011	0.217 ± 0.181	0.012 ± 0.006	0.023 ± 0.015	0.707 ± 0.107
Turbulence, 10 min	T1	9	0.019 ± 0.015	0.116 ± 0.023	0.019 ± 0.003	0.018 ± 0.008	0.586 ± 0.216
	T2	5	0.013 ± 0.005	0.106 ± 0.030	0.015 ± 0.005	0.015 ± 0.005	0.673 ± 0.153
	T3	8	0.019 ± 0.019	0.104 ± 0.059	0.018 ± 0.005	0.021 ± 0.009	0.627 ± 0.095
	T4	4	0.016 ± 0.002	0.074 ± 0.024	0.015 ± 0.003	0.020 ± 0.014	0.675 ± 0.126
	T5	4	0.016 ± 0.008	0.082 ± 0.012	0.022 ± 0.011	0.036 ± 0.025	0.678 ± 0.089
	T6	3	0.027 ± 0.009	0.105 ± 0.013	0.013 ± 0.004	0.022 ± 0.007	0.733 ± 0.050
Turbulence, 20 min	T1	6	0.027 ± 0.023	0.105 ± 0.035	0.022 ± 0.004	0.028 ± 0.013	0.580 ± 0.143
	T2	8	0.032 ± 0.017	0.158 ± 0.044	0.021 ± 0.006	0.023 ± 0.013	0.527 ± 0.109
	T3	6	0.022 ± 0.022	0.093 ± 0.042	0.021 ± 0.004	0.030 ± 0.012	0.600 ± 0.052
	T4	5	0.024 ± 0.020	0.111 ± 0.050	0.018 ± 0.003	0.022 ± 0.006	0.646 ± 0.086
	T5	2	0.040 ± 0.031	0.123 ± 0.035	0.022 ± 0	0.026 ± 0.011	0.656 ± 0.162
	T6	4	0.021 ± 0.013	0.142 ± 0.109	0.017 ± 0.001	0.017 ± 0.003	0.710 ± 0.082

Table A.3 (continued)

Type of stressor	Obs. time	No. of test fish reacting	Time to first reaction (s)	Duration of reaction (s)	Time to max.C-shape formation (s)	Time from max C-shape to end of reaction (s)	C-shape ratio (Head-tail distance: total body length)
Turbulence, 30 min	T1	14	0.026 ± 0.025	0.187 ± 0.025	0.013 ± 0.005	0.021 ± 0.018	0.662 ± 0.108
	T2	11	0.010 ± 0.005	0.118 ± 0.059	0.014 ± 0.009	0.028 ± 0.024	0.588 ± 0.132
	T3	6	0.013 ± 0.005	0.119 ± 0.050	0.012 ± 0.002	0.018 ± 0.008	0.747 ± 0.145
	T4	6	0.037 ± 0.048	0.106 ± 0.041	0.012 ± 0.009	0.022 ± 0.012	0.743 ± 0.182
	T5	8	0.023 ± 0.014	0.113 ± 0.041	0.015 ± 0.009	0.029± 0.013	0.740 ± 0.085
	T6	6	0.014 ± 0.003	0.111 ± 0.024	0.011 ± 0.003	0.017 ± 0.006	0.698 ± 0.110
MS-222, 200 mg/L	T1	6	0.011 ± 0.007	0.385 ± 0.321	0.020 ± 0.011	0.031 ± 0.032	0.702 ± 0.100
	T2	8	0.023 ± 0.018	0.222 ± 0.176	0.014 ± 0.007	0.043 ± 0.030	0.704 ± 0.147
	T3	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.0 ± 0
	T4	2	0.036 ± 0.014	0.147 ± 0.049	0.046 ± 0.048	0.086 ± 0.023	0.853 ± 0.176
	T5	1	0.524	0.132	0.050	0.082	0.849
	T6	6	0.049 ± 0.068	0.171 ± 0.113	0.013 ± 0.003	0.033 ± 0.015	0.749 ± 0.119
MS-222, 100 mg/L	T1	9	0.022 ± 0.018	0.155 ± 0.114	0.016 ± 0.006	0.024 ± 0.010	0.664 ± 0.161
	T2	11	0.022 ± 0.022	0.166 ± 0.105	0.012 ± 0.006	0.027 ± 0.015	0.727 ± 0.155
	T3	1	0.012	0.134	0.016	0.048	0.642
	T4	2	0.084 ± 0.031	0.269 ± 0.083	0.02*	0.016*	0.865*
	T5	7	0.029 ± 0.025	0.098 ± 0.037	0.014 ± 0.003	0.024 ± 0.010	0.801 ± 0.204
	T6	8	0.020 ± 0.009	0.156 ± 0.145	0.023 ± 0.014	0.029 ± 0.014	0.649 ± 0.145

*n=1; only one fish reacted and formed a C-shape.

Table A.4. Behavioral reaction measurements of responding fathead minnows (*Pimephales promelas*) after exposure to turbulence or tricaine methanesulfonate (MS-222)

Values are mean ±SD

Type of stressor	Obs. time	No. of test fish reacting	Tine to first reaction (s)	Duration of reaction (s)	Time to max.C-shape formation (s)	Time from max C-shape to end of reaction (s)	C-shape ratio (Head-tail distance: total body length)
Control	T1	10	0.012 ± 0.004	0.135 ± 0.095	0.014 ± 0.006	0.021 ± 0.012	0.655 ± 0.181
	T2	10	0.013 ± 0.009	0.080 ± 0.026	0.017 ± 0.012	0.030 ± 0.028	0.665 ± 0.128
	T3	7	0.062 ± 0.086	0.099 ± 0.036	0.011 ± 0.007	0.059 ± 0.059	0.678 ± 0.159
	T4	9	0.018 ± 0.016	0.109 ± 0.051	0.016 ± 0.021	0.050 ± 0.044	0.713 ± 0.216
	T5	10	0.013 ± 0.011	0.098 ± 0.045	0.018 ± 0.011	0.038 ± 0.038	0.621 ± 0.219
	T6	3	0.047 ± 0.085	0.088 ± 0.022	0.011 ± 0.004	0.014 ± 0.005	0.646 ± 0.268
Turbulence, 10 min	T1	9	0.010 ± 0.003	0.142 ± 0.139	0.009 ± 0.003	0.015 ± 0.007	0.687 ± 0.152
	T2	9	0.016 ± 0.019	0.122 ± 0.098	0.030 ± 0.060	0.032 ± 0.027	0.701 ± 0.156
	T3	8	0.070 ± 0.105	0.118 ± 0.112	0.043 ± 0.062	0.032 ± 0.015	0.706 ± 0.209
	T4	7	0.051 ± 0.099	0.140 ± 0.071	0.029 ± 0.044	0.042 ± 0.039	0.686 ± 0.162
	T5	8	0.010 ± 0.002	0.087 ± 0.039	0.017 ± 0.017	0.014 ± 0.011	0.682 ± 0.167
	T6	9	0.013 ± 0.008	0.100 ± 0.059	0.029 ± 0.060	0.034 ± 0.035	0.757 ± 0.174
Turbulence, 20 min	T1	8	0.014 ± 0.006	0.099 ± 0.053	0.014 ± 0.004	0.063 ± 0.062	0.653 ± 0.190
	T2	10	0.049 ± 0.109	0.135 ± 0.108	0.021 ± 0.025	0.085 ± 0.102	0.735 ± 0.154
	T3	7	0.105 ± 0.176	0.182 ± 0.117	0.029 ± 0.024	0.125 ± 0.118	0.624 ± 0.292
	T4	7	0.048 ± 0.083	0.103 ± 0.036	0.015 ± 0.013	0.064 ± 0.053	0.732 ± 0.107
	T5	9	0.013 ± 0.004	0.098 ± 0.051	0.010 ± 0.004	0.069 ± 0.063	0.657 ± 0.141
	T6	9	0.039 ± 0.084	0.093 ± 0.063	0.011 ± 0.004	0.042 ± 0.038	0.655 ± 0.132

Table A.4. (continued)

Type of stressor	Obs. time	No. of test fish reacting	Tine to first reaction (s)	Duration of reaction (s)	Time to max.C-shape formation (s)	Time from max C-shape to end of reaction (s)	C-shape ratio (Head-tail distance: total body length)
Turbulence, 30 min	T1	5	0.013 ± 0.003	0.096 ± 0.058	0.016 ± 0.006	0.030 ± 0.018	0.664 ± 0.216
	T2	7	0.027 ± 0.024	0.083 ± 0.027	0.015 ± 0.010	0.020 ± 0.011	0.597 ± 0.150
	T3	8	0.029 ± 0.033	0.118 ± 0.058	0.035 ± 0.022	0.041 ± 0.034	0.603 ± 0.215
	T4	6	0.009 ± 0.005	0.096 ± 0.061	0.012 ± 0.007	0.017 ± 0.010	0.774 ± 0.171
	T5	7	0.027 ± 0.028	0.059 ± 0.049	0.014 ± 0.009	0.021 ± 0.007	0.710 ± 0.177
	T6	6	0.024 ± 0.023	0.084 ± 0.071	0.020 ± 0.021	0.023 ± 0.010	0.778 ± 0.175
MS-222, 100 mg/L	T1	9	0.010 ± 0.005	0.105 ± 0.058	0.025 ± 0.037	0.031 ± 0.027	0.682 ± 0.131
	T2	8	0.013 ± 0.006	0.068 ± 0.027	0.013 ± 0.004	0.031 ± 0.022	0.683 ± 0.167
	T3	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.0 ± 0
	T4	0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.0 ± 0
	T5	7	0.013 ± 0.005	0.101 ± 0.046	0.013 ± 0.006	0.061 ± 0.057	0.705 ± 0.167
	T6	7	0.015 ± 0.008	0.037 ± 0.031	0.011 ± 0.005	0.025 ± 0.012	0.694 ± 0.129

INTERNAL DISTRIBUTION

1. M. S. Bevelhimer
2. M. A. Brown
3-7. G. F. Cada
8. C. C. Coutant
9. S. G. Hildebrand
6. G. K. Jacobs
7. M. K. McCracken
12. G. W. Morris
13-17. M. G. Ryon
18. M. J. Sale
19-20. D. A. Wolf
21-22. ESD Library
23. ORNL Central Research Library
24. ORNL Laboratory Records–RC
25. ORNL Laboratory Records–OSTI

EXTERNAL DISTRIBUTION

26. Jim Ahlgrimm, EE-2B, US Department of Energy, Office of Wind & Hydropower Technologies, Room 5H/072 FORS, 1000 Independence Ave., SW, Washington DC 20585
27. Paul Bernhardt, Reliant Energy, Inc., 225 Greenfield Parkway, Suite 201, Liverpool, NY 13088
28. Peggy Brookshier, U.S. Department of Energy, Idaho Operations Office, 850 Energy Drive MS-1220, Idaho Falls, ID 83401-1563
29. Stephen Brown, Grant County PUD, P.O. Box 878 Ephrata, WA 98823
30. Tom Carlson, Pacific Northwest National Laboratory, 620 S.W. 5th Avenue, Suite 810, Portland, OR 97204
31. Peter Christensen, R2 Resource Consultants, 15250 NE 95th Street, Redmond, WA 98052
32. Bill Christman, Chelan County PUD, 327 North Wenatchee Ave., P.O. Box 1231, Wenatchee, WA 98801
33. Ron Corso, Mead & Hunt, Inc., 904 Farley Court, S.E., Vienna, VA 22180-5916
34. Dennis D. Dauble, Pacific Northwest National Laboratory, Battelle Boulevard, P.O. 999, Richland, WA 99352
35. Doug Dixon, EPRI, 7905 Berkeley Drive, Gloucester Point, VA 23062
36. Steve Doret, Stone & Webster Consultants, Inc., One Bowdoin Square, Boston, MA 02114
37. Laurie L. Ebner, U.S. Army Corps of Engineers, P.O. Box 2946 , Portland, OR 97208-2946
38. John W. Ferguson, National Marine Fisheries Service, Northwest Fisheries Center, 2725 Montlake Boulevard, East Seattle, WA 98112
39-43. John V. Flynn, EE-2B, US Department of Energy, Office of Wind & Hydropower Technologies, Room 5H/088 FORS, 1000 Independence Ave., SW, Washington DC 20585
44. Peter Goldman, EE-2B, US Department of Energy, Office of Wind & Hydropower Technologies, Room 5H/072 FORS, 1000 Independence Ave., SW, Washington DC 20585
45. Scott Hendricks, Georgia Power Company, Environmental Laboratory, 5131 Maner Road, Smyrna, GA 30080
46. Jennifer Hill, Federal Energy Regulatory Commission, 888 First Street, N.E., Washington, DC 20426
47. Richard T. Hunt, Idaho National Engineering & Environmental Laboratory, 2525 Fremont Ave., Idaho Falls, ID 83415-3830
48. Mike Langeslay, U.S. Army Corps of Engineers, P.O. Box 2946 , Portland, OR 97208-2946
49. Charles R. Liston, Consultant, 168 Ralston Road, Bedford, KY 40006
50. Edward Meyer, National Marine Fisheries Service, 525 N.E. Oregon St., Suite 500, Portland, OR 97232-2737

51. Lawrence B. Moore, Southern Services Company, 42 Inverness Center Parkway, Birmingham, AL 35242
52. Thomas R Murphy, Federal Hydro Projects, Bonneville Power Administration, PGF/6th Floor, P.O. Box 3621, Portland, OR 97208-3621
53. Mufeed Odeh, U.S. Geological Survey, Department of Interior, One Migratory Way, P.O. Box 796, Turner Falls, MA 01376
54. Joe O'Hagan, California Energy Commission, PIER Environmental Area, 1516 9th St., MS 50, Sacramento, CA 95814-5512
55. Forrest Olson, CH2M Hill, P.O. Box 91500, Bellevue, WA 98009
56. Rock D. Peters, U.S. Army Corps of Engineers, 333 S.W. First Avenue, P.O. Box 2946, Portland, OR 97208-2946
57. William L. Preslan, Coastal and Hydraulics Laboratory, Engineer Research and Development Center, Waterways Experiment Station, 3909 Halls Ferry Road, Vicksburg, MS 39180
58. Marshall Richmond, Pacific Northwest National Laboratory, P.O. Box 999 / MS K9-33, Richland, WA 99352
59. Wayne Rogers, Synergics, Inc., 191 Main Street, Annapolis, MD 21401
60. Lee Sheldon, NLink Corp., 510 SW 3rd Ave., Suite 200, Portland, OR 97204-2507
61. Brennan T. Smith, Tennessee Valley Authority, Navigation & Hydraulic Engineering, P. O. Box 1649, Norris, TN 37828
62. Garold L. Sommers, Idaho National Engineering & Environmental Laboratory, 2525 Fremont Ave., Idaho Falls, ID 83415-3830
63. Fotis Sotiropoulos, School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA 30332-0355
64. Steve Wenke, Avista Corp., 1411 East Mission Avenue, Spokane, WA 99220-3727
65. Rod Wittinger, U.S. Army Corps of Engineers, 333 SW First Avenue, P.O. Box 2946, Portland, OR 97208-2946

A Strong Energy Portfolio for a Strong America
Energy efficiency and clean, renewable energy will mean a stronger economy, a cleaner environment, and greater energy independence for America. By investing in technology breakthroughs today, our nation can look forward to a more resilient economy and secure future.

Far-reaching technology changes will be essential to America's energy future. Working with a wide array of state, community, industry, and university partners, the U.S. Department of Energy's Office of Energy Efficiency and Renewable Energy invests in a portfolio of energy technologies that will:

- Conserve energy in the residential, commercial, industrial, government, and transportation sectors
- Increase and diversify energy supply, with a focus on renewable domestic sources
- Upgrade our national energy infrastructure
- Facilitate the emergence of hydrogen technologies as vital new “energy carriers.”

The Opportunities

Biomass Program
Using domestic, plant-derived resources to meet our fuel, power, and chemical needs

Building Technologies Program
Homes, schools, and businesses that use less energy, cost less to operate, and ultimately, generate as much power as they use

Distributed Energy & Electric Reliability Program
A more reliable energy infrastructure and reduced need for new power plants

Federal Energy Management Program
Leading by example, saving energy and taxpayer dollars in federal facilities

FreedomCAR & Vehicle Technologies Program
Less dependence on foreign oil, and eventual transition to an emissions-free, petroleum-free vehicle

Geothermal Technologies Program
Tapping the Earth's energy to meet our heat and power needs

Hydrogen, Fuel Cells & Infrastructure Technologies Program
Paving the way toward a hydrogen economy and net-zero carbon energy future

Industrial Technologies Program
Boosting the productivity and competitiveness of U.S. industry through improvements in energy and environmental performance

Solar Energy Technology Program
Utilizing the sun's natural energy to generate electricity and provide water and space heating

Weatherization & Intergovernmental Program
Accelerating the use of today's best energy-efficient and renewable technologies in homes, communities, and businesses

Wind & Hydropower Technologies Program
Harnessing America's abundant natural resources for clean power generation

To learn more, visit www.eere.energy.gov