Environmental Toxicology and Chemistry, Vol. 17, No. 2, pp. 282–285, 1998
© 1998 SETAC
Printed in the USA
0730-7268/98 $6.00 + .00

INDIVIDUAL VARIATION IN THE SWIMMING PERFORMANCE OF FISHES: AN OVERLOOKED SOURCE OF VARIATION IN TOXICITY STUDIES

ALAN S. KOLOK,*†‡§ ERIC P. PLAISANCE,† and ASSAF ABDELGHANI‡§
†Department of Biological Science, P.O. Box 2021, Nicholls State University, Thibodaux, Louisiana 70310, USA
‡Department of Environmental Health Sciences, Tulane University of Public Health and Tropical Medicine, 1501 Canal Street, New Orleans, Louisiana 70112, USA
§Tulane-Xavier Center for Bioenvironmental Research, 1430 Tulane Avenue, New Orleans, Louisiana 70112, USA

(*Received* 24 *February* 1997; *Accepted* 14 *May* 1997)

Abstract—A commonly used indicator of sublethal stress in fish is impaired swimming performance. Analysis of performance data usually employs a simple comparison, in which the mean of a stressed group of fish is compared to that of a control group. Although such a comparison is satisfactory in many cases, a comparison emphasizing individual variation in performance can yield valuable information unattainable by a means comparison. In this experiment, we determined critical swimming speeds of subadult male fathead minnows before and after exposure to contaminated sediments from Devil's Swamp, Louisiana, USA. The data were then analyzed using a means comparison and an individual approach to illustrate the differences in explanatory power between the two approaches.

Keywords—Performance repeatability Swimming performance Sediment contamination Heavy metals

INTRODUCTION

The swimming performance of fish is a whole-animal activity that has been successfully used as an indicator of sublethal stress associated with exposure to toxic chemicals [1–3 and references therein]. Although a number of different swimming performances can be quantitatively determined, the most frequently used measurement is critical swimming speed (U_{crit}) [3]. Critical swimming speed is a measurement in which the fish is forced to swim for a prolonged period of time against an incrementally increasing flow of water until it fatigues [4]. Significant reductions in U_{crit} have been documented after exposure to a number of organic and inorganic toxicants [1–3 and references therein].

Although U_{crit} has been frequently used as an indicator of sublethal stress, one problem associated with it is that the swimming performance of conspecific fish oftens varies considerably [5,6], even when the individuals are morphologically similar to each other. A large within-group variation makes it difficult to document significant differences between treatment and control means, which limits the utility of swim performance measurements as a reliable indicator of sublethal stress. Recent research involving reptiles and amphibians [7], and fish [8,9] has suggested that within-group variation in locomotor performance is not statistical noise, but is repeatable over time. This is significant as it suggests that experiments can exploit variation among individual fish as a repeatable source of variability, and that a simple means comparison may not be the best way to analyze swim performance data.

To analyze swim performance data using an individual approach, measurements must be taken on each fish before and after exposure to the stressor. Whether individual fish respond differently to the toxicant exposure can be determined from these data. To date, we are unaware of any published toxicity study that has used this approach. In this study, we determined the effect of exposure to contaminated sediments on the swimming performance of subadult male fathead minnows (*Pimephales promelas*). The resultant data were analyzed using a means comparison and an individual approach to compare the explanatory power of both approaches.

MATERIALS AND METHODS

Sediments were collected from a contaminated site (Devil's Swamp) and a reference site (Tunica Swamp) located northwest of Baton Rouge, Louisiana, USA. Sediments were collected, packed into 2-L jars, returned to the laboratory on ice, and maintained on ice or in the refrigerator (<10°C) until they were used in the experiments. Prior to use, 2 L of sediment was placed on the bottom of a 40-L aquaria filled with 30 L of dechlorinated tap water. Sediments were allowed to equilibrate with the water for a minimum of 4 d prior to addition of the fish.

Subadult male fathead minnows used in this study were obtained from a national supplier and were allowed to acclimate to laboratory conditions for at least 12 d prior to use in the experiments. During the acclimation period, all fish were maintained in dechlorinated fresh water at 24°C and were fed twice daily to satiation with high protein fish flakes and either live brine shrimp nauplii or frozen adult brine shrimp.

Critical swimming speeds were determined for each fish at 24°C using a previously described swim chamber [10]. A fish was placed in the clear acrylic chamber (7.7-cm i.d., 55 cm long) for approximately 1.5 h to habituate to a water velocity of 15 cm/s. After the habituation period, the fish was forced to swim to fatigue against a water velocity that increased 5 cm/s every 20 min until the fish fatigued. Fatigue was determined when the fish could no longer be persuaded to swim off the flow straighteners located at the end of the chamber.

After the initial swimming challenge, each fish was anesthetized with 3-aminobenzenesulfonic acid, fin-clipped, weighed, measured for length, and returned to a second clean

* To whom correspondence may be addressed (biol-ask@nich-nsunet.nich.edu).

15528618, 1998, 2, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5620170221 by Auburn University Libraries, Wiley Online Library on [23/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1. The mean (±SE) values for organic and inorganic contaminants (ng/g dry wt.) found in sediments from Tunica Swamp and Devil's Swamp, Louisiana, USA[a]

Contaminant	Devil's Swamp mean	SE	Tunica Swamp mean	SE
Arsenic	130	10	31.5	2.5
Barium	10,510	2,555	NA	—
Cadmium	860	20	27.5	12.5
Chromium	2,708	43	32.0	6.0
Cobalt	699	606	NA	—
Copper	1,950	160	288.5	43.5
Iron	511,431	13,240	48,618	2,636
Mercury	415	5	NA	—
Nickel	1,270	60	ND[b]	—
Lead	6,190	95	20.5	5.5
Zinc	4,455	655	NA	—
Hexachlorobutadiene	0.20	0.0005	ND[c]	—
Hexachlorobenzene	4.59	2.87	BQL[d]	—

[a] NA = not analyzed, ND = nondetectable, BQL = below quantifiable detection limit. Sample size = two for each contaminant.
[b] The detection limit for nickel is 5 ng/g.
[c] The detection limit for hexachlorobutadiene is 0.04 ng/g.
[d] The quantifiable detection limit for hexachlorobenzene is 2.4 ng/g.

40-L aquarium where the fish were allowed to recuperate for at least 3 d. Fin clips involved removal of a unique combination of the left and right pelvic fins, and the anal fin. In fathead minnows, removal of these fins has been successfully used as a mechanism for identifying individuals [11]. In the first experiment, the fish were moved into a clean 40-L aquarium without sediments. In the second experiment, the fish were moved into an aquarium containing approximately 2 L of Tunica Swamp sediments, and in the third experiment, fish were moved from the recovery tank into a 40-L aquarium containing 2 L of Devil's Swamp sediments. Fish were maintained in the experimental aquaria for 10 to 14 d. Fish were fed every day throughout the exposure, and water changes (one third of the tank) were conducted every other day. After 10 d of exposure, fish were netted from the aquaria, placed in the swim chamber, and challenged with a second bout of swimming. Only three swim performances could be conducted per day; therefore, the swimming measurements lasted for 4 d. After the second swim challenge the fish were anaesthetized, weighed, and measured for fork length.

After the experiment, aliquots of the contaminated and control sediments were collected from the aquaria and analyzed for contaminants previously isolated from Devil's Swamp sediments [12], namely 11 metals, hexachlorobenzene (HCB), and hexachlorobutadiene (HCBD). Sediment samples were analyzed for metals using U.S. Environmental Protection Agency (U.S. EPA) methods 3005A and 3050A [13]. Samples were air dried for 24 h, then oven dried at 80°C until constant weight. Dry ground samples were analyzed for metals using an inductively coupled plasma detector equipped with an ultrasonic nebulizer and an auto sampler (U.S. EPA method 6010A).

Hexachlorobenzene and HCBD were extracted from a 5-g sample of dried sediment. Hexachlorobenzene was extracted from oven-dry sediment using a super fluid extractor, followed by a cleanup in a florisil column. Hexachlorobutadiene was extracted by purge and trap (U.S. EPA extraction method 5030A). Extracts were analyzed for HCB and HCBD using capillary column gas chromatography equipped with an electron capture detector (U.S. EPA method 8121).

RESULTS

The contaminants found in the sediments from Devil's Swamp and Tunica Swamp are listed in Table 1. The sediments from Devil's Swamp contained statistically higher concentrations of arsenic, cadmium, chromium, copper, iron, and lead (*t* test, $p < 0.05$) than did the sediments from Tunica Swamp.

The 30 minnows used in these experiments had a mean body mass of 2.95 ± 0.13 g (SE) and a mean length of 61.2 ± 0.9 mm (SE). No significant differences were found between the mass or length of the fish in the three different exposure groups (analysis of variance $p < 0.05$). Furthermore the U_{crit}s (cm/s) of the pretreatment fish were not significantly correlated with body mass ($r^2 = 0.04$, $p = 0.30$) or length ($r^2 = 0.11$, $p = 0.08$).

The preexposure mean U_{crit}s of the minnows were not significantly different from each other (Kruskall–Wallis test, $p > 0.05$; Table 2). Furthermore, in the no-sediment and Tunica Swamp experiments, the mean U_{crit} was not significantly different between preexposure tests and postexposure tests

Table 2. The mean (±SE) body mass (BM), fork length (FL), and critical swimming speed (U_{crit}) of the fathead minnows exposed to no sediments, Tunica Swamp (LA, USA) sediments, and Devil's Swamp (LA, USA) sediments. An asterisk denotes a significant difference between the preexposure and postexposure U_{crit}s

Sediment	*n*	BM	FL	U_{crit} preexposure	U_{crit} postexposure
No sediment	10	2.7 (0.2)	6.1 (1.2)	44.7 (1.3)	46.3 (1.4)
Tunica Swamp	10	2.8 (0.2)	5.9 (1.4)	42.3 (1.8)	40.2 (1.3)
Devil's Swamp	9	3.4 (0.3)	6.4 (0.2)	42.8 (1.1)*	32.6 (3.7)

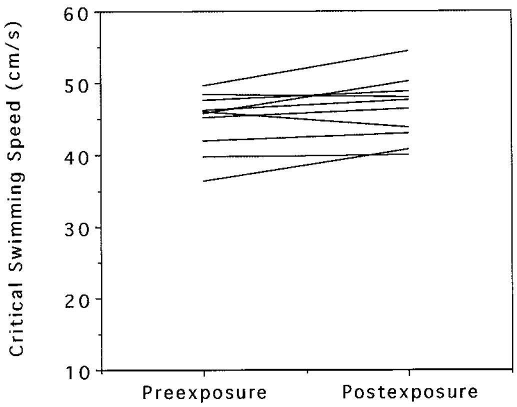

Fig. 1. The critical swimming speeds of 10 subadult male fathead minnows. Each individual was challenged with two bouts of swimming 10 to 14 d apart. Between the two bouts of swimming, fish were maintained in an aquarium without sediment.

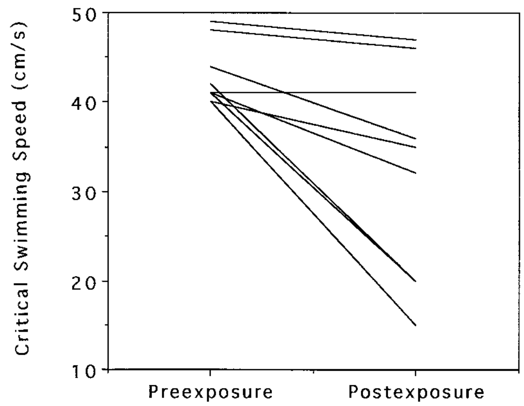

Fig. 3. The critical swimming speeds of nine subadult male fathead minnows. Each individual was challenged with two bouts of swimming 10 to 14 d apart. Between the two bouts of swimming, fish were maintained in an aquarium with 2 L of sediment from Devil's Swamp, Louisiana, USA.

(Mann–Whitney U test, $p > 0.05$; Table 2). In contrast, the mean U_{crit} of the fish exposed to Devil's Swamp sediments decreased significantly (Mann–Whitney U, $p < 0.05$; Table 2) after exposure.

The rank order of the control fish before exposure was significantly correlated to the rank order of the fish after exposure (Spearman rank order correlation coefficient, $r = 0.83$, $p < 0.05$; Fig. 1), indicating performance repeatability. In contrast, the rank orders of performance for the fish exposed to Tunica Swamp and Devil's Swamp sediments were not maintained after sediment exposure (Tunica Swamp $r = 0.382$, $p > 0.05$, Devil's Swamp $r = 0.644$, $p > 0.05$; 2 and 3). For the fish exposed to Tunica Swamp sediment, the five best performing individuals lost performance, whereas the remaining fish all gained in performance during the postexposure swim. For the fish exposed to Devil's Swamp sediment, three individuals experienced dramatic reductions (mean reduction = 53.0 ± 3.0%) in swim performance, whereas the remaining fish experienced relatively minor reductions (mean reduction = 10.3 ± 3.6 %) in performance (Fig. 3).

DISCUSSION

The primary objective of this study was to compare two different approaches used to ascertain the sublethal effects of exposure to contaminated sediments on the swimming performance of subadult male fathead minnows. Using a means comparison, we were able to document a significant difference between fish before exposure and after exposure to Devil's Swamp sediments. Variation around the mean is three times higher in the fish after exposure relative to before exposure (Table 2); however, in most studies measuring swim performance, little mention is made of performance variability except to point out the fact that substantial variation is often found in the performance measurement [5,6].

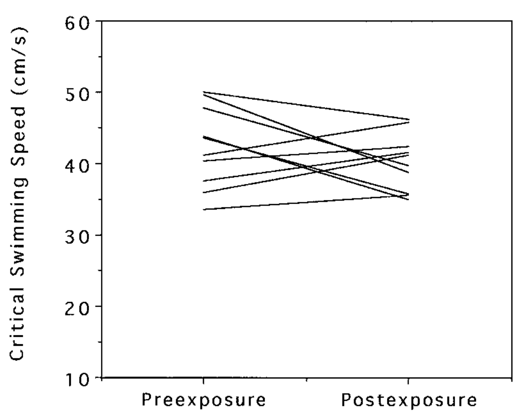

Fig. 2. The critical swimming speeds of 10 subadult male fathead minnows. Each individual was challenged with two bouts of swimming 10 to 14 d apart. Between the two bouts of swimming, fish were maintained in an aquarium with 2 L of sediment from Tunica Swamp, Louisiana, USA.

To use an individual approach to analyze performance data, a preexposure and postexposure performance measurement is conducted on each fish. Furthermore, swim performance must be established to be repeatable among nonstressed individuals. Individual swimming performances have been found to be repeatable in a number of reptiles and amphibians [7], as well as in juvenile largemouth bass [8] and northern squawfish [9]. In the current study, performance repeatability was also demonstrated for fathead minnows (Fig. 1). Interestingly, exposure of the fish to Tunica Swamp sediments altered the rank order of performance without influencing the mean U_{crit} for the preexposure and postexposure performances (Fig. 2).

Eight of the nine fish exposed to Devil's Swamp sediments experienced a drop in performance after exposure (Fig. 3). In addition, the decrease in performance varied widely from one individual to another, which resulted in a loss in performance repeatability between the fish before and after exposure. This result is quite different from that of other studies in which performance repeatability before and after a stressor was examined. For example, it was found that an acute reduction in water temperature reduced the swimming performance of juvenile largemouth bass; however, performance variability was maintained [8]. Similarly, a surgical manipulation reduced the swimming performance of northern squawfish; however, the relative rank order of the fish was maintained after surgery [9]. The lack of performance repeatability in the fish exposed to Devil's Swamp sediments suggests that individuals in the population are differentially resistant to the adverse effects of the contaminants. This is a result that would not have been identified using a means comparison approach.

Acknowledgement—This work was partially supported by a grant from the Nicholls State University Research Council and by a sub-

15528618, 1998, 2, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5620170221 by Auburn University Libraries, Wiley Online Library on [23/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

contract to ASK from the Tulane/Xavier Center for Bioenvironmental Research's U.S. DOE grant #DE-FG01-93-EW53023. We acknowledge the assistance of Albert Gros, Jr. in conducting the swim performances, and the assistance of Henry Bart and Laverne Taylor in the field collection of Devil's Swamp and Tunica Swamp sediments.

REFERENCES

1. Beitenger TL, McCauley WR. 1990. Whole-animal physiological processes for the assessment of stress in fishes. *J Great Lakes Res* 16:542–575.
2. Little EE, Finger SE. 1990. Swimming behavior as an indicator of sublethal toxicity in fish. *Environ Toxicol Chem* 9:13–19.
3. Heath WB. 1995. *Water Pollution and Fish Physiology,* 2nd ed. CRC, Boca Raton, FL, USA.
4. Beamish FWH. 1978. Swimming capacity. In Hoar WS, Randall DJ, eds, *Fish Physiology,* Vol 7. Academic, New York, NY, USA, pp 101–187.
5. Berry CR Jr, Pimentel R. 1985. Swimming performance of three rare Colorado River fishes. *Trans Am Fish Soc* 114:397–402.
6. Watenpaugh DE, Beitinger TL. 1985. Swimming performance of channel catfish (*Ictalurus punctatus*) after nitrite exposure. *Bull Environ Contam Toxicol* 34:754–760.
7. Bennett AF. 1987. Intraspecific variation: An underutilized resource. In Feder ME, ed, *New Directions in Ecological Physiology.* Cambridge University Press, New York, NY, USA, pp 147–166.
8. Kolok AS. 1992. The swimming performances of individual largemouth bass (*Micropterus salmoides*) are repeatable. *J Exp Biol* 170:265–270.
9. Kolok AS, Farrell AP. 1994. Individual variation in the swimming performance and cardiac performance of northern squawfish, *Ptychocheilus oregonensis. Physiol Zool* 67:706–722.
10. Kolok AS. 1991. Photoperiod alters the critical swimming speed of juvenile largemouth bass, *Micropterus salmoides* acclimated to cold water. *Copeia* 1991:1085–1090.
11. Kolok AS, Oris JT. 1995. The relationship between specific growth rate and swimming performance in male fathead minnows (*Pimephales promelas*). *Can J Zool* 73:2165–2167.
12. Thiyagarajah A, Hartley WR, Major SE, Broxson MW. 1996. Gill histopathology of two species of buffalo fish from a contaminated swamp. *Mar Environ Res* 42:261–266.
13. U.S. Environmental Protection Agency. 1994. Test methods for evaluating solid waste. Volume 1A: Laboratory manual physical/chemical methods. Office of Solid Waste and Emergency Response, Washington, DC.

15528618, 1998, 2, Downloaded from https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5620170221 by Auburn University Libraries, Wiley Online Library on [23/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License