STRESS RESPONSES OF WALLEYE AND WALLEYE X SAUGER HYBRIDS

Terence P. Barry, Masako Ochiai, Jeffrey A. Malison, and James A. Held, Department of Animal Sciences, University of Wisconsin-Madison, 1675 Observatory Dr., Madison, WI 53706, USA, tpbarry@wisc.edu.

Introduction. Hybrids of female walleye (Sander vitreus) and male sauger (S. canadensis, WxS) grow faster and convert feed more efficiently than purebred walleyes when reared under intensive culture conditions. There is great interest among North American aquaculturists in producing food-sized WxS hybrids to supply the needs of retail markets and restaurants (Malison 1990; Siegworth and Summerfelt 1990, 1992).

We postulated that the growth and performance advantages of WxS hybrids over walleyes are related to differences in responsiveness to stress. This idea was suggested by observations that hybrids are much more tolerant of disturbances associated with routine husbandry procedures, such as feeding and tank cleaning, than purebred walleye which are highly excitable and sensitive to noise, shadows, and movements (Malison et al., 1990; Siegworth and Summerfelt 1990, 1992; Held and Malison 1996). The goals of the present investigation were to (1) obtain baseline information on the physiological stress responses of purebred walleye, including determining the effects of time of day on baseline cortisol levels and the effects of acclimation temperature on their responses to acute stress, and (2) compare the physiological stress responses of purebred and hybrid walleye.

Methods. Fish. Walleye (271 mm mean total length, TL) were the offspring of wild brood fish collected from Rock Lake, WI. WxS hybrids (262 mm TL) were the progeny of female Rock Lake walleye and male Mississippi River sauger. The fish were held in separate flow-through 220-l circular fiberglass tanks at either 15 ± 0.5°C or 21 ± 0.5°C. The photoperiod was 12 h light/12 h dark with lights on at 0600 hrs. The fish were fed dry pelleted feed (Ziegler Brothers, Inc., Gardner, PA) at a rate of approximately 1% of body weight per day. Food was withheld 24 h before sampling.

Acute stress tests, diurnal rhythm, and temperature effect. Unstressed controls consisted of five fish that were netted from the holding tank, anesthetized in 50 mg/l unbuffered tricaine methanesulfonate (MS-222), and bled via the caudal vasculature. To evaluate the effect of time of day on baseline cortisol levels, five control fish acclimated at 21°C were sampled every 3-4 hours over a 24-h period (8 times total). To evaluate the effects of acclimation temperature on the acute stress response, groups of walleye were acclimated for one month at either 15°C or 21°C and subjected to the acute stress protocol described below starting at 0900 hrs. The acute stress tests were conducted as follows: fish were netted, held out of the water for one minute, and then randomly divided among seven 110-l tanks. At 7 min, 15 min, 30 min, 1 h, 3 h, 6 h and 24 h post-handling, all of the fish from one 110-liter tank were netted, anesthetized, and bled. The total length and weight of each fish were recorded.

Analytical methods. The blood was allowed to clot for 1 hour, and then centrifuged at 9000xg for 5 min. The serum was collected and stored at -40°C until analysis. Serum cortisol levels were measured with an ELISA validated for use in walleye. Glucose was measured using a diagnostic kit (510-DA, Sigma Chemical Company, St. Louis, MO). Chloride was measured using a Corning (Medfield, MA) model 925 chloride analyzer. All samples were assayed in duplicate.

Statistics. All data are presented as mean ± SEM. The data were analyzed by analysis of variance, followed by protected least-squares difference tests (P<0.05).

Results. Diurnal rhythm. Cortisol concentrations rose significantly from less than 5 ng/ml during the daylight hours, to a peak of over 36 ng/ml at midnight (Fig. 1).

![Cortisol Levels](image)

**Fig. 1.** Diurnal changes in baseline serum cortisol levels in purebred walleye.

Effect of temperature. At both acclimation temperatures, cortisol rose rapidly from less than 4 ng/ml, to peaks of over 150 ng/ml at 30 min. Cortisol returned to baseline by 3 hr (Fig. 2). Fish acclimated at 21°C, had higher and faster cortisol responses than fish acclimated at 15°C (Fig. 2). Significant differences between acclimation temperatures were detected at 15 min, 30 min, 1 hr, and 3 hr post-stress (Fig. 2).

At both acclimation temperatures, baseline glucose levels were approximately 60 mg/dl. At 15°C, glucose concentrations rose to a peak of 165 ± 3 mg/dl by 15 min, then fell to 127 ± 5 mg/dl by 1 hr where they remained steady until 6 hr. At 21°C, glucose concentrations rose more slowly to a higher peak of 190 ± 8 mg/dl by 1 hr, then fell to 128 ± 5 mg/dl by 3 hr, and declined further to 109 ± 8 mg/dl by 6 hr.

Serum chloride levels were 129 ± 3 mEq/L in the 15°C fish and 135 ± 1 mEq/L in the 21°C fish.
fish. No differences in chloride levels were detected in response to acute stress, or between treatment groups.

![Graph showing cortisol levels over time with temperature differences](Image)

**Fig. 2.** Effects of acclimation at 15°C and 21°C on the cortisol stress response in walleye.

**Purebred vs. hybrid.** Cortisol, glucose and chloride did not differ between purebred and hybrid walleye. Cortisol peaked at approximately 200 ng/ml within 15-30 min in both species, and returned to baseline levels by 3 h post-stress (data not shown). In both groups of fish, serum glucose remained elevated through 6 h, and returned to baseline levels by 24 h (data not shown).

**Discussion.** Contrary to our hypothesis, there were no differences in primary or secondary stress responses between hybrid and purebred walleye. We conclude, therefore, that the performance differences between these groups are not related to differences in their ability to respond to an acute handling stressor. There can be a major psychological component to the stress response, and individuals can show robust physiological stress reactions at only the impression of a threat. Given the marked behavioral and performance differences between purebred and hybrid walleyes, and our observations that both groups of fish appear to have the same machinery for responding to stress, we predict that hybridization alters the expression of neuronal pathways in the brain that control the psychological perceptions of stress.

Walleye showed a significant diurnal rhythm in baseline cortisol concentrations. This is a common phenomenon in vertebrates, with cortisol levels generally positively correlated with the activity patterns of the animal. The nighttime rise in cortisol in walleyes probably reflects its normal nocturnal feeding cycle.

Cortisol rose faster and higher, and declined more rapidly, in walleye acclimated at 21°C than it did in fish acclimated at 15°C. Temperature effects on enzymes controlling cortisol biosynthesis and peripheral metabolism probably explain these results. A reduced stress response may be one explanation for the benefits of handling and transporting walleye at cooler water temperatures. At both temperatures, glucose levels remained elevated longer than cortisol suggesting that hormones besides glucocorticoids (e.g., catecholamines) may be the primary regulators of glucose homeostasis in stressed walleye. Chloride levels did not change significantly in response to acute stress in walleye suggesting that stress-induced osmoregulatory dysfunction may not be as large a problem in walleye husbandry as it is in the culture of salmonids. The stress-induced changes observed in walleye were similar to those reported by Barton and Zitzow (1995).

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