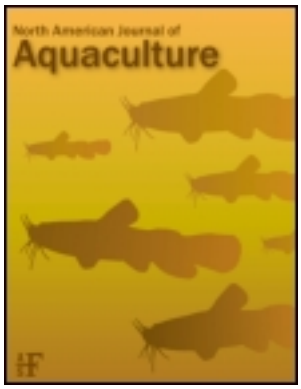

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Physiological Stress Response of Yellow Perch Subjected to Repeated Handlings and Salt Treatments at Different Temperatures

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COMMUNICATON

Physiological Stress Response of Yellow Perch Subjected to Repeated Handlings and Salt Treatments at Different Temperatures

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Abstract

Yellow Perch *Perca flavescens* were subjected to handling stress and salt treatments at different temperatures to determine their physiological changes. Yellow Perch, held at 8–10°C, were divided into three groups with four replicates and subjected to water temperatures of 14, 20, and 26°C to acclimate. Then they were subjected to acute handling twice within separate intervals, in addition to a salt treatment at a salinity of 5‰ for each of the fish groups. Plasma cortisol was used as a stress indicator, and blood samples were taken periodically for plasma cortisol concentration analysis. An increase in plasma cortisol after handling occurred in all groups, but the lowest level of plasma cortisol was in fish subjected to 20°C. We concluded that the optimum water temperature for handling Yellow Perch that results in minimal stress may be 20°C. Salt treatment after handling further stimulated the stress response and increased plasma cortisol levels. Research is needed to identify the optimal salinity to use for Yellow Perch and other fish species when handling fish during common aquaculture practices.

Fish exposed to stressors can exhibit physiological and behavioral changes; these alterations are often referred to as the stress response. The stressors are environmental factors, such as temperature extremes, salinity, water-borne pollutants, social interactions, or aquaculture practices such as handling and sorting (Stratholt et al. 1997). A fish's response to stress represents the perception of an altered state and is characterized by many responses; the primary response includes the release of catecholamines (Reid et al. 1998) and stimulation of the hypothalamic–pituitary–interrenal (HPI) axis to release the corticosteroid hormones into the circulation (Wendelaar Bonga 1997; Barton 2002; Lowe and Davison 2005; Hight et al. 2007; Hosoya et al. 2007; Pankhurst 2011), and secondary stress responses include changes in metabolism, respiration, acid–base status, hydromineral balance, immune function, and cellular responses (Mommsen et al. 1999). When the stressor is acute and short term, the response pattern is stimulatory, and the fish immune response shows an activating phase that specially enhances innate responses; but, if it is chronic, the immune response shows suppressive effects, and therefore the chance of infection may be enhanced (Tort 2011).

The predominant corticosteroid in teleost fishes is cortisol, which has long been used to quantify the stress response (Romero 2002). Under stressful conditions, cortisol and catecholamines are important for several reasons, including central nervous system stimulation and blood glucose elevation; cortisol mobilizes energy to provide metabolic substrates to adjust physiology and behavior aimed at restoring the organism to homeostasis (Rottmann et al. 1992; Vijayan et al. 1997; Mommsen et al. 1999; Barton 2002; Skomal and Mandelman 2012).

Fish immersion in salt water has been theorized to prevent loss of blood ions due to acute stress events (Wedemeyer 1972; Carmichael et al. 1984). While changes in salinity can induce a stress response in fish (Fiol and Kültz 2007), some studies have reported that fish immersion in isotonic saline water after a stressful event can help to reduce the stress and decreasing recovery time (Barton and Peter 1982; Barton and Zitzow 1995; Reubush and Heath 1997). Measurement of the corticosteroid

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stress response after salt treatment has shown conflicting results; some studies recorded an increase in the plasma cortisol level of fish treated with salt (Harrell 1992; Barton and Zitzow 1995), while other studies noted a decrease in cortisol response (Mazik et al. 1991; Carneiro and Urbinati 2001).

The response of fish to typical stressors in aquaculture practices has been extensively examined in the literature (Barton 2002; Acerete et al. 2004; Bertotto et al. 2011). Most of these studies have been carried out on some major aquaculture species, but other species such as Yellow Perch *Perca flavescens* have received less attention. The Yellow Perch is an important potential candidate species for aquaculture in North America and is highly prized as a recreational and food fish. Its culture is limited by its high sensitivity to handling and disturbances in intensive culture conditions (Head and Malison 2000). In order to overcome this limitation, more information on the cortisol stress response in perch is required.

The objective of this study was to develop a greater understanding of the stress response in Yellow Perch through the assessment of the effect of handling stress under different temperatures on the plasma cortisol level in Yellow Perch and to characterize the physiological changes associated with environmental exposures to varying temperatures and salinities.

METHODS

Experimental Fish

Yellow Perch (48 ± 10 g, mean ± SD) were obtained from the Aquaculture Research Center, The Ohio State University South Centers, Piketon, Ohio. Fish were held at a water temperature of 8–10°C in 800-L experimental tanks before transfer. Fish were fed twice with a commercial diet at a rate of 2.5% of average body weight daily. Two weeks prior to experimentation, fish were transferred to twelve 400-L experimental tanks (80 fish/tank) to acclimate them to the experimental system and the target temperatures of 14, 20, and 26°C. Fish were acclimated to the final temperatures by increasing the temperature in each tank gradually until the target temperature was reached for each group, and then the final temperature was maintained.

Experimental Design

First handling stressor at different temperatures.—Three experimental groups (four replicates in each) represented the fish acclimated to the different temperatures: 14, 20, and 26°C (Figure 1). Weighing fish from all groups tank by tank using standard practices was used as the handling stressor. Before weighing the fish, half of the water from the tank was siphoned into an empty tank for holding the weighed fish. After the fish were weighed, they were returned to the original tank by netting them from the second tank and transferring them by means of a net, and then the water was returned to the tank. The same method and timing of the handling stressor were applied to all tanks. Handling time was monitored and recorded for each tank. This procedure was carried out at 1000 hours and the timing for one feeding was adjusted to take place 1 h after handling. During this study, fish were sampled before handling (time = 0, prehandling samples), immediately after handling (i.e., 10 min after handling was completed), and 24 h posthandling (Figure 1).

Salt treatment study.—One hour after the samples were collected for the handling stress test, the remaining fish in experimental groups (two tanks from each experimental group and two other tanks as control) were subjected to a daily salt treatment at a salinity of 5‰ for 6 d. The salt treatment procedure was performed daily by adding 5‰ to treat fish in tanks for 2 h: a preweighed amount of salt for each tank was dissolved in water of the same temperature in a large bucket; water flow in each tank was stopped just before the salt water was added. Half of the salt water was added to each tank at the beginning of the trial, and then the other half was added an hour later. Water flow was restored at the end of 2 h at the rate of 1 L/min. Fish were sampled at 72 and 144 h after the last salt treatment.

Second handling stressor.—After the last samples were collected from the salt treatment and control groups, the fish groups were subjected to handling stress using the same protocol as in the first handling stress trial. Samples were collected immediately after handling and again 24 h later. Salt treatment was performed daily in the same manner as before and the last samples were obtained 144 h after the second handling.

Data Collection and Analysis

Blood sampling.—Fish were quickly captured and immediately placed in a bucket containing water and a lethal dose (400 mg/L) of tricaine methanesulfonate (Syndel Laboratories, Vancouver, British Columbia). Blood samples, collected from the caudal vein using a 5-mL heparinized syringe, were obtained within 2 min of the fish being captured. The blood was then stored on ice, and then plasma was separated by centrifugation (1,000 × *g* for 10 min) at 4°C, removed, and stored in 1.5-mL microcentrifuge tubes at −80°C for subsequent analysis.

Determination of plasma cortisol.—Total plasma cortisol levels were determined using an enzyme-linked immunosorbent assay (ELISA kit; NEOGEN, Lexington, Kentucky) according to the manufacturer's instructions, and plates were read with a BioTek microplate reader (BioTek Instruments, Winooski, Vermont) at an absorbance of 650 nm.

Statistical analysis.—An ANOVA was performed with the SPSS Statistical Package using GLM (SPSS 2004). The Duncan's multiple range test was used for testing mean differences and a *t*-test was used for determining statistical differences between groups at a significance level of $P < 0.05$.

RESULTS

Plasma Cortisol Levels after the First Handling Stressor

The total plasma cortisol concentrations in the prehandling samples from Yellow Perch subjected and acclimated to 20°C and 26°C were approximately two- to threefold higher than in those subjected to 14°C (Table 1).

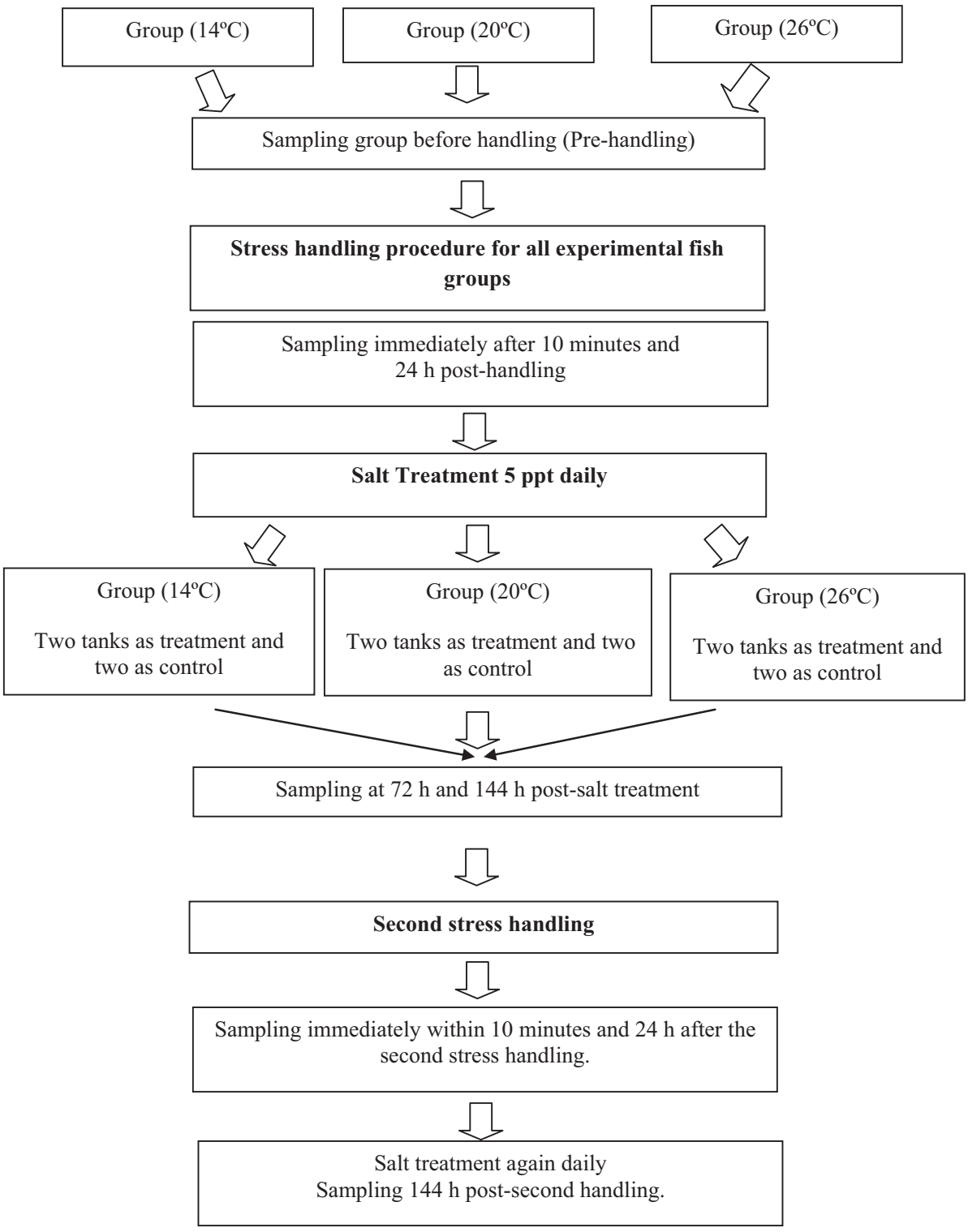

FIGURE 1. Experimental design and sampling schedule used to assess handling stress responses in Yellow Perch. Bold text indicates the times at which the stressors were applied.

TABLE 1. Plasma cortisol concentrations (ng/mL) of plasma in Yellow Perch subjected to handling and salt treatment (ST; 5‰) at different temperatures; values are mean ± SD. Values followed by the same letter are not significantly different from each other ($P < 0.05$).

Handling time	Water temperature		
	14°C	20°C	26°C
Prehandling	33.25 ± 18.6 y	92.0 ± 8.4 z	110.0 ± 51.0 z
Immediately after first handling	169.75 ± 12.5 z	107.75 ± 53.5 y	157.50 ± 13.6 z
24 h after first handling	72.50 ± 45.9	85.70 ± 54.4	78.48 ± 43.6
72 h after first handling with ST	119.75 ± 29.9 zy	79.75 ± 51.9 y	165.50 ± 27.0 z
72 h after first handling with ST: controls	111.75 ± 19.5 y	103.50 ± 45.9 y	184.25 ± 10.2 z
144 h after first handling with ST	128.25 ± 19.1 y	126.00 ± 7.7 y	166.75 ± 21.1 z
144 h after first handling with ST: controls	106.0 ± 37.7	147.25 ± 31.4	107.25 ± 85.9
Immediately after second handling	185.50 ± 7.0	187.25 ± 1.7	189.75 ± 7.9
24 h after second handling with ST	142.50 ± 24.2 zy	116.75 ± 34.7 y	166.00 ± 10.1 z
24 h after second handling with ST: controls	163.50 ± 31.4	168.00 ± 23.1	156.50 ± 24.3
144 h after second handling with ST	110.75 ± 57.9	118.75 ± 47.1	126.50 ± 26.9
144 h after second handling with ST: controls	131.00 ± 40.1	86.75 ± 71.6	92.25 ± 43.7

Plasma cortisol levels increased by approximately 0.5- to 5.0-fold immediately after handling in all fish groups, but increased levels were more prominent in Yellow Perch groups held at 14°C and 26°C than in the group exposed to 20°C (Table 1). No significant differences between the three groups in cortisol levels were detected 24 h after handling. However, cortisol levels were decreased by approximately 50% 24 h after handling in all groups compared with cortisol levels measured immediately (10 min) after handling.

Plasma cortisol levels in the control groups at 72 and 144 h in the daily salt treatment experiment had increased significantly compared with those measured in the groups without salt treatment at 24 h after handling. Also, there was an increase in all treatment groups, except for the plasma cortisol level of the group exposed to 20°C (Table 1).

Plasma Cortisol Levels after the Second Handling Stressor

The highest plasma cortisol levels for all Yellow Perch groups occurred immediately after the second handling, and there was no significant difference between groups. All three groups had nearly similar cortisol levels comparised with all previous cortisol levels. Plasma cortisol levels showed a slight decrease 24 and 144 h after the second handling in all groups, and a significant decrease was recorded in fish exposed to 20°C (Table 1).

General ANOVA of plasma cortisol concentrations for all groups showed high significance with time ($P = 0.000$), temperature ($P = 0.006$), and time versus temperature ($P = 0.020$) (Table 2). The *t*-test values were highly significant ($P = 0.000$) for the comparisons between time and salt, temperature and salt, and temperature and time (Table 3). The general mean determined for temperatures over all times showed that plasma cortisol in the group subjected to 26°C was significantly different from the two other groups, and cortisol levels in fish subjected to 14°C and 20°C were not statistically different (Table 1).No fish mortalities were observed during acclimation or throughout either of the experiments.

TABLE 2. General ANOVA results of plasma cortisol concentrations (ng/mL) in Yellow Perch subjected to three different temperatures and two handling stressors (significance: $P < 0.05$).

Source of variation	df	Mean square	*F*-value	*P*-value
Time	11	12,070.27	8.72	0.000
Temperature	2	7,406.88	5.35	0.006
Time × temperature	22	2,567.32	1.86	0.020
Error	108	1,384.37		
Total	144			

DISCUSSION

Cultured fish in intensive rearing facilities are continuously exposed to management practices, such as handling or transportation, which elicit stress responses (Davis et al. 2002). Repeated or prolonged exposure of fish to common stressors activates the HPI axis leading to increased plasma cortisol and an increased risk of disease (Wendelaar Bonga 1997; Mommsen et al. 1999).

TABLE 3. Analysis (*t*-test) of plasma cortisol concentrations (ng/mL) in Yellow Perch subjected to three different temperatures and two handling stressors (significance: $P < 0.05$).

Pairs compared	df	*t*-value	*P*-value
Time–salt	143	18.80	0.00
Temperature–salt	143	8.46	0.00
Temperature–time	143	15.17	0.00

In the present study, Yellow Perch had relatively high cortisol levels at different water temperatures before handling. The prehandling plasma cortisol levels in the groups held at 20°C and 26°C were two- to threefold higher than in the Yellow Perch exposed to 14°C. After handling, all groups exhibited a one- to fivefold increase in plasma cortisol, which then decreased within 24 h after handling. Other studies have also illustrated that Yellow Perch responded with initial increases in plasma cortisol right after handling, and then decreased gradually (Haukenes and Barton 2004; Hosoya et al. 2007; Haukenes et al. 2008). Acerete et al. (2004) also observed that cortisol levels in Eurasian Perch *P. fluviatilis* increased more than threefold after transportation. Transportation procedures induced rapid elevations in plasma cortisol from 100 to 160 ng/mL within 15–30 min of capture and loading in cultured juvenile Red Drum *Sciaenops ocellatus* (Robertson et al. 1988). Striped Bass *Morone saxatilis* after handling showed a 3.5-fold mean increase in cortisol level up to 400 ng/mL (Cech et al. 1996). In Golden Perch *Macquaria ambigua*, plasma cortisol increased to 240 ng/mL after 30 min of netting and confinement stress (Carragher and Rees 1994).

In this study, Yellow Perch held at 20°C showed the lowest increase in cortisol when first handled, and those held at 26°C and 14°C exhibited significantly higher cortisol levels when handled. This result suggests that Yellow Perch are minimally stressed at a temperature of 20°C. A temperature of 22°C appeared best for growth of Yellow Perch in tank culture systems regardless of the fish stocks (Brown et al. 2002), whereas a temperature of 28°C appeared sufficiently high to represent chronic stress conditions in Yellow Perch (Tidwell et al. 1999). Scott and Crossman (1973) and Brown et al. (2009) reported optimum temperature ranges from 21°C to 24°C for Yellow Perch with an upper lethal limit of 26.5°C for growth. The significantly higher cortisol level in Yellow Perch subjected to handling at14°C may be attributed to the cold water, which could be considered as an additional stress factor other than handling, since the preferred summer temperature of Yellow Perch ranges from 17.6°C to 25°C (Ferguson 1958; Krieger et al. 1983).

There was a significant increase in plasma cortisol from the first handling in Yellow Perch after salt treatment. This is attributed to the increase in water salinity, which is considered to be a stress factor due to alteration of the normal environment. Salinity stimulated the stress response and increased the cortisol level, which may help promote salinity acclimation (Fiol and Kültz 2007; Kammerer et al. 2010). Using saline water (0.5% NaCl) as recovery medium did not attenuate the corticosteroid responses of Walleye *Sander vitreus* to handling, but salt may have allowed the fish to recover more quickly (Barton and Zitzow 1995; Forsberg et al. 2001). Some studies reported that fish immersion in isotonic saline water after a stress event can help to reduce the stress and decrease recovery time (Barton and Peter 1982; Reubush and Heath 1997). In aquaculture practice, aquaculturists usually treat fish with salt after handling to eliminate bacteria and disease. Based on current results, this practice may increase the level of stress at the same time, as indicated by increased plasma cortisol. The salinity of 5‰ may be high for treating Yellow Perch after handling, and research is needed to identify the optimal dosage to treat Yellow Perch and other fish species after handling for common aquaculture practices. Also, lower cortisol levels occurred in groups that were subjected to 20°C during the period of salt treatment, and similar results were observed after the second handling stressor. From this study, we concluded that a water temperature of around 20°C may be desirable for handling Yellow Perch, but salt treatment after handling further stimulates the stress response and increases the circulating cortisol level. Thus, research is needed to identify the optimal salinity to treat Yellow Perch and other fish species after handling during common aquaculture practices.

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REFERENCES

Acerete, L., J. C. Balasch, E. Espinosa, A. Josa, and L. Tort. 2004. Physiological responses in Eurasian Perch (*Perca fluviatilis*, L.) subjected to stress by transport and handling. Aquaculture 237:167–178.

Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517–525.

Barton, B. A., and R. E. Peter. 1982. Plasma cortisol stress response in fingerling Rainbow Trout, *Salmo gairdneri* Richardson, to various transport conditions, anaesthesia, and cold shock. Journal of Fish Biology 20:39–51.

Barton, B. A., and R. E. Zitzow. 1995. Physiological responses of juvenile Walleyes to handling stress with recovery in saline water. Progressive Fish-Culturist 57:267–276.

Bertotto, D., C. Poltronieri, E. Negrato, J. Richard, F. Pascoli, C. Simontacchi, and G. Radaelli. 2011. Whole body cortisol and expression of HSP70, IGF-I and MSTN in early development of sea bass subjected to heat shock. General and Comparative Endocrinology 174:44–50.

Brown, P. B., J. E. Wetzel, J. Mays, K. A. Wilson, C. S. Kasper, and J. Malison. 2002. Growth differences among stocks of Yellow Perch, *Perca flavescens,* are temperature dependent. Journal of Applied Aquaculture 12(1):43–56.

Brown, T. G., B. Runciman, M. J. Bradford, and S. Pollard. 2009. A biological synopsis of Yellow Perch (*Perca flavescens*). Canadian Manuscript Report of Fisheries and Aquatic Sciences 2883.

Carmichael, G. J., J. R. Tomasso, B. A. Simco, and K. B. Davis. 1984. Characterization and alleviation of stress associated with hauling Largemouth Bass. Transactions of the American Fisheries Society 113:778–785.

Carneiro, P. C. F., and E. C. Urbinati. 2001. Salt as a stress response mitigator of Matrinxã, *Brycon cephalus* (Günther), during transport. Aquaculture Research 32:297–304.

Carragher, J. F., and C. M. Rees. 1994. Primary and secondary stress responses in Golden Perch, *Macquaria ambigua*. Comparative Biochemistry and Physiology 107A:49–56.

Cech, J. J., Jr., S. D. Bartholow, P. S. Young, and T. E. Hopkins. 1996. Striped Bass exercise and handling stress in freshwater: physiological responses to recovery environment. Transactions of the American Fisheries Society 125:308–320.

Davis, C. R., M. S. Okihiro, and D. E. Hinton. 2002. Effects of husbandry practices, gender, and normal physiological variation on growth and reproduction of Japanese Medaka, *Oryzias latipes*. Aquatic Toxicology 60:185–201.

Ferguson, R. G. 1958. The preferred temperature of fish and their midsummer distribution in temperate lakes and streams. Journal of the Fisheries Research Board of Canada 15:607–624.

Fiol, D. F., and D. Kültz. 2007. Osmotic stress sensing and signaling in fishes. FEBS (Federation of European Biochemical Societies) Journal 274:5790–5798.

Forsberg, J. A., R. C. Summerfelt, and B. A. Barton. 2001. Physiological and behavioral stress responses of Walleyes transported in salt and buffered-salt solutions. North American Journal of Aquaculture 63:191–200.

Harrell, R. M. 1992. Stress mitigation by use of salt and anesthetic for wild Striped Bass captured for brood stock. Progressive Fish-Culturist 54:228–233.

Haukenes, A. H., and B. A. Barton. 2004. Characterization of the cortisol response following an acute challenge with lipopolysaccharide in Yellow Perch and the influence of rearing density. Journal of Fish Biology 64:851–862.

Haukenes, A. H., B. A. Barton, and H. Bollig. 2008. Cortisol responses of Pallid Sturgeon and Yellow Perch following challenge with lipopolysaccharide. Journal of Fish Biology 72:780–784.

Head, A. B., and J. A. Malison. 2000. Effects of lighting spectrum and disturbance level on the growth and stress responses of Yellow Perch *Perca flavescens*. Journal of the World Aquaculture Society 31:73–80.

Hight, B. V., D. Holts, J. B. Graham, B. P. Kennedy, V. Taylor, C. A. Sepulveda, D. Bernal, D. Ramon, R. Rasmussen, and N. C. Lai. 2007. Plasma catecholamine levels as indicators of the post-release survivorship of juvenile pelagic sharks caught on experimental drift longlines in the Southern California Bight. Marine and Freshwater Research 58:145–151.

Hosoya, S., S. C. Johnson, G. K. Iwama, A. K. Gamperl, and L. O. B. Afonso. 2007. Changes in free and total plasma cortisol levels in juvenile Haddock (*Melanogrammus aeglefinus*) exposed to long-term handling stress. Comparative Biochemistry and Physiology 146A:78–86.

Kammerer, B. D., J. J. Cech Jr., and D. Kültz. 2010. Rapid changes in plasma cortisol, osmolality, and respiration in response to salinity stress in tilapia (*Oreochromis mossambicus*). Comparative Biochemistry and Physiology 157A:260–265.

Krieger, D. A., J. W. Terrell, and P. C. Nelson. 1983. Habitat suitability information: Yellow Perch. U.S. Fish and Wildlife Service Biological Services Program FWS/OBS-82/10.55. Available: purl.access.gpo.gov/GPO/LPS101936. (December 2012).

Lowe, C. J., and W. Davison. 2005. Plasma osmolarity, glucose concentration and erythrocyte responses of two Antarctic nototheniid fishes to acute and chronic thermal change. Journal of Fish Biology 67:752–766.

Mazik, P. M., B. A. Simco, and N. C. Parker. 1991. Influence of water hardness and salts on survival and physiological characteristics of Striped Bass during and after transport. Transactions of the American Fisheries Society 120:121–126.

Mommsen, T. P., M. M. Vijayan, and T. W. Moon. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9:211–268.

Pankhurst, N. W. 2011. The endocrinology of stress in fish: an environmental perspective. General and Comparative Endocrinology 170:265–275.

Reid, S. G., N. J. Bernier, and S. F. Perry. 1998. The adrenergic stress response in fish: control of catecholamine storage and release. Comparative Biochemistry and Physiology 120C:1–27.

Reubush, K. J., and A. G. Heath. 1997. Effects of recovery water salinity on secondary stress responses of hybrid Striped Bass fingerlings. Progressive Fish-Culturist 59:188–197.

Robertson, L., P. Thomas, and C. R. Arnold. 1988. Plasma cortisol and secondary stress responses of cultured Red Drum (*Sciaenops ocellatus*) to several transportation procedures. Aquaculture 68:115–130.

Romero, L. M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General and Comparative Endocrinology 128:1–24.

Rottmann, R. W., R. Francis-Floyd, and R. Durborow. 1992. The role of stress in fish disease. Southern Regional Aquaculture Center, Publication 474, Stoneville, Mississippi.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 184.

Skomal, G. B., and J. W. Mandelman. 2012. The physiological response to anthropogenic stressors in marine elasmobranch fishes: a review with a focus on the secondary response. Comparative Biochemistry and Physiology 162A:146–155.

SPSS. 2004. SPSS base 13.0: user's guide. SPSS, Chicago.

Stratholt, M. L., E. M. Donaldson, and N. R. Liley. 1997. Stress induced elevation of plasma cortisol in adult female Coho Salmon (*Oncorhynchus kisutch*) is reflected in egg cortisol content, but does not appear to affect early development. Aquaculture 158:141–153.

Tidwell, J. H., S. D. Coyle, J. Evans, C. Weibel, J. McKinney, K. Dodson, and H. Jones. 1999. Effect of culture temperature on growth, survival, and biochemical composition of Yellow Perch *Perca flavescens*. Journal of the World Aquaculture Society 30:324–330.

Tort, L. 2011. Stress and immune modulation in fish. Developmental and Comparative Immunology 35:1366–1375.

Vijayan, M. M., C. Pereira, E. G. Grau, and G. K. Iwama. 1997. Metabolic responses associated with confinement stress in tilapia: the role of cortisol. Comparative Biochemistry and Physiology 116C:89–95.

Wedemeyer, G. 1972. Some physiological consequences of handling stress in the juvenile Coho Salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada 29:1780–1783.

Wendelaar Bonga, S. E. 1997. The stress response in fish. Physiological Reviews 77:591–625.