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Effect of Aeration Type and Circulation on Stress Indices of Paddlefish Held in Rectangular Tanks for 24 Hours

Steven D. Mims
Richard J. Onders
Changzheng Wang

ABSTRACT. Paddlefish, *Polyodon spathula,* is an emerging aquaculture species that filter-feeds on zooplankton and grows well in polyculture with channel catfish, *Ictalurus punctatus,* in the southern region of the United States. Only a few private hatcheries exist that produce juvenile paddlefish for stocking in grow-out ponds; therefore, often the juveniles have to be transported for 8 to12 hours. The objective of this study was to determine the effect of different aeration and circulation practices on stress indices of juvenile paddlefish over a 24-hour period. Twelve rectangular polyethylene tanks (170 L capacity) were randomly assigned into four treatment groups: diffused air with circulation (A-C), diffused air without circulation (A-NC), compressed oxygen with circulation (O_2-C), and compressed oxygen without circulation (O_2-NC). Twenty juvenile paddlefish (210 ± 24 g) were stocked into each tank that was supplied with either oxygen or air. Submersible pumps were used in the tanks for circulation. Blood samples were taken from twenty fish to use as baseline data. At 4, 8, 12, and 24 hours after stocking, blood samples were taken from five fish in each group. At each sampling time, five new fish were used. Plasma cortisol, lactate, and glucose levels were quantified. Overall, O_2-NC group gave the lowest levels of plasma cortisol, whereas the A-C group had the highest level of plasma cortisol.

Steven D. Mims, Richard J. Onders, Aquaculture Research Center, Kentucky State University, Frankfort, KY 40601.

Changzheng Wang, Community Research Service, Kentucky State University, Frankfort, KY 40601.

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Plasma lactate and glucose levels decreased over the 24 hours and were not significantly different ($P \geq 0.05$) among treatments. Fish in tanks without circulation had significantly lower ($P < 0.05$) plasma cortisol levels than in tanks with circulation. Fish with circulation (i.e., O_2-C and A-C groups) appeared to be disturbed by the circulation as they tried to swim against the water current. On the other hand, fish in the O_2-NC and A-NC groups were calm and remained in an inverted position. Use of oxygen with no circulation was the least stressful method of holding the fish and is recommended for live transportation of juvenile paddlefish. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <http://www.HaworthPress.com> © 2006 by The Haworth Press, Inc. All rights reserved.]*

KEYWORDS. Paddlefish, aeration, circulation, cortisol, glucose, lactate

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INTRODUCTION

Paddlefish, *Polyodon spathula*, is a freshwater chondrostean fish, phylogenetically related to sturgeons. It is native in the river systems of the Gulf of Mexico drainage of North America including about 26 states in the United States (Hubbs and Lagler 1958; Gengerke 1986). Paddlefish, a filter-feeder, is an emerging aquacultural species that grows well in polyculture with channel catfish, *Ictalurus punctatus*, in the southern region of the United States (Mims 2001; Schardein et al. 2002; Schardein 2003). A few private hatcheries produce juvenile paddlefish for stocking in grow-out ponds, but often the fish must undergo a long transport time of 8 to12 hours.

Physical disturbances to fish during the transport process include handling, confinement, and stocking. These disturbances generally cause stress responses that can trigger physiological changes, and thus ultimately affect the health of the fish (Barton and Iwama 1991). Barton et al. (1998) were the first to report elevation in plasma cortisol and lactate of hatchery-reared juvenile paddlefish under severe confinement conditions (i.e., held in cages and disturbed frequently). Elevation in circulating corticosteroid is the physiological response of the hypothalamic-pituitary-interrenal axis due to stress (Hazon and Balment 1998). Further, secondary responses from elevated cortisol often include increase in plasma lactate due to anaerobic metabolism, and generation of lactic acid in the white muscle, as well as plasma glucose, due to mobilization of energy reserves. Plasma cortisol, lactate, and glucose can be quantitatively

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measured and can be useful parameters in monitoring stress in paddlefish during transportation.

There are some key biological differences between paddlefish and other finfish, which must be considered in developing transportation procedures. Paddlefish are continuous swimmers and must open their mouth as they swim to ventilate their gills, known as ram ventilation. Paddlefish in confinement will usually float belly up and remain lethargic. Common transport practices for paddlefish have included aeration via air compressor or compressed/liquid oxygen in combination with circulation via recirculation or agitation in order to supply moving, well-oxygenated water around the immobile fish. However, after periods of long transportation (> 8 hours) with subsequent stocking, survival was variable, suggesting that transport stress affected the health of the fish. Better understanding of the stress responses of paddlefish to aeration and circulation practices will help to develop transportation guidelines. The objective of this study was to determine the effect of aeration and circulation practices on the stress indices of juvenile paddlefish held in rectangular tanks over a 24-hour period.

MATERIALS AND METHOD

Fish

Juvenile paddlefish (7 months old; 210 ± 24 g) were progenies from wild Ohio River broodstock, reared in ponds on prepared diets as described by Mims (2001) at the Aquaculture Research Center (ARC), Kentucky State University, Frankfort. Paddlefish were not fed for 48 hours before they were collected with a bag seine from a 400-m^2 pond. Two hundred and sixty fish were placed into an 1125-L live tank supplied with oxygen and taken to an indoor facility to conduct the experiment. Fish were acclimated for 30 minutes to dechlorinated tap water, which replaced the pond water in the live tank. This simulated the transfer of paddlefish from ponds into live tanks filled with well water.

Twelve rectangular polyethylene tanks (170 L capacity) were randomly assigned into one of the four groups of three tanks each. Group A-NC was supplied with diffused air without circulation; Group A-C received diffused air with circulation; Group O_2-NC received compressed oxygen without circulation; and, Group O_2-C received compressed oxygen with circulation. Submersible pumps were used for water circulation in A-C

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and O_2-C Groups. The aeration system was on for 1 hour before any fish were placed into the tanks.

Each tank received 20 fish (0.5 fish/ L) and was covered for the study. Twenty additional fish were used for baseline data representing 0 hour.

Water temperature was maintained between 10-13°C in a temperature-controlled environment (via thermostat set at 11°C). Total ammonia, nitrite, alkalinity, pH, dissolved oxygen, and water temperature were measured and recorded at 0 hour and each additional time of 4, 8, 12, and 24 hours when the fish were sampled. After 24 hours, fish from each treatment were placed into one of four circular tanks (3.7-m diameter; 2-m depth) and observed for 24 hours before they were released into a pond.

Experimental Sampling and Analytical Procedures

At each sampling time, five fish from each tank were individually removed, immediately sampled for blood, measured for length and weight, fin clipped, and returned to its designated tank. Fin clipping indicated a previously sampled fish and these fish were not used again for further blood sampling. Blood was collected into a 3-mL vacutainer® (Pre-analytical Solutions, Franklin Lakes, New Jersey[1]) containing lithium heparin with a 21-gauge needle inserted into the caudal vein. The collected blood was placed immediately on ice and then centrifuged (2400 g) for 15 minutes before plasma was collected and stored at −20°C until analyses.

Plasma cortisol was measured by radioimmunoassay using a Coat-A-Count® Cortisol test kit (TKCO2; DPC, Los Angeles, California). Pre-testing of the kit gave cortisol levels comparable to those reported by Barton et al. (1998). Plasma lactate was determined enzymatically using Sigma Diagnostic® Procedure 735 (Sigma, St. Louis, Missouri). Plasma glucose was determined by spectrophotometric measurement using Sigma Diagnostic® pre-mixed ortho-toluidine reagent (Sigma, St. Louis, Missouri). Water temperature and dissolved oxygen (DO) were measured using an YSI Model 59 DO meter (YSI Instruments Co., Yellow Springs, Ohio). Total ammonia-nitrogen (TAN), nitrite-nitrogen, and alkalinity were determined according to outline procedures for HACH DR/2000 spectrophotometer (HACH Co., Loveland, Colorado), using water samples collected from each tank at each time period. The pH was determined at each time period using a pH meter (Oakton Instrumentation,

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Singapore). Un-ionized ammonia was calculated based on TAN, water temperature, and pH according to Emerson et al. (1975).

Data Analysis

Data at each sampling time were analyzed using the GLM procedure of SAS (SAS Institute, 1990). Treatment and tank were in the model. Tank effect was used as the error term to test the treatment effect. Orthogonal contrasts were used to detect the difference due to aeration, circulation, and the interaction between aeration and circulation. The probability level for statistical significance was set at $P < 0.05$.

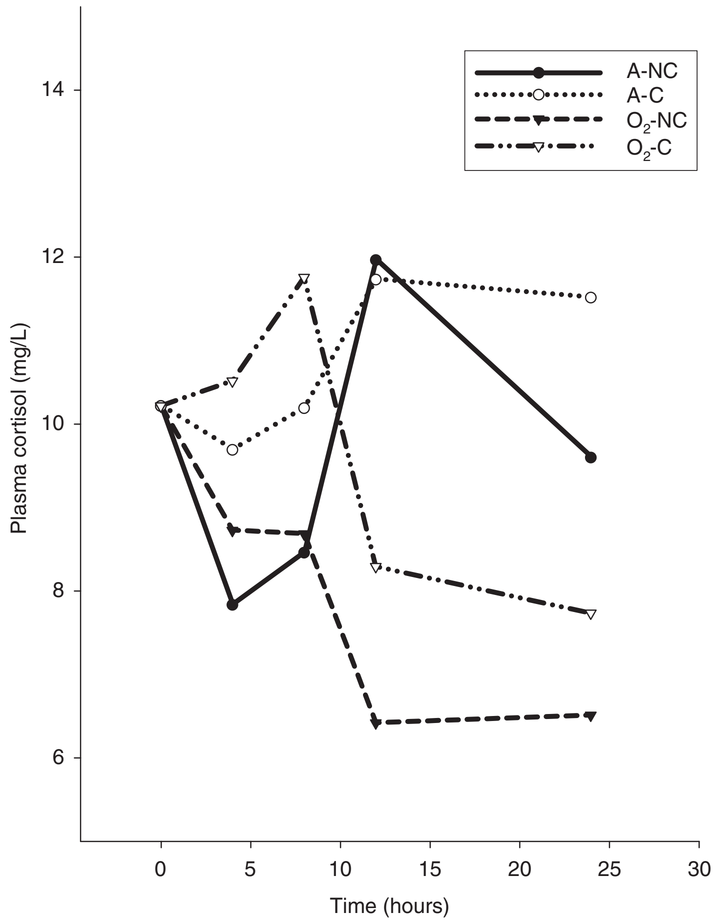
RESULTS

Plasma cortisol was 10.3 ± 1.77 ng/mL for the baseline group at 0 hour. This value represents an average about one hour from the time that the fish were initially disturbed (i.e., seined and transported to study site) and then stocked in the study. Overall, O_2-NC group gave the lowest levels of plasma cortisol, whereas the A-C group had the highest level of plasma cortisol (Figure 1). Cortisol level tended to decrease over time for O_2-NC and O_2-C groups, but it stayed elevated up to 24 hours for A-NC and A-C groups ($P < 0.01$). Cortisol level was higher for the O_2-C and A-C groups than for the O_2-NC and A-NC groups ($P < 0.01$). There was no significant interaction between aeration and circulation in plasma cortisol. Plasma cortisol levels from fish in NC treatments were significantly lower after 4 and 8 hours than from those of C treatments. Thereafter, plasma cortisol levels from fish in O_2 treatments were significantly lower after 12 and 24 hours than those of fish in A treatments. The O_2-NC treatment also had the lowest levels of plasma cortisol (ca. 6.4 ng/mL) after 12 and 24 hours compared to those or other treatments (range of 7.7-12.0 ng/mL).

Plasma lactate declined gradually from120 ± 9.3 to 29 ± 4.6 mg/dL for all groups during the 24-hour period, but there were no significant differences in plasma lactate levels among the groups at each time period (Figure 2). Plasma glucose also decreased from 40 ± 5.4 to 29 ± 2.7 mg/dL, but there were no significant differences for plasma glucose levels among treatments at each time period (Figure 3).

Dissolved oxygen level was approximately 9.0 ± 1.02 mg/L for A-NC and A-C groups with minimal fluctuation between sampling times (Figure 4). On the contrary, DO was more than 19.0 mg/L for O_2-NC group with

FIGURE 1. Mean concentration of cortisol (SEM = ± 0.89 ng/mL; n = 3) in the blood plasma of juvenile paddlefish held under four live-transport groups: air without circulation (A-NC), air with circulation (A-C), oxygen without circulation (O_2-NC) and oxygen with circulation (O_2-C).



only a slight decline towards the end of the experiment. For the A-C group, DO was 15.3 mg/L initially but decreased to 10.7 mg/L after 12 hours. Water temperature ranged from 10.3 to 13.1°C, pH ranged from 7.4 to 7.9, nitrite 0.013 to 0.047 mg/L, un-ionized ammonia ranged from 0.001 to 0.036 mg/L, and alkalinity 66 to 109 mg/L. There were no significant differences among groups in these parameters, which were within safe levels for paddlefish (Mims et al. 1999).

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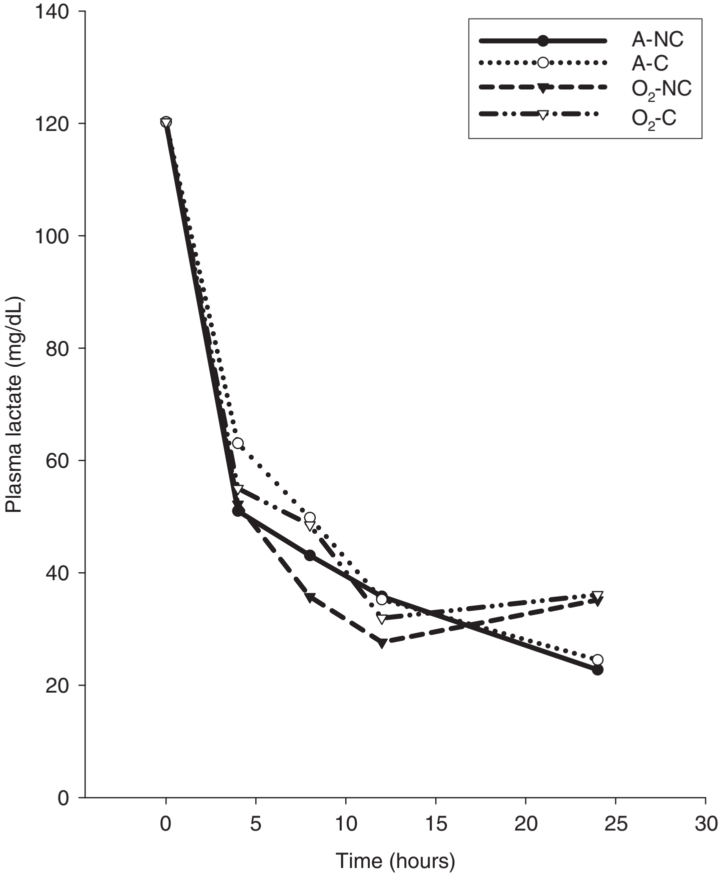
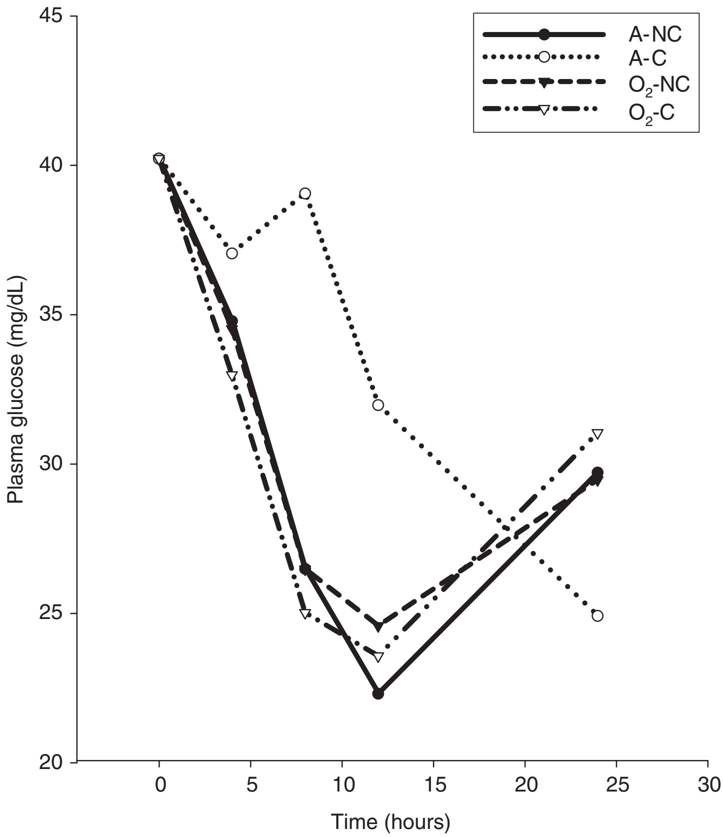
FIGURE 2. Mean concentration of lactate (SEM = ± 4.65 mg/dL; n = 3) in the blood plasma of juvenile paddlefish held under four live-transport groups: air without circulation (A-NC), air with circulation (A-C), oxygen without circulation (O_2-NC) and oxygen with circulation (O_2-C).



Fish Response

During the course of the study, fish in the O_2-C and A-C groups tried to swim against the water current, suggesting that the circulation causes some disturbance to the fish. On the other hand, fish in the O_2-NC and A-NC groups were calm and remained in an inverted position, which is a normal response for paddlefish in confinement. At the end of the study,

FIGURE 3. Mean concentration of glucose (SEM = ± 3.72 mg/dL; n = 3) in the blood plasma of juvenile paddlefish held under four live-transport groups: air without circulation (A-NC), air with circulation (A-C), oxygen without circulation (O_2-NC) and oxygen with circulation (O_2-C).



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each group was placed into a circular tank filled with water. Ten to twenty percent of the fish from the O_2-C and A-C treatments initially appeared disoriented. These fish swam at the surface with their paddles above the surface known as "billing." Within 15-20 minutes, they were able to submerge and resume normal swimming behavior. Billing was not observed for fish from the other groups without circulation. All the fish used in the experiment survived 24 hours after treatment.

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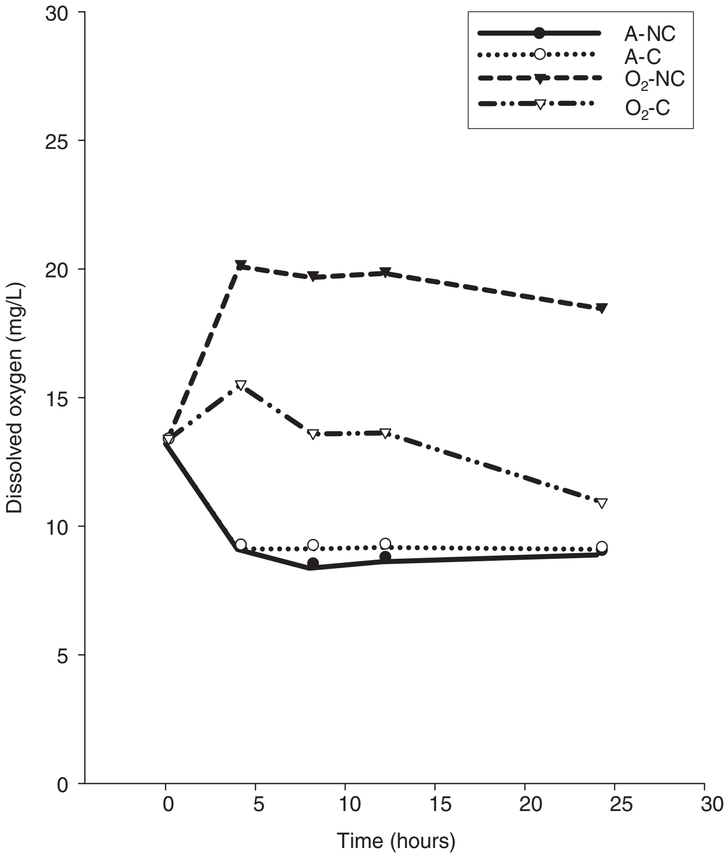
FIGURE 4. Mean concentration of dissolved oxygen (SEM = ± 1.02 mg/L; n = 3) in the water of the four live-transport groups: air without circulation (A-NC), air with circulation (A-C), oxygen without circulation (O_2-NC) and oxygen with circulation (O_2-C).



DISCUSSION

It is well known that cortisol secreted from interrenal tissue is stimulated in response to stress such as handling, confinement, crowding, and other physical disturbances (Hazon and Balment 1998; Barton 2000) and is known to be the principal steroid released during stress for paddlefish (Barton et al. 1998). However, juvenile paddlefish have demonstrated

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lower magnitudes of cortisol increase due to physical disturbances compared to levels in most teleostean fishes (Barton and Iwama 1991; Barton et al. 1998). This maybe explained by the finding of Rahn (1997) that the interrenal tissue is dispersed throughout the length of the kidneys in paddlefish in contrast to that of teleostean fishes, where the interrenal tissue is concentrated around the posterior cardinal vein in the head kidney (Chester-Jones and Mosley 1980).

Plasma cortisol levels observed in this study ranged from 6.4 ± 0.94 to 11.8 ± 0.89 ng/mL in response to the experimental conditions. This is in agreement with cortisol levels (2.2 ± 0.6 to 11±1.8 ng/mL) in the blood plasma of juvenile paddlefish handled with dip nets for 30 seconds and placed in circular tanks, but were well below levels of those in confined for 6 hours with severe handling (6.2 ± 1.6 to 74 ± 6.3 ng/mL) (Barton et al. 1998). These results suggest that transportation practices studied in this experiment only caused moderate stress responses in paddlefish.

Circulation is often recommended for transporting live fish (Johnson 2000). It is known that circulation will help to remove CO_2 and provide even distribution of O_2. In this study, levels of plasma cortisol were elevated in both circulation groups after 4 and 8 hours. It seemed that circulation actually lowered DO, and caused an increase in plasma levels of cortisol, which correlated with a higher stress level. This might have resulted from the fact that circulation forced the fish to bump into each other and the walls of the tank.

Plasma lactate and glucose were low in magnitude likely due to the low cortisol levels, indicating a minimal effect on the metabolism of these fish. In comparison, Barton et al. (1998) reported nearly a three-fold increase in lactate but no significant change in glucose under a 6-hour severe confinement with handling. This suggests that frequent handling caused more physiological stress than confinement and crowding alone.

Adequate supply of oxygen is critical for the success of transporting any live fish. In this study, cortisol in the groups supplied with compressed oxygen, were lower between 12 and 24 hours, of transportation, compared to the groups supplied with air. Dissolved oxygen was 12.7 mg/L for the group with circulation, but it was elevated to 19.1 mg/L without circulation. This oxygen supersaturated condition appeared especially beneficial to paddlefish because paddlefish do not have an active buccal pumping mechanism found in other fishes (Bemis et al. 1997). The circulation in this case might have accelerated the dissipation of oxygen, resulting in lowered DO among the groups with circulation.

The fish swimming responses after release from confinement suggests that a physical disturbance due to the circulating water could have caused

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disorientation (i.e., a type of vertigo) of the fish. Such disorientation could hinder normal swimming and cause the fish to become stranded at the shoreline or at the bottom of the pond. The inability to properly swim could interfere with ram ventilation, resulting in suffocation and mortality.

In conclusion, paddlefish, like other fishes, should be live-transported using a procedure to reduce stress and ensure arrival of healthy fish at their destination. The results from this study suggest that juvenile paddlefish should be supplied with oxygen without circulation during live transportation up to 24 hours. This information will be useful in the development of live-transport protocol. Further research in loading rates and water temperature regimes needs to be studied for more intensive transportation of juvenile paddlefish.

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REFERENCES

Barton, B. A. 2000. Stress. Pages 892-898 *in* R.R. Stickney, editor, Encyclopedia of Aquaculture. John Wiley & Sons, New York, New York.

Barton, B. A., and G. K. Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases 1:3-26.

Barton, B. A., A. B. Rahn, G. Feist, H. Bollig, and C. B. Schreck. 1998. Physiological stress responses of the freshwater chondrostean paddlefish (*Polyodon spathula*) to acute disturbances. Comparative Biochemistry and Physiology Part A 120: 355-363.

Bemis, W. E., E. K. Findeis, and L. Grande. 1997. An overview of Acipenseriformes. Environmental Biology 7:773-787.

Chester-Jones, I., and W. Mosley. 1980. The interrenal gland in Pisces. Part 1. Structure. Pages 396-472 *in* I. Chester-Jones and Henderson, I. W., editors, General, Comparative and Clinical Endrocrinology of the Adrenal Cortex. Academic Press, London, England.

Emerson, K., R. C. Russo, R. E. Lund, and R. V. Thurston. 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. Journal of Fisheries Research Board of Canada 32:2379-2383.

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Gengerke, T. W. 1986. Distribution and abundance of paddlefish in the United States. Pages 22-35 *in* J. G. Dillard, L. K. Graham and T. R. Russell, eds. The Paddlefish: Status, Management and Propagation. North Central Division, American Fisheries Society, Special Publication 7, Bethesda, Maryland.

Hazon, N., and R. J. Balment. 1998. Endocrinology. Pages 441-463 *in* David Evans, ed. The Physiology of Fishes 2nd Edition. CRC Press, Boca Raton, Florida.

Hubbs, C. L., and K. F. Lagler. 1958. Fishes of the Great Lakes region. Cranbrook Institute of Science 26:1-213.

Johnson, S. K. 2000. Live transport. Pages 496-501 *in* R.R. Stickney, ed. Encyclopedia of Aquaculture. John Wiley & Sons, New York, New York.

Mims, S. D. 2001. Aquaculture of paddlefish in the United States. Aquatic Living Resources 14:391-398.

Mims, S. D., W. L. Shelton, F. S. Wynne, and R. J. Onders. 1999. Production of Paddlefish. Southern Regional Aquaculture Center (SRAC) Publication 437. Southern Regional Aquaculture Center, Stoneville, Mississippi.

Rahn, A. B. 1997. The Distribution, Ultrastructure, and Steroidogenic Activity of the Interrenal Tissue in the Paddlefish, *Polyodon spathula*. Master's Thesis, Department of Biology, University of South Dakota, Vermillion, South Dakota.

SAS Institute, Inc. 1990. SAS User's Guide: Statistics, Version 6 edition. SAS, Inc., Cary, North Carolina.

Schardein, M. J. 2003. Polyculture of Paddlefish, *Polyodon spathula*, with Channel Catfish, *Ictalurus punctatus*, in the Southern Region of the United States. Master's Thesis, Kentucky State University, Frankfort, Kentucky.

Schardein, M. J., S. Dasgupta, and S. D. Mims. 2002. Growth, weight, and survival of paddlefish, *Polyodon spathula*, stocked at two densities in channel catfish, *Ictalurus punctatus*, ponds. Journal of the Kentucky Academy of Science 63(2):93-96.