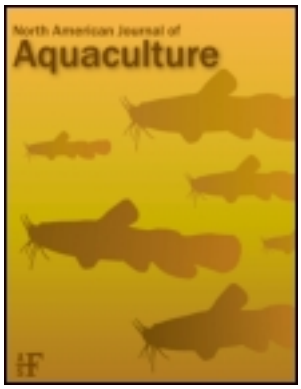

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COMMUNICATION

Evaluation of the Cortisol Stress Response in a Marine Perciform Fish, the San Pedro *Oplegnathus insignis*

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Abstract

The San Pedro (also known as the Pacific beakfish) *Oplegnathus insignis* is a species of perciform fish found in the eastern Pacific Ocean. In northern Chile, San Pedro are an important food fish currently being evaluated for aquaculture. The purpose of this study was to conduct an initial evaluation of the cortisol stress response in captive-bred San Pedro. The fish were subjected to confinement stress by crowding them into a low volume of water (231 kg/m^3) for 90 min. Blood was collected over time for the determination of plasma cortisol. Confinement resulted in a significant increase in plasma cortisol, from a resting concentration of 24.9 ng/mL to 120.7 ng/mL after 10 min into the stress experience. After 20 and 60 min of stress, cortisol concentrations plateaued at 225.3 ng/mL and 243.7 ng/mL, respectively, followed by a decrease to 56.1 ng/mL by 90 min. These results indicate a rapid and robust cortisol stress response in this species. This is the first evaluation of the San Pedro stress response, and these data will serve as the baseline for future evaluations of San Pedro stress physiology and the development of aquaculture techniques suitable for this species.

Among the native fishes of Chile, a marine rockfish commonly known as San Pedro (or Pacific beakfish) *Oplegnathus insignis*, is at present being developed as a potential species for use in aquaculture. This perciform fish belongs to the family Oplegnathidae, known as knifejaws, and is distributed from Paita, Peru, to Antofagasta, Chile, in the eastern Pacific Ocean (Chirichigno 1974). Its market value as a food fish is mainly based on the quality of its meat, high consumer demand, and attractive pricing, all of which support the possibilities of occupying a niche in international seafood markets. This last attribute is based primarily on the existence of resources from the same genus, *O. fasciatus* and *O. punctatus*, in Asia (Korea and Japan) that are highly ranked in their respective markets and are currently also under research aimed at developing technology for their culture.

In Chile, the research aimed at establishing the culture technology for San Pedro is ongoing and has been developed thus far by the Corporación Privada para el Desarrollo de la Universidad Arturo Prat, with the support of research and development funds from the State of Chile. The investigation has led to significant progress in understanding juvenile production under controlled conditions, having resolved problems relating to the collection, conditioning, and spawning of broodstock; incubation at the optimal conditions and ontogenetic development; and establishment of specific foods and feeding protocols for the larval and juvenile stages (Muñoz et al. 2009). However, there are still gaps to be addressed for the solid development of a process that is replicable, scalable, and transferable to industry.

Research foci requiring continued effort include optimizing factors that maximize growth during juvenile and grow-out

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stages. Such efforts involve the manipulation of environmental variables such as temperature, diet, and management to generate better performance in terms of biomass production. Research into the development of optimal culture techniques for new species has generated considerable attention in recent years concerning the potential detrimental effects of stress on fish and on the fish stocks used in aquaculture (Schreck 2010). Crowding, handling, sorting, grading, and transportation are among the many potential stressors a fish may experience in the culture environment. Such stressors are often detrimental and have been linked to increased oxygen consumption, diversion of energy from growth and development, suppressive effects on immune function, and mortality (Wendelaar Bonga 1997; Kassahn et al. 2009). Stress agents deregulate homeostasis, influencing all levels of organization and affecting a variety of metabolic pathways (Wendelaar Bonga 1997).

Stress has both direct and indirect components, depending on how they affect organizational structure and function. Direct effects are those that affect the fish at the organism level, such as alterations of physiological functions, hormones, or cellular mechanisms, for example, alterations in circulating cortisol and glucose and changes in ionic balance and hematological profile (Barton 2002). Indirect effects operate at the population or community level, affecting the energy availability of fish through alterations of trophic relationships (Barton and Iwama 1991). In freshwater and euryhaline teleost fish, responses to stressors that probably will be encountered in aquaculture environments have been well described (Barton and Iwama 1991; Wendelaar Bonga 1997). Furthermore, the resulting increase in plasma cortisol concentration following stress is well established as a measure of the degree of stress a fish experiences (Barton 2002). However, numerous factors are known to affect the intensity of cortisol responsiveness, including environmental factors, physiological status, rearing history, and genetics (Barton 2002; Martínez-Porchas et al. 2009). Understanding these effects is critical to the interpretation of cortisol concentrations with regard to the degree of stress a fish may be experiencing. Before these effects can be understood, resting (prestress) and maximal (poststress) circulating cortisol concentrations must be established as the scale for comparison.

Differences in prestress and poststress concentrations of circulating cortisol can be substantial among species. For example, pallid sturgeon *Scaphirhynchus albus* are reported to have prestress cortisol levels of 0.7 ng/mL and poststress concentrations of 10.7 ng/mL (Webb et al. 2007), while prestress and poststress cortisol concentrations of rainbow trout *Oncorhynchus mykiss* have been reported as 28.6 and 253 mg/L, respectively (Benfey and Biron 2000). Although the cortisol stress response is well studied in these and several other species of cultured fish, the cortisol stress response in San Pedro has not been previously documented. Understanding the stress response in these fish is important to the successful development of culture techniques for optimizing fish health and production efficiency. The present research provides the first description of prestress and poststress plasma cortisol concentrations in San Pedro to serve as a baseline for future evaluations of stress conditions.

METHODS

Experimental animals.—Juvenile San Pedro (5–10 g) were obtained from the Center of Reproduction (CORDUNAP) on the Huayquique Campus of the University Arturo Prat, where they had been maintained in a flow-through 1000-L tank receiving filtered natural saltwater (saline, 34.5–35 ppt); dissolved oxygen was between 6 and 8 mg/L, pH was between 6.5 and 7.5, temperature was controlled between 17°C and 19°C, and the water exchange rate was 270 L/h. No significant fluctuations in these parameters were observed in the months preceding the experiment. During this time, the fish were fed daily with a prepared feed containing 50% protein and 8–10% lipid and were subjected to a continuous light–dark cycle of 12 h:12 h. The sex of individual fish could not be determined by external examination.

Stress challenge.—During the stress challenge, blood was collected from caudal vessels into heparinized syringes. Prestress blood (approximately 100–200 μL) was collected from 10 anesthetized fish in less than 5 minutes total time. The fish were rapidly netted from the acclimated communal tank and immediately anesthetized with 200 mg/L tricaine methanesulfonate (MS-222). Approximately 100 fish were simultaneously netted from the communal tank (stocking density = 3.4 kg/m^3) and moved to an adjustable-diameter mesh enclosure inside another 1000-L tank. The volume of water in the tank was immediately reduced from 35 cm to 4 cm deep, until the fish could not maintain their orientation. The diameter of the mesh enclosure was reduced as fish were removed for sampling so that the remaining fish were kept in constant contact with each other at a density of approximately 231 kg/m^3. Air stones were placed at the periphery of the enclosure to maintain adequate dissolved oxygen. The fish were maintained under these conditions with aeration for 90 min.

At each time point during the stress challenge, blood collection was limited to 2 min after netting to ensure that cortisol levels represented the associated times poststress. This was accomplished by rapidly anesthetizing the fish with 200 mg/L tricaine methanesulfonate, after which two people took blood samples. As a result, 3–4 fish were sampled at 5, 10, 20, 30, 40, 50, 60, and 90 min after initiation of the confinement stressor. After blood collection, sampled fish were returned to the original communal tank for recovery and the mesh enclosure in the experimental tank was adjusted to maintain fish in constant contact with each other as previously described. Plasma was kept on ice, then separated from whole blood by centrifugation for 6 min at 6,000 × *g* at 4°C, and frozen (–80°C) for later analysis. Plasma cortisol was analyzed by a validated ELISA in the Instituto de Biotecnología de Tarapacá-CORDUNAP.

Cortisol analysis.—Quantitative determination of cortisol in standards and San Pedro plasma was conducted by ELISA (ADI-900-071:, Enzo Life Sciences, USA). This ELISA kit

uses a monoclonal antibody to cortisol to bind competitively to plasma cortisol or exogenous cortisol bound to alkaline phosphatase. Cortisol standards and assay solutions were prepared according to the manufacturer's directions. One hundred microliters of standards or plasma was consequently assayed in a 96-well microtiter plate. The standard curve was generated from eight cortisol standards: 0, 1.6, 3.1, 6.3, 12.5, 25.0, 50.0, and 100.0 ng/mL. Plasma samples determined to have cortisol concentrations exceeding that of the highest concentration standard were diluted accordingly and assayed again. Absorbance was measured at 405 nm with a Sunrise microplate reader (Tecan Group Ltd., Switzerland).

Parallelism in dilution curves of cortisol standards and plasma was determined by calculating the within-dilution coefficient of variation (CV) for the serially diluted plasma according to the guidelines established by Plikaytis et al. (1994). Within-dilution CVs for serially diluted San Pedro plasma were less than 15% and met the criteria for parallelism with the standard dilution curve (Plikaytis et al. 1994). Precision and reproducibility of the ELISA, as estimated by intra- and interassay CV, were determined from assays of plasma samples having low, moderate, and elevated cortisol levels and calculated as CV. Intra- and interassay CVs for the ELISA were less than 10% and 17%, respectively. Accuracy of the ELISA, calculated as the percent of exogenous cortisol recovered from cortisol-supplemented plasma samples, averaged 98.8% when added cortisol amounts were low (87.9% recovery) or high (109.7% recovery).

Statistical analysis.—Statistical differences among plasma cortisol levels at different time points were assessed by one-way analysis of variance (ANOVA) with a GraphPad Prism 5.0 (GraphPad Software, USA). Each fish served as the experimental unit, and variation among fish was used as the experimental error in tests of significance. Significant differences ($P < 0.01$) between treatment plasma cortisol levels measured during the stress challenge were determined by using the Bonferroni post hoc test. Plasma cortisol values are presented as means ± SEs.

RESULTS

All fish were exposed to the continuous confinement stress throughout the experiment in an effort to elicit a peak cortisol response. At time 0, prestress concentration of cortisol averaged 24.9 ± 3.9 ng/mL (Figure 1). After 10 min of the fish's exposure to the confinement stressor, the concentration of circulating cortisol increased significantly ($P < 0.01$), reaching 120.7 ± 22.3 ng/mL. After 20 min of confinement, plasma cortisol levels increased to 225.7 ± 11.5 ng/mL ($P < 0.01$). Subsequently, plasma cortisol levels at 20 and 60 min remained higher than at 0 and 10 min levels, reaching 243.7 ± 8.4 ng/mL at 60 min. By 90 min of confinement, circulating plasma cortisol levels had decreased significantly ($P < 0.01$), to 56.1 ± 11.6 ng/mL. Plasma levels at 90 min were not significantly different ($P > 0.01$) from those at time 0.

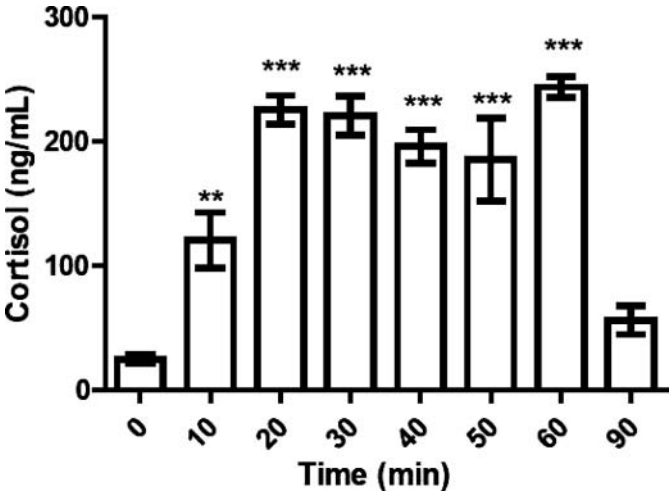

FIGURE 1. Mean ± SE plasma cortisol concentration of San Pedro during a 90-min confinement stress ($n = 3$–4). Asterisks indicate significant differences ($P < 0.01$) between treatments at a given time point.

DISCUSSION

Management practices used daily in aquaculture necessarily generate stressors, to which the fish being grown may respond dissimilarly. When a new species is exposed to such stressors, some fish will not withstand the culture environment and will die, while others are able to be successfully manipulated for commercial aquaculture use. In the Corporación Privada para el Desarrollo de la Universidad Arturo Prat, work is ongoing to develop procedures allowing the San Pedro to be grown under typical aquaculture conditions. As with any new species, this work has been complicated by a variety of unknowns. Addressing these unknowns, such as how well this fish performs in a captive environment, what environmental parameters are necessary for optimal performance, and how well San Pedro tolerate stressors associated with aquaculture management practices, provides critical information needed to develop a successful aquaculture industry. The present research provides important basic information regarding prestress and poststress concentrations of circulating cortisol that will serve as a reference for future studies addressing the ability of San Pedro to tolerate stressors commonly associated with aquaculture.

Subjecting San Pedro to an environment of confinement stress created by low water volume and crowding resulted in a characteristic increase in plasma cortisol concentrations in all fish sampled. From the time the stressor was initiated, basal levels increased significantly, reaching a plateau after 20 min that was maintained for 60 min. Similar, cortisol stress responses have been observed in other species (Davis et al. 1984; Demers and Bayne 1997; Barcellos et al. 1999; Geslin and Auperin 2004; Biswas et al. 2008). Peak plasma cortisol concentrations in most fish species occur within 30–60 min after exposure to a stressor (Barton and Iwama 1991), although exceptions to this characteristic pattern have been described. Vijayan and Moon (1994) reported that the sea raven *Hemitripterus americanus*, a

sedentary, benthic marine fish, took 4 h to reach peak cortisol levels after an acute stressor.

Prestress plasma cortisol concentrations in San Pedro (29.9 ng/mL) are somewhat higher relative to that of several other fish species examined. Barton (2002), reviewing stress in fishes, published the pre- and poststress concentrations of plasma cortisol of 12 species. Prestress levels in these species ranged from 1.0 to 11 ng/mL. Even so, the prestress level observed for San Pedro in the present study is comparable with prestress levels in the related striped knifejaw *O. fasciatus*, which inhabits the western Pacific shores of Japan, Korea, Taiwan, and Hawaii. In studies examining the effects of photoperiod (Biswas et al. 2008) and clove oil anesthesia (Park et al. 2009), prestress cortisol concentrations were reported between 20.5 and 45 ng/mL, respectively. In the present study, prestress samples were collected in less than 5 min to minimize the detection of a plasma cortisol increase attributable to handling. Taken with the values reported for striped knifejaw, prestress levels in this family appear to be somewhat higher than other species.

In the present study, cortisol levels in San Pedro peaked at around 244 ng/mL. Differences in the magnitude of cortisol stress responses among fish species is common. Barton's review describes poststress concentrations that ranged from as low as 3.0 ng/mL in pallid sturgeon to as high as 229 ng/mL for walleye *Sander vitreus* (Barton 2002). The response in San Pedro is thus on the high end of the range presented. However, no results from marine fish were reported for comparison (Barton 2002). Peak cortisol levels after stress in sea ravens were reported to be 206 ng/mL (Vijayan and Moon 1994), and net pen confinement of sockeye salmon *Oncorhynchus nerka* resulted in a poststress cortisol responses greater than 250 ng/mL (Donaldson et al. 2011). In another marine species, the gilthead seabream *Sparus aurata*, cortisol concentrations of fish held at low densities averaged 130 ng/mL 1 h after exposure to an acute handling stress, but was significantly less when fish were held at high densities for 14 d before being tested (Barton et al. 2005). Peak plasma cortisol concentrations in striped knifejaw after acute handling and confinement stress reportedly range from 110 to 170 ng/mL (Biswas et al. 2008; Park et al. 2009). Several factors can influence the stress response, including prior stress exposure and the intensity and duration of the stressor. In the present study, the stressor was administered continuously for 90 min at a very high density (231 kg/m^3) in low volumes of water to ensure that the maximal circulating cortisol concentration was detected, as indicated by the plateau between 20 and 60 min.

Peak levels of circulating cortisol were observed 60 min after the initiation of the confinement stress in San Pedro. However, between 60 and 90 min after initiation of the stressor, there was a significant decrease in circulating cortisol levels, reaching a concentration similar to that of time-0 fish. In their review, Martínez-Porchas et al. (2009) suggest several possible reasons for observations of weak or decreasing cortisol response after exposure to chronic stress. One possible explanation could be an adaptation of the fish to confinement, i.e., an effect of allostasis (Schreck 2010). This type of coping response has been described as potentially involving a characteristic cortisol response at the onset, where the animal may perceive the stress as more severe, but conclude with compensation to the new condition (Precht 1958). Schreck (2010) suggests examples of this type of response in fish could include living in environmental conditions close to the fish's tolerances or being crowded in fish culture. Given this possible explanation, the response observed in the San Pedro might suggest this species could adapt to increased confinement relatively quickly.

Alternatively, the decrease in cortisol may have been caused by exhaustion of the endocrine system as a result of prolonged hyperactivity (Hontela et al. 1992) or an attempt to avoid tissue damage (Wendelaar Bonga 1997). In which case, a decreased sensitivity of interrenal tissue to the actions of corticotropin (ACTH) or other pituitary hormones might be the root cause (Vijayan and Leatherland 1990; Mommsen et al. 1999). Rapid increases in cortisol during stressful procedures followed by decreases to basal concentrations after chronic exposure have been reported for red drum *Sciaenops ocellatus* and common dentex *Dentex dentex* (Morales et al. 2005), carp *Cyprinus carpio* (Pottinger 1998), and rainbow trout (Barton et al. 1987). The results presented here are the first for San Pedro; more research clearly is required before the precise mechanisms can be ascertained.

Conclusion

This is the first evaluation of San Pedro stress response. Intense chronic confinement for 90 min resulted in a high, sustained cortisol stress response in San Pedro at 20 and 60 min, followed by a decrease in plasma cortisol by 90 min. The observed levels of cortisol pre- and poststress are within the range of values observed for other species and provide a baseline for future evaluations of stress physiology and the development of aquaculture conditions and techniques suitable to this species.

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REFERENCES

Barcellos, L. J. G., S. Nicolaiewsky, S. M. G. De Souza, and F. Lulhier. 1999. Plasmatic levels of cortisol in the response to acute stress in Nile tilapia, *Oreochromis niloticus* (L.), previously exposed to chronic stress. Aquaculture Research 30:437–444.

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 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., L. Ribas, L. Acerete, and L. Tort. 2005. Effects of chronic confinement on physiological responses of juvenile gilthead sea bream, *Sparus aurata* L., to acute handling. Aquaculture Research 36:172–179.

Barton, B. A., C. B. Schreck, and L. D. Barton. 1987. Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. Diseases of Aquatic Organisms 2:173–185.

Benfey, T. J., and M. Biron. 2000. Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvenis fontinalis*). Aquaculture 184:167–176.

Biswas, A. K., M. Seoka, K. Ueno, A. S. K. Yong, B. K. Biswas, Y. S. Kim, K. Takii, and H. Kumai. 2008. Growth performance and physiological responses in striped knifejaw, *Oplegnathus fasciatus,* held under different photoperiods. Aquaculture 279:42–46.

Chirichigno, F. N. 1974. Clave para identificar los peces marinos del Perú. [Key to the identification of the marine fishes of Peru.] Informe IMARPE (Instituto del Mar del Perú), 44, Chucuito Callao, Peru.

Davis, K. B., M. A. Suttle, and N. C. Parker. 1984. Biotic and abiotic influences of corticosteroid hormone rhythms in channel catfish. Transactions of the American Fisheries Society 113:414–421.

Demers, N. E., and C. J. Bayne. 1997. The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. Developmental and Comparative Immunology 21:363–373.

Donaldson, M. R., S. G. Hinch, D. A. Patterson, J. Hills, J. O. Thomas, S. J. Cooke, G. D. Raby, L. A. Thompson, D. Robichaud, K. K. English, and A. P. Farrell. 2011. The consequences of angling, beach seining, and confinement on the physiology, post-release behaviour and survival of adult sockeye salmon during upriver migration. Fisheries Research 108:133–141.

Geslin, M., and B. Auperin. 2004. Relationship between changes in mRNAs of the genes encoding steroidogenic acute regulatory protein and P450 cholesterol side chain cleavage in head kidney and plasma levels of cortisol in response to different kinds of acute stress in the rainbow trout (*Oncorhynchus mykiss*). General and Comparative Endocrinology 135:70–80.

Hontela, A., J. B. Rasmussen, C. Audet, and G. Chevalier. 1992. Impaired cortisol stress response in fish from environments polluted by PAHs, PCBs, and mercury. Archives of Environmental Contamination and Toxicology 22: 278–283.

Kassahn, K. S., R. H. Crozier, H. O. Pörtner, and M. J. Caley. 2009. Animal performance and stress: responses and tolerance limits at different levels of biological organization. Biological Reviews of the Cambridge Philosophical Society 84:277–292.

Martínez-Porchas, M., L. R. Martínez-Córdova, and R. Ramos-Enriquez. 2009. Cortisol and glucose: reliable indicators of fish stress? Pan-American Journal of Aquatic Sciences 4:158–178.

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.

Morales, A. E., G. Cardenete, E. Abellán, and L. García-Rejón. 2005. Stress-related physiological responses to handling in common dentex (*Dentex dentex* Linnaeus, 1758). Aquaculture Research 36:33–40.

Muñoz, A., C. M. Marchant, and M. Futugawa. 2009. Optimización de la tecnología de producción de juveniles de San Pedro (*Oplegnathus insignis*). [Optimization of the production technology for juvenile San Pedro (*Oplegnathus insignis*).] Final Report, Código 05CN11PPD-01, Cordunap, Proyecto de Innovación Precompetitiva, CORFO (Corporación de Fomento de la Production), Santiago, Chile.

Park, M. O., S. Y. Im, D. W. Seol, and I. S. Park. 2009. Efficacy and physiological responses of rock bream*, Oplegnathus fasciatus,* to anesthetization with clove oil. Aquaculture 287:427–430.

Plikaytis, B. D., P. F. Holder, L. B. Pais, S. E. Maslanka, L. L. Gheesling, and G. M. Carlone. 1994. Determination of parallelism and nonparallelism in bioassay dilution curves. Journal of Clinical Microbiology 32:2441–2447.

Pottinger, T. G. 1998. Changes in blood cortisol, glucose and lactate in carp retained in anglers' keepnets. Journal of Fish Biology 53:728–742.

Precht, H. 1958. Concepts of the temperature adaptation of unchanging reaction systems of cold-blooded animals. Pages 50–78 *in* C. L. Processor, editor. Physiological adaptations. Lord Baltimore Press, Baltimore, Maryland.

Schreck, C. B. 2010. Stress and fish reproduction: the roles of allostasis and hormesis. General and Comparative Endocrinology 165:549–556.

Vijayan, M. M., and J. F. Leatherland. 1990. High stocking density affects cortisol secretion and tissue distribution in brook char, *Salvelinus fontinalis*. Journal of Endocrinology 124:311–318.

Vijayan, M. M., and T. W. Moon. 1994. The stress response and the plasma disappearance of corticosteroid and glucose in a marine teleost, the sea raven. Canadian Journal of Zoology 72:379–386.

Webb, M. A. H., J. A. Allert, K. M. Kappenman, J. Marcos, G. W. Feist, C. B. Schreck, and C. H. Shackleton. 2007. Identification of plasma glucocorticoids in pallid sturgeon in response to stress. General and Comparative Endocrinology 154:98–104.

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