Available online at www.sciencedirect.com
SCIENCE DIRECT®
Aquaculture 232 (2004) 665–678
Aquaculture
www.elsevier.com/locate/aqua-online

Physiological indicators of divergent stress responsiveness in male striped bass broodstock

Chongmin Wang, William King V, L. Curry Woods III*

Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742, USA

Received 24 April 2003; received in revised form 7 August 2003; accepted 12 August 2003

Abstract

Future expansion of the hybrid striped bass (*Morone saxatilis*) industry is limited in part by continued reliance upon wild sources for seedstock and the absence of domesticated broodstocks. Inherent in the development of a domesticated stock is the ability to identify and select individuals with superior performance attributes. Since the response to stress is a characteristic that may be of value in a culture environment, we evaluated a population of male striped bass for relative stress responsiveness. Male broodstock comprised of three families (MD, MD36 and NC) were exposed to a 1-min net challenge monthly for six consecutive months and then bled 1-h following the stressor. Mean plasma cortisol was highest (295 ± 20.4 ng/ml) in July and reached its lowest level (88 ± 10.9 ng/ml) in December at the end of the study suggesting that the fish adapted to the stressor. When fish were ranked for stress responsiveness based on their mean cortisol levels, high responders (HR; $n = 6$) could be distinguished from low responders (LR; $n = 6$) on the first three sample dates. When the fish were segregated by family, NC fish had significantly lower post-stress plasma cortisol and glucose levels when compared to MD and MD36 fish suggesting that the stress response is a genetically linked trait in striped bass as shown for other species. Selected HR fish were significantly longer than LR fish throughout the study but their weights were not significantly different. However, LR individuals had significantly better condition than HR fish throughout the study suggesting that there may be differences in energy partitioning between the two groups. Selected HR fish injected with bovine corticotropin releasing hormone (bovine corticotropin releasing hormone (bCRH); 50 μg/kg BW) had significantly greater plasma cortisol levels than injected LR fish 1 and 3 h following injection. Cortisol in bCRH-injected LR individuals did not change from pre-injection values. Plasma glucose in bCRH-injected animals followed a profile commonly observed following a stressful event, but there was no difference between HR and LR individuals. These results indicate

* Corresponding author. Tel.: +1-301-405-7974; fax: +1-301-405-9059.
E-mail address: lw60@umail.umd.edu (L.C. Woods).

doi:10.1016/j.aquaculture.2003.08.008

that post-stress plasma cortisol levels are useful for identifying divergent stress responsiveness in male striped bass.

Keywords: Striped bass; *Morone saxatilis*; Stress responsiveness; Cortisol; Glucose

1. Introduction

Stress is unavoidable in the aquaculture environment. Exposure of fish to common stressors such as handling, netting and confinement activates the hypothalamo-pituitary interrenal axis resulting in increased blood levels of catecholamines and cortisol (Wendelaar Bonga, 1997). Catecholamines induce rapid, short-term elevations in blood glucose primarily through the glycogenolytic pathway (Vijayan and Moon, 1992; Vijayan et al., 1997) while cortisol is involved, possibly with other hormones, in the longer-term mobilization of non-carbohydrate energy stores (Wendelaar Bonga, 1997) from muscle protein and by increases in plasma free fatty acids (Mazeaud et al., 1977). Cortisol is often associated with the detrimental effects of stress including: decreased growth rates, reproductive dysfunction (Pankhurst and Van Der Kraak, 1997) and increased incidence of disease (Barton and Iwama, 1991; Noga et al., 1994). In freshwater fish, the response to stress may also include: loss of electrolytes, decreases in hematocrit and hemodilution from catecholamine-induced increases in gill permeability (Randall and Perry, 1992) or increased loss of ions through the urine (McDonald and Milligan, 1997).

Research conducted in several vertebrate species has shown a marked divergence in the physiological responsiveness to stress. Because stress adversely affects growth and disease resistance, considerable effort has been made to evaluate stress responsiveness in economically significant animals including the chicken (Carsia and Weber, 1986), turkey (Brown and Nestor, 1973) and the pig (Hennessy et al., 1988). Results of confinement studies in rainbow trout, *Oncorhynchus mykiss*, (Pottinger et al., 1992) and Atlantic salmon, *Salmo salar*, (Fevolden et al., 1991) have indicated that the cortisol response to stress in teleost fish is a highly individualized trait. Some individuals display a consistently high cortisol stress response while others have a consistently low cortisol response. The reasons for these differences are presently unclear. Furthermore, selective breeding programs have demonstrated that stress responsiveness is heritable and that individual responsiveness is stable over time (Fevolden et al., 1991; Pottinger and Carrick, 1999a).

It has been suggested that fish with a lower degree of stress responsiveness may perform better in terms of growth, reproduction or disease resistance than fish with a higher degree of responsiveness. This postulate is intuitively attractive since fish with a lower stress responsiveness presumably would have more energy available for growth or reproduction than fish with a higher stress responsiveness. However, results of stress studies in which growth has been included as an endpoint are ambiguous. In some studies, low cortisol responders grew at faster rates than high cortisol responders (Fevolden et al., 2002) while in other studies, the high cortisol responders had better growth rates or simply

weighed more than the low cortisol responders (Pottinger and Carrick, 1999a). A recent report indicated that selecting rainbow trout for high and low stress responsiveness offered no significant advantage or disadvantage in terms of reproductive performance although the fish were not subjected to stress during the spawning trials (Pottinger and Carrick, 2000).

Currently hybrid striped bass aquaculturists rely on wild broodstock to produce fish for growers. Due to legal and logistical constraints, the capture and spawning of wild fish is difficult and the results are highly variable. If individuals displaying superior performance characteristics could be identified, they would be excellent candidates for a selective breeding and domestication program. By investigating stress responsiveness in males, we can take advantage of recently developed sperm cryopreservation protocols (Jenkins-Keeran and Woods, 2002a,b; He and Woods, 2003) to bank promising genetic material for future use. With these concepts in mind, the aim of this study was to identify the relative stress responsiveness of males in a research population of striped bass.

2. Methods

2.1. Experimental fish

Three year-old male striped bass ($n = 56$) broodstock from three families (MD, MD36 and NC) were used for the study. The MD and MD36 were half siblings sharing a wild female parent of Chesapeake Bay stock. The male parent for the MD group was also wild while the male for the MD36 group was domesticated. The NC fish were full and half siblings produced at the North Carolina State University's Pamlico Aquaculture Field Laboratory as a cross between a wild Roanoke River female and two captive bred males of unknown geographic lineage. The fish were implanted with a subcutaneous passive integrated transponder (PIT; Avid, Norco, CA) and then weighed, measured for total length and placed into an 8600-l fiberglass tank that was part of a larger recirculating system. Pure oxygen was supplied to maintain dissolve oxygen levels at or near saturation and water temperature was maintained at 22 ± 1 °C throughout the study. Other water quality parameters and their measured ranges were: pH 7.5–8.1, total ammonia < 0.5 mg/l, nitrite < 0.2 mg/l, nitrate < 100 mg/l, salinity 5–6 ppt. calcium 150–200 mg/l. The fish were fed a specially formulated squid-meal based diet (Ziegler Brothers, Gardners, PA) twice daily ad libitum. Food was withheld from the fish 2 days prior to each sampling date.

2.2. Experiment 1: time course following a net challenge

To determine the time course dynamics of the response to a standardized net challenge, MD and MD36 fish ($n = 6$) were captured individually from their tank, held in a net for 1 min and then placed into a water bath containing buffered (pH = 7.8) MS-222 (70 mg/l; Finquel, Argent Laboratories, Redmond, WA). Once the fish were anesthetized, blood (1.5 ml) was collected into ice-cold heparinized syringes fitted with 21-gauge needles and the fish were moved to a separate 1600-l holding tank that was part of the same recirculating

system. One hour after the net stress, all of the fish were rapidly captured, anesthetized, bled and returned to their holding tanks. This procedure was repeated at 3, 6, 12, 24, and 48 h following the net stress. Blood was maintained on ice in syringes for no more than 1 h. Hematocrit was determined in duplicate heparinized capillary tubes using a hematocrit reader (Clay Adams, New York) after centrifugation (Autocrit Ultra3, Becton Dickinson, Sparks, MD). The remaining blood was transferred to microfuge tubes each containing 250 μg ammonium heparin. The plasma was collected by centrifugation (4 °C) at 10,000 × *g* and stored at − 20 °C. The plasma was assayed for cortisol, glucose, chloride, total protein and osmolality (see Assays).

2.3. Experiment 2: identification of high and low responders

To determine the relative stress responsiveness of our population of male striped bass, fish from three families ($n = 36$, 12 each from MD, MD36 and NC families) were subjected to the 1-min net challenge once per month for 6 consecutive months. Fish used in experiment 1 were excluded from this part of the study. Fish were challenged in pairs by holding individuals in nets (1/net) for 1 min. After the net challenge, the fish were released into a 1600-l holding tank (6 fish/tank). After 1 h (time of maximum plasma cortisol level determined from experiment 1), the fish were captured, anesthetized and bled as previously described. Body weight and length were measured after bleeding and the fish were placed back into the original holding tank for recovery. This process was repeated until all 36 fish were bled and measured. Blood was processed and the plasma stored as described for experiment 1. Post-stress plasma cortisol levels were determined for all samples from each fish and the individuals were ranked based on their mean cortisol for the entire 6-month study. The six fish with the lowest mean plasma cortisol were designated as low responders (LR) while the six fish with the highest mean plasma cortisol level were designated as high responders (HR). The selected HR and LR fish were maintained in a separate 8600-l tank.

2.4. Experiment 3: effect of bCRH on selected high and low responders

The HR ($n = 6$) and LR ($n = 6$) fish were randomly captured from their tank two at a time. They were anesthetized, bled (0.5 ml), weighed and injected with 50 μg/kg body weight of bovine corticotropin releasing hormone (bCRH) into the dorsal lymphatic sinus and placed into a 1600-l holding tank. The dosage was chosen based on a preliminary experiment with fish from our general striped bass broodstock population. At 1, 3, 6, and 24 h following the injection, the fish were captured, anesthetized and bled (1.5 ml) as described before. Hematocrit was determined and the plasma separated and stored for later analysis of cortisol and glucose.

2.5. Assays

Cortisol was quantified directly from plasma using an ELISA kit (DRG Diagnostics, Mountainside, NJ). Reproducibility was verified by assaying aliquots of a striped bass plasma pool within a single assay (intrassay C.V. = 7.9%; $n = 10$) and between assays

(interassay C.V. = 9.5%; $n = 10$). Glucose concentrations were measured using a micro-assay protocol and hexokinase/glucose-6-phosphate dehydrogenase (Sigma Diagnostics, St. Louis, MO) as the enzyme substrate. Plasma chloride levels were determined by coulometric titration with a chloridometer (Labconco; Kansas City, MO). Total protein was determined in microtiter plates using a modified Bradford procedure with bovine serum albumin as the standard (Bio-Rad; Hercules, CA). A vapor pressure osmometer (Wescor; Logan, UT) was used to measure plasma osmolality.

2.6. Calculations and statistical analysis

Data were analyzed with a repeated measures, two-way, mixed-model analysis of variance. The p value for significance was preset at 0.05. The test for homogeneity of variance and a likelihood ratio test were conducted to help select the best statistical model. Least square means comparisons were used to test for significant effects due to time, family, high or low responders as well as for potential interaction effects between variables. Results unless otherwise noted are reported as the mean ± the standard error

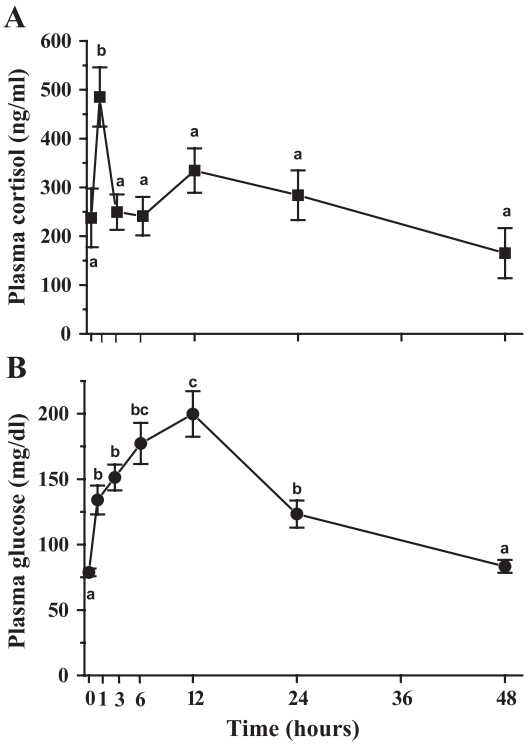

Fig. 1. Plasma cortisol (A) and glucose (B) in male striped bass sampled at various times following a 1-min net challenge. Each point represents the mean ± S.E.M. ($n = 6$). Points with different letter superscripts are significantly different from each other ($p < 0.05$).

Table 1
Mean ± S.E.M. hematocrit and plasma levels of protein, osmolality and chloride in male striped bass measured at various times following a 1-min net challenge

Time (h)	Parameter			
	Hematocrit (%)	Protein (mg/ml)	Osmolality (mmol/kg)	Chloride (mEq/l)
0	33 ± 0.6^{a}	27 ± 2.3^{a}	335 ± 11.0^{a}	143 ± 3.3^{a}
1	30 ± 0.8^{b}	23 ± 2.3^{ab}	317 ± 18.8^{ab}	133 ± 3.4^{ab}
3	29 ± 0.4^{b}	24 ± 1.4^{ab}	306 ± 12.0^{ab}	131 ± 2.3^{b}
6	29 ± 0.7^{b}	22 ± 1.3^{b}	288 ± 12.9^{b}	132 ± 1.9^{b}
12	29 ± 0.7^{b}	20 ± 1.3^{bc}	315 ± 14.2^{ab}	134 ± 2.6^{ab}
24	28 ± 1.2^{c}	16 ± 1.2^{d}	296 ± 14.2^{ab}	131 ± 2.9^{b}
48	25 ± 0.9^{c}	17 ± 1.4^{cd}	304 ± 14.2^{ab}	133 ± 2.0^{b}

Different letter superscripts indicate significant differences between sample times within the same parameter ($p < 0.05$).

of the mean (S.E.M.). All statistical analyses were conducted using Statistical Analysis Systems software for the microcomputer (SAS Institute, Cary, NC).

3. Results

3.1. Experiment 1: time course following a net challenge

Plasma cortisol increased significantly to reach its maximum level 1 h following the net challenge (Fig. 1A). Plasma glucose increased significantly by 1-h post-stress and reached maximum levels by 6 h. Glucose then declined gradually and reached pre-stress values 48 h after the stressor (Fig. 1B). Hematocrits of stressed fish decreased within 1 h and dropped significantly again 24 h after the net challenge (Table 1). Plasma

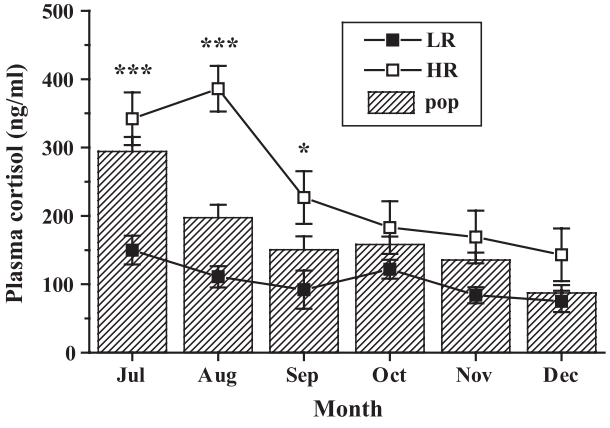

Fig. 2. Plasma cortisol in male striped bass sampled 1 h following a 1-min net challenge performed monthly for 6 consecutive months. Each point ($n = 6$) or bar ($n = 36$) represents the mean ± S.E.M. Asterisks denote significant differences between high (HR) and low responders (LR) within a sampling time, pop = experimental population.

Table 2
Mean ± S.E.M. hematocrit and plasma levels of protein, osmolality and chloride in male striped bass sampled monthly 1 h following a 1-min net challenge

Month	Parameter			
	Hematocrit (%)	Protein (mg/ml)	Osmolality (mmol/kg)	Chloride (mEq/l)
July	35 ± 0.5^{a}	48 ± 0.8^{a}	339 ± 2.8^{ab}	120 ± 1.0^{a}
August	36 ± 0.7^{ab}	48 ± 0.7^{a}	333 ± 1.8^{a}	131 ± 1.3^{b}
September	37 ± 0.8^{b}	34 ± 0.4^{b}	343 ± 1.4^{bc}	129 ± 0.7^{b}
October	38 ± 0.5^{b}	40 ± 0.5^{c}	346 ± 2.4^{c}	127 ± 0.4^{c}
November	37 ± 0.4^{b}	35 ± 0.5^{b}	336 ± 2.3^{a}	125 ± 1.4^{cd}
December	38 ± 0.5^{b}	31 ± 1.0^{d}	325 ± 3.0^{d}	122 ± 1.0^{ad}

Different letter superscripts indicate significant differences between months within the same parameter ($p < 0.05$).

osmolality and total protein each decreased significantly from pre-stress levels by 6 h. The concentration of protein in plasma decreased again 24-h post-stress while osmolality remained at 6-h levels through the end of the experiment (Table 1). Plasma chloride

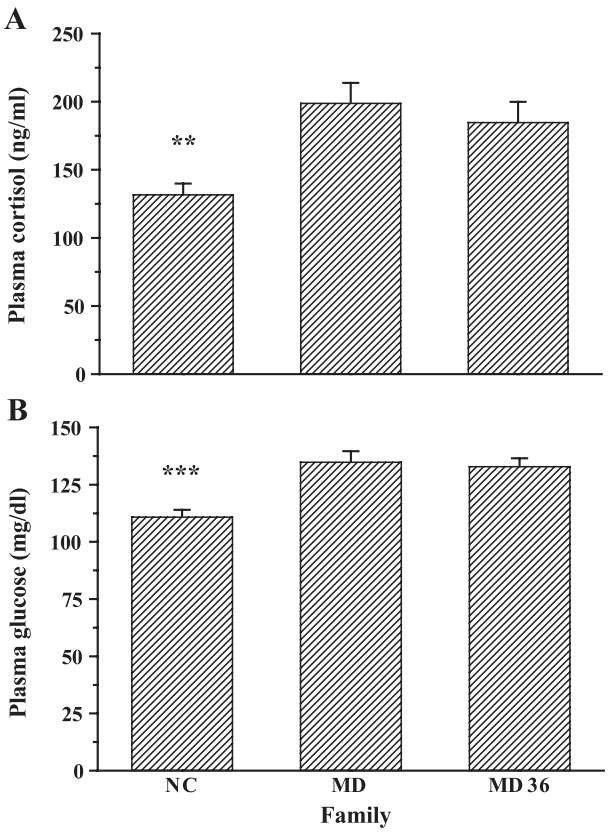

Fig. 3. Plasma cortisol (A) and glucose (B) of male striped bass segregated by family. Each bar ($n = 72$) represents the mean ± S.E.M. Asterisks denote significant differences between families, NC = North Carolina, MD = Maryland, MD36 = Maryland 36.

Table 3
Mean ± S.E.M. total lengths and weights of male striped bass in the experimental population and the selected high (HR) and low responders (LR) for each of the six sample months

Month	Length (mm)			Weight (kg)		
	LR	Population	HR	LR	Population	HR
July	542 ± 12.7	558 ± 5.1	568 ± 12.7	1.9 ± 0.13	2.1 ± 0.06	2.2 ± 0.18
August	542 ± 9.5*	567 ± 5.1	579 ± 9.5	1.9 ± 0.13	2.4 ± 0.07	2.1 ± 0.13
September	556 ± 9.5*	579 ± 5.1	592 ± 9.5	2.5 ± 0.16	2.7 ± 0.07	2.7 ± 0.16
October	567 ± 10.4*	592 ± 5.1	603 ± 10.4	2.7 ± 0.17	2.9 ± 0.07	2.9 ± 0.17
November	580 ± 9.1*	604 ± 5.1	616 ± 9.1	2.9 ± 0.16	3.1 ± 0.08	3.2 ± 0.16
December	593 ± 9.5*	618 ± 5.1	636 ± 9.5	3.1 ± 0.18	3.3 ± 0.08	3.5 ± 0.18

* Indicates significant difference in length between HR and LR within 1 month ($p < 0.05$).

levels decreased significantly 3 h following the challenge where they remained throughout the experiment.

3.2. Experiment 2: identification of high and low responders

Mean plasma cortisol levels in the experimental population decreased significantly from July through September where they remained until December when the lowest mean cortisol levels were observed (Fig. 2). Cortisol levels of the entire population in December were about one-third as high as levels measured in July. Individuals selected as HR could be distinguished from LR fish in July, August and September.

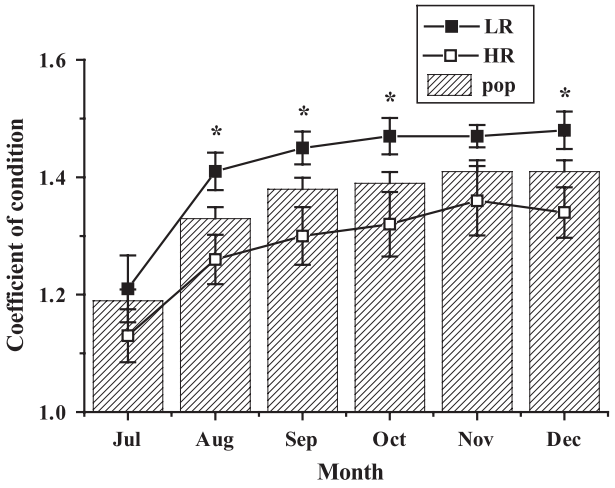

Fig. 4. Coefficient of condition for selected high (HR) and low (LR) responding male striped bass during the 6-month study. Each point ($n = 6$) or bar ($n = 36$) represents the mean ± S.E.M. Asterisks denote significant differences ($p < 0.05$) between high (HR) and low responders (LR) within a sampling time. Coefficient of condition = 100 (weight/length^3); pop = experimental population.

Hematocrits increased significantly from July to September where they remained through December (Table 2). Total protein decreased throughout the experiment to reach its lowest levels in December. Plasma osmolality tended to increase through October, but then decreased to reach its lowest level in December. Chloride levels increased initially by August, but then decreased to return to July values by December. There were no statistical differences in hematocrit, plasma glucose, protein, osmolality or chloride between selected HR and LR fish during the 6-month study (data not shown).

When data for each fish on every sampling date of the 6-month study were compiled for cortisol and glucose and then segregated by family, NC fish had significantly lower plasma cortisol and glucose levels when compared to MD and MD36 fish (Fig. 3).

Fish selected as HR and LR were of similar size at the beginning of the study (Table 3). By August, and for the remainder of the experiment, HR individuals were significantly longer but not heavier when compared to LR fish. The coefficient of condition was

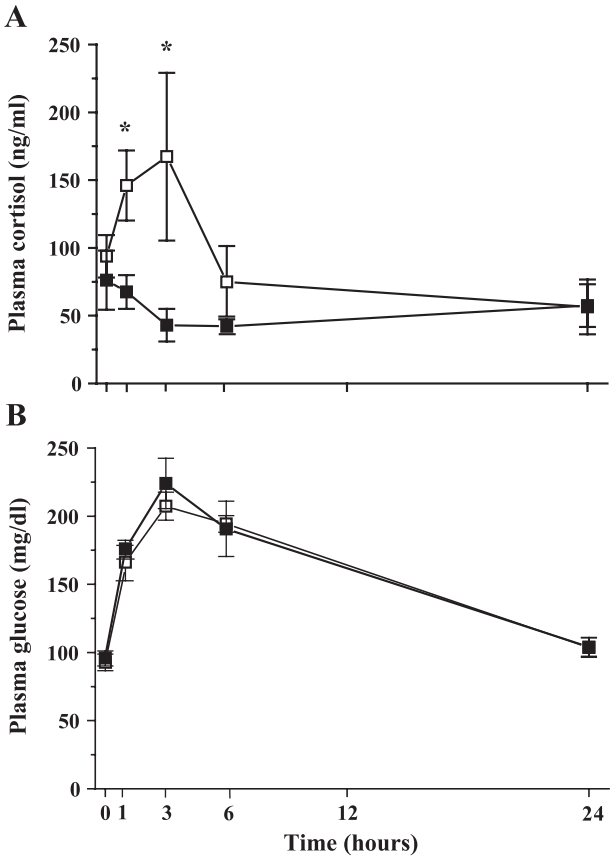

Fig. 5. Plasma cortisol (A) and glucose (B) of selected high (open squares) and low (filled squares) responding male striped bass sampled at various times following a single injection of bovine corticotropin releasing hormone (bCRH; 50 μg/kg body weight). Each point represents the mean ± S.E.M. ($n = 6$). Asterisks denote significant differences ($p < 0.05$) between groups within a sampling time.

significantly greater in LR fish than HR fish for each sample month except July and November (Fig. 4).

3.3. Experiment 3: effect of bCRH on selected high and low responders

Selected HR fish had significantly greater plasma cortisol levels 1 and 3 h following a single injection of bCRH when compared to LR animals (Fig. 5A). Plasma cortisol of bCRH-injected LR fish did not change from pre-injection values. Plasma glucose of HR and LR fish increased significantly 1 h following injection and reached peak levels after 3 h but there was no significant difference in glucose between HR and LR fish (Fig. 5B).

4. Discussion

The results of the present study demonstrate that individual striped bass exhibit divergent cortisol stress responsiveness which is stable over time. These findings corroborate previous research which documented differences in sensitivity to stress in other teleosts including rainbow trout (Fevolden et al., 1991; Pottinger et al., 1992, 1994; Pottinger and Carrick, 1999a,b), Atlantic salmon (Fevolden et al., 1991) and their progeny (Fevolden et al., 1991; Pottinger and Carrick, 1999b). Mean post-stress plasma cortisol levels were the most useful for identifying fish with high or low stress responsiveness. Although mean glucose levels followed a trend similar to cortisol, there were no differences between HR and LR fish for glucose during the 6-month study. It is possible that the inability to distinguish HR from LR fish on the basis of post-stress glucose levels was related to sampling the fish 1-h post-stress rather than after glucose levels had maximized. Furthermore, since factors other than stress, including nutrition, can directly affect blood glucose and blood, cortisol levels are widely used as a reliable index for activation of the stress response (Barton and Iwama, 1991), we chose to rank the animals based on cortisol responsiveness (Pottinger and Carrick, 1999b).

Mean plasma cortisol levels decreased progressively during the 6-month study, suggesting that the fish were adapting to the net challenge. High plasma cortisol combined with low plasma chloride suggests that the fish were most sensitive to the net stressor in July when they were handled for the first time. As the experiment progressed, mean cortisol dropped significantly to reach its lowest level at the end of the study in December. Although the fish were acclimated to the new tanks for over 7 weeks prior to the initiation of this study and they were not handled or disturbed during this period, they were naïve to the netting procedures necessary for effective capture and processing for the experiment. Since striped bass seem to be particularly sensitive to conditions in an artificial culture environment, it was not unusual to expect that they would have an exaggerated stress response once the study commenced. However, we observed that during the last third of the study the fish resumed eating 1 day following sampling while in the beginning of the study, they would not accept food for several days after sampling. It may be that late in the study, fish began recovering sooner from the net challenge and regained their appetite in parallel to recovery. This observation is in agreement with current knowledge concerning stress and appetite suppression (Bernier and Peter, 2001). In mammals, it has been

demonstrated that repeated exposure to the same stressor may cause habituation which is reflected in reduced plasma corticosteroid and adrenocorticotropic hormone (ACTH) levels (Martí and Armario, 1998).

Hematocrit and mean plasma levels of protein, osmolality and chloride levels changed during the 6-month study, but were not useful for distinguishing HR from LR individuals. Presumably the 1-h post-stress sampling time was too soon to detect any changes in electrolyte composition. Previous work has shown that osmoregulatory disruptions often take hours to appear and may last for days or weeks depending on the duration and severity of the stressor (Mazeaud et al., 1977; Wendelaar Bonga, 1997). Our experimental fish were maintained in water with moderately high hardness and 5 g/l salinity and so it was rather surprising to measure depressed chloride levels 48 h following the net challenge. Although we observed no morbidity or mortality during the course of these studies, this finding emphasizes the need to follow previous recommendations to handle striped bass in water with moderate hardness and salinity (Mazik et al., 1991). Plasma protein decreased to its lowest value 24 h following the net challenge. The mechanism for this drop is unclear, but may at least partly be due to hemodilution (McDonald and Milligan, 1997). Research in rats has shown that the stress of immobilization resulted in a >45% decrease in the rate of protein synthesis in liver, kidney, brain, lungs and adrenals (Klasing, 1985). Glucocorticoids have also been shown to mediate protein synthesis and degradation in the liver where a number of enzymes are involved in amino acid catabolism (Klasing, 1985; Kaplan, 2000).

In addition to individual differences in stress responsiveness, our population of striped bass also displayed family differences in their physiological response to stress similar to results reported for salmonids (Pickering and Pottinger, 1989; Pottinger and Carrick, 1999b). When fish were ranked on the basis of mean cortisol levels, five of the six LR fish were from the NC family, while all of the HR striped bass came from either the MD or MD36 families. It is interesting to note that in a strain evaluation study, Maryland striped bass were the most difficult to handle and were the most likely fish to jump from tanks (Jacobs et al., 1999) Although no North Carolina strains were included in that study, this observation coupled with our results suggests that Maryland fish may have less tolerance for culture conditions than other strains. Our results suggest that the NC family of fish have the genetic potential for low cortisol stress responsiveness and may make a good choice for selective breeding studies.

Similar to the results of the previous studies, the growth performance data were equivocal. For example, in rainbow trout, high cortisol responders have been shown to be significantly larger (Pottinger and Carrick, 1999a) or slower growing (Fevolden et al., 2002) than corresponding low responders. In the present study, HR fish tended to be longer, but they were not significantly heavier than LR fish and there were no differences in specific growth rate between HR and LR individuals (data not shown). However, the LR fish had significantly better condition at many of the sample dates throughout the study indicating that they had a better than average weight at a particular length (Busacker et al., 1990). This suggests the possibility that fish with lower stress responsiveness may be better able to shunt energy away from maintenance of a stress response and into somatic growth better than HR individuals; however, further study is required to verify this result. Diversion of energy under stressful conditions reduces the energy available for other

requirements, including somatic growth. This effect is demonstrated by the increase in oxygen consumption of stressed fish and the elevated plasma glucose levels consistently associated with stress (Barton and Iwama, 1991). Alternatively, since most of the LR individuals were from the NC family, it may be that increased weight at a given length is a characteristic typical of this particular family of fish.

Fish selected as HR and LR based on their mean cortisol level were injected with bCRH to verify their cortisol and glucose responsiveness. CRH-injected HR individuals had significantly greater cortisol levels 1 and 3 h after injection when compared to LR fish whose cortisol did not change from pre-injection values. A previous study investigating the effect of ACTH injection on dexamethasone-suppressed rainbow trout showed that HR individuals could be distinguished from LR individuals at all sample times (0–3 h) post-injection (Pottinger and Carrick, 2001). Although a preliminary study with animals from our general broodstock population indicated that 50 μg bCRH/kg was sufficient to stimulate interrenal production of cortisol, it may be that this dose of a heterologous CRH was insufficient to stimulate striped bass displaying low cortisol responsiveness. Plasma glucose of LR and HR individuals increased significantly and concurrently following bCRH injection to levels observed following an acute stressor suggesting that cortisol and glucose responsiveness may be controlled by independent mechanisms. We have observed that the stress of netting and handling for sham injection can cause modest increases in plasma glucose (up to 110 mg/dl) in our striped bass presumably through catecholamine-induced glycogenolysis (Van Raaij et al., 1995). Since cortisol levels did not change in LR fish following bCRH injection, it seems unlikely that glucose was synthesized through cortisol-assisted gluconeogenesis, the generally accepted mechanism for longer-term increases in blood glucose (Van Raaij et al., 1996). The increases in glucose observed here following bCRH injection may be due in part to CRH-activated catecholamine metabolism as shown in mice (Dunn and Berridge, 1990).

In conclusion, individual male striped bass with divergent stress responsiveness can be identified using cortisol as a physiological indicator. It is still unclear whether selecting fish for their stress responsiveness has any practical value that could be exploited in terms of growth performance.

Acknowledgements

We gratefully acknowledge the assistance of Dr. Larry Douglass of the University of Maryland, Department of Animal and Avian Sciences, who assisted with experimental design and statistical analyses. We also thank Dan Theisen, Shuyang He and Daniel Castranova who helped with sampling and fish husbandry. This study was funded by a Maryland Agricultural Experiment Station (MAES) Competitive Grant to L.C. Woods III.

References

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1, 3–26.

Bernier, N., Peter, R., 2001. The hypothalamic-pituitary-interrenal axis and the control of food intake in teleost fish. Comp. Biochem. Physiol., Part B Biochem. Mol. Biol. 129, 639–644.

Brown, K., Nestor, K., 1973. Some physiological responses of turkeys selected for high and low adrenal response to cold stress. Poultry Sci. 52, 1948–1954.

Busacker, G.P., Adelman, I.R., Goolish, E.M., 1990. In: Schreck, C.B., Moyle, P.B. (Eds.), Methods for Fish Biology. American Fisheries Society, Bethesda, MD, pp. 363–388.

Carsia, R., Weber, H., 1986. Genetic dependant alterations in adrenal stress response and adrenocortical cell function of the domestic fowl (*Gallus domesticus*). Proc. Soc. Exp. Biol. Med. 183, 99–105.

Dunn, A.J., Berridge, C.W., 1990. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? Brain Res. Rev. 15, 71–100.

Fevolden, S.-E., Refstie, T., Røed, K.H., 1991. Selection for high and low cortisol response in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 95, 53–65.

Fevolden, S.-E., Røed, K.H., Fjalestad, K.T., 2002. Selection response of cortisol and lysozyme in rainbow trout and correlation to growth. Aquaculture 205, 61–75.

He, S., Woods III, L.C., 2003. Effects of glycine and alanine on short-term storage and cryopreservation of striped bass (*Morone saxatilis*) spermatozoa. Cryobiology 46, 17–25.

Hennessy, D., Stelmasiak, T., Johnston, N., Jackson, P., Outch, K., 1988. Consistent capacity for adrenocortical response to ACTH administration in pigs. Am. J. Vet. Res. 49, 1276–1283.

Jacobs, J.M., Lindell, S., Van Heukelem, W., Hallerman, E.M., Harrell, R.M., 1999. Strain evaluation of striped bass (*Morone saxatilis*) under controlled conditions. Aquaculture 173, 171–177.

Jenkins-Keeran, K., Woods III, L.C., 2002a. An evaluation of extenders for the short-term storage of striped bass milt. N. Am. J. Aquac. 64, 248–256.

Jenkins-Keeran, K., Woods III, L.C., 2002b. The cryopreservation of striped bass semen. J. World Aquac. Soc. 33, 70–77.

Kaplan, N.M., 2000. The adrenal glands. In: Griffin, J.E., Ojeda, S.R. (Eds.), Textbook of Endocrine Physiology. Oxford Univ. Press, New York, pp. 328–356.

Klasing, K.C., 1985. Influence of stress on protein metabolism. In: Moberg, G.P. (Ed.), Animal Stress. American Physiological Society, Bethesda, MD, pp. 269–280.

Martí, O., Armario, A., 1998. Anterior pituitary response to stress: time related changes and adaptation. Int. J. Dev. Neurosci. 16, 241–260.

Mazeaud, M.M., Mazeaud, F., Donaldson, E.M., 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fish. Soc. 106, 201–212.

Mazik, P.M., Simco, B.A., Parker, N.C., 1991. Influence of water hardness and salts on survival and physiological characteristics of striped bass during and after transport. Trans. Am. Fish. Soc. 120, 121–126.

McDonald, G., Milligan, L., 1997. Ionic, osmotic and acid–base regulation in stress. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P., Schreck, C.B. (Eds.), Fish Stress and Health in Aquaculture. Cambridge Univ. Press, Cambridge, UK, pp. 119–144.

Noga, E.J., Kerby, J.H., King, W., Aucoin, D.P., Giesbrecht, F., 1994. Quantitative comparison of the stress response of striped bass (*Morone saxatilis*) and hybrid striped bass (*Morone saxatilis* × *Morone chrysops* and *Morone saxatilis* × *Morone americana*). Am. J. Vet. Res. 55, 405–409.

Pankhurst, N.W., Van Der Kraak, G., 1997. Effects of stress on reproduction and growth of fish. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P., Schreck, C.B. (Eds.), Fish Stress and Health in Aquaculture. Cambridge Univ. Press, Cambridge, UK, pp. 73–93.

Pickering, A., Pottinger, T., 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiol. Biochem. 7, 253–258.

Pottinger, T.G., Carrick, T.R., 1999a. A comparison of plasma glucose and plasma cortisol as selection markers for high and low stress-responsiveness in female rainbow trout. Aquaculture 175, 351–363.

Pottinger, T.G., Carrick, T.R., 1999b. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. Gen. Comp. Endocrinol. 116, 122–132.

Pottinger, T.G., Carrick, T.R., 2000. Indicators of reproductive performance in rainbow trout *Oncorhynchus mykiss* (Walbaum) selected for high and low responsiveness to stress. Aquac. Res. 31, 367–375.

Pottinger, T.G., Carrick, T.R., 2001. ACTH does not mediate divergent stress responsiveness in rainbow trout. Comp. Biochem. Physiol., Part A 129, 399–404.

Pottinger, T.G., Pickering, A.D., Hurley, M.A., 1992. Consistency in the stress response of individuals of two strains of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 103, 275–289.

Pottinger, T.G., Moran, T.A., Morgan, J.A.W., 1994. Primary and secondary indices of stress in the progeny of rainbow trout (*Oncorhynchus mykiss*) selected for high and low responsiveness to stress. J. Fish Biol. 44, 149–163.

Randall, D.J., Perry, S.F., 1992. Catecholamines. In: Hoar, W.S., Randall, D.J., Farrell, A.P. (Eds.), Fish Physiology, vol. XIIB. Academic Press, New York, pp. 255–300.

Van Raaij, M.T.M., van den Thillart, G.E.E.J.M., Hallemeesch, M., Balm, P.H.M., Steffens, A.B., 1995. Effect of arterially infused catecholamines and insulin on plasma glucose and free fatty acids in carp. Am. J. Physiol. 268, R1163–R1170.

Van Raaij, M.T.M., van den Thillart, G.E.E.J.M., Vianen, G.J., Pit, D.S.S., Balm, P.H.M., Steffens, A.B., 1996. Substrate mobilization and hormonal changes in rainbow trout (*Oncorhynchus mykiss*, Walbaum) and common carp (*Cyprinus carpio* L.) during deep hypoxia and subsequent recovery. J. Comp. Physiol., B 166, 443–452.

Vijayan, M.M., Moon, T.W., 1992. Acute handling stress alters hepatic glycogen metabolism in food-deprived rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 49, 2260–2266.

Vijayan, M., Pereira, C., Grau, E., Iwama, G., 1997. Metabolic responses associated with confinement stress in tilapia: the role of cortisol. Comp. Biochem. Physiol., Part C Pharmacol. Toxicol. 116, 89–95.

Wendelaar Bonga, B.S.E., 1997. The stress response in fish. Physiol. Rev. 77, 591–625.