

Clinical Pathology and Histopathology Characteristics of Net-Stressed Striped Bass with "Red Tail"

CRAIG A. HARMS

*Department of Companion Animal and Special Species Medicine, College of Veterinary Medicine
North Carolina State University, Raleigh, North Carolina 27606, USA*

CRAIG V. SULLIVAN

*Department of Zoology, College of Agriculture and Life Sciences,
Campus Box 7617, North Carolina State University, Raleigh, North Carolina 27695, USA*

RONALD G. HODSON

*University of North Carolina Sea Grant College Program,
Campus Box 8605, North Carolina State University, Raleigh, North Carolina 27695, USA*

MICHAEL K. STOSKOPF

Department of Companion Animal and Special Species Medicine, College of Veterinary Medicine

Abstract.—Simulated artificial spawning of striped bass *Morone saxatilis* while undergoing repeated net confinements was used to study the pathogenesis of the hyperemia of the fins and ventrum frequently observed when these fish are handled. Alterations in plasma chemistry that accompanied this external manifestation included markedly reduced osmolality, sodium, and chloride and increased plasma cortisol, total CO₂, and anion gap. Hemolysis was visually detectable in plasma from five of six net-stressed fish but not in plasma from the six control fish. Blood smears of treated fish contained spherocytes and erythrocyte fragments and exhibited anisocytosis. Histologically, the caudal fins of treated fish were characterized by mononuclear vasculitis and dilated, congested vasculature occluded with organized thrombi. Splenic architecture of net-stressed fish was indistinct and contained diffusely coalesced erythrocytes. Conclusive evidence for disseminated intravascular coagulation was not detected by activated clotting time, estimated thrombocyte count, fibrin degradation products, or histopathology.

Wild striped bass *Morone saxatilis* subjected to capture, repeated handling, and confinement for artificial spawning often develop red fins and ventrum, particularly pronounced in the caudal fin, giving rise to the colloquial descriptive term, "red tail." Pathophysiology of this phenomenon has received little attention in the literature. To characterize selected clinical pathological and histopathological features of this syndrome, we induced "red tail" in domestic striped bass by simulating handling stress associated with artificial spawning (Rees and Harrell 1990). Variables investigated included hematocrit, hemolysis, estimated white cell count, estimated thrombocyte count, fibrin degradation products (FDP), red and white cell mor-

phology, activated clotting time (ACT), plasma osmolality, plasma cortisol, plasma electrolytes (Na, K, and Cl; total carbon dioxide, TCO₂; and anion gap, AGAP), aerobic cultures of spleen and caudal kidney, and histopathology of major organs and the caudal fin.

Methods

Wild female striped bass captured from the mouth of the Roanoke River, North Carolina, and conditioned to captivity for 2 years were held in 38,000-L tanks with flow-through well water as previously described (Hodson and Sullivan 1993). All fish had fully grown post-vitellogenic oocytes at the start of the experiments. Water quality variables noted included temperature at 18°C, salinity 0‰, hardness 350 mg/L as CaCO₃, dissolved oxygen at saturation, and negligible ammonia nitrites and nitrates. Six treatment fish were selected to be confined in 780-L net-pens within the 38,000-L tanks and subjected to 10 min of net stress two to three times daily for 3 d. Net stress consisted of further confining the six treatment fish within a net (98 L) held quietly near the water surface. Six control fish were selected at large from the same tank.

At the conclusion of the stress period, fish were anesthetized with quinaldine sulfate at 70–100 mg/L, weighed, measured (total length), and bled from the caudal peduncle. Three milliliters of blood were drawn with no anticoagulant; 2 mL were placed in an FDP tube and 1 mL was placed in an ACT tube. An additional 2.5 mL were drawn in heparinized syringes to evaluate hematocrit, blood

smears, and plasma chemistry. Activated clotting time tubes were immediately gently inverted, then at 20 s and every 10 s thereafter until a solid clot had formed. Fibrin degradation products tubes stood at room temperature 30 min and were centrifuged for serum collection. Serum was frozen at -20°C for 2 weeks before analysis. Blood smears were made within 1 min of collection, hematocrit was determined within 30 min, and plasma was harvested within 30 min and frozen at -20°C before analyses which were completed within 4 weeks. Blood smears were stained with Diff-Quick[®] (American Scientific Products, McGraw Park, Illinois).

All treatment fish and three control fish were euthanized with an overdose of quinaldine sulfate. Swabs of spleen, liver, and trunk kidney were collected aseptically, plated onto blood agar, and incubated at room temperature. Wet mounts of gill filaments were examined immediately. Tissues fixed in 10% neutral buffered formalin included caudal fin, heart, swim bladder, cranial kidney, spleen, liver, duodenum, pancreas, gill arch, and ovary. Fixed tissues were processed routinely for paraffin sections and stained with hematoxylin and eosin for histological examination.

Hemolysis was estimated visually from hematocrit tubes. Thrombocytes per 100 white blood cells (WBC) were counted on blood smears (Stoskopf 1993). Plasma osmolality was determined on an osmometer (Advanced Micro-Osmometer, Model 3MO, Advanced Instruments, Inc., Needham Heights, Massachusetts). Plasma electrolytes (Na, K, Cl, TCO_2) were measured on an automated analyzer (Monarch Plus, Instrumentation Laboratory, Lexington, Massachusetts). Anion gap was calculated as follows: $(\text{Na} + \text{K}) - (\text{Cl} + \text{TCO}_2)$. Plasma cortisol was determined with an automated fluorescence polarization immunoassay (Abbott Laboratories, North Chicago, Illinois) previously validated for use in striped bass (Noga et al. 1994). Fibrin degradation product measurements were attempted with a commercial kit (Fibrin(ogen) Degradation Products (FDP) Detection Set, Baxter Healthcare Corp. Miami, Florida) with rabbit anti-human fibrinogen as the primary antibody. Measurements of treated fish and control fish were statistically compared by the Wilcoxon signed rank test, except for presence or absence of hemolysis, for which the chi-square test with Yates correction was used.

Results

Significant changes in clinical pathology were the pronounced decreases in osmolality, Na, and

TABLE 1.—Median weight, length, and clinical pathology values of striped bass subjected to net stress on three consecutive days and values for controls. Probabilities listed were determined from a Wilcoxon signed rank test, except as noted for presence or absence of hemolysis, for which a chi-square test with Yates correction was used; NS = not significant; $N = 6$ for both treatment and control groups.

Variable	Treatment (quartiles)	Control (quartiles)	P
Weight (kg)	1.28 (1.11–1.60)	1.08 (0.80–1.30)	NS
Total length (cm)	54.0 (52.0–57.0)	52.0 (44.5–54.5)	NS
Hematocrit (%)	47.5 (35.0–53.0)	42.0 (34.0–47.5)	NS
ACT ^a (s)	80 (58–110)	95 (85–120)	NS
Thrombocytes/ 100 WBC ^b	60 (24–109.75)	92 (64–142)	NS
Cortisol ($\mu\text{g}/\text{dL}$)	81.43 (72.58–104.07)	24.38 (13.16–27.21)	<0.01
Osmolality (mOsm)	291 (270–307)	344 (334–357)	<0.01
Na (mmol/L)	116 (107–129.5)	168 (164–168.5)	<0.01
K (mmol/L)	4.2 (2.8–4.4)	2.7 (2.3–3.2)	NS
Cl (mmol/L)	60.5 (54.5–71.25)	140.0 (139.8–141.5)	<0.01
Total CO_2 (mmol/L)	18.0 (14.2–22.0)	11.0 (9.0–13.2)	<0.05
AGAP ^c (mmol/L)	42.0 (34.0–48.5)	17.2 (15.8–20.6)	<0.01
Hemolysis (number of fish)	5	0	<0.05 ^d

^a Activated clotting time.

^b White blood cells.

^c Anion gap.

^d Chi-square test.

Cl and increases in cortisol, TCO_2 , and AGAP in treatment fish (Table 1). Although not statistically significant, there was a decreasing trend in thrombocyte count, with four of six treatment fish having less than 70 thrombocytes/100 WBC, and five of six control fish with more than 70. All treatment fish developed moderate to severe hyperemia of fins and ventral skin. One of six treatment fish had *Saprolegnia* sp. growth around the head, and two of six had unidentified mesenteric nematodes. One treatment fish yielded a pure culture of *Pseudomonas vesicularis* from the spleen. All treatment and control fish examined had pale livers, possibly a result of the manufactured diets fed to the captive brood stock (Brown et al. 1993). Anisocytosis, spherocytes, and red blood cell fragments were common on blood smears from treatment fish (five of six) but absent from controls. Lymphocytes

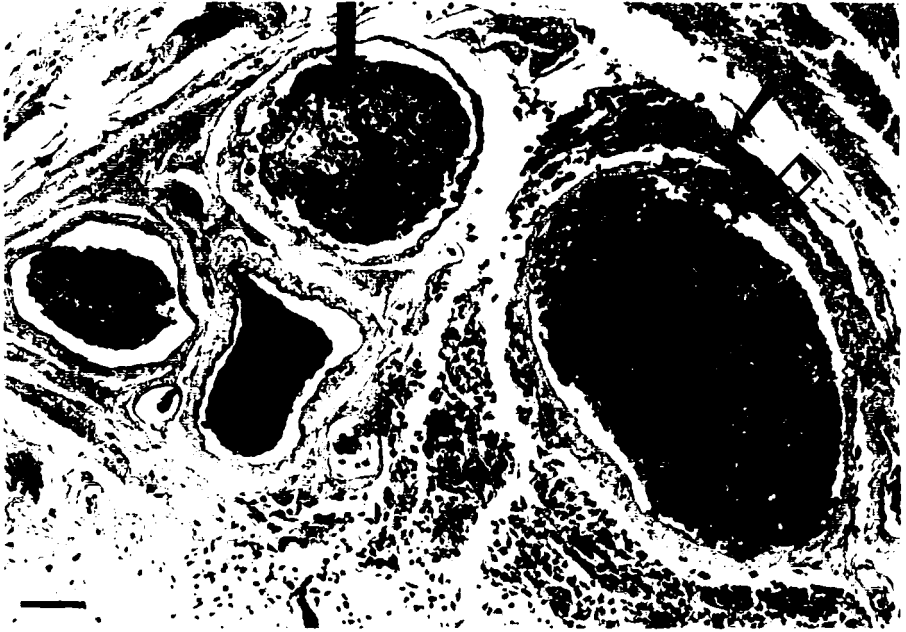


FIGURE 1.—Caudal fin cross section of net-stressed striped bass. Vasculature is dilated, congested, and occluded with organized thrombi (large solid arrow) consisting of coalesced thrombocytes and erythrocytes. Vasculitis is indicated by the disrupted endothelium (open arrow) and mononuclear perivascular inflammation (flared solid arrow). Bar = 26 μ m.

from treatment fish were reactive, with deep blue cytoplasm and pseudopodia, whereas in control fish they were quiescent.

The caudal fin vasculature of all stressed fish was dilated, congested, and occluded with organized thrombi composed of thrombocytes and coalesced erythrocytes (Figure 1). Mononuclear vasculitis with different degrees of intimal hyperplasia were present in the caudal fins of five of six stressed fish, with interstitial perivascular hemorrhage in the single remaining fish. The caudal fin blood vessels of control fish infrequently contained erythrocytes in the plane of section, and vascular endothelial cells were intact (Figure 2). Hepatic congestion was present in one treatment fish, splenic congestion in two treatment fish, and interrenal congestion and hemorrhage in one treatment fish. Vascular changes were not noted in other organs of treatment fish, and none were noted in control fish. The spleen of stressed fish contained diffusely coalesced red cells and indistinct melanomacrophage centers with macrophages frequently spreading from the centers into the surrounding stroma. Splenic architecture of the stressed fish was indistinct. In contrast, the spleen of control fish contained normal distinct red cells and more discreet melanomacrophage centers,

with clearly distinguishable areas of red pulp and white pulp. Hepatocytes of both treatment and control fish had vacuolated cytoplasm consistent with lipid storage, believed to be of nutritional origin. Gills of both treatment fish and control fish had histologic evidence of epitheliocystis (three of six treatment fish, one of three controls), trichodinids (five of six treatment fish, two of three controls) and telangiectasia (two of six treatment fish, one of three controls).

Discussion

Changes in fish blood clotting systems have been reported in response to environmental stressors (confinement, agitation, poor water quality, decompression, and hook and line) (Casillas and Smith 1977; Woodward et al. 1979; van Vliet et al. 1985) and infectious agents (Miller and Levin 1984; MacMillan et al. 1989; Salte et al. 1991). Disseminated intravascular coagulation (DIC) has been demonstrated in farmed Atlantic salmon *Salmo salar* with "Hitra disease" (Salte et al. 1987; Salte and Norberg 1991). Although we were unable to provide conclusive evidence of DIC in striped bass with "red tail" by the relatively insensitive methods of ACT, thrombocyte counts, or histopathology, intravascular coagulation local-



FIGURE 2.—Caudal fin cross section of control striped bass. Vasculature is not dilated, is virtually devoid of circulating cells, and lacks any indication of vasculitis. Dark object at right is a partial cross section of a fin ray. Bar = 31 μm .

ized to the fins was detected histologically. Fibrin degradation product measurements, which are useful indicators of DIC in mammalian species, were unsuccessful because of an apparent lack of cross-reactivity of the assay's rabbit anti-human fibrinogen antibody with striped bass fibrinogen. Other measures of clotting function (e.g., prothrombin time, partial thromboplastin time, thrombin time) were not attempted. Decreases in thrombocyte counts were not statistically significant; however, it is possible that the threshold of a consumptive coagulopathy had not been attained in all treatment fish within the allotted time.

Plasma cortisol increases in response to confinement have been previously documented for striped bass (Noga et al. 1994). Control of ion exchange across the gills involves a complex interaction of acid-base and electrolyte balance with neuroendocrine effectors, including cortisol (Evans 1975; McDonald et al. 1989; Perry and Laurent 1989); stress-induced perturbations in the system lead to osmoregulatory dysfunction (Harrell 1992). Salt has been shown to mitigate plasma corticosteroid and chloride responses in striped bass during transport (Harrell 1992). Increasing salinity to reduce osmotic electrolyte loss by the fish is the rationale behind this generally successful treatment of "red tail." Hemolysis and altered

red cell morphology observed in the present study were likely secondary to markedly lowered plasma osmolality. Release of intracellular proteins and electrolytes from lysed red cells could play a mitigating role in maintaining plasma osmotic pressure in the face of Na and Cl efflux.

Histologically, the red color of fins and skin in striped bass with "red tail" clearly results from vascular congestion, localized intravascular thrombi, and occasional perivascular hemorrhage. The dilatation of microvasculature, along with thrombus formation, may indicate sluggish peripheral blood flow suggestive of shock. Blood stasis causes local anoxemia, hypercapnia, and acidosis, which can cause endothelial injury (Slappendel 1989). Immunosuppression in affected striped bass was suggested by saprolegniasis in one fish, and the culture of *Pseudomonas vesicularis* from another, but sepsis was not a consistent feature of the red tail syndrome. The spleen appeared highly active in clearing damaged red cells from circulation. Hemolysis for any cause (most likely osmotic in this case) can lead to intravascular coagulation because of release of thromboplastic materials (Slappendel 1989). Thrombus formation in peripheral microvasculature may have been promoted by blood stasis, endothelial damage, and hemolysis. Although thrombus for-

mation was localized rather than disseminated and crude measures of clotting function (ACT, estimated thrombocyte count) did not provide evidence of DIC. conditions favored its development had they continued.

Although reddened fins are classically considered indicators of septic or stressed fish (Gratzek 1992), the pathophysiology has not been thoroughly investigated. Peripheral erythema, particularly of the fins, is considered one of the most common pathological conditions of the circulatory system resulting from toxic or septic insults (Ferguson 1989), yet histologic descriptions are rare. This report supplies some preliminary observations of the phenomenon in striped bass stressed by frequent handling.

Acknowledgments

We thank the Pamlico Aquaculture Field Laboratory staff and students for their assistance, L. Khoo and E. Noga for corroborating fin histopathology interpretation, J. Wright for photomicrography assistance, and T. Mashima for review of the manuscript.

References

- Brown, P. B., M. E. Griffin, and M. Randall White. 1993. Experimental and practical diet evaluations with juvenile hybrid striped bass. *Journal of the World Aquaculture Society* 24:80-89.
- Casillas, E., and L. S. Smith. 1977. Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology* 10:481-491.
- Evans, D. H. 1975. Ionic exchange mechanisms in fish gills. *Comparative Biochemistry and Physiology* 51A:491-495.
- Ferguson, H. W. 1989. *Systemic pathology of fish*. Iowa State University Press, Ames.
- Gratzek, J. B. 1992. *Aquariology: the science of fish health management*. Tetra Press, Moor Plains, New Jersey.
- 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.
- Hodson, R. G., and C. V. Sullivan. 1993. Induced maturation and spawning of domestic and wild striped bass, *Morone saxatilis* (Walbaum), broodstock with implanted GnRH analogue and injected hCG. *Aquaculture and Fisheries Management* 24:389-398.
- MacMillan, J. R., D. Mulcahy, and M. L. Landolt. 1989. Cytopathology and coagulopathy associated with viral erythrocytic necrosis in chum salmon. *Journal of Aquatic Animal Health* 1:255-262.
- McDonald, D. G., Y. Tang, and R. G. Boutilier. 1989. Acid and ion transfer across the gills of fish: mechanisms and regulation. *Canadian Journal of Zoology* 67:3046-3054.
- Miller, T. K., and J. Levin. 1984. The effects of multiple injections of bacterial endotoxin on blood coagulation in the toadfish, *Opsanus tau*. *Biological Bulletin (Woods Hole)* 166:189-205.
- Noga, E. J., J. H. Kerby, W. King, D. P. Aucoin, and F. Giesbrecht. 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*). *American Journal of Veterinary Research* 55:405-409.
- Perry, S. E., and P. Laurent. 1989. Adaptational responses of rainbow trout to lowered external NaCl concentration: contribution of the branchial chloride cell. *Journal of Experimental Biology* 147:147-168.
- Rees, R. A., and R. M. Harrell. 1990. Artificial spawning and fry production of striped bass and hybrids. Pages 43-72 in R. M. Harrell, J. H. Kerby and R. V. Minton, editors. *Culture and propagation of striped bass and its hybrids*. American Fisheries Society, Southern Division, Striped Bass Committee, Bethesda, Maryland.
- Salte, R., P. Nafstad, and T. Asgard. 1987. Disseminated intravascular coagulation in "Hitra disease" (hemorrhagic syndrome) in farmed Atlantic salmon. *Veterinary Pathology* 24:378-385.
- Salte, R., and K. Norberg. 1991. Disseminated intravascular coagulation in farmed Atlantic salmon, *Salmo salar* L.: evidence of consumptive coagulopathy. *Journal of Fish Diseases* 14:475-480.
- Salte, R., K. Norberg, and O. R. Odegaard. 1991. Do extracellular products of *Aeromonas salmonicida* induce thrombosis by entering the fish coagulation system at factor X? *Journal of Fish Diseases* 14:401-406.
- Slappendel, R. J. 1989. Disseminated intravascular coagulation. Pages 451-464 in R. W. Kirk, editor. *Current veterinary therapy X: small animal practice*. Saunders, Philadelphia.
- Stoskopf, M. K. 1993. *Clinical pathology*. Pages 113-131 in M. K. Stoskopf. *Fish medicine*. Saunders, Philadelphia.
- van Vliet, K. J., G. L. Smit, J. J. Pieterse, J. J. Schoonbee, and J. H. J. Van Vuren. 1985. A thromboelastographic study of the effect of stress on the blood coagulation in *Cyprinus carpio* (Cyprinidae) and *Oreochromis mossambicus* (Cichlidae). *Comparative Biochemistry and Physiology* 82A:23-27.
- Woodward, J. J., E. Casillas, L. S. Smith, and B. G. D'Aoust. 1979. Rapid decompression stress accelerates fibrinolysis in fingerling salmon. *Journal of the Fisheries Research Board of Canada* 36:592-594.