Hematology and Blood Chemistry Reference Intervals for Yellow Perch (*Perca flavescens*) Raised in Recirculation Systems

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ABSTRACT

Determination of hematology and blood plasma biochemistry values is routinely used to assess the health of wild and domestic animals. Yellow perch (*Perca flavescens*) culture is a growing segment of the U.S. aquaculture industry and tools are needed to monitor the health status of these fish. This paper reports reference values for complete hematological and biochemistry profiles of normal, healthy yellow perch raised in recirculation culture conditions. The following hematologic values were determined: packed cell volume, plasma protein, erythrocyte, leukocyte, lymphocyte, neutrophil, monocyte, and thrombocyte numbers. A description of leukocyte morphology is presented. Additionally, the following plasma biochemical values were determined: total protein, albumin, globulin, creatinine, total bilirubin, alkaline phosphatase, aspartate aminotransferase, sodium, potassium, chloride, calcium, phosphorus, magnesium, glucose, and cholesterol. Reference values for a specific population of fish need to be determined prior to utilizing diagnostic blood samples from individuals. Developing diagnostic hematology for fishes can enhance yellow perch culture by providing a means for the early detection and identification of infectious disease and of sub-lethal conditions that may affect production performance.

INTRODUCTION

Yellow perch (*Perca flavescens*) are an important game fish throughout much of the Northeast and Midwestern United States and Canada. Contamination of natural waters by pollutants and an increased consumer demand for fresh seafood has led to the aquaculture production of yellow perch. The culture of yellow perch is a rapidly emerging segment of aquaculture in the United States (Schmitz 1999) and has great economic potential, especially in recirculation aquaculture systems (Kelly 2000, Mallison 2000). As more producers cultivate yellow perch, it will become increasingly important to accurately evaluate the health of these fish and to develop tools, such as diagnostic hematology, to monitor the health status of fish during their production cycle.

Diagnostic evaluation of blood parameters has been used extensively for many mammalian, avian, and reptilian species. The rapidly growing aquaculture industry will increasingly need to utilize information of this type in order to assess the health status of cultured fishes. Unfortunately, hematology use in aquaculture remains limited in part due to the lack of

reliable reference blood values for most fish species. Accurate reference intervals have been developed for some species including hybrid striped bass and tilapia under different production settings (Hrubec *et al.* 1996, 1997a,b, 2000, 2001, Hrubec and Smith 2000) and for trout (biochemistry only, Wedemeyer and Nelson 1975), pacu (hematological only, Tocidlowski *et al.* 1997), and milkfish (Ram-Bhaskar and Srinivasa-Rao 1989).

Little is known about the blood response of yellow perch. There are few studies that have previously measured blood values in yellow perch (Toneys and Coble 1980, Nelson *et al.* 1988, Nelson and Mitchell 1992, van den Heuvel *et al.* 2000). Only a few parameters such as hematocrit, sodium, and chloride were determined as most of these studies were toxicological in nature and evaluated changes in other body systems. The objective of this study was to generate a complete comprehensive list of reference blood values for normal healthy yellow perch (*Perca flavescens*), raised to market size in a recirculation system, for later use as a diagnostic tool. This is the first paper to report full hematological and biochemical profiles for production yellow perch.

MATERIALS AND METHODS

Juvenile yellow perch were stocked into production recirculation systems as fingerlings and reared through their production cycle indoors in 10,219-L recirculation tanks with a rotating biological contactor filter and 10% freshwater exchange per day. At the end of their production cycle, when the fish were 17 months old, approximately 250 fish were removed from the production system and placed in a smaller 2,400-L circular tank with a slant tube clarifier and a trickle biofilter. The smaller tank had a freshwater replacement rate of 15% per day. The fish were placed in the smaller systems to allow for a more rapid and less stressful capture procedure. The fish were acclimated to the new tanks for 3 weeks. The photoperiod was approximately 14-h light and 10-h dark. Fish were fed daily to satiation with a commercial pelleted diet (Rangen EXTR 400 40% protein 10% fat, Rangen Inc., Buhl, ID, USA). The following water quality parameters were determined daily: temperature, pH, ammonia, alkalinity, hardness, nitrite, nitrate, and dissolved oxygen. Ranges for water quality over the duration of the study are shown in Table 1 and are representative of water quality observed daily in these tank systems.

Table 1. Water quality parameters for yellow perch (Perca flavescens) reared in a recirculation culture system. Values are means ± standard deviations for the parameters on the days that fish were bled for hematological and blood biochemical determinations and are representative of the daily water quality values in the system.

Parameter	For Hematology Fish	For Chemistry Fish
Temperature (°C)	20.9 ± 0.01	20.7 ± 1.1
pH	7.8 ± 0.1	7.9 ± 0.2
NH_3 un-ionized (mg/L)	0.012 ± 0.012	0.011 ± 0.005
NO_2-N (mg/L)	0.031 ± 0.015	0.043 ± 0.043
NO_3-N (mg/L)	6.0 ± 2.6	2.8 ± 0.9
Alkalinity (mg/L)	252 ± 44	313 ± 48
Hardness (mg/L)	399 ± 10	433 ± 27
Dissolved Oxygen (mg/L)	7.4 ± 0.5	7.4 ± 0.3

Fish were netted rapidly and anesthetized in aerated tank water with buffered tricaine methanesulfonate (MS-222, Sigma Chemical Co., St. Louis, MO, USA) until they began to lose equilibrium, approximately 20 seconds. Individual fish were bled for either hematological determinations (23g needle, 1-mL syringe) or for biochemical determinations (23g needle, 3-mL syringe); in both cases, blood was collected from the caudal vessels. After blood samples were collected, the fish were weighed, measured, and checked for external and internal pathologic lesions. The sex of each fish was determined by internal observation of the gonads.

Blood for hematological determinations was transferred to an ethylenediamine-tetraacetic acid (EDTA) treated pediatric blood tube and held on ice until analysis (< 1 hour). Blood for biochemical determinations was collected into cold 3-mL heparinized blood tubes and centrifuged at 14,000 x g immediately. Plasma was collected and frozen at -10°C until analyzed. The following analytes were determined in the plasma with an Olympus AU-400 (Olympus America Inc., Melleville, NY, USA) automated clinical chemistry analyzer: total protein, albumin, creatinine, total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol, glucose, sodium, potassium, chloride, phosphorus, calcium, and magnesium. Globulin was calculated from the total protein value minus the albumin value.

Hematological analytes were determined from the EDTA anticoagulated blood. As we have observed for other fish species, EDTA was superior to heparin for yellow perch blood, both in preventing clot formation and preserving cellular morphology (Hrubec *et al.* 1996, 2000). Blood from the EDTA tube was drawn into microhematocrit tubes and the packed cell volume (PCV) determined after centrifugation at 10,000 x g for 5 min. Plasma protein was determined with a clinical refractometer using plasma from the microhematocrit tube. The total erythrocyte and leukocyte-plus-thrombocyte counts were determined manually with a Neubauer hemacytometer using Natt-Herrick's solution as a diluent stain (Natt and Herrick 1952). Blood smears, made within 45 minutes of sample collection, were stained with Wright-Geimsa stain and were used to determine the differential counts as follows. Leukocytes and thrombocytes were identified and counted on the blood smears until 200 leukocytes and a variable number of thrombocytes were enumerated. The percentages of each leukocyte type and of thrombocytes were multiplied by the total leukocyte-plus-thrombocyte number to give the final cell counts. Thrombocyte numbers were subtracted from the leukocyte-plus-thrombocyte count to give the total leukocyte count. This method of manually determining total leukocyte and differential counts has been recommended for use with avian (Zinkl 1986) and fish blood (Hrubec *et al.* 1996, 1997a,b, 2000, 2001), as the nucleated red cells prevent accurate enumeration using automated analysis (Huffman and Arkoosh 1997). Slight thrombocyte clumping was observed on the hemacytometer for some individuals; only fish with minimal thrombocyte clumping (< 4 cells clumped) were used for the differential counts to ensure accuracy of the counts.

Reference intervals were determined following the guidelines proposed by the National Committee for Clinical Laboratory Standards (NCCLS 1992). As suggested in these guidelines, the data were checked for outliers using the 1/3 difference/range ratio, and no outliers were identified. The values were then ranked and the high and low 2.5% were discarded. The range of the remaining values provided the reference interval.

RESULTS AND DISCUSSION

The physiologic and health status of an individual is reflected in the blood, producing variations in hematological and blood biochemical values. Clinical analysis of blood is a fundamental tool used in human

and veterinary medicine to diagnose and predict the outcome of disease and to monitor the effect of therapeutic, nutritional, and environmental management. Blood analysis is not used extensively as a diagnostic tool in fish medicine, partly due to the lack of reference intervals for various fish species, and also because changes associated with specific diseases and metabolic disorders are not well characterized. With sufficient background data, clinical analysis of individual blood samples could detect infectious diseases, metabolic disorders, and sub-lethal disease states affecting production performance.

Hematological and plasma biochemistry data from diseased individuals can be evaluated by direct comparison to a reference interval, which is the appropriate range of variation in a blood parameter from a defined population of individuals under specific conditions. The reference interval needs to be determined on a sufficient number of normal, healthy individuals under similar production conditions using standardized analytical techniques (NCCLS 1992). When the deviation in a blood parameter is large enough to fall outside the reference interval, it indicates the value may be aberrant and is most likely not due to individual variation for a given fish.

Few previous studies have determined blood parameters for yellow perch (Toneys and Coble 1980, Nelson *et al.* 1988, Nelson and Mitchell 1992, van den Heuvel *et al.* 2000). These studies are of limited relevance as they were primarily toxicological studies and only a few blood parameters were determined, and also because blood samples were collected and handled by differing methods prior to determination of the blood value. Some of the capture (hook and line and gill netting) and blood collection methods (severing the caudal peduncle) used in these studies are also unsuitable for diagnostic blood samples, as they result in significant alteration in the blood which will mask diseased states. The study with the closest sampling procedure to that used in our experiment only presented selected blood chemistry values for yellow perch after 16 hours of moderate or exhaustive exercise (Nelson and Mitchell 1992). Therefore, although the previous studies on yellow perch hematology are helpful in determining the effects of environmental factors and stress, they have limited diagnostic utility and even prevent meaningful comparison with the data presented in this paper.

The average mean weight of the yellow perch used for the hematological determinations was 125 +/- 14g with a total length of 22.3 +/- 0.8 cm. For

the biochemical determinations, the fish had a weight of 121 +/- 15g and length of 22.6 +/- 0.8 cm. The results of the hematological determinations from 52 fish are listed in Table 2. Values for plasma chemistry reference intervals, determined on 42 samples, are listed in Table 3. Overall, both the hematological and plasma chemistry values were similar to those reported previously for hybrid striped bass and tilapia reared in recirculation systems (Hrubec *et al.* 1996, 2000, Hrubec and Smith, 2000). The striped bass and tilapia from production systems exhibited wider ranges in value for the different leukocyte types, increased numbers of reticulocytes, increased plasma and total protein values, increased creatinine values, and a decreased plasma chloride concentration as compared to fish in lower density recirculation tanks. We did not compare blood values from the production yellow perch in this study to yellow perch maintained in low-density tank settings so were unable to determine if these same trends are apparent in yellow perch as well. However, based on our previous experience with fish hematological and plasma biochemical values, there is an indication that these trends are occurring in yellow perch as well.

Table 2. Hematological reference intervals for adult yellow perch (Perca flavescens) reared in a recirculation system.

Analyte	N	Reference Interval	Mean	Stds[1]
PCV[2] (%)	57	29-47	38.8	4.5
Plasma Protein (g/dl)	57	6.0-8.2	6.7	0.6
Erythrocytes (x 106/ml)	53	2.160-3.345	2.737	0.356
Leukocytes (#/ml)	53	52,590-186,490	113,914	39,086
Lymphocytes (#/ml)				
Small	53	36,800-153,420	85,630	31,728
Large	53	3,530-23,130	11,602	5,359
Neutrophils (#/ml)	53	1,860-35,950	12,430	8,837
Monocytes (#/ml)	53	670-12,640	4,252	2,874
Thrombocytes (#/ml)	53	38,270-118,510	72,972	21,299

[1]*Standard deviation,* [2]*Packed cell volume*

Table 3. Plasma biochemical values for adult yellow perch (Perca flavescens) reared in a recirculation system.

Analyte	N	Reference Interval	Mean	Stds[1]
Total Protein (g/dl)	42	3.7-5.0	4.5	0.4
Albumin (g/dl)	42	0.6-0.9	0.7	0.1
Globulin (g/dl)	42	3.1-4.2	3.7	0.3
Creatinine (mg/dl)	42	0.4-1.0	0.6	0.1
Total bilirubin (mg/dl)	42	0.3-0.4	0.3	0.1
ALP[2] (U/l)	42	50-114	82.2	24.9
AST[3] (U/l)	42	2-29	8.5	6.4
Glucose (mg/dl)	42	62-181	100.0	35.0
Cholesterol (mg/dl)	42	182-323	244.0	33.0
Sodium (mEq/l)	42	138-153	147.0	4.0
Potassium (mEq/l)	42	2.0-3.8	3.2	0.5
Chloride (mEq/l)	42	119-133	126.0	4.0
Calcium (mEq/l)	42	8.6-12.0	10.3	1.3
Phosphorus (mEq/l)	42	5.0-9.6	7.4	1.1
Magnesium (mEq/l)	42	1.7-3.4	2.7	0.4

[1] Standard deviation, [2] Alkaline phosphatase [3] Aspartate aminotransferase (SGOT)

The blood cells present in the yellow perch were typical of teleost fish and included erythrocytes, thrombocytes, and leukocytes (Fig. 1). Erythrocytes were oval to round with characteristic red cytoplasm and an elongate and centrally-located nucleus. Immature erythrocytes, or reticulocytes, demonstrated a blue-purple tinge to the normal eosinophilic cytoplasm (Fig. 1A, B, C). The yellow perch had increased numbers of reticulocytes (included in the erythrocyte count) with approximately 7 to 10 per field at 100x oil immersion compared to what we observe for most fish species. The cause for the apparent reticulocytosis is not known, but has been observed in other fish species exposed to elevated nitrite and nitrate (Grabda *et al.* 1974, Hrubec *et al.* 1996, 2000, Hrubec and Smith 2000).

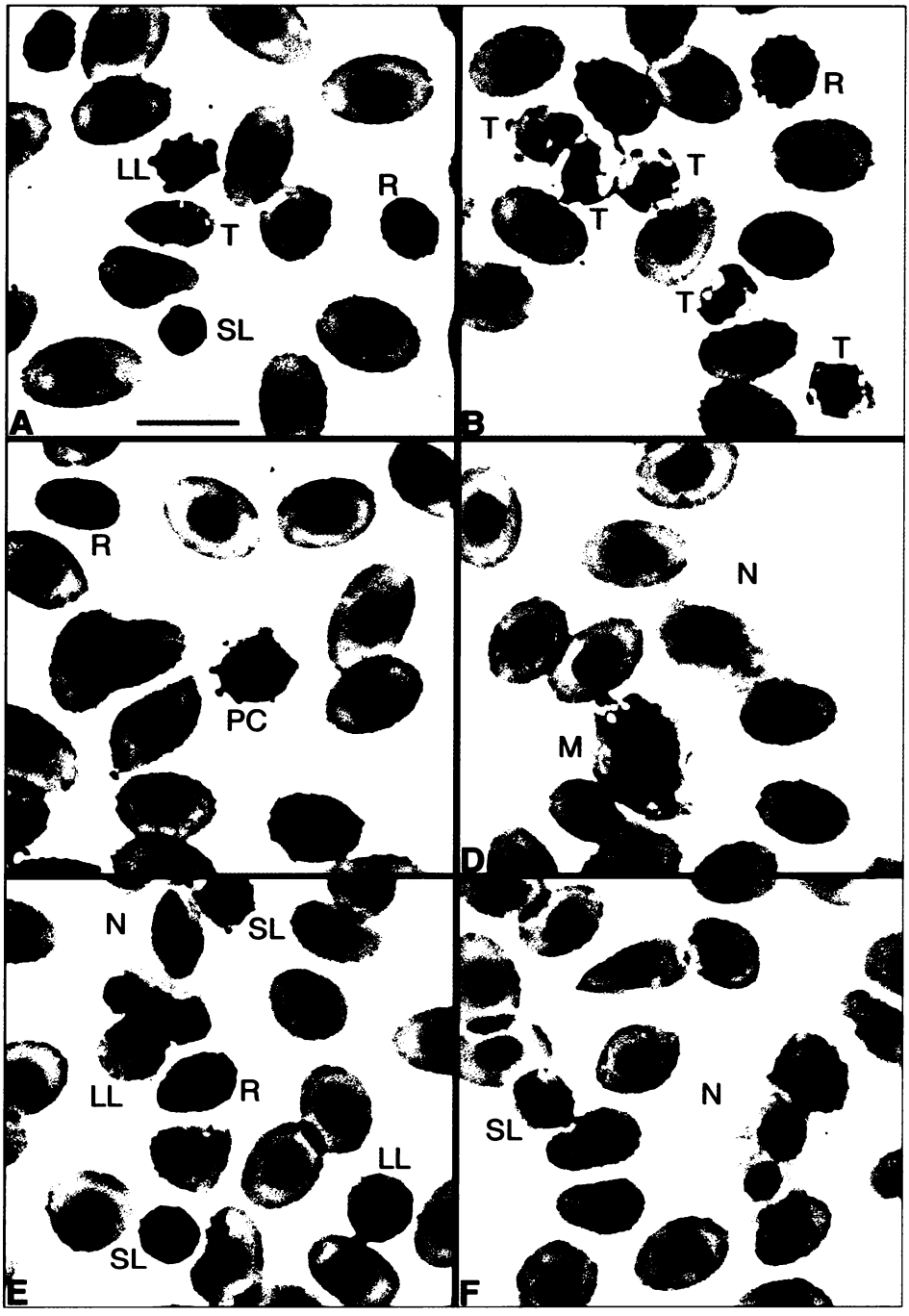

Figure 1. Characteristic blood cells from yellow perch reared in recirculation systems. Blood smears were made with EDTA anticoagulated blood stained with Wright's Geimsa stain. Cells are abbreviated as follows: LL – Large lymphocyte, SL – small lymphocyte, T – thrombocyte, R – reticulocytes (immature erythrocyte), PC – plasma cell (activated lymphocyte), M – monocyte, N – neutrophil. The blue bar in frame A is 10mm.

The thrombocytes were slightly smaller than the erythrocytes (Fig. 1A). They had clear cytoplasm and were variable in shape, being elongated, pyriform, oval, or round. Nuclear shape tended to follow cytoplasmic shape, although oval and round thrombocyte nuclei occasionally were lobulated (Fig. 1B), as described for striped bass and tilapia (Hrubec *et al.* 1996, 2000).

Leukocytes made up the remainder of the cell types seen in the blood and included small and large lymphocytes, neutrophils, and monocytes. No eosinophils or heterophils were observed. Small lymphocytes were the smallest cell present, with a rim of blue cytoplasm surrounding the round nucleus (Fig. 1A, E, F). Large lymphocytes had an abundant and darker blue cytoplasm and the nucleus was larger than observed in the small lymphocyte (Fig. 1A, E). Plasma cells were occasionally observed with a classic open nucleus, abundant dark blue cytoplasm and a clear cytoplasmic region adjacent to the nucleus presumably representing the Golgi as in mammalian plasma cells (Fig. 1C). Plasma cells were included in the large lymphocytes category for the differential counts.

Neutrophils were the largest cell present in the blood (Fig. 1D, E, F). The cytoplasm of the neutrophil was a translucent grey, containing no granules and infrequent vacuoles. Cytoplasmic shape was round to angular as the cellular borders often appeared slightly adherent to adjacent erythrocytes, distorting cellular shape. Nuclear shape of the neutrophil varied from round to horseshoe shaped and frequently segmented into two prominent lobes connected by a thin nuclear bridge (Fig. 1F). Monocytes had abundant dark blue cytoplasm that was frequently vacuolated (Fig. 1D). The round to kidney-bean shaped monocyte nucleus was large with prominent chromatin clumping.

Analysis of blood parameters can provide a wealth of information useful in analyzing the effects of disease and sub-optimal environmental conditions. Providing reference intervals for healthy adult yellow perch reared in recirculation systems furnishes veterinarians and fish health professionals the foundation to develop diagnostic hematology for this species. The number of studies that determine actual reference intervals for fish species is limited. The majority of blood values determined for fishes are reported in the literature as a mean value with a standard deviation. Historically, reference intervals were determined as two standard deviations from the mean, however, this method is only valid when blood values follow a normal distribution. It is incorrect to assume

that biological parameters are distributed normally, therefore non-parametric methods should be used to accurately determine reference intervals (Reed *et al.* 1971). Techniques to properly determine reference intervals have been established by the NCCLS (1992) and suggestions for their use and interpretation have been discussed (Lumsden 1998). As with mammalian species, reference intervals need to be determined for different populations of fish within a single species, as culture conditions and environmental variables affect blood values to the extent that they are outside the reference interval (Ram-Bhaskar and Srinivasa-Rao 1989, Hrubec *et al.* 1996, 1997a,b, 2000, 2001). Although the reference range for some of the blood parameters appears large, it is still possible to detect variation in hematologic values associated with pathological conditions (Hrubec *et al.* 1997b, and unpublished data).

Developing reference intervals is a necessary first step in determining which specific hematological changes can be associated with disease conditions. As the field of fish hematology develops, its usefulness to the aquaculture industry will increase. Information derived by standardized non-lethal diagnostic assays will be needed to enhance the culture of yellow perch and other fish species. Analysis of diagnostic blood samples can provide a means for early detection of infectious disease and assist in the identification of sub-lethal conditions affecting production performance. This should allow for more specific, timely, and effective disease treatments in the future.

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