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A Preliminary Study on the Hematology of Freshwater-Reared Seabass/Barramundi, *Lates calcarifer*

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Abstract

Thirty juvenile, freshwater-reared seabass/barramundi, *Lates calcarifer*, were examined for hematological parameters and serum protein levels. The seabass were siblings from an artificial spawning reared in a freshwater recirculating system in Queensland, Australia. Values for hemoglobin, hematocrit, erythrocyte count, and total and differential leucocyte counts were determined. There was wide individual variation, particularly in leucocyte values of healthy fish. The morphology and cytochemistry of blood cells are described.

Introduction

Seabass, or in Australia, barramundi, *Lates calcarifer*, are a highly desirable table and angling fish in Australia. Following commercially successful artificial breeding and larval rearing, barramundi have become the main finfish species used in tropical Australian aquaculture. Commercial production of cultured seabass in Queensland was estimated at 232 tonnes in 1992-93 (Lobegeiger 1994), worth US$1.83 million. There are two forms of seabass culture used: marine cages and floating cages in freshwater ponds. It is likely that future expansion of the industry to grow juveniles to market size will be restricted to the use of freshwater ponds. Though information on the biology, breeding and culture of seabass is available (Anon. 1984; Copland and Grey 1987; Pearson 1987), there remains a lack of fundamental physiological information. Only two records on hematology of seabass have been published (Danayadol and Boonranapanitkit 1984; Danayadol et al. 1987). In both studies,

the seabass were reared in seawater. The routine use of hematology in human and veterinary medicine is well established. Whilst hematology has been used in some finfish species as an indicator of physiological or pathological change (Blaxhall 1972), it is important to establish a range of normal values for each species for the purpose of health assessment (Blaxhall and Daisley 1973).

The aim of this study was to document various hematological and biochemical values of healthy juvenile seabass reared in freshwater.

Materials and Methods

Forty, 6-month old seabass, *L. calcarifer*, of 210-490 g live weight were purchased from a farm at Buderim, Queensland. They were siblings from an artificial spawning and had been reared in a north Queensland hatchery. Subsequent rearing occurred in a heated, freshwater recirculating system. After arrival at the laboratory in Brisbane the fish were maintained in a heated (22-25°C) freshwater recirculating system consisting of two 2,000-l cylindro-conical fiberglass tanks. During the 2-month acclimatization and holding period, the fish were fed a commercial seabass pelled diet twice daily *ad lib*. Water quality was measured daily to ensure the concentrations of dissolved oxygen, hydrogen ions and nitrogenous compounds remained within acceptable limits for health (Munro and Roberts 1989).

The fish were individually identified by anchor tags, with external streamers, inserted just ventral to the dorsal fin. Thirty healthy fish were randomly selected from the initial group for blood sampling.

For sampling, the fish were taken from the tanks with a dip net and immediately anesthetized in a 200 $mg \cdot l^{-1}$ benzocaine bath. One millilitre (ml) of blood was taken from the caudal vein using 2-ml plastic disposable syringes fitted with 0.8 x 38 mm hypodermic needles, and containing 3-4 mg of powdered ethylenediaminetetracetic acid (EDTA) (dipotassium salt). A further 0.5 ml, taken into a syringe without EDTA, was used to prepare blood films and for the determination of serum protein levles.

Hemoglobin concentration was measured using a cyanmethaemoglobin method. Erythrocyte nuclei were removed by centrifigation before reading in a Coulter Hemoglobinometer (Coulter Electronic Ltd., England). The method was standardized against commerial hemoglobin standards (Sigma Chemical Company, Sigma-Aldrich Pty. Limited). Erythrocyte counts were determined using an electronic cell counter (Coulter Electronic Ltd., England). In a preliminary experiment, erythrocyte counts determined electronically agreed with those determined manually using an improved Neubauer hemocytometer. Total leucocyte counts determined manually using an improved Neubauer hemocytometer. Blood was diluted 1:50 in buffered formol saline containing 0.5% new methylene blue and the number of leucocytes in 0.5 mm^3 counted. Hematocrit was determined by the microhematocrit method. Mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated. For differential leucocyte counts, 200 leucocytes were identified in methanol-fixed, May Grünwald-Giemsa (MGG)

(Dacie and Lewis 1968) stained blood films. The periodic acid-Schiff (PAS) reaction, myeloperoxidase reaction (Sigma Kit No. 391-A) and the sudan black stain were used to aid the differential classification of leucocytes. Cell sizes were measured with an eyepiece micrometer at x 1,000 magnification on MGG stained blood films. Fifty cells of each type were measured.

Total serum protein (TSP) and serum albumin values were determined in a discrete analyzer at 37°C on sera from 25 of the fish. TSP was assayed using biuret reagent at 540 nm and serum albumin using bromocresol green reagent at 600 nm. Serum globulin was calculated by difference.

Results

Hematology

The results of the analysis of blood samples from 30 seabass are given in Table 1.

Table 1. Hematological values for healthy seabass not previously bled and reared in freshwater. Thirty fish were sampled.

Parameter	Range	Mean	± 1 SE
Hemoglobin ($g \cdot dl^{-1}$)	6.0 - 9.5	8.1	± 0.2
Hematocrit (%)	26.0 - 40.0	33	± 0.7
Erythrocyte count x $10^{12} \cdot l^{-1}$	3.25 - 5.20	4.39	± 0.1
Mean cell volume (fl)	66 - 91	77	± 1
Mean cell hemoglobin (pg)	16.7 - 20.6	18.4	± 0.2
MCHC ($g \cdot dl^{-1}$)	20.0 - 27.0	24.1	± 0.3
Leucocyte count x $10^{9} \cdot l^{-1}$	6.5 - 56.0	24.8	± 2.2
Granulocytes x $10^{9} \cdot l^{-1}$	0.1 - 11.7	2.5	± 0.5
Granulocytes (%)	2 - 27	9	± 1
Lymphocytes x $10^{9} \cdot l^{-1}$	6.0 - 48.2	20.5	± 1.7
Lymphocytes (%)	60 - 93	84	± 2
Monocytes x $10^{9} \cdot l^{-1}$	0.1 - 6.9	1.9	± 0.3
Monocytes (%)	2 - 17	7	± 1
Total serum protein[1] ($g \cdot l^{-1}$)	43.0 - 58.0	50.3	± 0.7
Serum globulin[1] ($g \cdot l^{-1}$)	27.0 - 40.0	33.6	± 0.6
Serum albumin[1] ($g \cdot l^{-1}$)	15.0 - 18.0	16.7	± 0.2
Serum A/G ratio[1]	42 - 0.59	0.50	± 0.01

[1]Twenty-five fish only.

Morphology and Histochemistry of Blood Cells

The size of blood cells in stained blood films is given in Table 2. Classification of cells in films was based on the morphological descriptions given by Ellis (1977) and O'Connor (1984). Five types were identified and are described below.

Table 2. Sizes of blood cells from stained blood films of healthy seabass reared in freshwater. Fifty cells of each type were measured.

Cell type	Range (µm)	Mean (µm)	± 1 SE
Erythrocyte	10.5-13.0 x 5.0 - 6.5	11.4 x 5.7	± 0.05, 0.03
Lymphocyte	3.9 - 7.1	5.3	± 0.06
Monocyte	7.0 - 13.3	9.4	± 0.13
Granulocyte	6.2 - 12.3	9.6	± 0.12
Thrombocyte	7.7-12.6 x 4.0 - 6.0	9.1 x 5.1	± 0.09, 0.04

ERYTHROCYTES

The ellipsoidal erythrocytes had central nuclei which had their long axes parallel to the cells' long axes. The cytoplasm was an even pink, staining more intensely adjacent to the cell membrane. The nuclei stained purple with a thin band of chromatin on the inner surface of the nuclear membrane and scattered clumps of chromatin throughout, giving a granular appearance.

THROMBOCYTES

These cells were easily differentiated by their scant pale grey to colorless cytoplasm. Their shape ranged from round to oval, to "spiked". The central nuclei stained reddish-purple and had patterns of coarse reticular chromatin. Occassionally, clefts were present in oval nuclei. Immature thrombocytes (round and oval forms) had numerous fine, PAS-positive intracytoplasmic granules. Mature cells ("spiked" forms) occasionally contained two or three large PAS-positive cytoplasmic granules and clear cytoplasmic vacuoles. The cytoplasm was not stained with the peroxidase reaction or sudan black.

LYMPHOCYTES

Only small lymphocytes were observed. They tended to be oval, but usually had an irregular outline with small finger-like projections. Their dark purple, centrally placed nucleus was surrounded by a narrow rim of homogenous dark blue cytoplasm, and occupied 80% of the cell. These nuclei, with their pattern of dense clumped chromatin were more darkly stained than the other cell nuclei. The cytoplasm did not stain with sudan black, PAS or peroxidase reactions.

GRANULOCYTES

Granulocytes were round with smooth outlines. The pale grey cytoplasm contained very fine, scattered azurophilic and colorless granules. Cytochemically, the cytoplasm contained numerous fine PAS-positive granules; numerous, large, peroxidase-positive granules, ranging from round to rod-shaped; and many small granules which stained with sudan black. The nucleus occupied about 25% of the cell and was either eccentric or central. Nuclei were oval, slightly indented, folded, bow-shaped or segmented and stained dark reddish-purple with a coarse reticular pattern.

MONOCYTES

Monocytes were round with occasional cytoplasmic projections. The cytoplasm was deep blue and finely granular in appearance. Clear intracytoplasmic vacuoles were present. The large oval to bilobed nucleus was typically eccentric but could also be located centrally in the cell. The nucleus was reddish-purple and had a fine reticular chromatin pattern. Cytoplasmic granules staining with sudan black, PAS or peroxidase reactions, were absent.

Discussion

One constraint in the use of hematology in the assessment of health in fish is the wide variation in individual values (Barnhart 1969; Hattingh and Van Plentzen 1974; Van Vuren and Hattingh 1978). Factors that can cause variation are age, origin, stocking density (Klontz and Smith 1968), season, strain, method of capture, stage of sexual maturity (McCarthy et al. 1973) and diet (Barnhart 1969). The ranges of hematological parameters measured in this study were quite broad. This is surprising because the fish were genetically similar, of the same age and maintained in the same environment. In practical terms, these values support the findings of Ellis (1977) that leucocyte counts are impractical for diagnostic purposes. However, with further work, baseline values could be established to demonstrate specific changes in the blood picture resulting from disease, stress or toxic insult.

Extensive comparisons with other fish species will not be done as the aim of this study was to document a set of normal hematological values for juvenile seabass from freshwater. Inherent variations in hematological values, and variations resulting from different methods of analysis (Blaxhall 1972), make comparisons between species difficult. Erythrocyte values obtained in this study were similar to those reported by Danayadol and Boonranapanitkit (1984), who determined a mean hematocrit of 40% and total serum protein of 46 $g \cdot l^{-1}$ in juvenile seawater seabass. In this study, hemoglobin concentrations, erythrocyte counts and MCHC values were higher than those for rainbow trout (McCarthy et al. 1973; Miller et al. 1983), brown trout (Blaxhall and Daisley 1973), channel catfish (Breazile et al. 1982) and goldfish (Watson et al. 1963). The hematocrit of seabass blood was very similar to that of salmonids. The mean erthrocyte measurements of 11.4 x 5.7 µm are somewhat smaller than those measured in brown trout (Blaxhall and Daisley 1973) and goldfish (Watson et al. 1963). Rakitskaya (1982) and Larsson et al. (1976) have found that pelagic or fast-moving predatory fishes have higher erythrocyte counts and hemoglobin concentrations than sedentary fishes. They suggest this reflects the need for oxygen to carry out normal behavior of the animal. The values from the seabass, an active predatory fish, support this hypothesis.

In this study, a mean leucocyte count of 24.8 x 10^9 $cell \cdot l^{-1}$ was obtained as compared to 11.5 x 10^9 $cell \cdot l^{-1}$ for brown trout (Braxhall and Daisley 1973) and 14.0 x 10^9 $cell \cdot l^{-1}$ for rainbow trout (McCarthy et al. 1973). Though the range of leucocyte counts was very wide, 6.5 to 56.0 x 10^9 $cell \cdot l^{-1}$, a wide individual

variation in leucocyte numbers has been recorded in other fishes (Blaxhall and Daisley 1973; McCarthy et al. 1973). A genetic variation in the way individual fish respond to stress may explain this wide variation. Stress does change the type, proportion and number of leucocytes in the blood of fish (Blaxhall 1972; Robertson et al. 1987; Wiik et al. 1989). Although the husbandry and handling of the seabass in this study should have ensured all were physiologically normal at blood sampling, variations in food intake, level of dominance in the group (size variation) and handling may have been sufficient to cause stress in some individual fish. The morphology and cytochemistry of seabass leucocytes is similar to that of other fishes. Leucocytes and thrombocytes fitted the morphological criteria used by Ellis (1977). Typical of fish blood, lymphocytes were more numerous than neutrophil-like granulocytes. Eosinophilic and basophilic granulocytes are rare in many fish species (Ellis 1977; Hine et al. 1987). The lack of these granulocytes in the blood films of seabass examined in this study is not surprising.

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