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Hematological characteristics of the spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869) healthy and naturally infected by dactylogyrid monogeneans

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ABSTRACT

The aim of the present study was to obtain a basic knowledge of the hematology in order to determine changes in blood parameters of the spotted rose snapper *Lutjanus guttatus*. The morphological features of blood cells were described according to the observations made by light microscopy of Wright–Giemsa-stained blood films. The reference intervals and the mean value were determined for each hematological parameter evaluated in healthy fish and data were compared to those of naturally infected, with dactylogyrid monogeneans fish.

Infected fish showed a prevalence of 100% and a mean intensity of 246.6 parasites per fish. Mean values of HCT, WBC, thrombocytes percentage and eosinophils percentage were significantly higher ($P<0.05$) in the infected fish. In addition, lymphocytes percentage and total protein were significantly lower ($P<0.05$) in the infected fish compared to healthy fish. Only total WBC count, lymphocytes percentage and eosinophils percentage in infected fish were outside reference interval. The hematology of the spotted rose snapper of this study might serve as a basis for future studies and diagnosis. Changes observed in blood parameters in infected fish suggest that the immune system of *L. guttatus* was affected by the presence of the parasites.

1. Introduction

The spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869) is found in tropical and subtropical waters of the eastern Pacific from Mexico to Peru (Fischer et al., 1995). This is an important commercial fish species that has been in experimental culture for several years in Central America (Gutiérrez-Vargas and Durán-Delgado, 1999; Valverde-Chavarría and Boza-Abarca, 1999). In Mexico there is an increasing interest in spotted rose snapper cultivation by important fish sectors. Several grow-out trials are being carried out by more than 17 coastal Pacific fishery cooperatives, based on wild juveniles of snappers, including the spotted rose snapper (Avilés-Quevedo, 2006).

The studies for the experimental culture of spotted rose snapper have been developed mainly in the northwest region of Mexico. Nowadays the technology of juvenile massive production is ready to achieve a pilot-scale (Alvarez-Lajonchère et al., 2007). However, the occurrence of ectoparasites has risen as a potential problem for its development. In this regard, the culture of the spotted rose snapper has lead to the increase of several diseases produced by infestation of the ciliated protozoans, i.e. *Brooklynella hostilis* and *Cryptocaryon irritans* and a dinoflagellate *Amyloodinium ocellatum*. The last two groups of organisms attack the skin and gills causing the death of the fish in captivity (Fajer-Ávila et al., 2006).

Other abundant and potentially dangerous parasites are the dactylogyrid monogeneans, a heavy infection of which can cause gill epithelial hyperplasia, asphyxia and anorexia of the spotted rose snappers (Fajer-Ávila et al., 2007); and this in turn, might alter the fish blood parameters. Monitoring the health of fishes using hematological analysis is especially important as a direct or inferential indicator; because this gives information related to the tolerance to a stressor agent or/and illness of the fish (Schreck and Moyle, 1990; Del Rio-Zaragoza et al., 2008) such as parasites.

In order to facilitate such an approach, first it is necessary to thoroughly characterize the blood cells of the organisms and to use this knowledge to quantify normal blood parameters (Shigdar et al., 2007). In the present work, we studied blood parameters of spotted rose snapper healthy and naturally infected with dactylogyrid monogeneans to establish the interval which represents the health status of cultured fish.

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2. Materials and methods

2.1. Fish

Spotted rose snappers of the same cohort were obtained from the CIAD's Reproduction Laboratory where fish were cultivated in 7000 l tanks with 2000 fish per tank and fed with commercial diet. The fish were taken from two tanks. One tank had filtered seawater, daily clean and water exchange rate of 50%. From these tank were selected the healthy fish ($n = 30$), while the other tank did not have water exchange and daily cleaning, so infection by dactylogyrids monogeneans was present. The presence of parasites was confirmed based on the technique of Fajer-Ávila et al. (2007) and described below. From this tank naturally infected fish ($n = 10$) were selected and transferred to CIAD's Laboratory of Parasitology. The fish were kept for 15 days. 10 fish per tank of healthy fish group were maintained in 400 l circular tanks. Infected fish tank was isolated from the other tanks. All tanks received continuous natural seawater flow. Previously, the seawater was filtered and passed by a UV lamp. Fish were exposed to natural photoperiods; temperature, $25.6 \pm 1.5\,^{\circ}C$; salinity, 35‰; pH, from 7.6 to 7.9; and dissolved oxygen concentration, 5.3–6.6 mg/l. Fish were fed two times a day to apparent satiation level with commercial diet (45% protein, Purina. Mazatlan, Mexico). Every day, leftover food and faeces were removed from the tanks. To ascertain the presence or absence of adult parasites on the infected and uninfected fish, cotton thread traps were previously placed into tanks (Fajer-Ávila et al., 2007). Observation of parasite eggs in the traps was confirmed and analyzed by stereomicroscope (Leica Microsystems, Wetzlar, Germany). Healthy fish did not show pathological sign caused by bacteria or another infection. Intensity of parasites on infected fish was confirmed at sampling time. When the acclimation period ended, fish were not fed for 24 h before the initial blood samples were taken. Weight and fork length of each fish were measured and condition factor (CF) was calculated as $(\text{weight/fork length3}) \times 100$ (Lagler, 1978).

2.2. Collection of blood samples

Healthy fish ($n = 30$) and naturally infected fish ($n = 10$) were carefully handled to minimize stress. Fish were anaesthetized with 0.5 ml/l of 2-phenoxyethanol (Sigma, St. Louis, MO, USA) in less than three minutes blood samples were collected from the caudal vein using 1-ml non-anticoagulant insulin syringes (Terumo Mexico, DF, Mexico). Immediately after sampling, one blood drop was used for a blood smears. Then blood sample was placed into two Eppendorf tubes: right away from the first tube with no anticoagulant, two capillary were used for measured (1) coagulation time (CT) and (2) sedimentation rate (SR) and hematocrit (HCT). Leftover blood was centrifuged for 10 min and serum was stored in a $-20\,^{\circ}C$ freezer for further analysis of total protein concentration The second tube had K_2EDTA (BD Microtainer Franklin lakes, NJ, USA) to prevent coagulation. This tube was used for hemoglobin (HGB), total red blood cell count (RBC), and total white blood cell count (WBC).

2.3. Hematological analysis

CT was measured with a blood sample in a not heparinised 2/3 filled capillary tube (Modulohm A/S, Herlev, Denmark) and placed in 45° inclination. CT was considered until the blood stop running through the tube (Del Rio-Zaragoza et al., 2008). SR was calculated with a heparinised 2/3 filled capillary tube (Corning, Mexico, DF, Mexico). The tube was sealed and left in vertical position for 60 min. After this, plasma and erythrocytes sediment was measured using a digital vernier (Mytutoyo, Mexico, DF, Mexico) and reported in mm/h (Del Rio-Zaragoza et al., 2008). The same tube was placed for 10 min in a micro-hematocrit centrifuge (SOL-BAT P600, Mexico, DF, Mexico), and the packed cell was measured using a hematocrit reader and reported as percentage (Del Rio-Zaragoza et al., 2008). HGB in erythrocytes was determined using the cyanmethaemoglobin method (hemogloWiener reactive, Wiener Lab.) following manufacturer's instructions. Total RBC count was measured as follows; a blood sample was taken with a Thoma pipette (Marienfeld-SupeRior, Lauda-Königshofen, Germany), and diluted with Hendricks's (1952) solution. The diluted sample was placed in a Neubauer hemacytometer (Marienfeld-SupeRior, Lauda-Königshofen, Germany) and then the RBC was counted using a microscope (LEICA DMLB-10, Guadalajara, Jalisco, Mexico). The Natt and Herrick's (1952) method was used for total WBC-plus-thrombocyte count. The leukocytes cellular differentiation was observed through blood smears treated with a Wright–Giemsa stain (Hycel, Mexico, DF, Mexico) in an optical microscope. The images were processed and cells were measured using the Image-Pro Plus 3 software (Media Cybernetics, Bethesda, MD, USA). Sixty digital images of an average of 203 ± 100 cells were captured from each blood smear. From these images, WBC (lymphocytes, granulocytes, monocytes) and thrombocytes were counted and reported as percentage. Size reference intervals of each cell type were determined recording the smallest and the biggest cell found (Del Rio-Zaragoza et al., 2008). The following indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by standard formulas with data of HCT, RBC and HGB. Total protein was measured based on a colorimetric Biuret assay (BioSystems, Barcelona, Spain) following manufacturer's instructions and using bovine serum albumin as standard.

2.4. Gill analysis

After blood collection, healthy fish and naturally infected fish with dactylogyrid monogeneans were analyzed. Monogeneans (*Euryhaliotrema* spp. and *Haliotrematoides* spp.) on the fish gills were quantified as a group and where not identified to species level. The right gill of the fish was used to quantify parasites (number of parasites was not multiplied by two). The four right side gills of each fish were placed into Petri dishes with marine water and parasites were counted using a stereomicroscope (Leica MZ 9.5, Microsystems, Wetzlar, Germany). Prevalence and mean intensity of infection were determined as defined by Bush et al. (1997).

2.5. Statistical analysis

All measurements were done by triplicate for each fish. Results are expressed as means ± standard deviation (SD). Normality tests and one-way ANOVAs ($P < 0.05$) were applied to all data, using SigmaPlot 9 software (Systat Software, Inc., San Jose, CA, USA). All percentage data were arcsine transformed before statistical comparisons. Non normal data were analyzed by the Kruskal–Wallis test. To determine significant differences among groups, a multiple comparison analysis '*a posteriori*' Holm-Sidak and Dunn's tests were carried out. Reference intervals for each blood parameter were produced using statistical software (Analyse-it Method evaluation edition for Microsoft Excel version 2.11). This software follows the guidelines proposed by International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) expert panel (EPTRV) and Clinical and Laboratory Standards Institute (CLSI) C28-A2 protocols.