Development and health status of *Centropomus undecimalis* parasitized by *Rhabdosynochus rhabdosynochus* (Monogenea) under different salinity and temperature conditions

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Abstract

This study evaluated the correlation of hematological parameters with the mean abundance of the monogenean helminth *Rhabdosynochus rhabdosynochus* in *Centropomus undecimalis* reared at different temperatures and salinities. The experimental conditions were: 28 °C/0 ppt (parts per thousand); 28 °C/15 ppt; 28 °C/32 ppt; 25 °C/0 ppt; 25 °C/15 ppt; and 25 °C/32 ppt. The prevalence was 100.0% in fish at 28 °C/15 ppt, 28 °C/32 ppt and 25 °C/15 ppt, which was significantly different (p < 0.05) from those at 25 °C/32 ppt. The red blood cell (RBC) count, hematocrit and total leukocyte (WBC) count were significantly higher in fish at 28 °C/15 ppt and 28 °C/32 ppt. The mean abundance of *R. rhabdosynochus*, hematocrit and RBC showed positive correlations (P < 0.05) with temperature (ρ = 0.3908; ρ = 0.4771 and ρ = 0.2812). Mean abundance showed negative correlations with hemoglobin (ρ = -0.3567) and mean corpuscular hemoglobin concentration (MCHC) (ρ = -0.2684). No correlation between abundance and salinity was detected among the experimental conditions (ρ = -0.0204). The low numbers of monogeneans recorded (min -1 and max -33) explain the few changes to fish health. This suggests that these experimental conditions may be recommended for development of rearing of *C. undecimalis* in Brazil, without any influence or economic losses from *R. rhabdosynochus*.

Keywords: Common snook, parasitology, water quality, hematology, Monogenea, *Rhabdosynochus*.

Resumo

Este estudo avaliou a correlação dos parâmetros hematológicos com a abundância média de helmintos monogenea *Rhabdosynochus rhabdosynochus* em robalo-flecha, *Centropomus undecimalis*, cultivado em diferentes temperaturas e salinidades. As condições experimentais foram: 28 °C/0 ‰; 28 °C/15 ‰; 28 °C/32 ‰; 25 °C/0 ‰; 25 °C/15 ‰; 25 °C/32 ‰. A prevalência (P) foi de 100,0% nos peixes de 28 °C/15 ‰, 28 °C/32 ‰ e 25 °C/15 ‰, que foi significativamente diferente (p < 0.05) dos peixes de 25 °C/32 ‰. O número de eritrócitos, hematocrito e leucócitos totais foram significativamente maiores nos peixes mantidos a 28 °C/15 ‰ e 28 °C/32 ‰. A abundância média de *R. rhabdosynochus*, hematocrito e número de eritrócitos mostraram correlação positiva (p<0,05) com a temperatura (ρ = 0,3908; ρ = 0,4771 e ρ = 0,2812, respectivamente). Houve correlação negativa da abundância média com a hemoglobina (ρ = -0,3567) e a concentração de hemoglobina corpuscular média (CHCM) (ρ = -0,2684). Não houve correlação entre a abundância e a salinidade entre os tratamentos (ρ = -0,0204). O baixo número de Monogenea registrado (mín: 1 e máx: 33) explica as poucas alterações na saúde dos animais avaliados. Isso sugere que essas condições experimentais de cultivo podem ser recomendadas para um futuro desenvolvimento do cultivo de *C. undecimalis* no Brasil, sem que haja influência e perdas econômicas associadas a mortalidade, por parasitos *R. rhabdosynochus*.

Palavras-chave: Robalo, parasitologia, qualidade de água, hematologia, Monogenea, *Rhabdosynochus*.
Introduction

The common snook, *Centropomus undecimalis* (Bloch, 1792), is distributed in tropical and subtropical areas of the west coast of the Atlantic Ocean, from Cape Canaveral, Florida, USA, to southern Brazil (ALVAREZ-LAJONCHERE & TSUZUKI, 2008). It is considered to be a diadromous, euryhaline, stenothermic and estuarine-dependent fish found in rivers, estuaries and coastal lagoons and along rocky shores (McMICHAEL et al., 1989; POPE et al., 2006). The common snook is considered to be the largest and also the fastest growing snook of all the species of the genus *Centropomus* in the Americas, and is a sporting fish of high market value (TUCKER, 2005). The species has received high priority with regard to conservation and aquaculture issues, due to its ecological and economic characteristics (WINNER et al., 2010).

With the intensification of fish farming, sanitary problems have become more frequent, making it necessary to periodically monitor fish health conditions. On this view, hematological investigations contribute to the evaluation of the organism's state of defense for facing the common challenges in aquaculture, in which fish are exposed to high stocking densities, parasites, changes to environmental quality and to the physical-chemical parameters of the water, and unbalanced diet (RANZANI-PAIVA et al., 2013).

Hematological parameters are recognized as secondary indicators of stress when increased red blood cell count, hematocrit, hemoglobin concentration and leukopenia are observed (WEDEMEYER et al., 1990; WOJTASZEK et al, 2002; PIERSON et al., 2004). For *C. undecimalis*, studies relating to hematology under fish-farming conditions are scarce. However, some laboratory studies have been conducted on the congeneric species *C. parallelus* (RANZANI-PAIVA et al., 2008; SANTOS et al., 2009, 2012; MARTINS et al., 2010; BARBOSA et al., 2011; SILVA et al., 2011). Recently, Dotta et al. (2015) evaluated the hematological parameters of the mutton snapper (*Lutjanus analis*) reared in cages, and observed significant increases in the hematocrit and total numbers of leukocytes and monocytes after 30 days of rearing, in comparison with fish in their natural environment.

In South America, knowledge of the parasitic fauna of farmed marine fish can still be considered to be at an initial stage despite the great ichthyological diversity and number of potential host species. Most research has been in relation to taxonomic aspects of helminth species, mainly developed in Brazil, Chile, Peru and Argentina (LUQUE, 2004).

Few studies have described and identified parasites in the common snook. Tavares & Luque (2003) identified a new species of *Acantholochothus* (Copepoda: Bomolochidae) in the coastal zone of the state of Rio de Janeiro, Brazil. The trematode *Acanthocollaritrematidae umbilicatum* Travassos, Freitas & Bührnheim 1965 (Digenea: Acanthocollaritrematidae) was collected in the coastal zone of the state of Rio de Janeiro, Brazil. The trematode species of *Bucephalus* sp. (Digenea), *Cucullanus* sp. (Nematoda) and *Lernanthropidae* (Crustacea). Several studies have shown the influence of salinity on the growth and survival of marine fish (DENDRINOS & THORPE, 1985; MCCORMICK et al., 1989; IMSLAND et al., 2001). Additionally, temperature has been found to have a significant effect on numerous physiological processes in fish (BRETT & GROVES, 1979). It is considered to be one of the most important environmental parameters, given that it directly affects the metabolism, oxygen consumption, growth and survival of marine organisms (JIAN et al., 2003).

In this context, studies relating to sanitary aspects of the common snook are required in order to gain an understanding of the state of health of this fish under farmed conditions and in fluctuating environments. The aim of this study was to evaluate the relationship between hematological parameters and the mean abundance of gill parasites in the common snook reared at different temperatures and salinities in a recirculating aquaculture system.

Materials and Methods

Fish and experimental design

A total of 540 juveniles (2.9 ± 0.5 g) of *C. undecimalis* were obtained from the Marine Aquaculture Laboratory (LAPMAR/UFSC) and were transferred to the Aquaculture Laboratory (LAQ), State University of Santa Catarina (UDESC), located in Laguna, Santa Catarina, Brazil, where the experiment was conducted.

The fish were maintained for 90 days with a combination of different temperatures (25 and 28 °C) and salinities (0, 15, 32 ppt), divided into six treatments with three replicates each: 28 °C/0 ppt; 28 °C/15 ppt; 28 °C/32 ppt; 25 °C/0 ppt; 25 °C/15 ppt; and 25 °C/32 ppt. Each experimental unit was stocked with 30 juveniles (150 fish/m³).

Six recirculation systems (one for each combination of temperature and salinity) were used. Each system had three tanks of 200 L connected to a macroenvironment of 150 L capacity, provided with a biological and mechanical filter, activated carbon, heaters and an ultraviolet filter in the return pipe.

The fish were fed until they reached apparent satiation, twice a day, using a commercial feed for marine fish (61.7% CP – crude protein and 11.6% EE – ethereal extract). The dissolved oxygen concentration, temperature, pH and salinity conditions were monitored daily using a HANNA® multiparameter device (HANNA® Instruments, USA; model HI 9828). N-NH₃, N-NO₂, N-NO₃, and PO₄ were measured every week using a colorimetric method (Alfakit®, Brazil; model AT100P), and alkalinity was measured by means of an acid titration kit.

Samples collection

After experimental period, 12 fish per treatment (four fish per replicate) were collected for hematological and parasitological analyzes, totaling 72 animals. All procedures were performed in accordance with the principles of the Ethics Committee for Animal Experimentation of the University of the State of Santa Catarina - CETEA/ UDESC, protocol number 1.10.13.
Hematological analysis

Blood was withdrawn from the caudal vein with syringes containing EDTA 5% to evaluate the hematocrit percentage by microhematocrit method (RANZANI-PAIVA et al., 2013); hemoglobin concentration by the cianometahemoglobin (RANZANI-PAIVA et al., 2013); total erythrocyte count (RBC) in a Neubauer chamber after dilution 1:200 in saline solution 0.9%, mean corpuscular volume (MCV); and mean corpuscular hemoglobin concentration (MCHC) (RANZANI-PAIVA et al., 2013). The total number of leukocytes (WBC) and thrombocytes were obtained by indirect method from extensions stained with May-Grünwald-Giemsa-Wright (MGGW) (ISHIKAWA et al., 2008).

Parasitological analysis

Immediately after blood collection, fish were sacrificed by cerebral concussion (CONCEA, 2013) and the mucus from the body surface was collected by scraping with blade and observed by light microscopy for detection of parasites. The gills were collected according to Jerônimo et al. (2011), for parasites observation and quantifications. The quantified Monogenea specimens were mounted on blades with Hoyer’s for easy viewing of sclerotized structures, important for identification at species level (bars, anchors and hooks - haptor), vagina and the male copulatory complex (penis and accessory piece). Some individuals were stained with Gomori trichrome to visualize the internal structures. After staining, they were clarified with Faia Cresote and mounted on permanent slides with Canada balsam (EIRAS et al., 2006). Prevalence rate, mean intensity and mean abundance were calculated according to Jerônimo et al. (2011) for parasites observation and quantifications. The quantified Monogenea specimens were mounted on blades with Hoyer’s for easy viewing of sclerotized structures, important for identification at species level (bars, anchors and hooks - haptor), vagina and the male copulatory complex (penis and accessory piece). Some individuals were stained with Gomori trichrome to visualize the internal structures. After staining, they were clarified with Faia Cresote and mounted on permanent slides with Canada balsam (EIRAS et al., 2006). Prevalence rate, mean intensity and mean abundance were calculated according to Jerônimo et al. (2011), for parasites observation and quantifications. The quantified Monogenea specimens were mounted on blades with Hoyer’s for easy viewing of sclerotized structures, important for identification at species level (bars, anchors and hooks - haptor), vagina and the male copulatory complex (penis and accessory piece). Some individuals were stained with Gomori trichrome to visualize the internal structures. After staining, they were clarified with Faia Cresote and mounted on permanent slides with Canada balsam (EIRAS et al., 2006). Prevalence rate, mean intensity and mean abundance were calculated according to Jerônimo et al. (2011), for parasites observation and quantifications.

Statistical analysis

The data were checked for normality of distribution and homoscedasticity by means of the Kolmogorov-Smirnov and Bartlett tests, respectively. The treatments were compared using Tukey’s test (p < 0.05). The means were subjected to factorial analysis of variance (ANOVA) and the means were compared using T ukey’s test (p < 0.05). The data were transformed (Y\text{arcsine}) as needed. The variables also were subjected to Pearson correlation analysis.

Results

The water quality parameters were maintained at acceptable levels for the species. There were no significant differences among the treatments with regard to dissolved oxygen concentration (6.2 ± 1.0 mg.L\(^{-1}\)), pH (7.8 ± 0.2), N-NH\(_4\) (0.1 ± 0.1 mg.L\(^{-1}\)), N-NO\(_3\) (0.1 ± 0.1 mg.L\(^{-1}\)), N-NO\(_2\) (0.6 ± 0.3 mg.L\(^{-1}\)) or PO\(_4\) (1.3 ± 0.5 mg.L\(^{-1}\)). Significant differences were only observed in relation to the parameters that are directly affected by increased salinity, with higher values for higher salinities, such as alkalinity (0 ppt: 47.9 ± 5.3; 15 ppt: 66.0 ± 9.2; and 32 ppt: 76.6 ± 8.3 mg.L\(^{-1}\) CaCO\(_3\)). The parameters obtained during this study were 28.0 ± 0.4 °C and 24.9 ± 0.9 °C for water temperature and 0.3 ± 0.1 ppt, 14.9 ± 1.5 ppt and 32.5 ± 3.5 ppt for salinity.

No parasites were found on the skin and fins but the monogenean Rhabdosynochus rhabdosynochus Mizelle and Blatz, 1941 (Dactylogyridae: Diplectanidae) was observed on the gills. The highest prevalence (100%) was observed in fish at 28 °C/15 ppt, 28 °C/32 ppt and 25 °C/15 ppt, being significantly different (p < 0.05) from the fish at 25 °C/32 ppt (75%). The mean intensity of infection differed significantly among the experimental conditions, such that the lowest values were among the fish at 28 °C/0 ppt (9.2 ± 10.2) and the highest were among those at 28 °C/15 ppt (27.1 ± 6.2). The mean abundance was lower (p < 0.05) among fish at 28 °C/0 ppt and 25 °C/32 ppt (7.7 ± 9.8 and 8.2 ± 7.3, respectively) (Table 1).

The average values for weight and length were greater from the fish maintained at higher temperature and did not differ with regard to salinity (Table 2). Significant difference on weight and length of examined fish could be associated with temperature and salinity conditions once no difference was observed among fish maintained in 28 and 25 °C. The hematological analysis showed that there were higher values for RBC, hemocrit and WBC in fish at 28 °C/15 ppt and 28 °C/32 ppt than among those at 25 °C/15 ppt (Table 2). Hemoglobin and MCHC were higher (p < 0.05) in the fish at 25 °C/0 ppt (11.4 ± 2.0 and 57.8 ± 20.8 respectively) than in the fish maintained at 28 °C/15 ppt and 28 °C/32 ppt. Total thrombocytes were lower in the fish at T6 (2.9 ± 1.8), being significantly different from those at 28 °C/0 ppt and 28 °C/15 ppt (7.1 ± 2.7 and 6.3 ± 3.1). No difference in MCV was found among the experimental conditions.

The mean abundances of R. rhabdosynochus, hemocrit and RBC showed positive correlations (p < 0.05) with temperature (ρ = 0.3908; ρ = 0.4771 and ρ = 0.2812 respectively) (Table 3).

| Temperature/salinity | P (%) | MI | MA | PN |
|----------------------|-------|----|----|----|
| 28 °C/0 ppt          | 83.3\(^a\) | 9.2 ± 10.2\(^a\) | 7.7 ± 9.8\(^b\) | 1-33 |
| 28 °C/15 ppt         | 100\(^b\)   | 27.1 ± 6.2\(^b\) | 27.1 ± 6.2\(^b\) | 11-30 |
| 28 °C/32 ppt         | 100\(^b\)   | 16.8 ± 6.5\(^b\) | 16.8 ± 6.5\(^b\) | 6-29 |
| 25 °C/0 ppt          | 91.7\(^b\)  | 14.5 ± 9.9\(^b\) | 13.3 ± 10.4\(^b\) | 4-35 |
| 25 °C/15 ppt         | 100\(^b\)   | 11.8 ± 5.3\(^b\) | 11.8 ± 5.3\(^b\) | 4-23 |
| 25 °C/32 ppt         | 75\(^b\)    | 10.9 ± 6.2\(^b\) | 8.2 ± 7.3\(^b\) | 2-20 |

Means with the same letters are not significantly different.
Mean abundance showed negative correlations with hemoglobin ($\rho = -0.3567$) and MCHC ($\rho = -0.2684$). There was no correlation between abundance and salinity among the experimental conditions ($\rho = -0.0204$).

**Discussion**

Monogenean parasites are mostly found parasitizing both wild and cultivated fish and are considered to be important pathogenic agents in aquaculture (TURGUT, 2012). They feed on the epithelial cells from the body surface and gills, or blood in some cases (EIRAS, 1994). The genus *Rhadyosynochus* was originally described by Mizelle & Blatz (1941) in *C. undecimalis* in East Florida, USA. In the present study, we recorded *R. rhadyosynochus* in the gills of common snook, but other species can be found in Brazil, such as *R. bargisi, R. budronii* and *Rhadyosynochus* sp. in common snook from Itamaracá, Pernambuco (KRITSKY et al., 2001). Abdallah et al. (2012) described *R. guaduensis* on the gills of common snook examined in Guandu river, state of Rio de Janeiro. Kritsky et al. (2010) proposed a redescription of the genus based on the fish specimens examined from Centropomidae fish in Florida, USA, and Mendoza-Franco et al. (2008) described *R. alterinstitus, R. lituparum, R. velucris* and *R. siliqua* parasitizing the gills of *Centropomus nigricans* and *Centropomus robalito*, collected on the coast of Mexico.

Several factors influence monogenean egg ecosion and depend on the parasite species. Turbulence, photoperiod, temperature and mucus released by the host can stimulate ecosion and determine the success of parasitism (EIRAS, 1994).

Temperature is considered to be one of the most important factors in determining parasite abundance (OZTURK &
ALTUNEL, 2006). The fish examined in this study showed a positive correlation between monogenean abundance and temperature. Fish maintained at 28 °C were more parasitized than those kept at 25 °C, independently of the salinity.

In fact, several studies have focused on the correlation between temperature and parasite abundance and/or egg eclosion (GANNICOTT & TINSLEY, 1998). These authors observed greater viability of eggs of Discocotyle sagittata in rainbow trout (Oncorhynchus mykiss) kept at 13-18 °C. Similarly, Özer & Erdem (1999) reported higher prevalence of Dactylogyrus anchoratus in Cyprinus carpio in summer (25.5–26.2 °C) than in winter (13.6–12.7 °C). Our results are also supported by the findings of Barker & Cone (2000), who reported that prevalence and abundance had a positive correlation with water temperature (10–24 °C).

In this study, salinity did not influence parasite abundance, and this may have been related to the fact that the common snook is considered to be a euryhaline and diadromous species (McMICHAEL et al., 1989; POPE et al., 2006). These fish can be found inhabiting environments of variable salinities (McMICHAEL et al., 1989) and consequently the parasite species may have developed the same resistance over the years due to coevolution.

In the study by Violante-González et al. (2010), parasite richness in C. nigrescens was evaluated at five different sites in a coastal lagoon located in Guerrero, Mexico. At sites where salinity was 15 ppt, the authors recorded greater diversity of the monogeneans R. alterinstitus and Cornutobapta nigrescensi than at sites with salinity of 6 ppt.

Few studies on fish hematology in relation to sanitary issues have been conducted in Brazil, especially in relation to C. undecimalis. Fujimoto et al. (2009) reported that this metazoan parasite was found in common snook from Bragança, Pará, in northeastern Brazil. Nevertheless, other studies have focused on C. parallelus (RANZANI-PAIVA et al., 2008; SANTOS et al., 2009, 2012; MARTINS et al., 2010; BARBOSA et al., 2011; SILVA et al., 2011). The variations in the hematological parameters in freshwater and marine fishes may be related to length, gender or maturation (RANZANI-PAIVA & SOUZA, 2004).

In analyzing the hematological parameters, the fish kept at a lower temperature (25 °C) showed decreased RBC counts and hematocrit levels without positive correlation with the abundance of R. rhudosynochus. In contrast, Fujimoto et al. (2009) observed decreased values for RBC and hematocrit in parasitized common snook.

The relationships between hematocrit and RBC, and between hemoglobin and hematocrit, are known as hematometric indices. These are used to classify anemia, which is an unpaired condition of the blood relating to transporting oxygen to tissues (RANZANI-PAIVA et al., 2013). In this study, these parameters did not show any correlation with parasite abundance, except for hemoglobin, which that showed a negative correlation.

Hematological alterations can be related to the eutrophication level of the environment, as observed by Seriani et al. (2013) in common snook examined in Cananéia and São Vicente, state of São Paulo. Fish from São Vicente, which was the most eutrophicated region, showed the highest values for hematocrit, mean corpuscular volume, WBC and RBC.

In the present study, fish were maintained under good conditions in the recirculation system under controlled water quality. The low numbers of parasites observed (1 to 33) may have been strongly related to the experimental cultivation conditions. It can be inferred that a recirculation system is recommended for commercial maintenance of common snook, avoiding economic losses caused by monogeneans, since the water quality is monitored.

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