

INFLUENCE OF ECTOPARASITES ON HEMATOLOGICAL PARAMETERS OF WILD AND CULTURED DUSKY GROUPER FROM SOUTHEASTERN BRAZIL

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ABSTRACT

This study evaluated the occurrence of ectoparasites and its influence on the blood parameters of wild and cultured dusky grouper *Epinephelus marginatus* from Southeastern Brazil. Hematological and parasitological analyses were made on 147 groupers, obtained from wild (n = 73) and aquaculture origins (n = 74), during cold (n = 70) and hot (n = 77) seasons between July 2009 and June 2010. Two species of ectoparasites were identified: *Pseudorhabdosynochus beverleyburtonae* (Monogenea: Diplectanidae) on the gills and *Neobenedenia melleni* (Monogenea: Capsalidae) on the body surface of the fish. Positive and negative correlations between mean abundances of *N. melleni* and *P. beverleyburtonae*, respectively, with red blood cells were observed in fish from both origins during hot season. Positive correlations between mean abundances of *P. beverleyburtonae* and thrombocytes in fish from both origins and negative correlations between mean abundance of *N. melleni* in cultured fish and thrombocytes were observed during hot season. Also during hot season, positive correlations between total count of white blood cells and mean abundance of *N. melleni* in cultured fish was found. This study demonstrates that parasitism by *N. melleni* and *P. beverleyburtonae* had influence on hematological parameters of wild and cultured dusky grouper *E. marginatus* in Southeastern Brazil.

Keywords: *Epinephelus marginatus*; blood; parasites; *Pseudorhabdosynochus*; *Neobenedenia*

INFLUÊNCIA DE ECTOPARASITOS SOBRE OS PARÂMETROS HEMATOLÓGICOS DE GAROUPA VERDADEIRA SELVAGEM E CULTIVADA NO SUDESTE DO BRASIL

RESUMO

Este estudo avaliou a ocorrência de ectoparasitos e seus efeitos sobre os parâmetros hematológicos da garoupa verdadeira *Epinephelus marginatus* selvagem e de aquicultura no Sudeste do Brasil. Análises hematológicas e parasitológicas foram realizadas em 147 garoupas, coletadas no ambiente natural (n = 73) e em cativeiro (n = 74), durante as estações fria (n = 70) e quente (n = 77) entre julho de 2009 e junho de 2010. Duas espécies de ectoparasitos foram identificadas: *Pseudorhabdosynochus beverleyburtonae* (Monogenea: Diplectanidae) nas brânquias e *Neobenedenia melleni* (Monogenea: Capsalidae) na superfície corporal dos peixes. Correlações positiva e negativa entre abundâncias médias de *N. melleni* e *P. beverleyburtonae*, respectivamente, com eritrócitos, foram observadas em peixes de ambas origens durante a estação quente. Correlação positiva entre abundâncias médias de *P. beverleyburtonae* e trombócitos em peixes de ambas as origens e correlação negativa entre abundância média de *N. melleni* em peixes cultivados e trombócitos foram observadas durante a estação quente. Ainda durante a estação quente, correlação positiva entre a contagem total de leucócitos e abundância de *N. melleni* em peixes cultivados foi encontrada. Este estudo demonstra que o parasitismo por *N. melleni* e *P. beverleyburtonae* influenciou nos parâmetros hematológicos da garoupa verdadeira *E. marginatus* selvagem e cultivada no Sudeste do Brasil.

Palavras chave: *Epinephelus marginatus*; sangue; parasitos; *Pseudorhabdosynochus*; *Neobenedenia*

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INTRODUCTION

Hematology is commonly used to evaluate the physiological status of fish (TAVARES-DIAS and MORAES, 2004), assisting on determining pathological conditions that may affect the homeostasis collaborating with diagnosis of adverse conditions (TAVARES-DIAS *et al.*, 1999). Blood parameters are closely related to animal's response to the environment, thus, alterations on these parameters may occur in fish exposed to culture conditions when compared to wild animals.

Whereas hematological parameters are susceptible to changes in the aquatic environment, they can help to understand the process of animals' adaptation to their environment (RANZANI-PAIVA and SILVA-SOUZA, 2004). Under culture conditions, the stress of capture, transport, overcrowding, inadequate food, and bad water quality may decrease the resistance against diseases and alter blood variables values (MORAES and MARTINS, 2004). Changes in blood parameters can also occur due to intense parasitism (TAVARES-DIAS and MORAES, 2004), condition that may be favored in fish farms.

Hematological analysis can provide valuable information for monitoring health conditions in wild and cultured fish (TAVARES-DIAS and MORAES, 2004). Understanding patterns of blood parameters variation in natural environment specimens allows better monitoring on specimens kept in captivity (RANZANI-PAIVA and SILVA-SOUZA, 2004). By using hematology routinely in disease investigations, it may be possible to understand the health status of the fish. Thus, the aim of the present study was to evaluate the influence of parasitism on the hematological parameters in wild and cultured dusky grouper *Epinephelus marginatus* during cold and hot seasons.

MATERIAL AND METHODS

Fish sample

A total of 147 groupers were collected in Ubatuba, coast of São Paulo (23°26'20"S, 45°01'37"W), Southeastern Brazil, between July/2009 and June/2010, during cold (n = 70) and hot (n = 77) seasons (Ethic Committee 23080.029981/2009-76). Cultured fish (n = 74) were

kept at a density of nine fish per tank in 8 m³ cages made of multifilament nylon with 25 mm mesh size, attached to a "long line" system 20 m parallel to the coast and 4.0 ± 0.5 m deep. Cages were cleaned every 30 days to maintain adequate water circulation. Fish were fed with trash fish, that had their head and viscera removed. Wild fish (n = 73) were caught with baited traps and kept in a 3,000 L tank with closed recirculation system and constant aeration for 10 days, where they were fed every 2 days until sampling. For all samples the time between capture and examination was standardized. Fish were weighed and measured to the nearest 0.1 g and 0.1 cm, respectively. The mean water temperature and salinity in the culture area were monitored throughout the studied period.

Hematological analysis

After biometry, the fish were anesthetized in a benzocaine solution (50 mg L⁻¹) and approximately 2.0 mL of blood were collected by caudal venous puncture with syringes containing EDTA 10%. An aliquot of the collected blood was used to determine the hematocrit percentage (GOLDENFARB *et al.*, 1971). Later, blood smears were stained with May-Grunwald/Giemsa by the ROSENFELD (1947) method for the differential count of the white blood cells and total counts of thrombocytes and white blood cells. Total count of red blood cells was performed using a hemocytometer after a 1:200 dilution in sodium chloride solution (0.85%) and total count of white blood cells and thrombocytes were calculated by the indirect method, from blood smears as suggested by ISHIKAWA *et al.* (2008).

Parasitological analysis

After fish euthanasia, mucus from body surface was scrapped and gills were removed and subsequently fixed according to EIRAS *et al.* (2006) and observed under a stereomicroscope to verify the occurrence of parasites. Monogenea were stained with Gomori's trichrome solution, mounted on permanent slides with Canada balsam or mounted directly in Hoyer's medium for identification according to JAHN and KUHN (1932) and YAMAGUTI (1965) and the redescrptions of WHITTINGTON and HORTON (1996) and SANTOS *et al.* (2000). Prevalence, mean

abundance, and mean intensity of infection were calculated according to BUSH *et al.* (1997) and analyzed using the software Quantitative Parasitology 3.0® (REICZIGEL and RÓZSA, 2005).

Statistical analysis

Data were tested for normality and homocedasticity, using Kolmogorov-Smirnov and Bartlett tests, respectively, and upon compliance were submitted to analysis of variance (ANOVA) using a significance level of 5%. Tukey's test was used for mean comparison and data transformations were used according to pertinence. Mean prevalence rates of ectoparasites

were compared by chi-square analysis, at a significance level of 5%. Data descriptive statistic was assessed and correlation between variables was obtained using Spearman's rank correlation coefficient.

RESULTS

Mean water temperature in the studied period was 24.05 ± 1.48 °C, reaching maximum value in February/10 (27.80 °C) and minimum in June/10 (21.80 °C). Salinity remained stable, ranging from 33.26 to 36.30. The biometrical values of wild and cultured grouper in cold and hot seasons are presented in Table 1.

Table 1. Total length and weight \pm standard deviation (minimum and maximum values in parentheses) of wild and cultured dusky grouper *Epinephelus marginatus* from Ubatuba, São Paulo, Brazil during cold and hot seasons. N: Number of analyzed fish.

Season	Origin	N	Total length (cm)	Weight (g)
Cold	Wild	35	31.26 \pm 3.87 (26.5–42.0)	450.78 \pm 190.51 (248.75–1117.81)
	Cultured	35	25.35 \pm 4.75 (13.0–36.5)	258.59 \pm 127.43 (25.49–650.13)
Hot	Wild	38	27.61 \pm 3.51 (20.9–34.8)	292.33 \pm 118.91 (82.50–629.39)
	Cultured	39	31.93 \pm 5.11 (21.0–46.0)	486.67 \pm 240.29 (127.02–1392.89)

Two species of ectoparasites were identified: *Pseudorhabdosynochus beverleyburtonae* Oliver, 1984 Kritsky and Beverley-Burton, 1986 (Monogenea: Diplectanidae) on the gills and *Neobenedenia melleni* (MacCallum, 1927) Yamaguti, 1963 (Monogenea: Capsalidae) on the body surface.

P. beverleyburtonae showed 100% prevalence in both wild and cultured fish during cold and hot

seasons, with the exception of cultured fish during hot season (97.4%), with no significant differences in mean abundances and mean intensity of infection (Table 2) between seasons. The ectoparasite *N. melleni* had higher prevalence in fish from both origins and mean intensity of infection in cultured fish during hot season, reaching the highest intensity value (Table 2).

Table 2. Parasitological indices of wild and cultured dusky grouper *Epinephelus marginatus* from Ubatuba, São Paulo, Brazil, during cold and hot seasons. IF: infected fish; EF: examined fish; P: prevalence (%), MA: mean abundance, MI: mean intensity of infection.

Season	Origin	Monogenea	IF/EF	P	MA	MI
Cold	Wild	<i>P. beverleyburtonae</i>	35/35	100 ^a	1031.43 \pm 1615.68 ^a	1031.43 \pm 1615.68 ^a
		<i>N. melleni</i>	15/35	42.9 ^a	0.63 \pm 0.88 ^a	1.47 \pm 0.74 ^a
	Cultured	<i>P. beverleyburtonae</i>	35/35	100 ^a	1200.40 \pm 1679.02 ^a	1200.40 \pm 1679.02 ^a
		<i>N. melleni</i>	20/35	57.1 ^a	2.74 \pm 5.45 ^a	4.8 \pm 6.54 ^a
Hot	Wild	<i>P. beverleyburtonae</i>	39/39	100 ^a	710.77 \pm 745.12 ^a	710.77 \pm 745.12 ^a
		<i>N. melleni</i>	34/39	87.2 ^b	6.03 \pm 8.65 ^a	6.91 \pm 8.94 ^a
	Cultured	<i>P. beverleyburtonae</i>	37/38	97.4 ^a	561.42 \pm 1150 ^a	576.59 \pm 1162.94 ^a
		<i>N. melleni</i>	30/38	78.9 ^b	29.82 \pm 39.98 ^b	37.77 \pm 41.58 ^b

Different letters indicate significant difference by chi-square test for prevalence and Tukey's test ($P < 0.05$) for mean abundance and intensity of parasites between cold and hot season.

Higher values of hematocrit were observed in wild grouper ($P<0.05$) when compared to cultured grouper during cold season whereas no significant differences between origins of fish during hot season were observed (Table 3). Red blood cells number was significantly higher ($P<0.05$) in cultured fish during hot season when compared to fish from cold season (Table 3). Wild grouper showed significantly higher thrombocytes number ($P<0.05$) during cold season when compared to fish from hot season (Table 3). White blood cells number was significantly higher ($P<0.05$) in wild fish during hot season when compared to fish from cold season (Table 3).

The most abundant cells in differential count of white blood cells were lymphocytes followed by neutrophils and monocytes, except for cultured grouper during cold season, when it was observed the lowest number of lymphocytes ($P<0.05$) and the highest number of neutrophils ($P<0.05$) (Table 3). A small amount of PAS positive cells and eosinophils were found. There were no recorded basophils. Relative values can be assigned as approximately 50.14% lymphocytes, 29.3% neutrophils, 19.48% monocytes, 1.1% PAS positive cells and 0.97% eosinophils in wild fish and 45.59% lymphocytes, 32.4% neutrophils, 21.12% monocytes, 0.85% eosinophils and 0.04% PAS positive cells in cultured fish.

Table 3. Hematological parameters (mean \pm standard deviation) in wild and cultured *Epinephelus marginatus* from Ubatuba, SP, Brazil, during cold and hot seasons. HTC: hematocrit; RBC: red blood cells; WBC: white blood cells.

Parameters	Cold season		Hot season	
	Origin		Origin	
	Wild	Cultured	Wild	Cultured
HTC (%)	36.2 \pm 6.77 ^a	31.4 \pm 4.13 ^b	35.5 \pm 7.10 ^a	35.2 \pm 6.90 ^{ab}
RBC ($10^6 \mu\text{L}^{-1}$)	1.79 \pm 0.42 ^a	1.75 \pm 0.32 ^a	1.94 \pm 0.81 ^{ab}	2.28 \pm 0.95 ^b
Thrombocytes ($10^3 \mu\text{L}^{-1}$)	18.55 \pm 13.16 ^a	12.83 \pm 9.65 ^{ab}	10.31 \pm 6.97 ^b	11.84 \pm 7.68 ^b
WBC ($10^3 \mu\text{L}^{-1}$)	19.61 \pm 8.93 ^a	22.34 \pm 14.22 ^a	37.96 \pm 28.24 ^b	37.41 \pm 26.45 ^{ab}
Lymphocytes ($10^3 \mu\text{L}^{-1}$)	47.17 \pm 21.77 ^a	29.24 \pm 11.89 ^b	46.58 \pm 24.34 ^a	68.50 \pm 36.83 ^a
Neutrophils ($10^3 \mu\text{L}^{-1}$)	23.39 \pm 13.79 ^a	36.70 \pm 18.06 ^b	31.78 \pm 21.91 ^a	24.62 \pm 14.47 ^a
Monocytes ($10^3 \mu\text{L}^{-1}$)	17.96 \pm 9.97 ^{ab}	20.82 \pm 10.98 ^b	18.23 \pm 7.45 ^a	21.14 \pm 10.65 ^{ab}

Different letters indicate significant difference by Tukey's test ($P<0.05$) between cold and hot season.

Positive correlation between mean abundances of *P. beverleyburtonae* and *N. melleni* was observed in cultured fish during cold season and negative correlation between mean abundances of both parasites was observed in wild fish during hot season (Table 4). In relation to weight, positive correlation with mean abundances of *P. beverleyburtonae* was found in wild fish during hot season (Table 4). Additionally, positive correlations between mean abundances of *N. melleni* and weight and length were also found in cultured fish during hot season (Table 4).

In relation to ectoparasites and hematological parameters, positive correlation between mean abundances of *N. melleni* and red blood cells were observed in fish from both origins during hot season. On the other hand, negative correlations

between mean abundances of *P. beverleyburtonae* and red blood cells were observed in fish from both origins during this season (Table 5). Positive correlations between mean abundances of *P. beverleyburtonae* and thrombocytes in fish from both origins were observed during hot season, and negative correlations between mean abundance of *N. melleni* in cultured fish and thrombocytes was also observed during this season (Table 5). Also during hot season, positive correlations between total count of white blood cells and mean abundance of *N. melleni* in cultured fish was found (Table 5).

In relation to differential count of white blood cells, during cold season, negative correlation between lymphocytes and mean abundance of *P. beverleyburtonae* in wild grouper was found

(Table 5). Negative correlation between neutrophils and mean abundances of *N. melleni* and *P. beverleyburtonae* in cultured fish were observed (Table 5). Also during cold season, positive correlations between monocytes mean abundances

of *N. melleni* in cultured fish was found (Table 5). On the other hand, during hot season, negative correlation between monocytes and mean abundance of *N. melleni* in wild fish was observed (Table 5).

Table 4. Spearman's correlation coefficients between mean abundances of *Pseudorhabdosynochus beverleyburtonae* and *Neobenedenia melleni*, length and weight in wild and cultured *Epinephelus marginatus* from Ubatuba, SP, Brazil, during hot and cold season.

Season	Origin	Monogenea	<i>Pseudorhabdosynochus beverleyburtonae</i>	<i>Neobenedenia melleni</i>	Length	Weight
Cold	Wild	<i>P. beverleyburtonae</i>	1.000	0.067 ^{ns}	0.174 ^{ns}	0.175 ^{ns}
		<i>N. melleni</i>	0.067 ^{ns}	1.000	-0.029 ^{ns}	-0.078 ^{ns}
	Cultured	<i>P. beverleyburtonae</i>	1.000	0.503*	0.165 ^{ns}	0.156 ^{ns}
		<i>N. melleni</i>	0.503*	1.000	0.037 ^{ns}	0.036 ^{ns}
Hot	Wild	<i>P. beverleyburtonae</i>	1.000	-0.372*	0.267 ^{ns}	0.320*
		<i>N. melleni</i>	-0.372*	1.000	-0.204 ^{ns}	-0.203 ^{ns}
	Cultured	<i>P. beverleyburtonae</i>	1.000	-0.136 ^{ns}	0.270 ^{ns}	0.261 ^{ns}
		<i>N. melleni</i>	-0.136 ^{ns}	1.000	0.401*	0.504*

* Significant correlation ($P < 0.05$); ^{ns} Non-significant correlation ($P \geq 0.05$).

Table 5. Spearman's correlation coefficients between hematological parameters and mean abundances of *Pseudorhabdosynochus beverleyburtonae* and *Neobenedenia melleni* in wild and cultured *Epinephelus marginatus* from Ubatuba, SP, Brazil, during hot and cold season. HTC: hematocrit; RBC: red blood cells; WBC: white blood cells.

Season	Origin	Monogenea	HTC (%)	RBC ($10^6 \mu\text{L}^{-1}$)	Thrombocytes ($10^3 \mu\text{L}^{-1}$)	WBC ($10^3 \mu\text{L}^{-1}$)	Lymphocytes ($10^3 \mu\text{L}^{-1}$)	Neutrophils ($10^3 \mu\text{L}^{-1}$)	Monocytes ($10^3 \mu\text{L}^{-1}$)
Cold	Wild	<i>P. beverleyburtonae</i>	-0.014 ^{ns}	0.185 ^{ns}	0.179 ^{ns}	0.155 ^{ns}	-0.346*	0.321 ^{ns}	0.087 ^{ns}
		<i>N. melleni</i>	-0.129 ^{ns}	0.060 ^{ns}	0.218 ^{ns}	-0.122 ^{ns}	-0.161 ^{ns}	0.168 ^{ns}	0.027 ^{ns}
	Cultured	<i>P. beverleyburtonae</i>	0.266 ^{ns}	-0.035 ^{ns}	-0.137 ^{ns}	-0.172 ^{ns}	0.243 ^{ns}	-0.373*	0.272 ^{ns}
		<i>N. melleni</i>	0.105 ^{ns}	-0.076 ^{ns}	-0.034 ^{ns}	-0.164 ^{ns}	0.138 ^{ns}	-0.334*	0.357*
Hot	Wild	<i>P. beverleyburtonae</i>	0.148 ^{ns}	-0.333*	0.650*	-0.205 ^{ns}	-0.230 ^{ns}	0.154 ^{ns}	0.166 ^{ns}
		<i>N. melleni</i>	-0.156 ^{ns}	0.342*	-0.297 ^{ns}	0.118 ^{ns}	-0.067 ^{ns}	0.197 ^{ns}	-0.335*
	Cultured	<i>P. beverleyburtonae</i>	-0.102 ^{ns}	-0.348*	0.518*	-0.161 ^{ns}	-0.206 ^{ns}	0.164 ^{ns}	0.084 ^{ns}
		<i>N. melleni</i>	0.198 ^{ns}	0.593*	-0.328*	0.476*	0.119 ^{ns}	-0.126 ^{ns}	0.073 ^{ns}

* Significant correlation ($P < 0.05$); ^{ns} Non-significant correlation ($P \geq 0.05$).

DISCUSSION

Many parasites can eventually live on the host without causing damage, which is related to the balance between the parasite, host and environment (MORAES and MARTINS, 2004). When this balance is broken, epizootic events can occur. Damage in the host depends on the type and amount of parasites, type of injury and health status of the host (TAVARES-DIAS *et al.*, 1999), as well as physiological disorders. Parasitism is a stressor that impairs the health of fish and may

cause changes to the hematological values (FUJIMOTO *et al.*, 2009).

Neobenedenia melleni is one of the most commonly monogeneans reported for grouper (*Epinephelus* spp.) and is responsible for significant losses in aquaculture. Its pathogenicity is directly linked to its attachment on the host and its hematophagous feeding, which causes severe anemia in infected fish (CARNEVIA, 1993). HIRAZAWA *et al.* (2010) observed an increase in the number of *N. melleni* (= *N. girellae*) as water

temperature increased. Moreover, life cycle is often faster and parasites can cause more severe infections at higher temperatures, corroborating the results found in cultured grouper in the present study.

In situations of high parasitic load, fish become weak and may present anemia, characterized by a reduced hematocrit percentage and red blood cells number (MARTINS *et al.*, 2004). In the present study, hematological changes characterizing anemia were not observed. This fact may be related to an attempt by the fish of keeping homeostasis through increased hematopoiesis. In addition, stress and increased environmental temperature can possibly increase the erythropoietic activity (LECKLIN and NIKINMAA, 1998).

Different species of fish, even if in the same genus, can exhibit variations in hematocrit percentage and red blood cells counts. Intra-specific variations can be attributed to different behavioral characteristics, habitat, diet and climate (TAVARES-DIAS and MORAES, 2004). ZHOU *et al.* (2009) found no significant difference in hematocrit levels of wild and cultured *Misgurnus anguillicaudatus*, but observed higher values of red blood cells in cultured fish. In *Channa argus*, GUL *et al.* (2011) observed higher values of hematocrit percentage and number of red blood cells in cultured fish. In the present study, higher values of hematocrit percentage was observed in wild fish during cold season when compared to cultured fish while no differences in hematocrit percentage between fish origins during hot season were found. Besides, no correlation between ectoparasites and hematocrit percentage was observed.

In *Centropomus undecimalis*, FUJIMOTO *et al.* (2009) found lower values of red blood cells count in infected fish, with no differences in the hematocrit percentage. In the present study, higher number of red blood cells was observed in cultured grouper during hot season accompanied by increased parasitism by *N. melleni*, that can be related to an increased erythropoiesis in these fish. Positive correlation was found between mean abundance of this parasite and red blood cells in fish from both origins during this season. In contrast, negative correlation between mean abundance of *P. beverleyburtonae* and red blood

cells were also observed in wild and cultured grouper during hot season.

Changes in thrombocytes and white blood cells are observed in teleost fishes infected with the most varied pathogens (TAVARES-DIAS and MORAES, 2004). In *Piaractus mesopotamicus* with multiple parasitic infections (TAVARES-DIAS *et al.*, 1999; TAVARES-DIAS *et al.*, 2000) lower number of thrombocyte count was observed. Similar results were observed in *Takifugu rubripes* infected by monogenean *Heterobothrium okamotoi* (GUITANG, 1998). According to KOZINSKA *et al.* (1999), thrombocytes are possibly deployed to contribute with defense mechanisms, since an increased activity of these cells reduces the susceptibility to infections. In the present study, higher number of thrombocytes was found in wild fish during cold season when compared with fish collected during hot season. Positive correlation between mean abundance of *P. beverleyburtonae* and thrombocytes were observed in both wild and cultured fish during hot season. On the other hand, negative correlation between mean abundances of *N. melleni* and thrombocytes was observed in cultured fish during this season for an unclear reason.

Seasonal effects on parasitism may be responsible for changes in white blood cells population, and increased amounts of these cells may indicate a better response of the body's immune system (TAVARES-DIAS and MORAES, 2004). In *Oreochromis aureus*, the number of neutrophils and lymphocytes varies significantly during dry season, which is influenced by the appearance of *Chilodonella hexasticha* (SILVEIRA and FAJER, 1988). These authors observed that during rainy season, the number of monocytes increases as a result of infection by monogenean and ciliate parasites. In the present study, higher number of white blood cells number was observed in wild fish during hot season when compared to fish from cold season.

The white blood cells count can be used to detect many fish diseases, because changes in the distribution frequency of these cells may reveal a pathological process (RANZANI-PAIVA and GODINHO, 1983). Lymphocytes are usually the most common white blood cells in the peripheral blood of several species of marine fish, such as fat snook *Centropomus paralellus* (RANZANI-PAIVA

et al., 2008), common snook *C. undecimalis* (FUJIMOTO *et al.*, 2009), and freshwater fish, such as Nile tilapia *Oreochromis niloticus* (GHIRALDELLI *et al.*, 2006), common carp *Cyprinus carpio* (GHIRALDELLI *et al.*, 2006), and pirarucu *Arapaima gigas* (TAVARES-DIAS *et al.*, 2007). It was also observed in the present study, since the most numerous cells in fish from both origins and seasons, except for cultured grouper during cold season, were the lymphocytes.

Environmental temperature is also a factor of influence on the immune response of fish (BLY and CLEM, 1992). Lymphocytes and neutrophils are white blood cells that respond the most temperature changes (TAVARES-DIAS and MORAES, 2004). At low temperatures, there is a reduction in the number of circulating white blood cells (BLY and CLEM, 1992), which can induce immunosuppression (BLY and CLEM, 1992). Neutrophils appear to be the most resistant white blood cells (BLY and CLEM, 1992), with important migrations activity (PEDDIE *et al.*, 2002).

Many authors have demonstrated higher number of neutrophils accompanied by lower number of lymphocytes in fish subjected to stress (FUJIMOTO *et al.*, 2009), such as parasitism or low water temperature (PICKERING, 1986). In *O. niloticus* heavily infected by *Ichthyophthirius multifiliis* and *Saprolegnia* sp., the percentage of neutrophils and monocytes were significantly higher in the infected group (TAVARES-DIAS and FAUSTINO, 1998). Although negative correlations between neutrophils and mean abundances of *N. melleni* and *P. beverleyburtonae* in cultured fish were observed in the present study, the lower number of lymphocytes accompanied of higher number of neutrophils in cultured fish during cold season can be related to the stress conditions caused by parasitism and lower water temperatures that occurred in this season.

In situations of infection or stressful stimuli, monocytes migrate from blood vessels to tissues, turning into macrophages. These will then present antigens to lymphocytes, thereby increasing its concentration (IWAMA and NAKANISHI, 1996). In *O. aureus* the number of monocytes increased with higher intensities of infection by *Cichlidogyrus* sp. (SILVEIRA and FAJER, 1988).

This increase may be due to the increased activity of cellular defense mechanisms, as suggested by SOPINSKA (1984) in infected *C. carpio*. In the present study, during cold season, positive correlation between monocyte and mean abundance of *N. melleni* in cultured fish was found corroborating the results of SOPINSKA (1984).

Seasonality may affect the population of several species of parasites (JERÔNIMO *et al.*, 2011). Different species of monogeneans that infect the same host species may adopt different reproductive strategies (WHITTINGTON and KEARN, 2011). KEARN *et al.* (1992) observed that *Benedenia seriolae* on the body surface and *Heteraxine heterocerca* on the gills of the same host, *Seriola quinqueradiata*, have markedly different reproductive rhythms. The authors attributed this difference to the fact that parasites have different sites of infection. In the present study, no significant differences were found in mean intensities of infection by *P. beverleyburtonae* during hot and cold seasons. Positive and negative correlations between mean abundances of *P. beverleyburtonae* and *N. melleni* were observed in cultured fish during cold season and in wild fish during hot season, respectively. These findings suggest that these parasites can have distinct reproductive rhythms in natural environment.

CONCLUSION

This study demonstrates that parasitism by *N. melleni* and *P. beverleyburtonae* had influence on hematological parameters of wild and cultured dusky grouper *E. marginatus* in Southeastern Brazil.

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