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Original paper

Metazoan endoparasites of *Myloplus nigrolineatus* (Characiformes: Serrasalmidae) from upper Amazon river basin, Brazil

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ABSTRACT. The success of Trematoda and Nematoda infection in fish involves a complexity of variables. The objective of this study was to report the parasitological descriptors (prevalence, mean abundance, and mean intensity) and community status of *Myloplus nigrolineatus* as well as to evaluate the relationship between abundance and richness of endoparasites with biometric parameters, sex, Kn the hosts, percentage cover native vegetation and water temperature. A total of 7,256 endoparasites were found: 861 digeneas and 6,395 specimens of nematodes were collected in the intestine from *Myloplus nigrolineatus*. One species of Digenea and five species of Nematoda were collected. The initial documentation of the prevalence (%) of endohelminth species in *M. nigrolineatus* revealed the following: *Dadaytrema oxicephala* (70%), *Chabaudinema americanum* (52%), *Cucullanus pinnai pinnai* (13%), *Myleusnema bicornis* (65%), *Procamallanus* (*Spirocamallanus*) *inopinatus* (9%) and *Rondonia rondoni* (35%). The mean intensity and mean abundance were 329.82 ± 416 and 315.48 ± 417, respectively. The trematode *Dadaytrema oxycephala* was considered a central and dominant species. The total abundance was explained by the variables total length, relative condition factor (Kn), percentage cover native vegetation and water temperature. Fish relative condition factor (Kn) and sex were not influenced by the parasite infection and did not impair the body condition of the hosts. The GLMM showed there is no relationship between abundance and richness of endoparasites with percentage cover native vegetation, while that every twenty centimeters more in the total length of the hosts, the abundance of endoparasites in the intestine increases, approximately, 2 specimens.

Keywords: upper tocantins basin, lotic environment, ichthyoparasite fauna, Digenea, Nematoda

Introduction

Freshwater fish serve as hosts for one or more parasite species [1]. The ichthyoparasitofauna can vary in composition depending on the position in the food chain and the trophic level of the host [2–4] of the age, length and sex of the fish [5] and the geographic dispersal potential of the host [6]. The size of the host fish has been demonstrated to influence the richness, as well as the parasitological descriptors prevalence, mean intensity, and mean abundance of parasites. Larger and heavier fish have

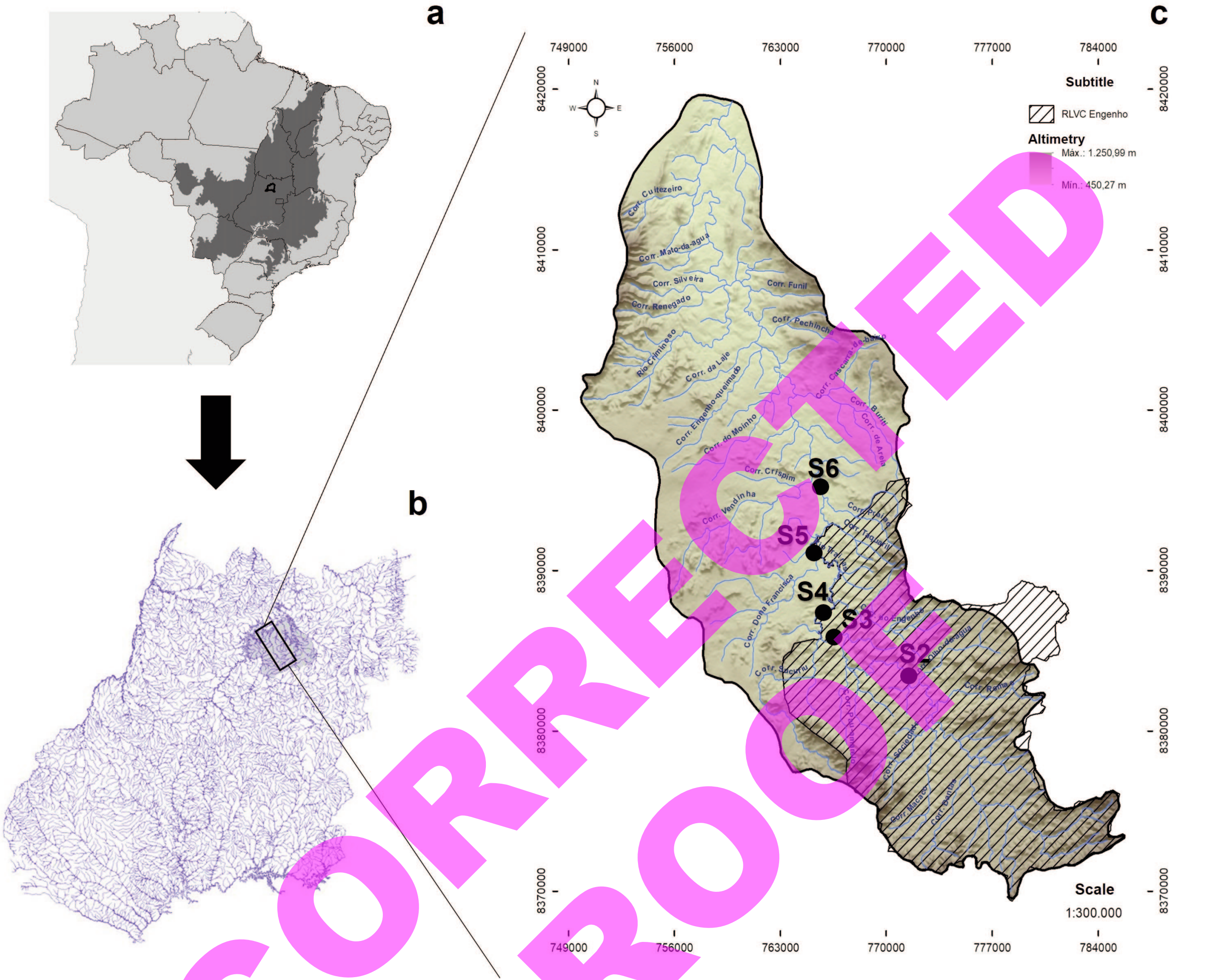

Figure 1. Sampling sites along the Traíras River basin, municipality of Niquelândia, State of Goiás, Brazil. (a) map of Brazil with delimitation of the Cerrado Biome; (b) map of the State of Goiás, with emphasis on the municipality of Niquelândia; (c) map of the Traíras River basin and location of sampling sites inside (S2 to S5) and outside (S6) in the Private Reserve Legado Verdes do Cerrado (dashed area), Niquelândia, Goiás, Brazil

been observed to harbor a greater number of parasites [2,4]. Thus, they are an important component of fish communities of natural environments for the understanding of the functioning of aquatic ecosystems [7].

Physical and chemical properties of water, such as temperature, pH, dissolved oxygen [8], habitat depth, seasons [9–11] and geographic factors [8] are among the abiotic factors that influence parasite communities. In addition, fish parasites from natural environments are indicators of ecosystem health [12,13].

A significant number of these biotic and abiotic factors are directly correlated with the welfare of fish. The length-weight relationship is employed to evaluate the physical condition of fish in their natural environment [14]. In the field of fisheries biology, the ratio between the observed weight and the expected weight is referred to as the relative condition factor (Kn). The standard or control value of Kn is equal to 1 [9,15]. Accordingly, environmental factors, food intake, and even parasitism can impact Kn [16,17].

The protection and conservation of riparian vegetation are of paramount importance for the maintenance of fish biodiversity [18,19]. This is since riparian vegetation provides protection and food resources for fish. Moreover, the accumulation of leaf, branch, and tree trunk remains in these aquatic ecosystems contributes to the fixation of micro and macroscopic organisms that are essential for the maintenance of the aquatic community [20].

This, in turn, is crucial for the maintenance of the life cycle of different groups of parasites [13].

The genus *Myloplus* is among the 16 extant genera of the order Characiformes [21]. The species *Myloplus nigrolineatus* Ota, Machado, Andrade, Collins, Farias & Hrbek 2020 was classified based on molecular criteria and morphophysiological characters [22]. Among the pacus, *M. nigrolineatus* is widely distributed in slow-flowing clear and dark water tributaries, such as backwaters and lakes, of the Amazon basin. Its diet consists primarily of aquatic and terrestrial plants [23]. They are diurnal, migratory, and form schools for external fertilization and spawning in areas of marginal vegetation in rivers [24,25]. Finally, the majority of pacu species are highly sought after in both commercial and subsistence fisheries.

To the best of our knowledge, this constitutes the first record of *M. nigrolineatus* for the upper Tocantins-Araguaia basin, as well as the first record of its associated endoparasites. The objective of this study was to report the parasitological descriptors (prevalence, mean abundance, and mean intensity) of *M. nigrolineatus* and to verify whether there is a relationship between the abundance and richness of endoparasites and the biometric parameters, sex, Kn of the host, as well as water temperature and percentage cover native vegetation. Although data is scarce in the literature on evaluating the effects of multiple possible factors to explain the level of parasitic infection in fish, the results provided here constitute the first step to unravel on the composition of the local parasitic fauna in Neotropical areas.

The hypothesis that the abundance and richness of endohelminths are related to biometric parameters, sex, Kn of the host, water temperature and percentage cover native vegetation was tested.

Materials and Methods

The study was carried out on the Traíras River (Fig. 1a–c), one of the main tributaries of the Maranhão River, both belonging to the Tocantins-Araguaia River Basin. The study area is in the municipality of Niquelândia, within the State of Goiás, Brazil [26]. Of the six sampling sites, five (S2, S3, S4, and S5) are situated within the boundaries of the Legado Verde do Cerrado Private Reserve, while S6 is located outside this reserve (Fig. 1c). This study was approved by the Chico Mendes Institute for Biodiversity Conservation – ICMBio (process No. 02010.000260/01-73) and by the Biodiversity Authorization and Information System – SISBIO (process No. 71279-1) and was developed in accordance with the principles adopted by the National Council for the Control of Animal Experimentation (CONCEA) and with approval from the Ethics Committee in the Use of Animals of State University of Goiás (No. 003 – CEUA/UEG).

A total of 23 specimens of *M. nigrolineatus* were collected during October 2019, January 2020, March 2021, March 2022 and July 2022 (see Table SII in Supplementary Material) by the mesh method (waiting nets) using meshes with openings between 12, 15, 25, 40, 50 and 80 mm between opposite nodes according to Oliveira and Tejerina-Garro [27] with modifications. Fish spcies were identified according to Melo et al. [28]. Afterwards, the fish were anesthetized in clove-oil-derived eugenol (250 mg L^{-1}) in a 13-L box (45.5 × 30 × 10 cm) and euthanized by hypothermia [29]. Next, the biometric parameters of fish, such as total length (TL) (cm), standard length (SL) (cm), total weight (TW) (g), were measured. Fish were stored in a polystyrene box with ice and transported to the laboratory and kept in a freezer (–20 ºC) until further analysis. All the fish were defrosted and necropsied for parasites analysis. The eyes, gills, muscles and visceras were carefully dissected and individualized in a Petri dish with physiological solution (0.8% NaCl) [30] and examined under a stereoscopic microscope (STEMI 508; Zeiss, UK).

Helminths were removed from the intestine using a stereomicroscope (STEMI 508; Zeiss). The study of the morphological and anatomical characteristics of the digeneans and nematodes specimens followed the protocol of Eiras et al. [30]. Identification and characterization of nematodes were based on dichotomous keys [31,32] and of the digeneans was based on previous study [32], updated by articles from new records [9,11,33–35]. Digeneans and nematodes were identified and recorded using a STEMI 508 stereomicroscope (Zeiss) associated with the AxioCam 105 color camera and the ZEN Blue 2.6 software. Life stage, morphological structures, and sex were determined on a light microscope (Olympus, US).

Relative body condition factor was determined by the following equation: Kn = (observed total weight / expected total weight) [16]. Prevalence, mean intensity and mean abundance of parasites were determined according to Bush et al. [36].

To analyze the structure of the *M. nigrolineatus*

Table 1. List of parasite species found in *Myloplus nigrolineatus* from the Traíras river, upper Tocantins river basin, Goiás

Parasite species	FP/FA	P%	MI	MA	SI	CS	BIV	BCV
Trematoda								
Digenea								
Dadaytrema oxycephala	16/23	70	53.81	37.43	I	Central	8.630	Dominant
Nematoda								
Chabaudinema americanum	12/23	52	40.17	20.96	I	Secondary	3.623	Dominant
Cucullanus pinnai pinnai	3/23	13	2	0.26	I	Satellite	0.011	Codominant
Myleusnema bicornis	15/23	65	31.93	20.83	I	Secondary	4.501	Dominant
Procamallanus (*Spirocamallanus*) *inopinatus*	2/23	9	1.5	0.13	I	Satellite	0.004	Subordinate
Rondonia rondoni	8/23	35	678.13	235.87	I	Secondary	27.188	Dominant

Explanations: FP/FA: fish parasitized/fish analyzed, P(%): prevalence of infection, MI: mean intensity, MA: mean abundance, I: intestine, SI: site of infection, CS: community status, BIV: Bush importance value, BCV: Bush classification value

endoparasite community, each parasite species (infrapopulation) was classified according to the hypothesis of Bush and Rolmes [37], which consists of classifying the species as central, secondary and satellites based on the prevalence. The dominance index (D) was calculated to verify the degree of dominance of each component in the parasite infracommunities [38]. Furthermore, the calculation of the Bush importance value (BIV) for each species of parasites found was classified according to Thul et al. [39].

Data related to water temperature (°C), dissolved oxygen – DO (mg L^{-1}), electrical conductivity of water (µs cm^{-1}), hydrogen potential (pH), were obtained with the use of a U-50 Horiba Multiparameter Probe. The values obtained were compared to those determined as maximum permitted values (V.M.P) by the Ministry of Environment of the Resolution of the National Council of the Environment-CONAMA no. 357/2005 (Class 2).

The classification of land use in the Traíras River Basin was performed through the vector map of the boundary of this Basin, made available by the geoprocessing database on the website of the State System of Statistics and Geographic Information of the state of Goiás (SIEG) (www.sieg.go.gov.br). The georeferencing of the image was done in QGIS software 3.16 through the points collected in the field, by a Global Positioning System (GPS) device. The landscape analysis was performed in this same software and used land use maps on the scale of 1:300,000 available. The landscape was classified into three categories: native vegetation (cerrado sensu stricto area and riparian forest area), anthropic area (agriculture and pasture) and water bodies according to Bonnet et al. [40] with modifications. Thus, each category was expressed as a percentage value with a circular buffer of 500 meters radius that was defined around each sampling site and the land use evaluated in this area for each point.

Student two-sample t-test was used to evaluate whether the hosts fish and sex differ in relation to the (Kn) [41] and, whether males and females of these differ in length. Furthermore, this t-test was used to assess whether the host's sex has any effect on the abundance of parasites. The relative condition factor (Kn) was also tested against the standard value Kn=1.00 using a one-sample t-test.: parasite abundance and parasite species richness. Regarding the predictors for both variables were fish length and Kn water temperature and percentage cover native vegetation. A generalized linear mixed model (GLMM) was applied for the analysis of abundance of parasites in *M. nigrolineatus*, to control for differences in sample sites which were taken as random effects and fish length, Kn, water temperature and percentage cover of native vegetation as fixed effects. Nevertheless, due to small sample size it was decided to test the

Table 2. Generalized Linear Mixed Model (GLMM) values showing the influence of total length, relative condition factor (Kn), percentage cover native vegetation and water temperature on endoparasite abundance (Log (x+1)) in *Myloplus nigrolineatus* from the Traíras river, upper Tocantins river basin, Goiás

Variable	R^2	AIC	Estimate	ci lower	ci upper	p-value	power
Total length (cm)	0.307	59.0	0.123	0.056	0.195	0.002*	0.861
Relative condition factor (Kn)	0.016	61.8	0.799	-1.695	3.425	0.543	0.091
Percentage cover native vegetation (%)	0.016	69.9	-0.011	-0.059	0.039	0.698	0.089
Water temperature (ºC)	0.003	67.1	0.026	-0.229	0.246	0.814	0.058

* Statistically significant values (p<0,05) are indicate in boldface type

individual effect of the predictor variables. A similar approach was employed to explain the variation in parasite species richness, but due to a small sample size and collinearity, the models could not be fitted because of a singular covariance-variance matrix. So, the model was simplified by excluding the random factor and using simple linear regression for each analysis. The best predictor was selected based on Akaike's information criteria. Otherwise, the power of each test was estimated to ensure that the conclusions were well supported. GLMM models were fitted using lme4 package, and power test based on pwr package in the R program [44]. The power of each test was presented to provide stronger support and evaluation of the analysis, considering the effect of a small sample size on the probability of rejecting a false null hypothesis [85]. A log transformation of abundance was used to generalized linear mixed model (GLMM). The significance level of $p<0.05$ was assumed for all analyses.

Results

A total of 7,256 parasites were collected in the *M. nigrolineatus* intestinal lumen from S2, S3, S4, S5 and S6 (Fig. 1). The results showed that 100% of male fish were infected with endohelminths (8/8), and 93% of females (14/15). A total of six species of parasites were identified, including *Dadaytrema oxycephala* (Diesing, 1836) Travassos, 1931 (Digenea), *Chabaudinema americanum* Díaz-Ungría, 1968; *Cucullanus pinnai pinnai* Travassos, Artigas & Pereira, 1928; *Myleusnema bicornis* Moravec & Thatcher, 1996; *Procamallanus (Spirocamallanus) inopinatus* Travassos, Artigas & Pereira, 1928 and *Rondonia rondoni* Travassos, 1920 (Nematoda). The overall prevalence of infection in *M. nigrolineatus* was 96% (22 parasitized/23 analyzed). The mean intensity and mean abundance were 329.82 ± 416 (ranging from 0 to 746 parasites per infected host) and 315.48 ± 417 (0 to 732 parasites per analyzed host) in *M. nigrolineatus*, respectively. When analyzed by taxon of endoparasites, the trematode *D. oxycephala* exhibited the highest prevalence (70%) when compared to the nematodes *M. bicornis* (65%), *C. americanum* (52%), *R. rondoni* (35%), *C. pinnai* (13%), and *P.* (*S*) *inopinatus* (9%). The nematode *R. rondoni* exhibited higher values for both mean intensity and mean abundance when compared to the other parasites, with respective values of 678.13 and 235.87. In terms of community status, only the species *D. oxycephala* (Digenea) was identified as central, while the nematodes C. *americanum*, *M. bicornis*, and *R. rondoni* were classified as secondary. The species *P.* (*S.*) *inopinatus* and *C. pinnai* were classified as satellites. Regarding the Bush importance value, four species were identified as dominant, with *C. pinnai* codominant and *P.* (*S.*) *inopinatus* classified as subordinate (Table 1).

The total parasite abundance did not differ significantly between sexes of fish hosts (independent samples t-test; $t = 0.891$; $df = 21$; $p = 0.383$). There was no difference in fish length between males and females (t-test for independent samples; $t = -0.501$; $df = 21$; $p = 0.622$). The relative condition factor (Kn) of *M. nigrolineatus* (1.00 ±

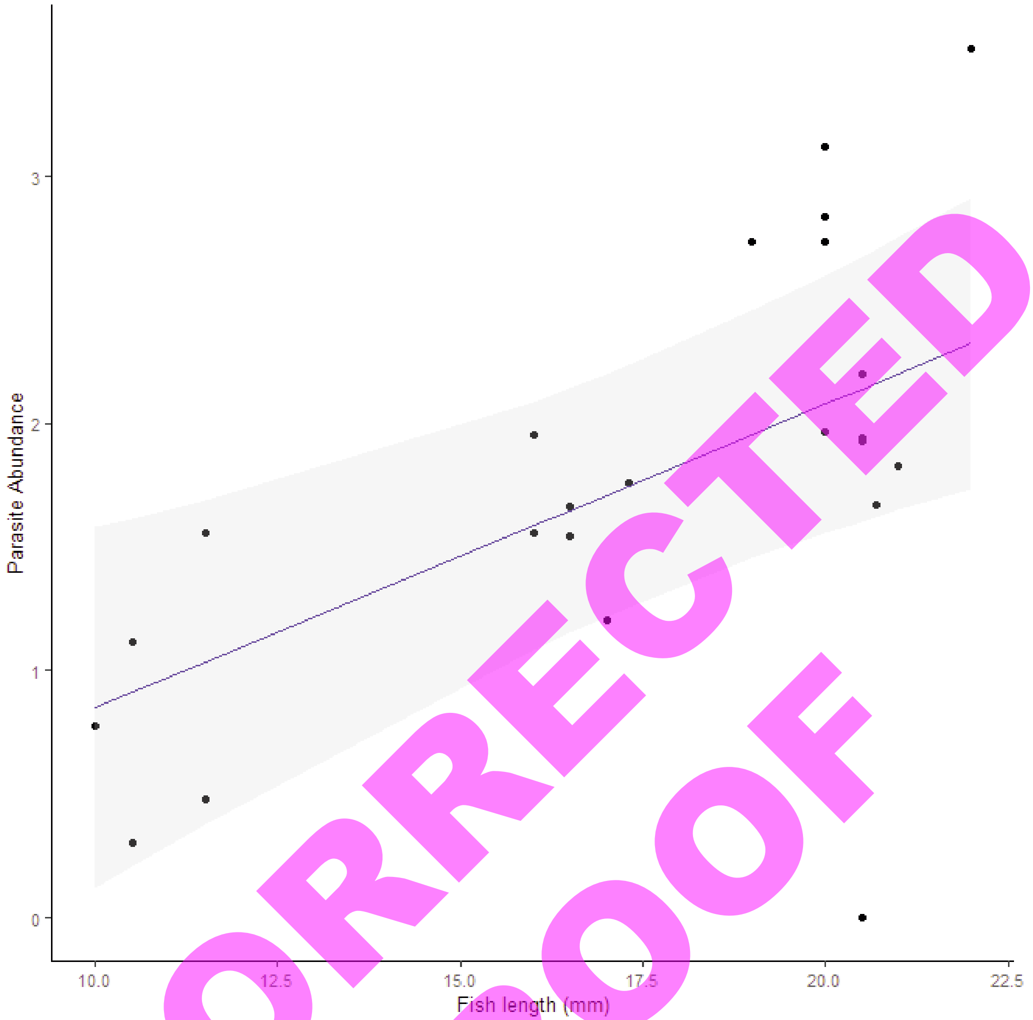

Figure 2. Generalized Linear Mixed Model (GLMM) of the relationship between parasite abundance (Log (x+1)) and length (cm), analyzed independently, of *Myloplus nigrolineatus* from the Traíras river, upper Tocantins river basin, Goiás. The GLMM analysis showed that the greater the length of the hosts, the greater the capacity for accumulating endoparasites in the hosts ($R^2 = 0.307$; $p = 0.002$).

0.15) showed no difference (t-test for one sample; t = 0.321; df = 22; p = 0.751) of the standard value Kn (Kn = 1.0). There was also no relationship between Kn and host sex (independent samples t-test; t = 0.350; df = 21; p = 0.729). Biometric parameters and somatic indexes of host fish *M. nigrolineatus* were recorded (see Table SII in the Supplementary Material).

Considering the isolated effects of total length and relative condition factor (Kn) of the hosts, percentage cover native vegetation and water temperature, the best model (AIC = 59.0) to explain parasitic abundance was based on total length of the hosts fish, controlling for the sampling point dependence (GLMM, $R^2 = 0.307$; $p = 0.002$) (Table 2). It was estimated that for each additional twenty centimeters in the total length of the hosts, there is an approximate two individuals increase more in the number of endoparasites present in the fish intestine (Fig. 2). However, no effect was observed for Kn ($R^2 = 0.016$; $p = 0.543$) (AIC = 61.8), percentage cover native vegetation ($R^2 = 0.016$; $p = 0.698$) (AIC = 69.9), or water temperature ($R^2 = 0.003$; $p = 0.814$) (AIC = 67.1) on the number of parasites (Table 2). Despite the low sample size, the dependency of total fish length is well supported by a higher than 0.8 of power (Table 2).

The best model (AIC = 69.2) to explain the endoparasite richness in *M. nigrolineatus* suggest a negative relation to water temperature (estimated effect = -0.262; $R^2 = 0.239$; $p = 0.018$) (Table 3), so a 10°C increase in water temperature may cause a decrease in nearly three endoparasite species (Fig. 3). Conversely, variables such as total length ($R^2 = 0.097$; $p = 0.147$) (AIC = 73.2), Kn ($R^2 = 0.013$; $p = 0.598$) (AIC = 75.2), and percentage of native

Table 3. Linear Regression Models values showing the influence of total length, relative condition factor (Kn), percentage cover native vegetation and water temperature on endoparasite richness in *Myloplus nigrolineatus* from the Traíras river, upper Tocantins river basin, Goiás

Variable	R^2	AIC	Estimate	ci lower	ci upper	p-value	power
Total length (cm)	0.097	73.2	0.089	-0.034	0.212	0.147	0.324
Relative condition factor (Kn)	0.013	75.2	0.893	-2.577	4.364	0.598	0.083
Percentage cover native vegetation (%)	0.012	75.2	-0.012	-0.058	0.035	0.608	0.082
Water temperature (ºC)	0.239	69.2	-0.262	-0.474	-0.050	0.018*	0.727

* Statistically significant values ($p<0,05$) are indicate in boldface type

vegetation cover ($R^2 = 0.013$; $p = 0.608$) (AIC = 75.2) were not considered ideal and significant for explaining the parasite richness (Table 3). Nevertheless, the relation to water temperature is weakly supported by a power of 0.727. The values for the parameters of temperature, dissolved oxygen, pH and conductivity of the water in the Traíras River at the five sampling sites were below

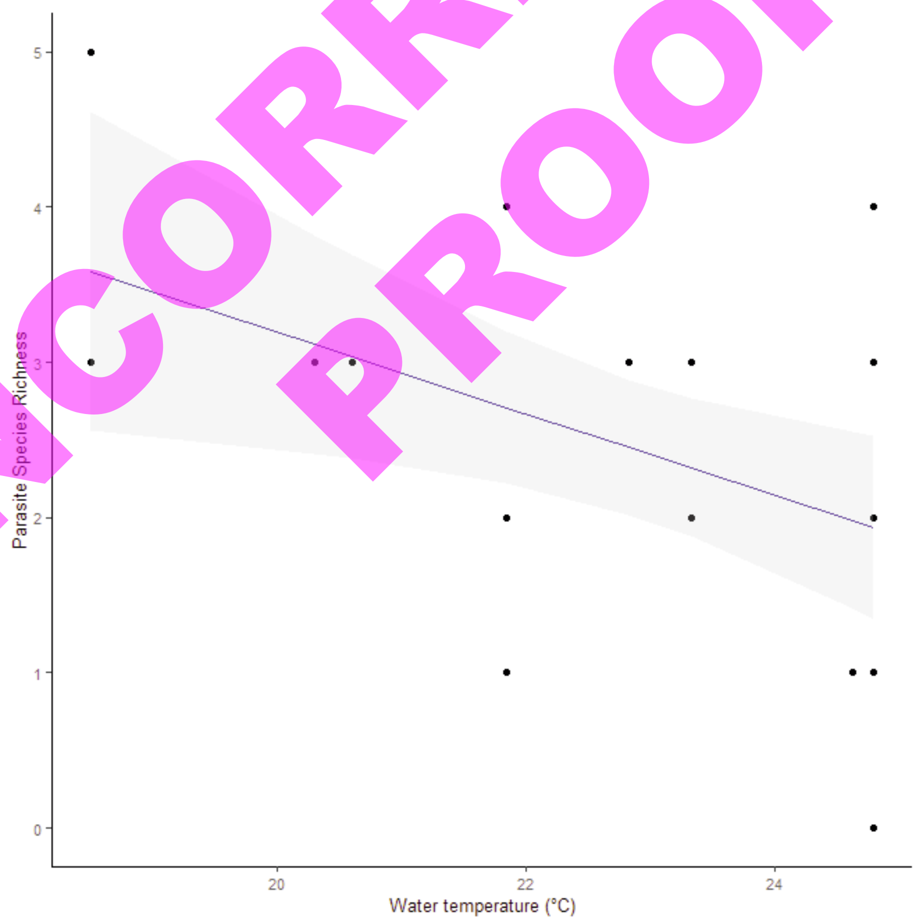

Figure 3. Linear Regression Models of the relationship between parasite richness and water temperature (ºC), analyzed independently, of *Myloplus nigrolineatus* from the Traíras river, upper Tocantins river basin, Goiás. The Linear Regression analysis showed that the higher the water temperature, the lower the endoparasites richness in the hosts ($R^2= 0.239$; $p=0.018$)

the maximum values allowed by Brazilian health standards (CONAMA 357/2005) (see Table SI in the Supplementary Material). The results showed that, by analyzing the multiple regression, the sampling sites showed little variation in relation to the limnological variables, and these had no effect on the abundance of parasites (R^2 = 0.383; p = 0.058).

Discussion

The structure of the parasite community of *M. nigrolineatus* in this study, as determined by the community status and the Bush importance value (I), revealed *D. oxycephala* to be the central species, three Nematoda species to be secondary, and the species *C. pinnai* and *P.* (*S.*) *inopinatus* to be considered satellites. The Bush importance value demonstrated the presence of four dominant species, with *C. pinnai* identified as a co-dominant species and *P.* (*S.*) *inopinatus* classified as subordinate (Table 1).

In the parasite-host relationship, several factors, including sex, physiological conditions, behaviour [45,46], host feeding habits, and population dynamics [46], have been demonstrated to influence parasitological descriptors. However, the present study did not find evidence that host sex influences the total abundance and prevalence of all endoparasites. The female fish of the species *Megaleporinus obtusidens* (Valenciennes, 1837) [47], *Piaractus mesopotamicus* (Holmberg, 1887) [48] and *Leporinus friderici* (Bloch, 1794) [49] exhibited a higher mean abundance parasite, whereas in *Serrasalmus rhombeus* (Linnaeus, 1776), male fish demonstrated a higher mean abundance [50].

In addition to sexual dimorphism, host fish length is related to endoparasite abundance. Our data indicated a higher mean abundance of endohelminths in larger individuals of *M. nigrolineatus*. Larger male fish have been observed to harbor a greater number of parasites due to a greater surface area and area for parasite attachment [2,4,12,50]. In larger individuals of *L. friderici* from the Igarapé Fortaleza, a tributary of the Jari River [33], *P. mesopotamicus* from the Aquidauana and Miranda Rivers in the Pantanal of Mato Grosso do Sul [48], *Trachelyopterus galeatus* Linnaeus, 1766 and *Acestrorhynchus heterolepis* Cope, 1878 in the western Amazon, the mean abundance of endoparasites was higher compared to smaller individuals [6]. Moreover, the internal environment of these fish is regarded as a more stable habitat for parasites, which often have greater longevity compared to those residing in smaller fish [12]. In contrast, the opposite was observed in *Metynnis lippincottianus* (Cope, 1870), which inhabits floodplain lakes in eastern Amazonia. In this case, the abundance of endoparasites was found to be greater in smaller individuals [51]. A similar dataset was presented by Baia et al. [52].

A higher mean abundance of endohelminths may affect fish health [33]. Nevertheless, some host-parasite relationship issues should be considered [34,45,53], as *M. nigrolineatus* in this study was parasitized by helminths from one species of Digenea and five species of Nematoda. Despite the presence of 7,256 specimens of endohelminths in the intestinal lumen, these fish showed no changes in welfare as measured by Kn. In addition to parasitism, other adverse ecophysiological conditions have been shown to cause stress and immunosuppression in fish [54]. In most cases, fish parasites from natural environments do not cause significant damage to their hosts. This may be due to the relatively short life cycle of the parasite, with fish being constantly infected and parasites being eliminated [9, 11]. The anostomid fish *L. friderici* [33], the cichlid *Cichlasoma bimaculatum* (Linnaeus, 1776) [9], and the serrasalmids *Mylossoma duriventre* (Cuvier, 1818) and *M. lippincottianus* from the Amazon River basin did not show impaired body condition due to parasite abundance [51].

Regarding the prevalence data of the endoparasites infracommunity of *M. nigrolineatus*, infection by *D. oxycephala* was observed in 70% of the specimens, followed by *M. bicornis* (65%), *C. americanum* (52%), *R. rondoni* (35%), *C. pinnai pinnai* (13%), and *P.* (*S.*) *inopinatus* (9%). Although this is the first documentation of these parasites in *M. nigrolineatus*, the endoparasite species in question have been previously described in fish from different locations in South America (Table 4). In addition, the genus *Cucullanus* sp. was recorded in the family Characidae *Hemibrycon surinamensis* Géry, 1962 in Igarapé Fortaleza, Amapá State [51], Cichlidae *Cichla piquiti* Kullander & Ferreira, 2006 of the Lageado UHE reservoir, Tocantins State [55] and Anostomidae *Megaleporinus obtusidens* (Valenciennes, 1837) in Lake Guaiba, State of Rio Grande do Sul [47].

The occurrence of *M. bicornis* in fishes of the

Table 4. Prevalence of *Dadaytrema oxycephala*, *Chabaudinema americanum*, *Cucullanus pinnai pinnai*, *Myleusnema bicornis*, *Procamallanus* (*Spirocamallanus*) *inopinatus* and *Rodonia rondoni* in fishes of South America

Group	Parasite species	Host	P%	Location	References
Digenea	*Dadaytrema oxycephala*	*Acestrorhynchus heterolepis*	11.9–28.6	Western Amazon	[6]
		Corydoras multiradiatus	18.7	Lakes of the Amazon River	[70]
		Myleus micans	77.3	Três Marias Dam, São Francisco River	[56]
		Mylossoma duriventre	6.7	Lower Jari River	[57]
		Nemadora humeralis	20–26.7	Western Amazon	[6]
		Ossancora asterophysa	4.4–51.1	Western Amazon	[6]
		Piaractus mesopotamicus	93.7	Miranda River	[48]
			91.78	Miranda and Aquidauna Rivers	[45]
			100	Aquidauna River	[48]
		Piaractus brachypomum	91.78	Lower Amazon River	[53]
			18.7	Lakes of the Amazon River	[70]
		Pimelodus blochii	15	Acre and Iaco Rivers	[71]
			2.5	Acre River	[58]
			1.67	Xapuri River	[58]
		Psectrogaster amazonica	30	Western Amazon	[6]
Nematoda	*Chabaudinema americanum*	*Colossoma macropomum*	< 10	Fish farms in Venezuela	[59]
		Piaractus brachypomum	< 10	Orinoco River Delta, Venezuela	[72]
		Piaractus mesopotamicus	< 10	Miranda and Aquidauna Rivers	[45]
	Cucullanus pinnai pinnai	*Ageneiosus militaris*	–	Paraná River, Brazil	[60]
		Hoplias malabaricus	4.8–9.5	Western Amazon	[6]
		Loricaria sp.	–	–	[61]
		Megalonema platanum	–	Paraná River, Paraguay	[62]
		Nemadoras humeralis	4.4–26.7	Western Amazon	[6]
		Ossancora asterophysa	1.3–15.6	Western Amazon	[6]
		Pimelodella gracilis	–	–	[61]
		Pimelodus ornatus	–	Paraná River, Brazil	[60]

Table 4. Prevalence of *Dadaytrema oxycephala*, *Chabaudinema americanum*, *Cucullanus pinnai pinnai*, *Myleusnema bicornis*, *Procamallanus* (*Spirocamallanus*) *inopinatus* and *Rodonia rondoni* in fishes of South America (cont)

Group	Parasite species	Host	P%	Location	References
Nematoda		*Prochilodus nigricans*	–	Western Amazon	[6]
		Psectrogaster amazonica	5–22.5	Western Amazon	[6]
		Pseudoplatystoma corruscans	–	Paraná River, Paraguay	[62]
		Zungaro zungaro	–	–	[61]
	Myleusnema bicornis	*Myleus ternetzi*	–	Freshwater fish from French Guiana	[63]
	Procamallanus (*Spirocamallanus*) *inopinatus*	*Acestrorhynchus heterolepis*	9.5–28.6	Western Amazon	[6]
		Astyanax altiparanae	73.3	Paraná River Basin	[64]
		Biotodoma cupido	2.5–37.5	Western Amazon	[6]
		Cichlasoma bimaculatum	5.4	Igarapé Fortaleza, Amazon River	[9]
		Colossoma brachypomum	1.7–25	Fish Farms, Amapá	[65]
		Hoplias malabaricus	4.8–28.6	Western Amazon	[6]
		Leporinus friderici	90	Paraná River Basin	[49]
			74	Traíras River, Upper Tocantins River	[66]
		Ossancora asterophysa	2.2–26.7	Western Amazon	[6]
		Prochilodus nigricans	2.5–20	Western Amazon	[6]
		Psectrogaster amazonica	2.5–20	Western Amazon	[6]
		Pygocentrus nattereri	22.37	Negro River	[67]
			100	Delta Lakes in Central Amazon	[11]
		Pygocentrus piraya	83.3	Três Marias Dam, São Francisco River	[68]
		Serrasalmus maculatus	7.1–38.1	Western Amazon	[6]
		Serrasalmus marginatus	5.49	Negro River	[69]
		Serrasalmus rhombeus	53	Traíras River, Upper Tocantins River	[66]
	Rondonia rondoni	*Colossoma macropomum*	71	Orinoco River Delta, Venezuela	[72]
		Piaractus mesopotamicus	73.97	Miranda and Aquidauna Rivers	[45]
			71	Fish Farm Station, Pirassununga	[73]

family Serrasalmidae was described for the host *Myleus ternetzi* (Norman, 1929), freshwater fish from French Guiana [63], while *Myleusnema brasiliense* Moravec & Thatcher, 1999 in *Myleus* sp. from the Serra da Mesa UHE reservoir, Tocantins River, Pará State [74]. However, the reliable location of this artificial reservoir is in the State of Goiás and not in Pará, and the fish order for the genus *Myleus* Müller e Troschel, 1844 is Characiformes [75] and not Cypriniformes, which casts even more doubt on the occurrence of this nematode by the authors. Nevertheless, the prevalence of *M. brasiliense* in *Myleus* sp. was 70%.

This study also assessed the relationship between the abundance and richness of *M. nigrolineatus* endoparasites and two additional environmental factors: the water temperature and percentage cover native vegetation. Water temperature was identified as the primary predictor of endoparasite richness in *M. nigrolineatus*. The northern region of the Goiás State has typical temperatures, with the mean water temperature of the Traíras River being approximately 24°C. The increase in global temperature associated with climate change has facilitated the maintenance of the life cycle of certain trematodes, including *Ithyoclinostomum dimorphum* (Diesing, 1850) Witenberg, 1925 and *Posthodiplostomum* sp. [6], and *D. oxycephala* [35], the latter species being found in *M. nigrolineatus*. On the other hand, a negative correlation between temperature and parasite abundance has been observed in marine [76] and freshwater [77] environments.

The conservation status of riparian vegetation has been shown to influence the abundance and richness of parasites [18,19, 78] however, our results showed that this variable did not influence the abundance and richness of endoparasites at the sampling sites. The highest prevalence of *D. oxycephala* (Paramphistomidae) was observed in site 3, which had 56.30% of native vegetation and 43.11% of anthropized areas, with a notable prevalence of pastures and cattle ranching. Members of the superfamily Paramphistomidae typically complete their life cycle in herbivorous animals, including ruminants, which represents a life strategy for these parasites [79]. Nevertheless, the life cycle of *D. oxycephala* remains poorly understood. What is known is that its larvae, known as metacercariae, encyst in aquatic plants [79]. The remarkable parasitism of *M. nigrolineatus* by this digenean can be attributed to the omnivorous feeding behaviour of the fish when consuming plants and invertebrates, as well as the behaviour of the metacercariae, the infective stage for fish, to encyst in aquatic macrophytes [70,71]. Furthermore, in the life cycle of Digenea, mollusks serve as the first intermediate hosts and play a secondary role as prey for fish [80]. Infection by *D. oxycephala* metacercariae has been documented in omnivorous fish in the Peruvian Amazon [70] and in the Acre and Iaco rivers in the state of Acre, western Brazil [71].

Regarding the abundance of nematodes found in *M. nigrolineatus* in our study, the species *M. bicornis* and *C. americanum* were most abundant in site 2, while *R. rondoni* and *P.* (*S.*) *inopinatus* were most abundant in site 3 and *C. pinnai* in site 4. Site 2 had the largest area of native vegetation cover (76.35%), followed by sites 4 and 5 with a percentage of 63.71%, while site 6 had the lowest percentage with 36.87%. The conservation of riparian vegetation is a critical factor influencing the composition of the macroinvertebrate fauna [18,19]. Insects and crustaceans have been shown to serve as intermediate or transport hosts for some nematodes [81,82] and they also serve as a food source for fish [11,45,72]. The prevalence of endoparasites may be reduced in lotic environments, due to the lack of intermediate hosts compared to lentic environments [83].

The omnivorous fish *M. lippincottianus* has been documented as a regular predator of microcrustaceans and other invertebrates. It also serves as a host for four nematode species in the Igarapé Fortaleza basin [51]. In contrast, anostomid *M. obtusidens* specimens from the Alto Paraná basin [17] were found to harbor seven parasite species, while those from Lago Guaíba, Rio Grande do Sul [47] were infected by only two parasite species. The transmission of parasites through the food chain contributes to the evolutionary success of different endoparasites species in natural fish populations [77,84].

The initial hypothesis was rejected due to the absence of differences between the abundance and richness of endoparasites in the Kn and sex of the fish, as well as in the cover of native vegetation. However, the hypothesis was accepted when it was found that the total length of the fish was an ideal measure to explain the abundance of parasites in *M. nigrolineatus*, and that larger individuals had a greater number of endoparasites. Furthermore, an inverse relationship was observed where an increase in water temperature led to a decrease in endoparasite richness in this host.

In conclusion, this is the first report of the richness and abundance of six endohelminth species in *M. nigrolineatus* in the upper Amazon River basin of Goiás, Brazil. This study contributes to the existing knowledge base on the geographic distribution of this host and represents the first investigation of its endohelminths. Therefore, our study supports the notion that successful infection involves multiple variables in addition to the biological characteristics of parasites. Further research is imperative to better understand the prey-predator-environment relationship in tropical ecosystems, thus highlighting the fundamental role of parasites in the food web.

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SUPPLEMENTARY MATERIAL

Table I. Physical and chemical parameters of the water by sampling sites from the Traíras river, upper Tocantins river basin, Goiás. VMP: maximum permitted values by CONAMA no. 357/2005 (Class 2), Temp: water temperature (ºC), DO: dissolved oxygen (mg L^{-1}), pH: hydrogen potential, Cond: electrical conductivity of water (µS/cm^{-1}). Results are express as mean ± SD

Sites	Parameters			
	Temp	DO	pH	Cond
VMP	–	>5 mg/L	6.0–9.0	–
S2	23.38 ± 2.8	8.17 ± 1.8	7.34 ± 0.8	110.60 ± 45.7
S3	23.01 ± 2.9	8.03 ± 1.6	7.47 ± 0.7	120.40 ± 61.2
S4	23.77 ± 2.8	7.90 ± 1.7	7.54 ± 0.7	141.10 ± 79.7
S5	24.66 ± 3.2	8.09 ± 0.9	7.67 ± 0.8	178.20 ± 99.1
S6	24.01 ± 1.6	8.37 ± 1.7	7.14 ± 0.5	178.20 ± 117.3

Table II. Biometric parameters and somatic indexes of male and female of Myloplus nigrolineatus from the Traíras river, upper Tocantins river basin, Goiás. TL: total length, SL: standard length, OW: observed weight, Kn: relative condition factor, M: male, F: female, no: no observation
*specimens collected at each site. Results are express as mean ± SD

Parameters	Site					
	Sex	S2 (5F, 4M)*	S3 (5F, 1M)*	S4 (4F, 2M)*	S5 (1M)*	S6 (1F)*
TL (cm)	M	17.6 ± 1.7	20 ± 0	16.1 ± 6.5	20.5 ± 0	no
	F	16.4 ± 5.6	18.8 ± 2.7	15.5 ± 5.3	n.o.	17 ± 0
SL (cm)	M	14.9 ± 1.4	16.5 ± 0	14.3 ± 5.5	17.5 ± 0	no
	F	13.3 ± 4.6	16 ± 2.1	13.5 ± 4.1	n.o.	14 ± 0
OW (g)	M	130.5 ± 41.7	196 ± 0	120.5 ± 98.3	228 ± 0	no
	F	128.2 ± 97.6	173.2 ± 65.1	116.5 ± 84.4	n.o.	104 ± 0
Kn	M	1.0 ± 0	1.03 ± 0	1.1 ± 0.3	1.12 ± 0	no
	F	0.9 ± 0.1	1.0 ± 0	1.1 ± 0.2	no	0.9 ± 0

Table III. Raw data of Myloplus nigrolineatus from the Traíras river, upper Tocantins river basin, Goiás. N: number of hosts, S: sampling sites, TL: total length, OW: observed weight, Kn: relative condition factor, Sex: sex of the hosts (M: male, F: female), n: number of parasites collected from each hosts, Log(n+1): log transformation of abundance, R: richness of parasites collected from each hosts, WT: water temperature (ºC), DO: dissolved oxygen (mg L^{-1}), pH: hydrogen potential, Cond: electrical conductivity of water (µS/cm^{-1}), CNV: cover native vegetation (%)

N	S	TL	OW	Kn	Sex	n	Log(n+1)	R	WT	DO	pH	Cond	CNV
1	S2	20.5	208	1.02	F	158	2.201397124	4	24.80	8.17	7.34	110.6	76.35
2	S2	16.5	110	1.00	M	45	1.662757832	2	24.80	8.17	7.34	110.6	76.35
3	S2	20.5	198	0.97	F	84	1.929418926	2	24.80	8.17	7.34	110.6	76.35
4	S2	16.5	101	0.91	M	34	1.544068044	2	24.80	8.17	7.34	110.6	76.35
5	S2	17.3	119	0.94	M	56	1.755874856	2	24.80	8.17	7.34	110.6	76.35
6	S2	20.5	192	0.94	F	0	0	0	24.80	8.17	7.34	110.6	76.35
7	S2	20	192	1.01	M	92	1.968482949	3	24.80	8.17	7.34	110.6	76.35
8	S2	10.5	23	0.75	F	1	0.301029996	1	24.80	8.17	7.34	110.6	76.35
9	S2	10	20	0.74	F	5	0.77815125	3	24.80	8.17	7.34	110.6	76.35
10	S3	20	194	1.02	F	1326	3.122870923	2	23.33	8.03	7.47	120.4	56.30
11	S3	22	259	1.04	F	3300	3.518645524	2	23.33	8.03	7.47	120.4	56.30
12	S3	20	196	1.03	M	686	2.836956737	3	23.33	8.03	7.47	120.4	56.30
13	S3	16	114	1.13	F	89	1.954242509	3	22.83	8.03	7.47	120.4	56.30
14	S3	20	197	1.04	F	541	2.733999287	5	18.50	8.03	7.47	120.4	56.30
15	S3	16	102	1.01	F	35	1.556302501	3	18.50	8.03	7.47	120.4	56.30
16	S4	10.5	41	1.33	F	12	1.113943352	2	21.85	7.90	7.54	141.1	63.71
17	S4	11.5	46	1.15	F	35	1.556302501	2	21.85	7.90	7.54	141.1	63.71
18	S4	11.5	51	1.28	M	2	0.477121255	1	21.85	7.90	7.54	141.1	63.71
19	S4	19	193	1.17	F	542	2.73479983	4	21.85	7.90	7.54	141.1	63.71
20	S4	21	186	0.85	F	66	1.826074803	3	20.60	7.90	7.54	141.1	63.71
21	S4	20.7	190	0.91	M	46	1.672097858	3	20.60	7.90	7.54	141.1	63.71
22	S5	20.5	228	1.12	M	86	1.939519253	3	20.30	8.09	7.67	178.2	51.68
23	S6	17	104	0.87	F	15	1.204119983	1	24.63	8.37	7.14	178.2	36.87

Table IV. Infection intensity of Myloplus nigrolineatus from the Traíras river, upper Tocantins river basin, Goiás. N: Number of hosts

	Digenea	Nematoda				
N	*Dadaytrema oxycephala* (Diesing, 1836)	*Chabaudinema americanum* Díaz-Ungría, 1968	*Cucullanus pinnai pinnai* Travassos, Artigas & Pereira, 1928	*Myleusnema bicornis* Moravec & Thatcher, 1996	*Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928	*Rondonia rondoni* Travassos, 1920
1	7	108	0	34	0	9
2	12	33	0	0	0	0
3	9	75	0	0	0	0
4	9	0	0	25	0	0
5	3	0	0	53	0	0
6	0	0	0	0	0	0
7	37	32	0	23	0	0
8	0	0	0	1	0	0
9	3	1	0	0	1	0
10	0	47	0	0	0	1279
11	0	67	0	0	0	3233
12	300	40	0	0	0	346
13	79	9	0	1	0	0
14	318	3	0	99	2	119
15	2	0	0	32	0	1
16	0	2	0	10	0	0
17	13	0	0	22	0	0
18	2	0	0	0	0	0
19	29	65	0	12	0	436
20	0	0	2	62	0	2
21	37	0	3	6	0	0
22	1	0	1	84	0	0
23	0	0	0	15	0	0