



Effect of burrowing cymothoid parasitism on loricariids

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ABSTRACT

Cymothoids belong to the order Isopoda and are ectoparasites of fishes, and their main parasitism strategies are by penetrating, burrowing, and lodging in the abdominal cavity of the hosts. Due to this complex parasitism strategy, they are considered highly host-specific parasites. We investigated the effects of parasitism of the burrowing cymothoid *Artystone* sp. on the loricariids *Hisonotus chromodontus* and *Curculionichthys luteofrenatus* in the Selma stream, a tributary of the Teles Pires river, Southern Amazon. The hypothesis under study is that parasitism causes negative effects on the feeding, reproduction, and length-weight relationship of the hosts. The presence of alternative hosts was also investigated. The parasitic interaction of *Artystone* sp. with *Curculionichthys luteofrenatus* and *Hisonotus chromodontus* was monitored for one year with standardized monthly collections, and was found to be highly specific when there were no other parasitized fish species. Parasitic castration caused by *Artystone* sp. occurred in *Curculionichthys luteofrenatus* and *Hisonotus chromodontus* hosts, and there was a higher prevalence of infestation in females. The weight-length relationship was lower in parasitized *Hisonotus chromodontus* hosts, indicating a negative effect on somatic increment, although all hosts had fully replete stomachs and gastrointestinal tracts. The greatest standard-length values for both species were observed in the parasitized hosts. The presence of parasitized young specimens with undifferentiated sex and immature males and females suggests that the parasitic interaction in both species starts at a young age.

1. Introduction

Ichthyoparasites of the subphylum Crustacea are generally external parasites (ectoparasite), which is common in representatives of the order Isopoda, subclass Branchiura, and class Copepoda, and found mainly in tropical waters (Rohde, 2005; Ah Yong et al., 2011). Isopods of the family Cymothoidae Leach, 1814 are obligate parasites and are considered the second largest family of ectoparasitic crustaceans of fishes (Bruce and Schotte, 2008). In the marine environment, the literature on the taxonomy and ecology of cymothoids is broad, as is the knowledge on the distribution of cymothoid species and their hosts, as well as the effect of parasite-host interactions on both cymothoids and host fish (Smit et al., 2014; Pawluk et al., 2015; Welicky et al., 2015,

2017, 2018). In freshwater environments, in-depth studies are few, temporally spaced, and mainly focused on records of the occurrence of the parasitism, with little information on the interaction itself, which is slowly advancing (Thatcher, 2006; Luque et al., 2013; Rosa et al., 2020; Virgilio et al., 2020).

Cymothoids are known as generalist parasites (Thatcher, 2006); however, recent studies have shown that their specificity can be influenced by latitude (Smit et al., 2014), and more recently, that burrowing cymothoids have high host specificity (Hata et al., 2017). They have four regions of attachment on hosts: body surface, oral cavity, opercular cavity, and within the abdominal cavity (Smit et al., 2014; Hata et al., 2017). This strategy of cymothoids to excavate the abdominal tissues, penetrate the cavity, and lodge in the abdomen caused the species

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Artystone trysibia Schioedte, 1866 to be considered an endoparasite in early studies of the species (Huizinga, 1972). Among the burrowing cymothoids, the genus *Artystone* comprises three species: *A. trysibia*, *Artystone minima* Thatcher, 2006, and *Artystone bolivianensis* Thatcher, 2006. These species have been recorded as parasitizing fish in Amazonian water bodies, mainly using hosts of the families Characidae and Loricariidae. Studies have revealed that cymothoids have the potential to castrate the host via mechanical processes (feeding on gonadal products) or by affecting gonadal maturation and influencing the growth rate of hosts, with direct negative effects on the somatic increment of parasitized fishes, as verified by analysis of the weight-length relationship (Azevedo et al., 2006; Fogelman and Grutter, 2008; Folgeman et al., 2009; Lafferty and Kuris, 2009).

The fish species *Hisonotus chromodontus* Britski and Garavello (2007) and *Curculionichthys luteofrenatus* (Britski and Garavello 2007) (Loricariidae: Hypoptopomatinae) are widely distributed in the Teles Pires river basin and are abundant in their environment (Ohara et al., 2017), but have only been the subject of taxonomic papers, apart from a study by Costa et al. (2021) that was the first to analyze the forensic interaction of Chironomidae larvae with these sympatric loricariids. In the present study, we recorded the interaction of *Artystone* sp. with *H. chromodontus* and *C. luteofrenatus*, investigated the existence of alternative hosts, and evaluated the effects associated with the interaction of these two fish species. We hypothesized that the parasite has direct negative effects on feeding rate, due to the stress caused by its presence in the abdominal cavity and reduced availability of physical space, and growth and reproduction, related to the energy expenditure caused by the parasite.

2. Material and methods

2.1. Study area, fish, and ectoparasite collection

The study area is a stretch of Selma stream (11°36'03.6" S, 55°25'41.4" W), which is a tributary of Teles Pires river, Tapajos basin (Fig. 1), located at a northern region of the state of Mato Grosso, Brazil. Laboratório de Ictiologia Tropical (LIT) of the Federal University of Mato Grosso, Sinop campus, has monitored this stretch of the Selma stream since 2010 with occasional collections of the ichthyofauna inventory. From May 2016 to February 2019, fish diversity was monitored quarterly with standardized collections using a hand net in active searches. The stretch of the Selma stream was sampled for 30 min by three samplers simultaneously, a methodology adapted from Mendonça et al. (2005). The loricariids *Curculionichthys luteofrenatus* and *Hisonotus chromodontus* sampling occurred monthly from April 2018 to March 2019, totaling one hydrological cycle (rainy-dry season). A minimum of 30 specimens of each host species was established for each collection.

After collection, the fish were placed in plastic bags with water and euthanized with a lethal dose of eugenol anesthetic diluted in 70 % ethanol. They were then fixed in 10 % formalin and after 3 days, transferred and kept in 70 % ethanol. Identification of loricariids (Supplementary Fig. 1) was performed according to Britski and Garavello (2007) and Roxo et al. (2015). Voucher specimens were listed in the Ichthyological collection of the Laboratório de Ictiologia Tropical at the Federal University of Mato Grosso, Sinop campus, under the acronym LIT (*C. luteofrenatus* - LIT1735, LIT1810 and *H. chromodontus* - LIT3143, LIT3144, LIT3145) and in the Coleção Ictiológica do Núcleo de

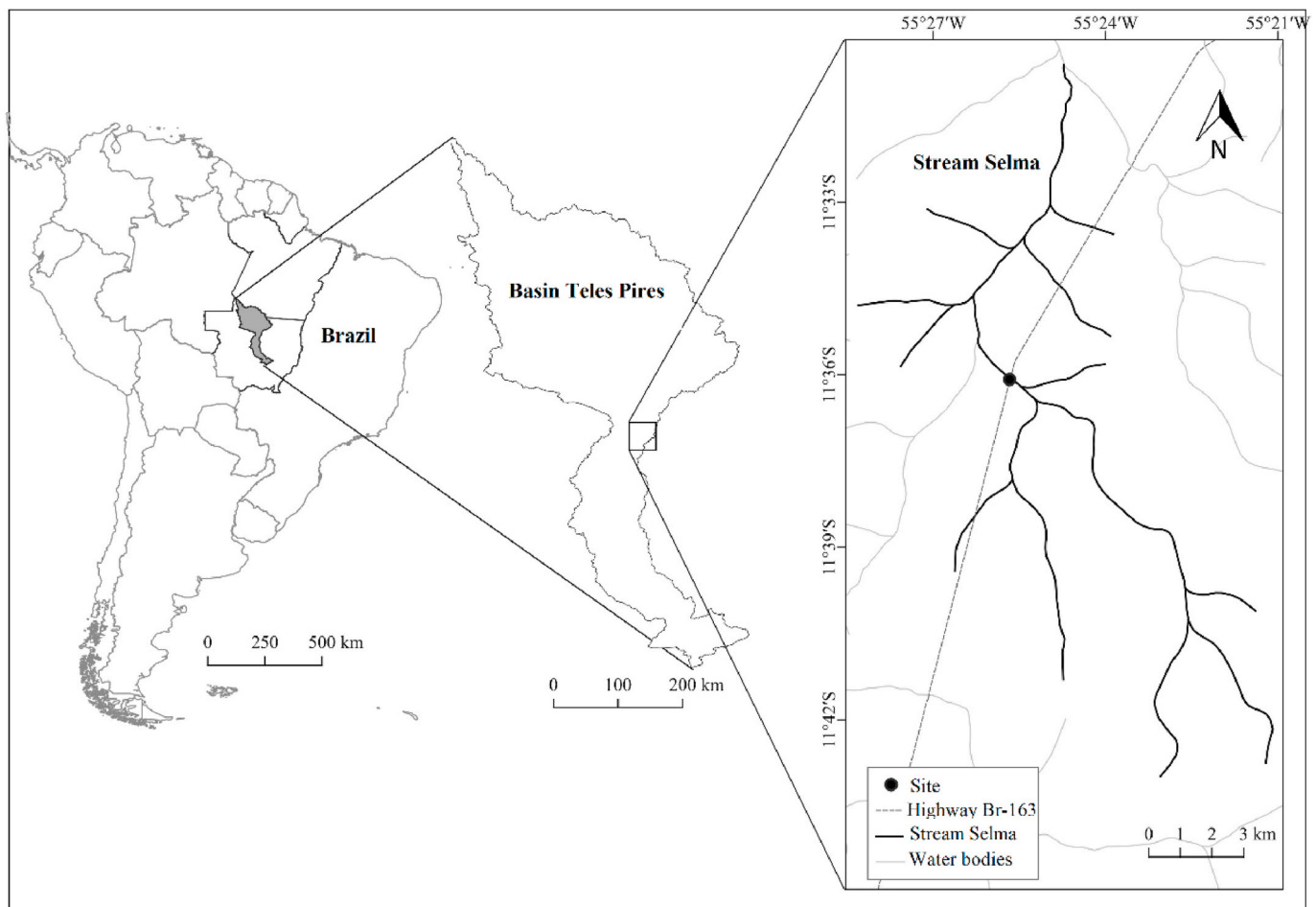


Fig. 1. Location of the sampling point for monitoring the ichthyofauna and studying the parasitic interaction of *Artystone* sp. and loricariids in the Selma stream, which is a tributary of Teles Pires River, Tapajos basin.

Pesquisas em Limnologia, Ictiologia e Aquicultura - Nupélia at the State University of Maringá, under the acronym NUP (*H. chromodontus* - NUP22680 and *C. luteofrenatus* - NUP22683).

Fish were individually numbered in the laboratory and the standard length (mm) and weight (mg) were measured. Prior to weighing, the parasites were removed from their abdominal cavity (Fig. 2). The degree of stomach repletion was estimated using a scale of 0–3, according to the degree of stomach filling (0 = empty stomach; 1 = food-filled stomach filled from 25 % to 50 %; 2 = food-filled stomach filled from 50 % to 75 %; 3 = food-filled stomach filled from 75 % to 100 %), adapted from Soares (1979) and Goulding et al. (1988). Information on sex (undifferentiated, female, and male) and reproductive phase (immature, developing, spawning capable, regressing, and regenerating) of the fish were obtained using a stereomicroscope with a camera for better visualization of the gonads using the scale proposed by Brown-Peterson et al. (2011).

All collections were carried out with authorization from the Secretaria de Estado do Meio Ambiente (SEMA) of the state of Mato Grosso (Authorization No. 855/2018). Animal capture was authorized by permit (Special Fishing License: LEP 352/2016 and LEP 855/2018) and a permit (# 18924-1) from ICMBio- Instituto Chico Mendes de Conservação da Biodiversidade.

2.2. Collection and identification of ectoparasites of the order Isopoda

The ectoparasite *Artystone* sp. was identified using identification keys proposed by Castro and Silva (1985), Salgado-Maldonado et al. (2000), Thatcher (2006), and Luque et al. (2013). The ectoparasites collected were numbered with the same number of the hosts, placed in Eppendorf microtubes, fixed, and preserved in 70 % ethanol according to Eiras et al. (2006).

2.3. Data analysis

Using all samples from the Selma stream (quarterly collections from May 2016 to February 2019), we determined species richness and calculated the percentage of individuals per family of the fish assembly

(Supplementary Table 1). We calculated the parasite prevalence index according to Bush et al. (1997) using the one-year (April 2018 to March 2019) monitoring data with the hosts *C. luteofrenatus* and *H. chromodontus* (Supplementary Table 2).

To test our hypothesis, we used a generalized linear mixed model (GLMM) to evaluate whether there were statistically significant differences in *Artystone* sp. prevalence between host species, sex, and reproductive phase of parasitized and non-parasitized hosts (Supplementary Table 3). Subsequently, we applied a Tukey-type post-hoc test for each of the factors (Supplementary Table 4). For the weight (without the parasite) and standard-length data of the parasitized and non-parasitized hosts, normality and homoscedasticity of the data were assumed based on the n of sampled hosts ($n > 30$) of the two species, and an analysis of covariance (ANCOVA) of the effect of parasitism on the weight-length relationship for each host species was performed.

All statistical procedures were performed in R software version 4.0.2 (R Core Team, 2020) with the packages *vegan* (Oksanen et al., 2019), *ggpubr* (Kassambara, 2020), *car* (Fox and Weisberg, 2019), *ggplot2* (Wickham, 2016), *dplyr* (Wickham et al., 2020), *MASS* (Venables and Ripley, 2002), *multcomp* (Hothorn et al., 2008), *lsmeans* (Lenth, 2016), and *lme4* (Bates et al., 2015).

3. Results

During the 12 months of collection aimed at studying parasite interactions with loriciariids, 695 specimens of *C. luteofrenatus* and 1834 specimens of *H. chromodontus* were collected. The prevalence of *Artystone* sp. was 2.87 % for *C. luteofrenatus* and 2.83 % for *H. chromodontus* (Supplementary Table 2), which was not significantly different between host species (Supplementary Table 4). For *C. luteofrenatus*, 10 % prevalence was observed in May 2018; in seven of the 12 months, the prevalence was 0 %. For *H. chromodontus*, January 2019 had the highest prevalence (22.72 %), and there was no month with 0 % prevalence. The lowest rate was observed in July 2018 (0.84 %). There was also no gradual increase or decrease from one month to the next, or one hydrological period where parasitism was greater relative to the others.

The parasites did not affect the feeding of the hosts once all were

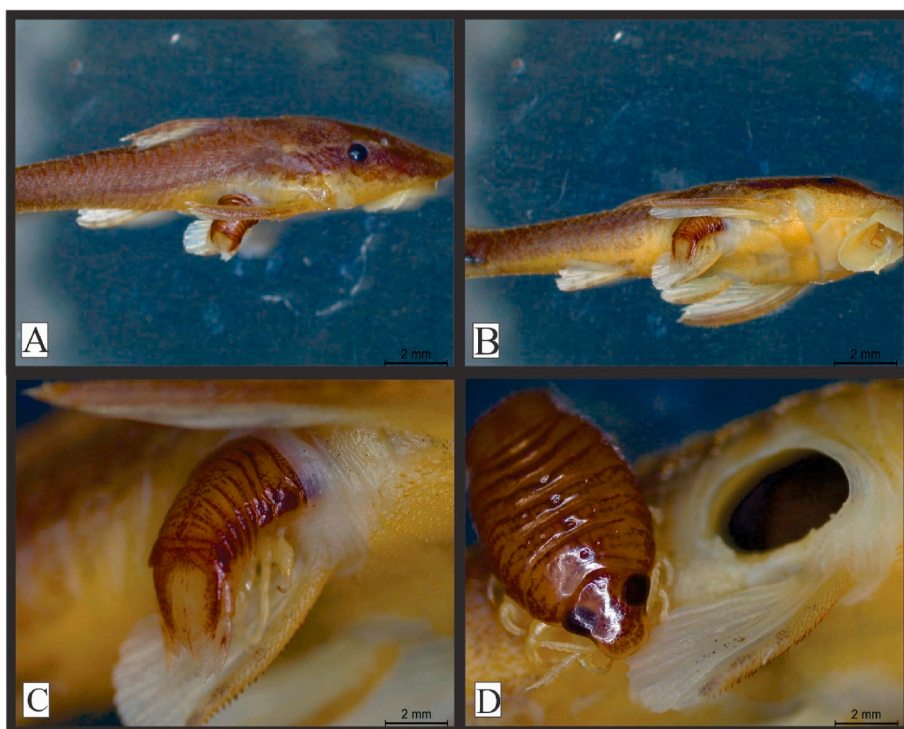


Fig. 2. Parasite-host interaction between *Hisonotus chromodontus* Britski and Garavello (2007) and *Artystone* sp. in the Selma stream, a tributary of Teles Pires river during monitoring of the interaction (2018–2019): (A) side view of a parasitized specimen photographed before biometrics and biology; (B) dorsal view of a parasitized specimen photographed before biometrics and biology; (C) approximate view of the parasite puncture site with the parasite still lodged in the host's abdomen; (D) parasite removed from the host next to the site it was inserted in the host.

satiated (stomach repletion grade equal to 3). The prevalence was significantly higher in females than in males (Supplementary Table 3 and Table 4, P = 0.05). Lesions and/or scarring were not observed in the gonads, regardless of sex. However, there were differences in the prevalence between the reproductive phases of the hosts. Immature, developmental, and regressional were the most parasitized and differed significantly from the others (Supplementary Table 3 and Table 4, P = 0.05).

The parasites negatively affected the length-weight relationship of the host *H. chromodontus* (Table 1, Fig. 3). The analysis showed a greater linear slope of the non-parasitized species for both species. However, only *H. chromodontus* differed significantly. This indicates a lower performance in the growth curve of the parasitized hosts compared to the non-parasitized hosts, that is, a lower somatic increment of the parasitized hosts of both species.

4. Discussion

Burrowing cymothoids exhibit high host specificity (Hata et al., 2017), despite being latitude-dependent, which is directly linked to organism diversity (Smit et al., 2014). For example, in tropical regions, the burrowing cymothoid *Artystone* spp. has been reported to parasitize fish species such as *Moenkhausia collettii* (Steindachner 1882), *Acestro-rhynchus falcatus* (Bloch 1794), *Hyphessobrycon heliacus* Moreira, Landim & Costa 2002, *Hyphessobrycon vilmae* Géry 1966, and *Crenicichla semicincta* Steindachner 1892 (Luque et al., 2013; Junoy, 2016; Oliveira et al., 2019; Rosa et al., 2020). In the present study, we found that *Artystone* sp. parasitizes only the two loricarids *C. luteofrenatus* and *H. chromodontus* in the Selma stream, although it has a richness of 56 species, including other loricarids such as *Curculionichthys* aff. *coxipone* Roxo et al. (2015) and *Parotocinclus dani* Roxo et al., 2015. This shows that specificity occurred in these two species in this locality.

Among the 20 families of fish in the Selma stream, the families Characidae and Loricariidae prevail with the highest percentage of specimens with 41.25 % and 34.01 %, respectively, among the total number of specimens collected during the four years of monitoring the ichthyofauna. Considering the abundance of Loricariidae and Characidae, the other registered hosts (Luque et al., 2013), and the study by Rosa et al. (2020), which identified alternative hosts for *A. minima*, there appears to be a high affinity between *Artystone* sp. and the loricarids *C. luteofrenatus* and *H. chromodontus* for the parasitic interaction observed in the Selma stream.

The preference for *C. luteofrenatus* and *H. chromodontus* could be explained by two main factors: First, the loricarids of the subfamily Hypoptopomatinae are scrapers that are small in size and always stay close to the bottom substrate (Casatti, 2002), a fact that facilitates

Table 1

Summary of the analysis of covariance (ANCOVA) of the presence of the parasite *Artystone* sp. (covariate) on the weight-length relationship in *Hisonotus chromodontus* Britski and Garavello (2007) and *Curculionichthys luteofrenatus* (Britski and Garavello 2007) in the Selma stream, a tributary of the Teles Pires river during the collection period from April 2018 to March 2019.

Source of variation	Degrees of freedom	F value	P value
<i>Curculionichthys luteofrenatus</i>			
Standard length	1	2443.1328	0.00*
Parasitized	1	1.2977	0.25
Standard length x Parasitized	1	1.5111	0.21
Error	508		
<i>Hisonotus chromodontus</i>			
Standard length	1	7118.0858	0.00*
Parasitized	1	3.9250	0.04*
Standard length x Parasitized	1	6.4984	0.01*
Error	985		

Significance was assessed at 5 % probability and significant p-values are highlighted (*) and in bold.

infestation by the mancae (young stage of Isopoda) on the host, as observed by Folgeman and Grutter (2008) in their laboratory study with *Anilocra apogonae* Bruce, 1987, which quickly infested the host *Cheilodipterus quinquelineatus* Cuvier & Valenciennes, 1828 while it was still considered a young individual. Second, in the monitoring collections at Selma stream, the Loricariidae family represented 34 % of the individuals collected, with 451 individuals in total. Of these, *C. luteofrenatus* and *H. chromodontus* represented 36 % and 46 % of the individuals, respectively, summing up to 82 % of the loricarids. As the burrowing cymothoid *Artystone* sp. is an ectoparasite of high specificity, its host must be abundant for it to remain in the environment.

The prevalence of *Artystone* sp. in the hosts *C. luteofrenatus* and *H. chromodontus* did not differ statistically, which is because they are similar species anatomically and morphologically and use the environment and food resources in a similar way (Casatti, 2002). The prevalence values we found are difficult to compare with other studies on burrowing cymothoids (e.g., Rosa et al. (2020) revealed a prevalence of 0.214 % of *A. minima*, and Oliveira et al. (2019) demonstrated a prevalence of 6.94 % of *A. trysibia*), because our result is the product of 12 consecutive months of sampling. When evaluated monthly, the prevalence of *Artystone* sp. ranged from 0 % to 10 % for *C. luteofrenatus* and from 0.84 % to 22.72 % for *H. chromodontus* among the 12 months.

The observed monthly frequency of *Artystone* sp., the parasitism strategy, the genus specificity for the hosts, and the fact that no other fish were found parasitized refutes the suggestion of accidental parasitism. While Rosa et al. (2020) observed a higher prevalence of *A. minima* in *Nannostomus beckfordi* Günther, 1872 in the rainy period, our monthly prevalence data suggest that there is no gradient of increasing or decreasing parasitism and that the dynamics of the interaction appear not to be influenced by climatic events such as precipitation, photoperiod, or air and water temperature. Our study presents a robust standardization of collections and periodicity, showing that ideas of a trend of higher parasitism in some specific hydrological periods should be considered with caution. If our collections were quarterly, as is the normal procedure, we would likely find a hydrological period of higher parasitism, but in monthly collections, this “pattern” is diluted.

In our hypothesis, there would be a negative effect on reproduction when the parasitized hosts *C. luteofrenatus* and *H. chromodontus* were in a mode of parasitic castration (Azevedo et al., 2006; Folgeman et al., 2009; Lafferty and Kuris, 2009). Mechanical/trophic parasitic castration was not observed as all hosts had their gonads whole and intact, and signs of scarring in the gonadal tissue were not found. Thus, we consider the occurrence of chemical castration due to competition for energy resources between the parasite and the host (Bonds, 2006; Lima et al., 2007).

For the vast majority of *C. luteofrenatus* and *H. chromodontus* specimens, it was possible to determine sex and reproductive phase, a result that is especially important as sex data for burrowing cymothoid hosts is scarce (e.g., Oda et al., 2015; Junoy, 2016; Rodríguez-Haro et al., 2017; Oliveira et al., 2019). As in the study by Rosa et al. (2020), which found a higher prevalence of infestation in females of *N. beckfordi* parasitized by *A. minima*, we found a higher and significant infestation of *Artystone* sp. in females of *C. luteofrenatus* and *H. chromodontus*. The higher infestation in female hosts is not a pattern for cymothoids. In a study by Azevedo et al. (2006) with *Cyphocharax gilbert* (Quoy and Gaimard, 1824) infested by *Riggia paranensis* Szidat, 1948 (by burrowing) and Folgeman et al. (2009) with *C. quinquelineatus* infested by *A. apogonae* (by external attachment), they found no difference in infestation between males and females. This may be a bottleneck for studies of cymothoids, with approaches involving the presence or absence of secondary sexual characteristics and the sex preference of the parasite.

Among the evaluated hosts of both species, there were parasitized young specimens that were sexually undifferentiated, or males and females in the immature reproductive stage, indicating that the interaction of *Artystone* sp. and its hosts *C. luteofrenatus* and *H. chromodontus* starts early in their life histories. This corroborates the findings of

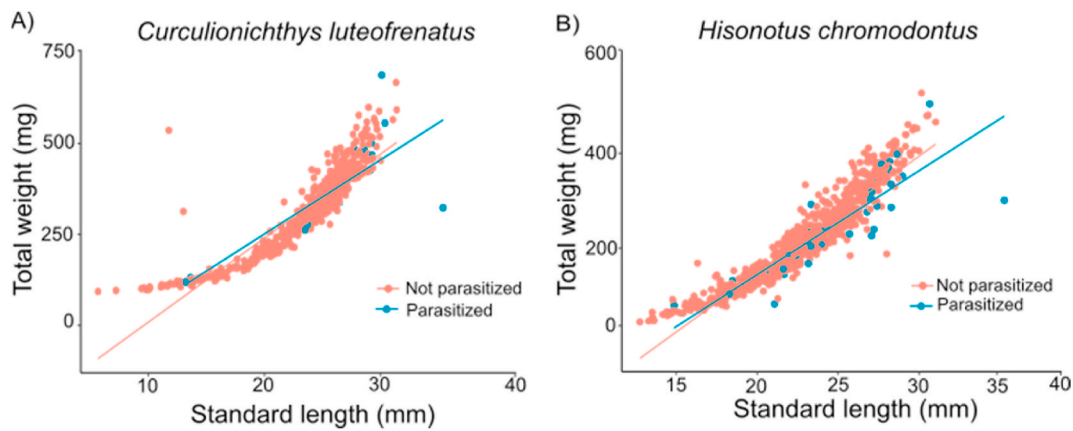


Fig. 3. ANCOVA regression of the length-weight relationship of the hosts *Hisonotus chromodontus* Britski and Garavello (2007) and *Curculionichthys luteofrenatus* (Britski and Garavello 2007) with the covariate parasitized (blue points) and not parasitized (red points) by the burrowing cymothoid *Artystone* sp. in the Selma stream, a tributary of the Teles Pires River, during one year of parasite interaction monitoring. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Folgeman and Grutter (2008), who observed a rapid infestation of *A. apogonae* (mancae of Isopoda) on the juvenile host *C. quinque-lineatus*.

In the hosts *C. luteofrenatus* and *H. chromodontus*, there was a significant prevalence of *Artystone* sp. in the immature, developmental, and regressional reproductive stages, enhancing the viability of the hypothesis of parasitic castration by energy tradeoff, since, if castrated, the hosts would be in a state of “dormancy” similar to the reproductive stages of regression and regeneration. There are streams in the literature that discuss damage to host fecundity as parasite castration (Hurd et al., 2001; Lima et al., 2007; Folgeman et al., 2009; Lafferty and Kuris, 2009), but the fact that the reproductive phases that differed were the immature, developmental, and regressional phases shows that once parasitized the host has an increase in its maintenance energy demand and it is unlikely to obtain sufficient energy resources for the fit-for-reproduction phase, especially females, putting the host in a loop between the developmental and regressional reproductive phases.

Therefore, our results support Rosa et al. (2020), in that females of *N. beckfordi* not reaching the reproductive stage capable of spawning is an indication of parasitic castration resulting from the interaction with *A. minima*. Thus, we found a parasitic castration effect of the burrowing cymothoid *Artystone* sp. on the hosts *C. luteofrenatus* and *H. chromodontus*. Despite the low prevalence of infestation, a castration effect on parasitized hosts over the years may compromise the host population.

We expected to find a “voluntary” feeding restriction due to a stressor, which in this study was the physical space occupied by the parasite inside the abdominal cavity. However, all hosts had fully replete stomachs and gastrointestinal tracts, so our hypothesis of a negative effect on feeding rate was refuted. Although the hosts were feeding, we found a negative effect on the length-weight relationship of the parasitized *H. chromodontus* hosts when compared to the non-parasitized hosts, which has been shown in other studies with cymothoids (Folgeman and Grutter, 2008; Folgeman et al., 2009; Rosa et al., 2020). However, this negative effect is not standard and needs to be further investigated, as stated by Azevedo et al. (2002) and (2006), and Lima et al. (2007), who with several years of collection of the host *C. gilbert* parasitized by *R. paranensis*, observed no differences between the growth of parasitized and non-parasitized hosts.

The raw data revealed that for both host species there was a minimum standard length (13 mm) for the parasites that can be explained by the fact that the genus *Artystone* is characterized by the “burrowing,” “penetrating,” or “piercing” parasitism strategy in the abdomen of the hosts (Smit et al., 2014; Hata et al., 2017), and due to the size of adult mancaes or isopods, the host needs to be sufficiently large for this parasite to lodge in the abdominal cavity. In this relationship, the

highest standard-length values were observed in the parasitized hosts for both species, even though the weight-length relationship itself was lower due to parasitism. This result corroborates other studies showing positive correlations between the growth of cymothoids and their hosts, which were also castrated because once the host invests energy only in growth, more space becomes occupied in the abdomen; thus, the parasite also grows and can reproduce (Adlard and Lester, 1995; Azevedo et al., 2006; Folgeman et al., 2009).

We can state that the parasitism of *Artystone* sp. negatively affects the weight-length relationship in both species of loriciids by increasing the subsistence energy expenditure, affecting the energy storage in the form of fat and muscle which decreases the host weight; there is also an effect on reproduction. It is valid to assume that even for the *C. luteofrenatus* hosts, in which the effect of parasitism was not statistically significant, the results of analyses of the weight-length relationship were similar to *H. chromodontus*, which was significantly affected by parasitism. Determining the effect of cymothoid parasitism on host feeding and growth, even with advanced techniques, is challenging and requires a combined interpretation of several factors (Welicky et al., 2017). Despite the importance of statistical analyses, this biological response occurs as shown in the data and analyses in the present study.

Studies on cymothoids that parasitize the oral cavity of marine fish have already provided robust data on the positive relationship between host size and cymothoid size (Welicky et al., 2019). In the study of Pawluk et al. (2015), who used the marine cymothoid *Ceratothoa italica* Schioedte & Meinert, 1883 parasite of *Lithognathus mormyrus* Linnaeus, 1758 and showed that host size is a good predictor of parasite size, even if the characteristics of males are smaller than those of females. However, with freshwater burrowing cymothoids, further advancement is required.

Studies on burrowing cymothoids can greatly advance our understanding of harmonic and disharmonic ecological relationships as well as question the idea that parasites are essentially opportunistic when extremely complex parasitism strategies exist, such as the one observed in our study. Analyzing the interaction of *Artystone* sp. with *H. chromodontus* and *C. luteofrenatus* in the Selma stream creates a rare opportunity to further the understanding of burrowing cymothoid parasitism, because although the parasite burrows and lodges in the abdomen of the host, a relationship has been established that allows the host to survive, feed, and grow, ensuring the permanence of the parasite and host in the environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2021.07.009>.

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