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Parasites of Nile Tilapia larvae *Oreochromis niloticus* (Pisces: Cichlidae) in concrete ponds in Guanacaste, Northern Costa Rica

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ABSTRACT: Tilapia is the second most important cultured species in the world fish culture but it can be affected by parasites. We conducted a cross-sectional parasitic study in tilapia larvae during sexual reversion for two seasons in Costa Rica. A total of 320 larvae from a concrete pond were necropsied and we found ten parasite species: *Ichtyobodo sp., Apiosoma sp., Chillodonella sp., Heteropollaria sp., Trichodina sp., Dactylogyrus sp., Girodactylus sp., Centrocestus sp.*, lasidies and glochidies (two larval forms). These were classified in five taxonomic groups (two subtypes of protozoa, two metazoan classes and a type of mollusk). Protozoans and monogeneans (except *Trichodina sp.)* had a higher prevalence in the rainy season, when water had more solid waste, while digeneans and molluscs were more prevalent in the dry season, with different infection dynamics over gills, skin, fins and head.

Key words: Prevalence; parasites; Nile tilapia; sex reversal process; Costa Rica.

RESUMEN: Parásitos de larvas de tilapia del Nilo *Oreochromis niloticus* (**Pisces: Cichlidae) en estanques en Guanacaste, Costa Rica.** La tilapia es la segunda especie de cultivo más importante en el mundo de la pesca, pero puede verse afectada por parásitos. Realizamos un estudio transversal de parásitos en larvas de tilapia durante la reversión sexual en dos temporadas en Costa Rica. Un total de 320 larvas de un estanque de concreto fueron necropsiados y encontramos diez especies de parásitos: *Ichtyobodo sp., Apiosoma sp., Chillodonella sp., Heteropollaria sp., Trichodina sp., Dactylogyrus sp., Girodactylus sp., Centrocestus sp.*, lasidios y gloquidios (dos formas larvarias). Estos se clasifican en cinco grupos taxonómicos (dos subtipos de protozoarios, dos clases de metazoarios y un tipo de molusco). Los protozoarios y monogeneos (excepto *Trichodina sp.*) tuvieron una mayor prevalencia en la época lluviosa, cuando el agua tenía más residuos sólidos, mientras que los digeneos y moluscos eran más frecuentes en la estación seca, con diferentes dinámicas de infección sobre las branquias, la piel, las aletas y la cabeza.

Palabras clave: Prevalencia; parásitos; tilapia del Nilo; proceso de reversión sexual; Costa Rica.

Interest on tilapia species cultivation began in Africa and Asia due to the low cost and high nutritional value of these animals, which were beneficial to feed the human population in these developing areas (Food and Agriculture Organization of the United Nations, 2007). Currently, tilapia is the second most important group of fish for the world fish culture. In 2014, it reached a production volume from 200 000 tones contributing China approximately 35% and Taiwan and Indonesia, being the main exporters of whole product and frozen fillet, while the trade in fresh fillets in Latin America has been led by Guatemala, Costa Rica and Honduras (Food and Agriculture Organization of the United Nations, 2015).

Tilapia is considered relatively resistant to diseases (Coward & Bromage, 2000), but it can be affected by different parasites. There are reports about protozoa presence as: *Ichthyophthirius multifiliis, Trichodina, Chilodonella, Ichtyobodo necatrix;* Monogenean: *Cichlidogyrus, Gyrodactylus, Dactylogyrus;* Nematodes*: Contracaecum* sp. and parasitic crustaceans such as*: Argulus, Ergasitus* and *Lernea* (Basson, Van-As & Paperna, 1983; Kazubski, 1986; Okaeme, Obiekezie, Lehman, Antai & Madu, 1988; Paperna 1991; Noga & Flower, 1995; Pariselle & Euzet, 1996; Tavares, Martins & Moraes, 2001; Martins, Marchiori, Nunes, & Rodrigues, 2010; Pariselle, Nyom & Bilong, 2013). Although, the greatest affection is recorded in fingerlings and larvae.

Some epidemiological research of parasites in tilapia have established that infections are increased in intensive culture systems because of high fish density affecting directly the population growth of parasite species (Garcia, Osorio-Saraiba & Constatino, 1993).

In different situations, if the parasites do not reach the level or amount necessary to cause an epizootic, they can weaken the host fish, or be the entry route for other infections. (Tesana, Thabsripair, Suwannatrai, Haruay, Piratae, Khampoosa, & Jones, 2014).

Some tilapia parasites are also important for public health (Sommerville, 1982; Tesana et al., 2014). For example, *Clonorchis sinensis* causes hepatomegalias, cachexia, ascites and cirrhosis. *O. viverrini* is the causative agent of obstructive jaundice, pancreatitis, cholangitis, and cholangiocarcinoma (Choi et al., 2006). Moreover, *C. formosanus* has been reported causing diarrhea, epigastric pain and indigestion (Chai et al., 2013).

In Costa Rica the cultivation of Nile tilapia is the main productive freshwater activity. In recent years it has experienced an industrial development, with annual productions exceeding the 20 000 MT (Metric Tonnes). The major companies are located in Guanacaste province (Costa Rican Institute of Fisheries and Aquaculture, 2014).

Despite the economic and cultural importance of tilapia for Costa Rica, studies that show the parasitic infectious agents dynamics that affect tilapia under production systems, are lacking. A digenean trematode *Centrocestus formosanus* was reported as the cause of death of tilapia fingerlings in the dry Pacific of Costa Rica (Arguedas, Dolz, Romero, Jiménez & León, 2010) determining experimentally part of helminth life cycle; although, there are no similar studies in Costa Rica. About wild fish, exists only an investigation (Sandlund, Daverdin, Choudhury, Brooks & Diserud, 2010), but findings remained at the project report level.

The present study aims to register parasites in Nile tilapia larvae during the process of sexual reversion.

MATERIALS AND METHODS

Study area: The research was carried out between January and December of 2014, in an aquaculture farm located in San Miguel, Cañas, Guanacaste, Costa Rica (10º20'00" N- 85º05'00"W), between 50 and 60m above sea level, with an average temperature of 27,9ºC and 2 266mm precipitation, in a tropical dry forest (Janzen, 1983).

Sampling fish: A total number of 320 tilapia larvae of approximately 0,85g of weight in process of sexual reversion (SR) were captured during the investigation of a concrete pond from 300m^2 (20m x 25m), 160 fish were caught in the month of April (dry season) and another 160 fish in October (rainy season). The sample size for the calculation of prevalence for each of the samples was made using EPIINFO software, version 6.0 (Dean, Dean, Burton, & Dicker, 1995), with a prevalence of 50%, an absolute error of 10% and 95% confidence level. The larvae were transferred separately in plastic bags provided with oxygen to the Laboratory for Analysis of Wastewater and Diseases Freshwater of the National Technical University, Cañas, Guanacaste, Costa Rica. Immediately, fish were examined for parasites under light microscope, and parasites did not identified, were referred to the Center for Research and Advanced Studies in Animal Health (CIESA), Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico, Toluca, State of Mexico, Mexico.

Parasitological examination: The 320 larvae were examined to observe parasites and microscopic alterations in body surface. The eyes, gills, skin and fins were analyzed under microscope at 40-100X. Infested fish were examined. Immediately they were into glass petri box physiological serum added to 0.65%. Moreover, parasites were picked up using forceps and Pasteur pipettes by the methodology recommended by Noga (2011). The found parasites were classified taxonomically (Paperna, 1996).

Statistic analysis: The prevalence of parasites was analyzed as follows:

The prevalence (%) of the parasites was estimated as the ratio between the number of infected fish and the number of examined fish expressed in percentages.

$$\text{Prevalence} = \frac{\text{Number of host species infested with a particular parasite species} \times 100}{\text{Total number of hosts examined.}}$$

The Chi square test (χ^2) was used to determine the degree of dependence between prevalence of the species found and sampling seasons. The differences were statistically significant if the p value was ≤ p 0,05.

Physicochemical parameters: Dissolved oxygen, pH and water temperature were measured with an YSI 85® multiparameter oxygenator and turbidity with the Secchi disk. Measurements were performed in triplicate (8, 13 and 16 hours) for 21 days (period of the sexual reversal process). The values were averaged each season.

RESULTS

Taxonomic groups: Ten species of parasites were recorded in five taxonomic groups: two protozoan subtypes, two metazoan types and one mollusk type. 1. Sarcomastigophora: *(Ichtyobodo* sp.*)=Costia;* 2. Ciliophora: *(Apiosoma* sp.*)=Glosatella* sp., *Chillodonella* sp., *(Heteropollaria* sp.*)=Epistylis* sp., *Trichodinas;* 3. Monogeneans: *(Dactylogyrus* sp. and *Girodactylus* sp.*)*; 4. Digeneans: *(Centrocestus* sp.*)*; 5. Molluscs (two larval forms, lasidies and glochidies).

Parasites prevalence between seasons: The parasite prevalence in dry and rainy seasons are showed in table 1. *Ichtyobodo* sp. had a prevalence on gills of 15% in dry season and 84% in rainy season, while, *Apiosoma* sp. with prevalence level of 84% and 100% respectively. The prevalences were significant in rainy season for both parasites species (χ^2 =11, 59, p≤0, 05).

Comparing *Ichtyobodo sp* and *Apiosoma* sp. prevalence identified from skin, *Ichtyobodo sp* registered 9% in dry season and 29% in rainy season, while *Apiosoma* sp. with prevalence level of 27% and 84% respectively. The prevalence were not statistically significant (χ^2 =0, 0063, p>0, 05).

In dry season *Chillodonella* sp. showed a prevalence level of 3% but higher in rainy season (61%). However, this parasite was not registered on skin any season at all.

Heteropollaria sp. was not found into gills, however, it was a protozoan with a lower prevalence on skin (3%) registered in dry season, compared with a 56% in rainy season.

Trichodina genus was not identified to level species. These ciliates were recovered from gills and skin. During dry season was registered a prevalence on gills of 82% and 53% in rainy season. The skin prevalence peaked in dry season (87%) when was compared with rainy season (57%), although, the prevalence were not statistically significant any season.

The two monogeneans recovered were *Dactylogyrus* sp. and *Girodactylus* sp. *Dactylogyrus* was found only into gills (5%) in dry season and rainy season 45% of prevalence. Contrary, *Girodactylus sp* was only registered on skin showing a higher prevalence level in rainy season (78%) than dry season (3%).

The unique digenean attached was *Centrocestus* sp. (metacercariae). In dry season this zoonotic trematode was registered from different locations (60% into gills, 1% on skin and 1% on fins). In contrast, in rainy season only was into gills (12%).

With regard to parasite molluscs, in both seasons were found lasidies and glochidies fixed from gills and fins. The lasidies prevalence were less than 5%, but 25% and 15% on head in dry and rainy seasons, respectively. Larvae of glochidies type were found in gills and fins, but their prevalence were less than 5% in both seasons too.

TABLE 1
Parasite prevalence in tilapia larvae organs from a concrete pond of 200m² during reversal sex process in the dry and rainy season.

Parasites	Location	Prevalence (%)	
		Dry Season	Rainy Season
Ichtyobodo sp.	Gill	15	84
(*Costia*)	Skin	9	29
Apiosoma sp.	Gill	84	100
(*Glossatella*)	Skin	27	84
Chillodonella sp.	Gill	3	61
	Skin	0	0
Heteropollaria sp.	Gill	0	0
(*Epistilys* sp.)	Skin	3	56
Trichodina sp.	Gill	82	53
	Skin	87	57
Dactylogyrus sp.	Gill	5	45
	Skin	0	0
Gyrodactylus sp.	Gill	0	0
	Skin	3	78
Centrocestus sp.	Gill	60	12
	Skin	1	0
	Fin	1	0
Lasidies	Gill	0	1
	Fin	3	2
	Head	25	15
Glochidies	Gill	1	2
	Fin	1	3

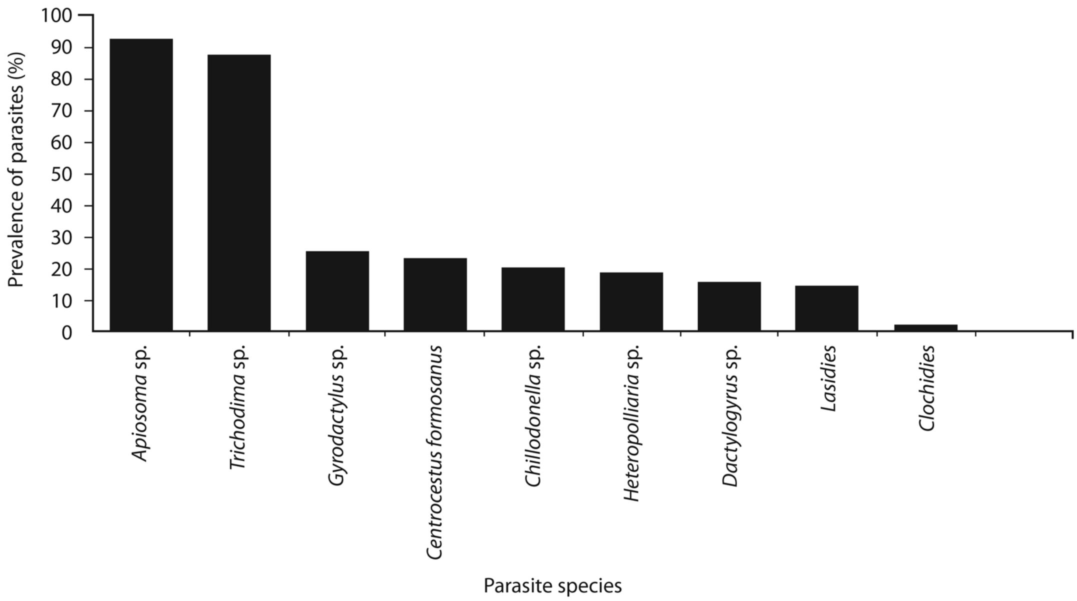

Fig. 1. Prevalence of parasites from Nile tilapia larvae during the period of study.

Parasites prevalence between species: The prevalence by species are shown in Fig. 1. *Apiosoma* sp. and *Trichodina* sp. showed the highest prevalence (92% and 87% respectively). While, lowest prevalence were registered by lasidies (14%) and glochidies (2%).

Physicochemical parameters of water: Table 2 shows the physicochemical parameters of water. The water temperature (ºC) was higher in dry season; likewise, the dissolved oxygen concentration, with a value of 4,4 mg/L, whereas, in rainy season was registered a value of 2mg/L. Turbidity had a value of 28cm, being highest in the rainy season.

TABLE 2
Physicochemical parameters of water from a concrete pond of $200m^2$ in the dry and rainy season during reversal sex process.

Parameter	Dry season (Mean)	Rainy season (Mean)
Dissolved oxygen (mg/L)	4,4	2
pH	7,6	4,8
Temperature **(ºC)**	31	21
Turbidity(cm)	28	55

DISCUSSION

In Costa Rica there are few studies to know the parasitic biodiversity that affects tilapia fish (Arguedas et al., 2010). This study is the first report for identification of parasite diversity on tilapia fish larvae under systems culture, in stage reversal sexual process, in which parasitic agents can cause high mortalities, or immunosuppressive (Conroy, 2005; Pádua, Ishikawa, Ventura, Martins, & Tavares, 2013).

In this research we detected 10 species of parasites, into 5 taxonomic groups, however, in another study in fish cultured in Brazil, 16 species were identified in four groups (Pantoja, Neves, Montagner, & Tavares-Dias, 2012). On the other hand, the calculated prevalence by wild fish (Sandlund et al., 2010) were lower than those recorded in our study, but they registered 50 species, although, the report was from 27 sites with different environments.

In regards protozoa, prevalence recorded for *Ichtyobodo* sp. are higher than prevalence reported by Williams & Jones (1994) who researched parasite biodiversity of cichlids. Paperna (1996) found *Ichtyobodo necator* (*Costia necatrix*) affecting juvenile cichlids. In our study, *Ichtyobodo* sp. the etiological agent of costiasis disease was found attached from gills and skin, hypothesizing to tilapia as a host of this protozoan, which could be dispersed around Costa Rica, through fingerlings sending among farms. *Ichtyobodo necator,* is a cichlid´s parasite reported in other fish families, producing high mortality in culture farm with different water temperatures (Deen, Hady, Kenawy, & Mona, 2015). This find agreed with our report of prevalence parasite in dry and rainy seasons, caused by temperature water differences.

The *Apiosoma* sp. prevalence, also reported as *(Glosatella* sp.*)* were increasing from dry season to rainy season, on gills and skin. According to Noga (2000) this ciliate had high prevalence in waste-water solids. This information agrees with our outcomes for the turbidity measurement obtained on the rainy season, reaching a 28 cm value.

The higher *Chillodonella* sp. prevalence in rainy season is similar to reported in *Ciprinus carpius* culture (Roberts, 1981) a serious epizootic problem during rainy months in freshwater fish (Monir et al., 2015). Moreover, it is correlated with hyperinfections from cultured tilapia in lowest temperature water to 22°C (Kazubski, 1986; Abdel-Baki & Quraishy, 2014), similar to our investigation. However, our findings showed to *Chillodonella* sp. was attached to the gills, in contrast with reported in several previous studies (Oldewage & Van As, 1987), who observed the parasite on skin tilapia hybrids.

Heteropollaria sp. (*Epistylis* genus) showed prevalence differences between seasons (Martins, Cardoso, Marchiori, & Benites, 2015), showing high values in rainy season, which agrees with our report. Probably, this protozoa has an optimal reproduction at lower temperature with hypoxic water conditions.

Trichodina affecting gills and skin, which confirms the adaptation degree of these protozoa (Basson & Vas As, 1989). The *Trichodina* high prevalence on fish in sex inversion phase could be associated to its simple reproduction cycle (Madsen, Buchmann, & Mellergaard, 2000), caused by limited water exchange and high fish number into concrete pond resulting a massive colonization. The *Trichodina* prevalence were different between seasons. In dry season were higher than rainy season, in contrast with study reported by Abd & Hassan (1999) who found high prevalence on winter from tilapia farms located in Saudi Arabia. We agree with Conroy (2005) who reports trichodines as fastidious parasites in tilapia sex inversion phase on fresh and salty water. We observed trichodines have opportunistic conditions due to high prevalence even under stress (critical values of water quality) registered in rainy season.

Dactylogyrus is a monogenean attached in gills, contrary *Gyrodactylus* usually on skin (Paperna, 1991; Williams & Jones, 1994). This information supports our results that both worms can be collected from gills and skin in the same fish. Moreover, these species have been associated with heavy infections under stress of negative water quality, in consonance with this research. These evidences suggest an extensive physiological adaptation to different environment factors. Another possible explanation is *Dactylogyrus* develops eggs in dry season, given birth in rainy season.

Centrocestus formosanus was an identified trematode found in dry season infecting gills, skin and fins. For this parasite difference of prevalence between seasons were similar to reported on *O. niloticus* cultured during flooding season in Vietnam farms (Thien, Dalsgaard, Thanh, Olsen, & Murrell, 2007), but minor to the trematodes diversity reported by Wiriya, Clausen, Inpankaew, Thaenkham, Jittapalapong, Satapornvanit, & Dalsgaard (2013) that determined in Nile tilapia to *Stellantchasmus falcatus*, *Haplorchis pumilio* and *Procerovum varium*. The findings about *C. formosanus* are of interest for health authorities, particularly because it is a new report for this zoonotic parasite in Costa Rica.

Studies to know the pathological impact of these parasites in Costa Rican aquacultural farms are necessary. Moreover, the spread and impact in wild fish populations.

In the present study, we revealed 10 species of parasites. *Ichtyobodo sp, Apiosoma sp, Chillodonella sp, Heteropollaria sp, Trichodina sp, Dactylogyrus sp*, *Girodactylus sp, Centrocestus sp*, lasidies and glochidies (two larval forms). The results indicate higher prevalence for protozoans and monogeneans in rainy season, while digeneans and molluscs are more prevalent in dry season, having different infection dynamics over gills, skin, fins and head.

The information obtained may provide strategies in aquaculture management to reduce potential economic losses in fry tilapia production caused by parasitic infection. It is important to check annually parasite population in Nile tilapia farms.

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