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ORIGINAL RESEARCH

Ecto-parasitic infection in Nile tilapia (*Oreochromis niloticus*) fry during male reversal in Veracruz, México

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Abstract The monogenean gyrodactylids and some ciliated protozoa species may cause ectoparasitic epidemics in tilapia hatcheries. An experimental framework was developed with fish reared during male reversal treatment. The infection dynamics were examined: mean intensity, prevalence, condition factor (*K*), fish growth and mortality. A total number of 3400 infected tilapia fish were transferred from high density (45 fish L^{-1}) to a low-density tank (2 fish L^{-1}). In the fish (0.012 ± 0.003 g) there were identified four ectoparasitic species; a worm, *Gyrodactylus cichlidarum* and 3 ciliated protozoa (*Trichodina* sp.,*Chilodonella* sp. and *Ambiphrya* sp.). The infection dynamic examined during 31 days showed that the parasitic load and parasite richness for the different ecto-parasites demonstrated positive interspecific correlations, in spite of that, non-synergistic or antagonistic interactions were manifested. The condition factor *K* was strongly affected ($P< 0.05$) by *G. cichlidarum* during the course of infections. The growth and survival recovered after 31 days at low density.

Keywords *Gyrodactylus cichlidarum* . Intensity . Prevalence . Protozoa ectoparasites . Tilapia nursery

Introduction

Intensive fish farming creates abnormal shoaling that promotes the outbreak of parasitic infections (Barber et al. 2000; Ward et al. 2005). Healthy fish had the habit of the shoal, while infected fish reduce this habit. The reduced shoaling tendency of infected hosts reduces the chances of transmission (Rahn et al. 2015). At low rearing density, uninfected fish may largely benefit from avoiding contact with infected fish as this reduces their own infection risk. High rearing density is an aquaculture tendency for business profit, however, at high density, the interaction fish-parasite is aggravated due to chronic social stress also by the increase in the transmission rate of parasites with a direct-life cycle such as protozoa and monogeneans.

Parasitic infections give indications of water quality since they commonly increase in polluted waters (Poulin 1992; Anirban et al. 2020). As pollution is no usual wild condition; natural aquatic bodies have a regulatory effect on the distribution of parasite communities that might be strongly affected by human impacts (Suliman and Al-Harbi 2015). Intensive nursery rearing causes water pollution that may also contribute to triggering parasitic manifestations (Barton and Iwama 1991; Chogale et al. 2015). The presence of abundant free-living microbes and skin inhabitants bacteria act as a common factor in ectoparasitic infections in polluted waters (Noga 2010; Kristmundsson et al. 2006). There exists evidence that ectoparasitic infections and related diseases resulted in poor fish growth (Barker et al. 2002; Faruk 2018), or mass mortalities (Popma and Lovshin 1995; Khan 2004; Faruk and Anka, 2017).

Monogeneans worms are one of the most common parasites in cultured fish, their communities reflect the ecosystem's health and environmental pollution (Sures et al. 2017; Mabrock et al. 2020). Trichodinids

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and monogeneans worms are commensal parasites feeding on the skin surface and gill tissues; their presence correlated well with bad water quality and their mode of attachment may promote other infections. Although the parasitic mechanisms between monogeneans and ciliated protozoa are clearly distinct, the mixed infections of identical host occur often in fish and are top causing agents leading to mortality (Sahoo et al. 2020), tilapia fingerlings possibly spread over synergistic interactions when mixed parasites infect the same body area (Abdel-Latif et al. 2020).

Ciliated protozoa skate the fish surfaces feeding on bacteria and mucus, acting primarily as commensal bugs. The protozoan *Ambiphrya* sp. is not pathogenic, but massive infections hamper the fish's respiration (Noga 2010). In Brazil, six species of trichodinids were identified as parasites of Nile tilapia (Maciel el al. 2018). The morbid mechanism of trichodinids is said to the way within which they infect their hosts since once the parasite is mounted firmly onto its host, the border of the aboral membrane creates a suction movement on the surface of the epithelial cells, that doubtless causes irritation to the tissues of the fish (Basson and Van As 2006). *Trichodina* sp. infects the host skin, adhering and suctioning on the animal tissue surface (Lom 1973; Jackson 1978; Lom and Dyková 1992; Yusni and Rambe 2019) it will become extremely virulent once proliferate massively over sick fish inflicting animal tissue dysplasia and hypertrophy (Kristmundsson et al. 2006; El-Tantawy and El-Sherbiny 2010). Thus, a high abundance of those parasites and their constant circular movements might seriously harm the epithelial tissue of their hosts, thereby triggering physiological alterations (Van As and Basson 1989).

Gyrodactylus cichlidarum Paperna 1968, has been studied taxonomically at the morphological and molecular level. This monogenean worm has been recognized as a prevalent pathogen of commercial tilapias and has invaded other native Cichlidae fish in the tropics by the introduction of exotic species (Mendoza-Franco et al. 2018). Gyrodactylids use a genuine injurious mechanism (the opisthaptoral apparatus) that penetrates the skin or gills to hook then get nourished on bacteria, cell debris or mucus from the host surfaces (Mo 1991). It has been documented that parasitic monogeneans infect specific host limited to particular taxonomic fish groups (Yusni and Rambe 2019), however, still not clear what is the promoting factor for mixed infections with protozoans. In spite of that, pathogenic monogenean epidemics are issues of concern in Mexican tilapia hatcheries (Paredes-Trujillo et al. 2016), the understanding of their infection dynamics, and how to get rid of them, is scarcely known. The little available data from commercial hatcheries suggests that poor practices (mainly extreme high rearing densities) leading to high ammonia levels above 1 mg L^{-1} may be the origins of parasitic outbreaks (Paredes-Trujillo et al. 2016), paradoxically local practices still using extreme high (up to 40 fish L^{-1}) densities. The aim of this study was to examine the infection dynamics of *Gyrodactylus cichlidarum* accompanied by other ciliated protozoa in naturally infected tilapia fingerlings at low rearing density.

Material and methods

Fish and rearing conditions

The experimental fish were 8 days post hatch (dph) tilapia fry 0.012 ± 0.003 g weight reared in high density (45 fish L^{-1}) in a hatchery located at the central region of Veracruz State. The tilapia fry were infected by 4 types of parasites, the monogenean *Gyrodactylus cichlidarum* and 3 ciliated protozoa (*Trichodina* sp. *Chilodonella* sp. and *Ambiphrya* sp.). Three thousand and four hundred (3400) infected fry were transferred to a 1700 L concrete tank with clean freshwater where the density was reduced to 2 fish L^{-1}, water was renewed continuously. The fry were treated for male reversal (Jo 1988) with a commercial feed of 52% protein added 17-α-metiltestosterona (MT), *ad libitum* 9 times per day.

Water quality

Water temperature (°C, glass thermometer), dissolved oxygen (mg L^{-1}; digital oxygen-meter) and pH (pH meter) were measured daily; ammonium-N (mg L^{-1}) was analysed weekly with a commercial kit (range 0.0-7.3 mg NH_4-N L^{-1}).

Parasitological examination

A total number of 150 fingerlings were examined for *G. cichlidarum* and ciliated protozoa. Biometrical parameters were weight (g) and survival (%). Fish samples (15 samples; n=10 fish) were randomly collected from the low density tank at 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 27, 29 and 31 days of rearing. Live fry were transported to lab facilities and kept alive not longer than 4 hours until its examination. Individual animals were quickly euthanatized by a neural lesion followed of weight measurements. The identification of the monogenean parasite was made on examination of specimens mounted unstained in Gray&Wess medium for the study of sclerotised structures (Kritsky et al. 2013) using a Leica microscope DM2500 with Nomarski interference contrast and based on re-description of *Gyrodactyluds cichlidarum* provided in García-Vázquez et al (2007).

Respect protozoans, they were identified at level of genus. The fins and gills were examined in wet mounts under the composite microscope. Mucus and skin scrapes were taken from the body surface and few drops of diluted Gentian violet were added for helping the visualization, counts and identification of protozoa (Conn 1930).

Infection parameters

These values were calculated by sample according following formulas (Bush et al. 1997):

Prevalence (%) = (Number of hosts infected with a particular parasite species / Total number of hosts examined in the sample) x 100

Mean intensity = Number of individuals of a particular parasite species / Infected hosts with that parasite species in the sample

$$\textit{Condition factor } K = [100\text{*Weight (g)}] / [\text{Total length (cm)}]^3$$

The Pearson's correlation test (Pearson's product correlation coefficient *r*) was used to analyse inter-specific associations between total individuals by each parasite species; correlations between total individuals by each parasite species; the total parasitic abundance (Σ ecto-parasite individuals= Σprotozoa + Σmonogeneans) by individual fish, and parasite species richness (number of types-species) versus growth parameters (body weight and condition factor). Log-transformed data was analysed by one-way ANOVA test at P< 0.05 (Statistica 7.0, www.statsoft.de).

Results

Occurrence of ecto-parasites

The fingerlings were infected with a mix of four ecto-parasitic types, the monogenean *G. cichlidarum* and 3 ciliated protozoans *Trichodina* sp., *Chilodonella* sp. and *Ambiphrya* sp. (Figure 1). Two of them very prevalent, *Trichodina* sp. and *G. cichlidarum* (See Figures 2a,b – day 1 of infection). The higher richness of 3 ± 1 types fish^{-1} occurred between days 11 to 13. *Trichodina* sp. and *Chilodonella* sp. disappeared on day 23 (Figures 2a and 3a), *Ambiphrya* sp. at day 25, while the monogenean lasted until day 31 (Figures 2b-3b).

Gyrodactylus cichlidarum

After transferring infected fish to a low rearing density, the monogenean occurred frequently in 87% of the samples, while in 61% of these samples, the prevalence was higher than 50%, particularly during the day-1 and day-13 (Figure 2b). At day 1 the prevalence was 75% with an intensity of 2±3 organisms/parasitized fish, after that, it was observed a cycle of 9 days in the number of worms with a maximum peak at day 5 of rearing. (Fig. 2b). Another significative peak, but of prevalence, occurred between days 19 and 21. *G. cichlidarum* disappeared from the host until day-31 (Figure 2b). The monogenean peaks between day-3 and day-7 correlated well with a significant decrease in K (Figure 4a).

Later, from the day-9 to day-18, occurred a slow increase in weight, but not of *K*, which showed a conspicuous leap from day-11, connected to the intensity decreases of *G. cichlidarum*. Between day-20 to day-31 was observed the recovering phase with marked increase in the growth and *K* when the infections were lessened. Finally, during days 20-31 were observed increases in the growth and *K*, when the infections by monogenean worms were remarkably low (Figures 4a-b).

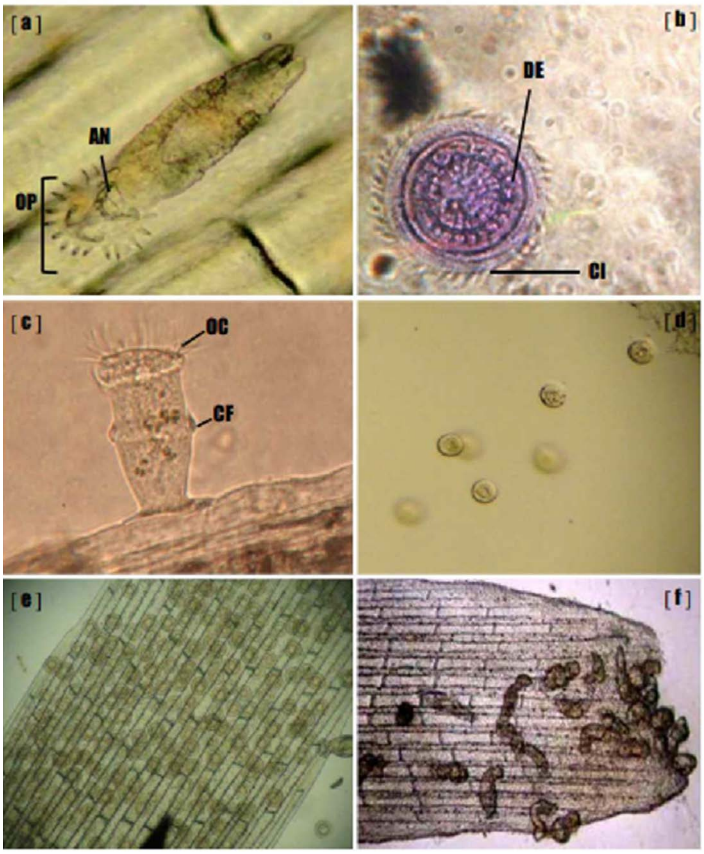

Fig. 1 *Gyrodactylus cichlidarum* and other ciliated protozoa found in infected tilapia fingerlings, *O. niloticus.* [a] *G. cichlidarum*, AN= Anchor, OP= Opistohaptor; [b] T*richodina* sp. CI= Cilia, DE= Denticle; [c] *Ambiphrya* sp. OC= Oral cilia, CF= Ciliary fringe; [d] *Chilodonella* sp. [e] Caudal fin infected with *Trichodina* sp. [f] Caudal fin infected with *G. cichlidarum*. Snapshots made with a digital camera (no scale).

Trichodina sp.

The protozoans occurred from 53 to 67% of the samples, with prevalence superior to 50% only in 1 or 2 samples.This protozoan was highly prevalent 100%, with intensity of 21 ± 20 organisms/parasitized fish at day 1 and disappeared from the infected fish on day-23. There was observed a cycle of 5 days with a peak in the number of protozoa at the middle (Figure 1a).

Chilodonella sp. and Ambiphrya sp.

These protozoa were less prevalent 36% for *Chilodonella* sp. and 10% for *Ambiphrya* sp. and intensities 6±7 and 1±0 organisms/parasitized fish, respectively at day 1. *Chilodonella* sp disappeared from the infected fish on day-23, while *Ambiphrya* sp disappeared until day-25 (Figures 3a-b). One peak of these protozoans occurred between day-9 and day-13 but without overlapping the *G. cichlidarum* peak (Figures 2b, 3a-b).

Host-parasite and parasite-parasite associations

The species richness (*G. cichlidarum, Trichodina* sp. *Chilodonella* sp. and *Ambiphrya* sp.) and parasitic abundance by species and total (Σ protozoa + Σ monogenea) were negatively correlated with the fish weight but the *K* was affected by *G. cichlidarum* abundance (Table 1). Concerning the interspecific parasitic associations, the 4 ectoparasitic species were positively correlated, excepted *Trichodina* sp *vs Ambiphrya* sp. (Table 2).

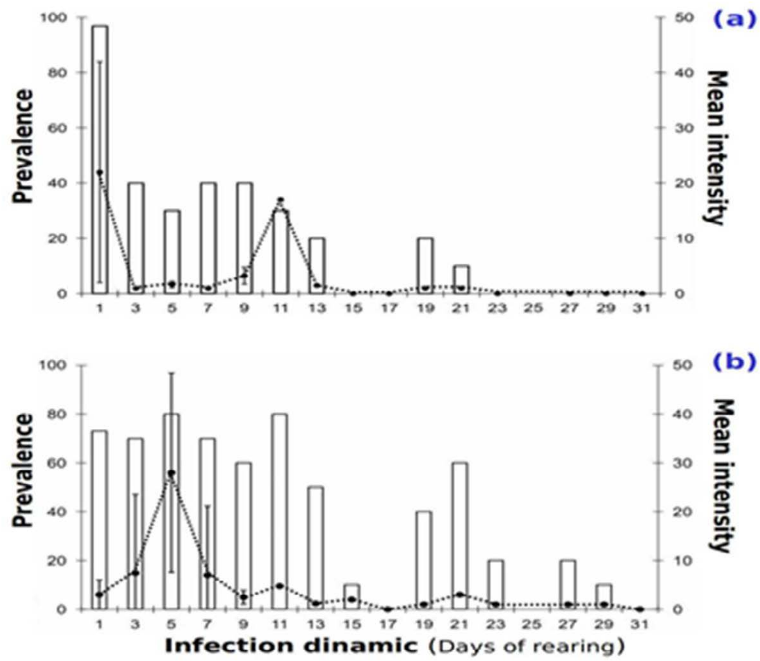

Fig. 2 Parasitic prevalence and average intensity of *Trichodina* sp. (a) and *Gyrodactylus cichlidarum* (b), in infected Nile tilapia (*Oreochromis niloticus*) fry after decreasing the stocking density during 31 days. Bars = Prevalence axis; Dotted-line = Mean intensity (± SD)

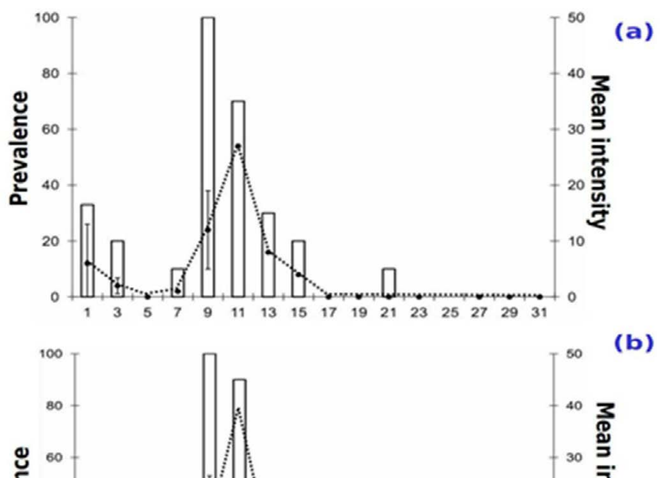

Fig. 3 Parasitic prevalence and average intensity of *Chilodonella* sp. (a) and *Ambiphrya* sp. (b), in infected Nile tilapia (*Oreochromis niloticus*) fry after decreasing the stocking density during 31 days. Bars = Prevalence axis; Dotted-line = Mean intensity (± SD)

Survival, growth and K

The overall survival of fish fry was 99.5%. The body weight (g) between day-1 to day-21 increased slowly from 0.012 ± 0.003 to 0.037 ± 0.012; thereafter a significant ($p<0.05$) increase to 0.065 ± 0.05 occurred at day-23 (Fig 4a). Finally, at day-31, the fry reached a marked ($p<0.05$) maximum weigh (0.17 ± 0.08 g) and length (20.6 ± 3.3 mm). The *K* varied from 1.49 ± 0.38 (day-1) to 1.71 ± 0.28 (day-31) and occurred significant ($p<0.05$) falls between day-3 to day-7 (Figure 4b).

Water quality

The average temperature was 29.5 ± 3 ºC, the dissolved oxygen was 4.2 ± 0.9 mg L^{-1}, pH was 7.82 ± 0.38 and ammonium-N was 1.0 ± 0.6 mg L^{-1} during 31 days of rearing. A high peak of *Trichodina* sp. at (Figure 2a) was recorded at day-1, corresponding to previous high density and remains of bad water quality effects at the hatchery.

Discussion

Rearing density and water quality

The reduction in the stocking density has been considered an advantageous tactic to promote weight gain of farmed fish. In this work decreasing the stocking density evidently improved the environment; so the water quality was beneficial according to previous studies (Rakocy 1989; Popma and Lovshin 1995; El-Shafai et al. 2004). However, after the infected fingerlings were transferred to low density and better water quality, the prevalence was 100% that must be the remaining infective effect of polluted water. At high fish density in commercial hatcheries, high ammonia levels above 1 mg L^{-1} are frequent. In a previous study *Trichodina* sp prevalence correlated with moderate ammonia 0.25 and 0.61 mg NH_4-N L^{-1} and; high nitrite 2.54 and 5.10 mg NO_2-N L^{-1} (Jiménez-García et al. 2012). The present study revealed no correlation between stocking density and parasite occurrence with the presence of good water and nutritional quality.

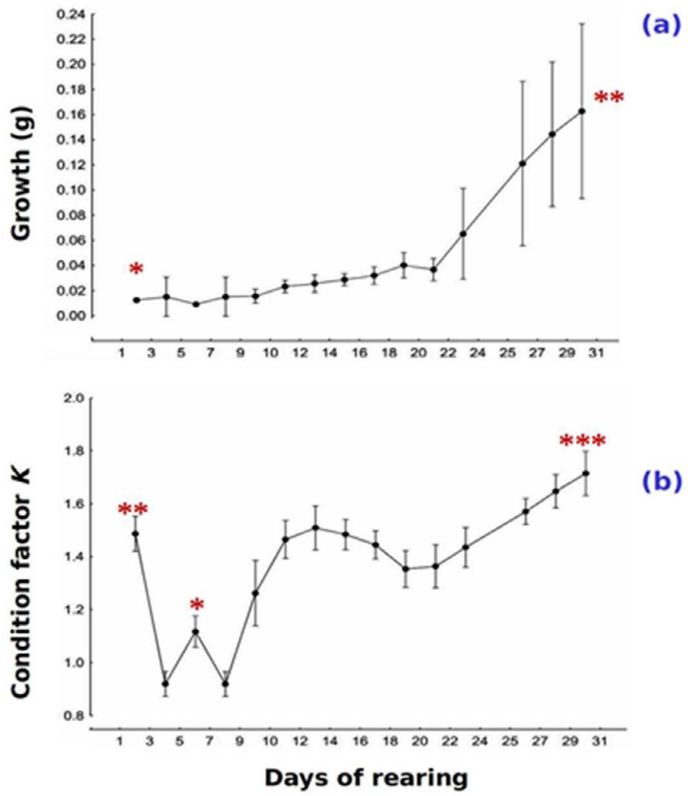

Fig. 4 Growth (a) and condition factor *K* (b); in infected tilapia fingerlings (*O. niloticus*) following a decrease in stocking density during 31 days. Bars = 0.95 confidence intervals.

The finding of prevalence and intensity of ectoparasites are often contradictory, as reported by Baidoo et al. (2015) who found prevalence and the mean intensity of parasites were relatively low in cage farmed tilapia in Ghana. But also recorded a low rate of prevalence and intensity of parasites on *O. niloticus* from fish ponds, attributed to good pond water and proper management practices. Suliman and Al-Harbi (2015) confirmed that there is a clear relationship between ectoparasites and water quality and nutritional quality. Moraes and Martins (2004) indicated that the presence of ectoparasites is related to water quality and pond management. Other authors also found a relationship between host, parasite, and the environment (Buchmann and Lindestrom 2002; Suliman and Al-Harbi 2015). On the other hand, Suliman and Al-Harbi (2015) revealed no correlation between stocking density and parasite occurrence with the presence of high water and nutritional quality. Johnson et al. (2011) reported a animal density approach to test the assumption that infective incidence rises proportionally with contact rates and host density. In our trial, decreasing the rearing density by 22 times may have eradicated the negative effects of abnormal shoaling by deceleration of the infection transmission rates that depended on direct contact (Bakke et al. 1992; Soleng et al. 1999; Buchmann and Lindenstrøm 2002; Huyse and Volckaert 2005).

Fish growth and condition factor K

In the present work, the claimed benefits from water quality barely enhanced the weight gained of infected

Table 1 Correlation coefficients (*r*) and probabilities (*p*) associations between fish biometrics with individual parasites, total parasitic abundance (Σ protozoa + Σ monogenea) and parasite species richness in infected tilapia fingerlings (*O. niloticus*) following a decrease in stocking density during 31 days

	Fish weight	Fish length	*K*
Chilodonella sp.	-0.218 0.002	-0.292 0.000	
Trichodina sp.	-0.273 0.000	-0.476 0.000	
Ambiphrya sp.	-0.186 0.009	-0.190 0.008	
Gyrodactylus cichlidarum	-0.322 0.000	-0.466 0.000	-0.269 0.000
Total parasitic abundance	-0.419 0.000	-0.601 0.000	
Parasite species richness	-0.427 0.000	-0.594 0.000	

Note: Original data were transformed (lnX+1) before analysis; Values *r* is upper and *p* is under

Table 2 Correlation coefficients (*r*) and probabilities (*p*) for the interspecific associations between total parasitic abundance (Σ protozoa + Σ monogenea) and parasite species richness in infected tilapia fingerlings (*O. niloticus*) following a decrease in stocking density during 31 days

	Chilodonella sp.	*Trichodina* sp.	*Ambiphrya* sp.	*Gyrodactylus cichlidarum*	Total parasitic abundance	Parasite species richness
Chilodonella sp.						
Trichodina sp.	0.243 0.001					
Ambiphrya sp.	0.710 0.000					
Gyrodactylus Cichlidarum	0.236 0.001	0.229 0.003	0.223 0.002			
Total parasitic abundance	0.661 0.000	0.395 0.000	0.636 0.000	0.680 0.000		
Parasite species richness	0.653 0.000	0.569 0.000	0.530 0.000	0.588 0.000	0.843 0.000	

Note: Original data were transformed (lnX+1) before analysis; Values *r* is upper and *p* is under fish during the first week. Although the survival was high, the final recovered growth was low compared with previous reports (Drummond et al. 2009; Jiménez-García et al. 2012). The studies of *in-situ* condition for infection dynamics are scarce, this case corroborates a hypothesis for reduction of density to control the prevalence of ectoparasitic infections, however still remains to be confirmed. The fingerlings grew just one-half of the range reported in other studies (Popma and Lovshin 1995; Chenyambuga et al. 2014) or were lower compared to fingerlings at 8 fish L^{-1} that grew from 0.02 g to 1.0 g (Rakocy 1989). The heterogeneous growth after day-23 was probably because of a "compensatory mechanism" after the reduction of the monogeneans that affected *K.* It has been suggested that overcrowding promotes "metabolic mechanisms" (stress) prompting heterogeneous fish growth (Fernandes and Volpato 1993; Moreira and Volpato 2004), however, this statement seemed contradictory for particular cohorts that grew uniformly at 18 fish L^{-1} with a low infection but elevated ammonia events in a closed recirculated system (Jiménez-García et al. 2012).

Ciliated protozoan

Trichodinids parasites infect gills and skin of the Nile tilapia *O. niloticus*, due to water quality and pond management (Moraes and Martins 2004). The report of Indahsari et al. (2019) is opposite to the claimed benefits of good water quality and low density, they found 100% prevalence in low stocking density dominated by *Trichodina nobilis,* attributed to its rapid life cycle of binary fission at cleavage speed of every 1⁄2 hour and the transmission of *Trichodina* is through the water as well as direct contact with infected fishes. In young fish, the *Trichodina* sp infection has a serious effect, particularly when the parasite intensity exceeds 25 parasites /microscope fields (Eissa 2004).

Monogenean worm

In the present study, *G. cichlidarum* seemed more harmful to the growth of tilapia fingerlings in spite of the 2-magnitude counts differences to the protozoan parasites. A sort of infective cycle was repeated every 5-days with prevalence and/or intensity peaks in the middle. Curiously, during the monogenean infection at low density, the co-infective protozoa did not overlap the worms peaks, however, contrasting field observations suggested that high stocking densities could be the promoter of overlapping prevalence peaks: e.g. a hatchery in South Eastern Mexico acquired a 35 days epidemic of gyrodactylids and trichodins and *G. cichlidarum* with some other protozoa have persisted during an annual cycle in four different tilapia types (Rubio-Godoy et al. 2012). In this study, *G. cichlidarum* abundance was more pernicious for fingerlings compared to the infection of the other three ciliated protozoa in sum, however, non-synergistic or antagonistic interactions (Noga 2010; Kristmundsson et al. 2006) were evident (e.g. mass mortality). Although cases of harmful synergy between trichodinids and gyrodactylids mixed infections were reported for farmed flatfish *Pseudopleuronectes americanus*, in agreement to this study case, the flatfish growth was renewed after the epidemic ended (Barker et al. 2002). To establish the ontogeny of the complex gyrodactylid infection accompanied mixed with protozoa, probably must be taken into consideration the initial parasite invader and the infective sequences.

Mixed ectoparasitic infections

To establish the ontogeny of the gyrodactylid infection mixed with protozoa, probably must consider the initial parasite invader and the infective sequences. Trichodinosis and gyrodactylosis induce aggravated pathological effects on fish leading to elevated mortality rates. Pernicious social interactions (such as bites, abrasion, damage gills, and stress), due to forced shoaling may have impaired the inexpert fingerlings defenses (Kebus et al. 1992; Barcellos et al. 1999; Ellis et al. 2002; Conte 2004). One of the foremost vital effects of mixed infections is that the pathogens would possibly diminish the fish's resistance to different diseases (Evans et al. 2007). for instance, the infection with ectoparasites might scale back the barrier immunity and act as a portal of entry for different co-infecting pathogens. This has been the case in the genus *Tilapia*, Xu et al. (2009) has demonstrated that the infestation of *Ichthyophthirius multifiliis* caused the destruction of the protecting mucose of fish and increased the susceptibility to *S. iniae* infection. In Thailand, occurred multiple outbreaks with serious mortality (20%–90%) in tiny fish between 1 g to 50 g fish

Nile Tilapia (*Oreochromis* sp.) and hybrid *Tilapia* (Surachetpong et al. 2017). Multiple and mixed infections were involved in these mortalities and several concurrent infections with bacteria, like *Flavobacterium* sp, *Aeromonas* sp and *Streptococcus* sp, and varied infestations by ectoparasites, like monogenean parasites as well as *Gyrodactylus* sp., and protozoans as *Trichodina* sp., were conjointly implicated (Abdel-Latif et al. 2020). At high fish densities, the mixed infections were characterized by elevate intensities and 100% prevalences: e.g. up to 15 monogeneans and hundreds of protozoans by individual fish at 40 fingerling L^{-1}.

The proper farming practices paradox in tilapia hatcheries

The influences of intensive rearing practices on the performance of tilapia fingerlings seem to be controversial. The practice of high stocking density in intensive tilapia nursery apparently obeys two main aspects: 1) the need to economize in the use of the special feed for male reversal and, 2) the recovery of the economic losses due to diseases and mass mortalities (an unorthodox management strategy in some rustic Mexican hatcheries). The rationale of such "strategy" remains to be assessed, because, high stocking densities increase the risks of parasitic epidemics, stress, and other harmful syndromes. High densities of fish often correlate with the prevalence of ectoparasitic infections, *Caligus* sp prevalence in Salmon cage farming is the evident example to this erroneous farming practice, on the other hand, we have demonstrated that controlled microbiota in closed systems could be a good strategy to control ectoparasitic infections (Jiménez-García et al. 2012). Trials for the control of trichodinosis and gyrodactylosis using garlic have been tested also in hatchery-reared *O. niloticus* (Abdelgalil and Aboelhadid 2011), further studies remain to demonstrated higher efficacy of alternative approaches. A research framework like this study may not be in accordance with realistic hatcheries operations, however, theobserved parasitic dynamics, suggested that to improve the fingerling's welfare, the stocking density must be conspicuously decreased. This statement agrees with Banerjee and Bandyopadhyay (2010) who suggested the correct control of density and water quality to avoid parasitic infections in ponds.

Conclusions

Nile tilapia fry (8 dph) reared at high density (45 fish L^{-1}) from a commercial hatchery located in the central part of Veracruz State were infected by the monogenean specie *G. cichlidarum* and ciliated protozoans *Trichodina, Chilodonella* and *Ambiphrya*. Thereafter, when the fish were transferred to an experimental low density of 2 fish L^{-1}, the apparent elimination of social stress and improvements in the water quality could offer to the fish the "favorable conditions" to fight and eradicate the parasitic infections in a period of 31 days.

Authors' contributions IJG conceived of the study and coordinated the experiment, IJG and CRRG performed analysis and interpretation of data, EFMF carried out the monogenean parasite identification, CRRG drafted the manuscript. All authors read and approved the final manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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