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Research Article

The effectiveness of sodium chloride and formalin in trichodiniasis of farmed freshwater tilapia *Oreochromis niloticus* (Linnaeus, 1758) in southeastern Mexico

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ABSTRACT. Trichodiniasis is one of the major diseases found in fish aquaculture worldwide causing massive fish mortality and costly effects. In this study, fresh smears of gills and skin were prepared and analyzed for the presence of trichodinids under light microscopy, revealing the presence of *Trichodina pediculus* Ehrenberg, 1831, *T. compacta* Van As & Basson, 1989, and *T. nigra* Lom, 1960 and *T. centrostrigeata* (Basson, Van As & Paperna, 1983). The effectiveness of treatment with sodium chloride and formaldehyde was evaluated for controlling infection caused by these parasites in tilapia *Oreochromis niloticus* Linnaeus, 1758 in aquaculture facilities in southeastern Mexico. A total of 240 fish were examined. The results showed that all doses using sodium were effective for controlling trichodinids and eliminated the parasites (100%) however, fish exposed to doses of formalin showed a higher level of stress than those exposed to sodium chloride. Bathing in formalin 0.250 mL L^{-1} for 10 min and 30 g L^{-1} of sodium chloride for 10 min, significantly reduced parasitic infection in tilapia under captive conditions ($P < 0.05$). This is the first record of a trichodinids species for native wild freshwater fish in a culture environment in southeastern Mexico since all previous records were from exotic cultured fish. The species found were *Trichodina pediculus*, *T. compacta*, *T. nigra* and *T. centrostrigeata* are new records of host and geographic location.

Keywords: *Trichodina*; Trichodinidae; ciliates; ectoparasites; treatments; aquaculture; Mexico

INTRODUCTION

The study of parasites as pathogenic agents for host fish is important in the food production and industrial process, in culture conditions. Infestation in fish increases when they are cultured intensively, as high density causes an increase in the parasite populations, which may cause epizootic conditions mainly in the case of parasites of the direct cycle such as certain protozoans (Woo, 1995). The larval stages of fish are the most sensitive to the development of protozoan diseases; within which the trichodiniasis can be mentioned for its frequency and negative effect in the vast majority of cultures which showed characteristic disease signs: rubbing on the sides or bottom on the pond, caudal fin erosion and hemorrhagic areas in the skin (Valladão *et al.*, 2013, 2014, 2015).

Several studies have demonstrated the sensitivity of parasites and some drug agents, *i.e.* formalin (formaldehyde) and sodium chloride, this is the case of the ciliate parasite *Anophryoides haemophila* of the American lobster *Homarus americanus* according to Speare *et al.* (1996). These authors determined, that formalin at 50 mg L^{-1} or low salinity of 8 for 1 h killed 100% of the parasites. Buchmann & Kristensson (2003) evaluated the efficacy of sodium percarbonate and formaldehyde bath treatments against *Gyrodactylus derjavini* infestations of rainbow trout *Oncorhynchus mykiss*. Other authors studied the efficacy of formalin, sodium chloride and the combination of substances (Policar *et al.*, 2011; Bradley *et al.*, 2013; Abd El-Gawad *et al.*, 2016). Chemotherapy of the ciliate *Trichodina* sp. on juvenile turbot *Colistium nudipinnis* Waite, 1910 with a recommended treatment for use in

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a commercial situation is 200 ppm formalin for 30 min (Diggles, 2000) also some authors reported teflubenzuron as a potential drug to be used in Brazilian aquaculture; which attends to important requirements, such as low toxicity and high efficacy in controlling trichodinids. infection in *Oreochromis niloticus*, and for *Piaractus mesopotamicus* (Venâncio *et al.*, 2015).

The larval stages of fish are most sensitive to the development of protozoan diseases; within which the trichodiniasis can be mentioned for its frequency and negative effect in the vast majority of cultures (Valladão *et al.*, 2016). Trichodinids have been found as parasites, especially in "stressed" fish in overpopulated conditions in supply pools with low oxygen environments (Valladão *et al.*, 2016). The cichlids and carps are commonly affected by trichodinids that cause "epizootic" blooms causing important economic losses in cultures (Paperna, 1996). Aquaculture must expand sustainably, reducing environmental impacts. However, some agents are essential for the maintenance of healthy stocks of fish. This industry faces serious challenges towards minimizing its dependency on chemicals to treat parasitic infections during intensive fish farming.

The Nile tilapia *O. niloticus* (Linnaeus, 1758) is among the most important groups of production of freshwater cichlid fish (FAO, 2012). These cichlids, in general, are the main hosts for trichodinids (Rodríguez-Santiago 2002; Valladão *et al*., 2013, 2014, 2015). To date, *Trichodina* spp. (Ciliophora, Oligohymenophorea) are ciliated protozoan parasites of skin and gills; they are pathogenic, causing damage to much freshwater fish, particularly larval stages of fishes, which are more susceptible to developing diseases. Its proliferation might be promoted by changes in the relationship among host, parasite, and environment caused by nutritional deficiency, poor water quality, and parasitic diseases (Khan, 2004); causing severe epidermal lesions and disease outbreaks (Martins *et al.*, 2010).

Studies of trichodinids in Mexico have been carried out on exotic cultured fishes such as *Carassius auratus* (Linnaeus), *Ctenopharyngodon idella* (Valenciennes), *Cyprinus carpio* (Linnaeus) (Cyprinidae), and *O. niloticus* (Linnaeus) (Cichlidae) (Aladro-Lubel *et al.*, 2006). In Mexico however; the records on protozoan parasites are specific for parasites ciliates, and they are limited to *Trichodina symmetrica* in *Sarotherodon hornorum* Trewavas, 1966 and cultured *O. mossambicus* Peters, 1852 (Chávez-Mancilla, 1985) and *T. wellborni* in the Cyprinids (Herróz-Zamorano, 1999). Few studies are reporting *Trichodina* spp. Armijo-Ortiz (1968) identified new records of species not previously identified for Mexico; *T. domerguei* Wallengren, 1897 and *T. symmetrica* Davis, 1947 in freshwater fish. Herróz-Zamorano (1999) identified *T. wellborni*, and Rodriguez-Santiago (2002) found *T. pediculus, T. centrostrigata, T. magna* and *T. nigra* in *Oreochromis niloticus*, which are new records of host and geographic location.

Most information about studies of fish parasites in Mexico is limited to helminths (Lamothe- Argumedo *et al.*, 1996). In most of the fish farms in Mexico, Trichodiniasis is reported by its frequency and mortality effects on tilapia (Rodríguez-Santiago, 2002) The identification of trichodinids becomes a valuable contribution to the knowledge of these protozoan parasites which affect high-value commercial species. Infestations caused by trichodinids are particularly significant in aquaculture because they are responsible for causing chronic mortality during cage production, change in larvae swimming behavior patterns, gill inflammatory infiltration, and necrosis (Valladão *et al.*, 2013, 2014, 2015, 2016). This study aimed to assess the effectiveness of sodium chloride and formaldehyde employed the *O. niloticus*/trichodinids infection model to examine these questions, concerning treatments on the host, in order to improve fish welfare in aquaculture conditions.

MATERIALS AND METHODS

Experimental fish, set up and experimental procedures

This study was performed at the Parasitology Laboratory of the Academic Division of Biological Sciences of the Universidad Juárez Autónoma de Tabasco. All procedures performed involving animals were under ethical standards according to Mexican laws (NOM-033-ZOO-1995). The efficacy of bath treatments using sodium chloride (10, 20, and 30 g L^{-1}) and formalin (0.200, 0.230, and 0.250 mL L^{-1}) was tested as described in Table 1. A total of 240 fish (total length 2-7 cm, weight 1-5 g) were placed in 3 L glass tanks equipped with aeration and dechlorinated clean freshwater, 10 fish per treatments and 3 replicates for each treatment including a control group were employed.

Each dose was diluted in 1 L of clean tap water for each treatment in their respective replicas. Fish were monitored during each treatment, assessing the animals' welfare, *i.e.*, lethargy, aggressiveness, change in skin color that indicate stress concerning the exposure to different doses and treatment times.

After each exposure of different doses, the fish were transferred to tanks of 20 L with clean filtered freshwater (divided into three sections). Controlled parameters

Table 1. Two control treatments (NaCl and formalin) against trichodinids in tilapia. T: time, R: replicate.

Treatment	Concentration	Time	Replicate
NaCl			
T1 (Control)	0	0	T1-R1; T1-R2; T1-R3
T2	10 g L^{-1}	3 min	T2-R1; T2-R2; T2-R3
T3	20 g L^{-1}	5 min	T3-R1; T3-R2; T3-R3
T4	30 g L^{-1}	10 min	T4-R1; T4-R2; T4-R3
Formaldehyde			
T1(Control)	0	0	T1-R1; T1-R2; T1-R3
T2	0.200 mL L^{-1}	2.5 min	T2-R1; T2-R2; T2-R3
T3	0.230 mL L^{-1}	3 min	T3-R1; T3-R2; T3-R3
T4	0.250 mL L^{-1}	10 min	T4-R1; T4-R2; T4-R3

included dissolved oxygen, temperature, and pH of the water in controls and treated groups; during and post-treatment using a potentiometer brand HI 98107 Hanna model and an oximeter (YSI model 55). These parameters were evaluated to assess whether a correlation between the chemical dose and possible changes in pH, dissolved oxygen, and water temperature existed, as well as to relate whether different doses applied altered the optimal range values mentioned for cichlids, and hybrids of both tilapias, in the CENDEPESCA Handbook on reproduction and cultivation of tilapia (Legal Guidelines for Health and Nutrition Aquaculture in Mexico) (García-Ortega & Calvario-Martínez 2008).

Trichodinids were observed under a Zeiss Stereoscopic Microscope for their taxonomic identification. The samples were dried under environmental conditions for further tinting. The silvery tint by Klein was used for detailed observation of the denticle structures, the fixation disk characteristics and the number of their constituents, and the hematoxyline tint by Harris was used for the observation of the nuclear apparatus as additional information. The methodologies reported by Lom (1958) and Wellborn (1967) were used.

Klein's technique was used to mount the stained slides. They were observed in detail under the compound microscope searching for trichodinids. Those that were wholly mature and well-shaped with all the constituents of the adhesive disk clear and well tinted were selected.

At 1,000x amplification, the denticle morphology of each one of the organisms is carefully observed: blade and ray, the degree of silvery impregnation of the center of the adhesive disk and presence or absence of chitin structures, etc. The angle described by the adoral spiral was also observed. To locate all of these characteristics taxonomically, they were compared to Lom & Dyková (1992) descriptions tentatively, followed by the review of the original studies for the determination of the species, for which the corresponding measurements were done as well.

Skin samples

Trichodinids were counted under the 10x objective, and the average value of five fields was determined as very low (<1), low (1-5), moderate (6-50), high (51-100) or very high (100+) based on Bunkley-Williams & Williams Jr. (1995).

Gill samples

Trichodinids were recorded on each gill (right and left). The prevalence of infection of each host was determined according to Bush *et al.* (1997) criteria: Prevalence = number of hosts parasitized by a species of parasite over the total number of hosts examined, expressed as a percentage.

Statistical analysis

Shapiro-Wilk's and Levene's tests were used to test for normality and homogeneity of the data (total abundance of trichodinids per host individual) (Zar, 1984). Kruskal-Wallis' (KW) non-parametric one way analysis of variance was used to determine whether the total abundance of trichodinids per host vary among the different concentrations of formalin (control, 0.200 mL L^{-1} 2.5 min^{-1}, 0.230 mL L^{-1} 3 min^{-1} and 0.250 mL L^{-1} 10 min^{-1}) and sodium chloride (control, 10 g L^{-1} 3 min^{-1}, 20g L^{-1} 5 min^{-1} and 30 g L^{-1} 10 min^{-1}). The Dunn's test (with Bonferroni correction) for post-hoc analyses were used. All statistical analyses were performed using the program InfoStat.

RESULTS

The identified species were *Trichodina pediculus*, *T. compacta*, *T. nigra* and *T. centrostrigeata* which were collected in the skin and gills of the fish. The highest prevalence of trichodinids was found on the skin (46%)

(10 ± 74 trichodinids/host) and gills (30%) (10 ± 20 trichodinids/host). The treatments at doses of 10, 20 and 30 g L^{-1} in 3, 5 and 10 min respectively were effective to control trichodinid's infection (Table 1).

Treatments with sodium chloride (NaCl) are shown in Fig. 1a. The prevalence of replicates of Treatment 1 (control) was 20 to 100%, while in treatments 2-4, the prevalence was reduced to zero on skin and gills. The total mean for Treatment 1 (control) of this substance was 50% (Fig. 1b).

In the analysis performed per infected organ, the highest prevalence was found on skin 46%, and lower for the gills (30%). The treatments of 10, 20, and 30 g L^{-1} in times of 3, 5 and 10 min, respectively were effective for the control of trichidinids as these parasites were eliminated from skin and gills (Figs. 1c-1e).

In the formaldehyde treatments, the prevalence of replicates of Treatment 1 (control) was 90% to 100%, with some variations for this parameter in treatments 2-4 (Fig. 2a). The total average for Treatment 1 (control) was 96.6%, whereas for treatments 2 to 4 were 60 to 0% (Fig. 2b) highlights the behavior of these values.

In Treatment 2 (0.200 mL L^{-1} for 2.5 min), the skin prevalence in the replicates was 6.6-23.3% (50%). In the gills, the prevalence of replicates was 0-23.3% with an average of 40% (Fig. 2c). In Treatment 3 (0.230 mL L^{-1} for 3 min), the skin replicas showed a prevalence of 3-10%, with a prevalence average (23%). For gills, the prevalence ranged from 3 to 13%, with an average prevalence for replicates of 20% (Fig. 2d). In Treatment 4 (0.250 mL L^{-1} for 10 min), there was no record of the presence of trichodinids in any of the replicas. The mean values for the infection intensity observed in the controls of the different doses; NaCl groups and formaldehyde treatments for skin and gills are displayed in Figure 2e and Table 2 respectively.

The highest infection intensities for skin and gills were present in Control 2 of the treatment with NaCl with a prevalence of 100% and an intensity of 11.1 parasites/skin and 3.2 for gills. In the treatment with formaldehyde in the Control group 3, the prevalence was 90% with an intensity of 22.5 parasites/skin, and 6.5 parasites/gills were registered.

During the analysis of the variances concerning average values of the infection intensities, for formaldehyde Treatment 1 (control) significant differences were observed among the mean doses of infection intensity for skin and gills respectively. In Treatment 1 (control), and Treatments 2 and 3, a decrease in the mean intensity values was shown for skin and gills during the formalin doses at 0.200 mL L^{-1} for 2.5 min and 0.230 mL L^{-1} for 5 min for skin and gills (Table 2).

In the analysis of the intensity of infection by fish sizes, the averages in Treatment 1 (control) with NaCl, the highest skin infection intensity was presented in Replicate 2, with fish sizes from 2.6-5.5 cm with an average intensity of infection of 15-20 trichodinids. In the case of formaldehyde, the highest infection intensity was found in Replicate 3 (4-6 cm), with an average intensity of 25-75 trichodinids per field (Table 2).

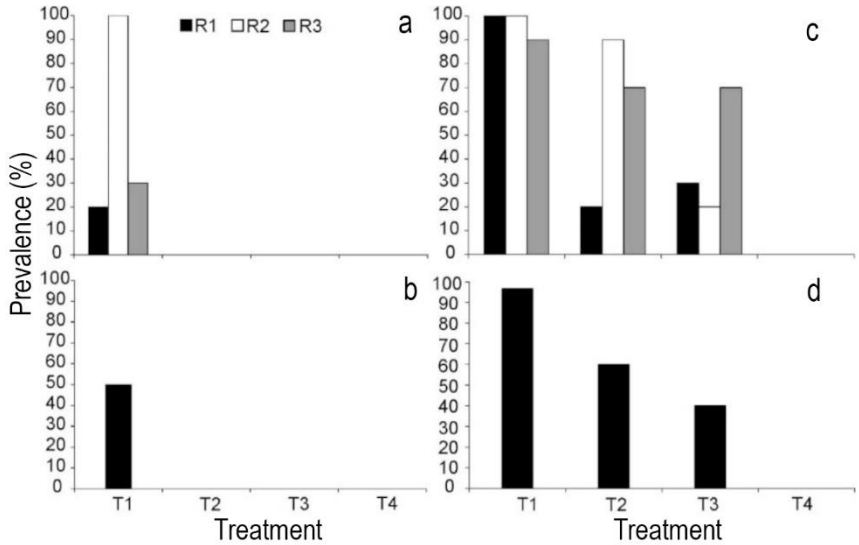

Figure 1. Prevalence of trichodinids. a) In treatment replicates with sodium chloride, b) total average prevalence in treatments with sodium chloride, c) in treatment replicates with formalin, and d) total average prevalence in treatments with formalin.

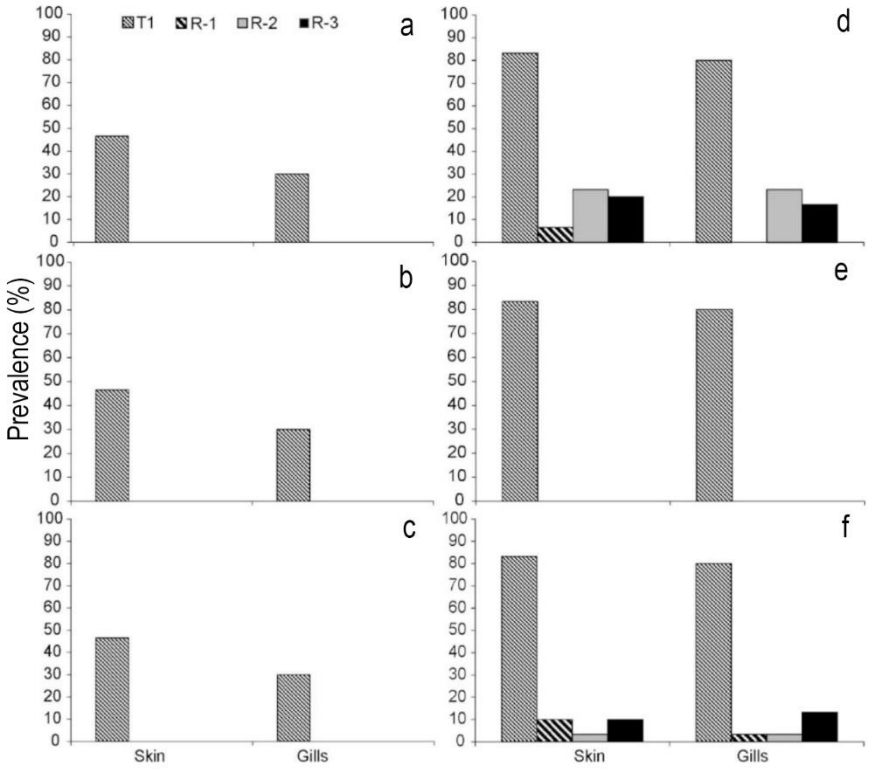

Figure 2. Prevalence of trichodinids in skin and gills in the four treatments using sodium chloride: a) Replicate 1, b) Replicate 2, c) Replicate 3. Prevalence of trichodinids in skin and gills in the four treatments using formalin: d) Replicate 1, e) Replicate 2, f) Replicate 3.

Table 2. Averages of size and range from minimum to maximum intensities of infection in examined fishes. R: replicate, NaCl: sodium chloride, Min: minimum, Max: maximum.

NaCl	Average size (cm)	Values of intensities Min-Max range		Formaldehyde	Average size (cm)	Values of intensities Min-Max	
		Skin	Gills			Skin	Gills
T1(0)-R1	2.35	1-5	0-1	T1(0)-R1	4.98	2-10	1-12
T1(0)-R2	4.17	1-20	1-20	T1(0)-R2	4.67	1-25	1-15
T1(0)-R3	5.66	2-5	1-2	T1(0)-R3	4.98	25-75	5-20
T2-R1	2.42	0	0	T2-R1	4.99	1-4	0
T2-R2	2.44	0	0	T2-R2	4.56	1-15	1-3
T2-R3	2.39	0	0	T2-R3	4.97	1-8	1-2
T3-R1	4.17	0	0	T3-R1	4.15	0-1	0-1
T3-R2	3.97	0	0	T3-R2	4.35	0-1	0-1
T3-R3	4.68	0	0	T3-R3	4.55	0-1	1-10
T4-R1	5.01	0	0	T4-R1	4.99	0	0
T4-R2	4.77	0	0	T4-R2	4.56	0	0
T4-R3	5.43	0	0	T4-R3	4.97	0	0

In Treatment 1 (control) of NaCl, the highest infection intensity occurred in Replicate 2 (fish from 4.8 to 5.5 cm) with an infection intensity of 5-20 trichodinids. In the case of formaldehyde, the highest infection intensity occurred in fish from 5.3 to 5.4 cm total length, with an average intensity of 15-20 trichodinids per field in the Replicate 3 of Treatment 1 (control). The organisms most susceptible to infection by trichodinds were fish (3-6 cm of total length).

In the values of water quality parameters (Table 3), it was observed that although there were slight variations in pH and oxygen, there were no variations

Table 3. Physical and chemical parameters measured during the treatments. The values obtained in each replicate and the averages of each parameter per treatment are shown.

Treatments	Parameter	Unit	R1	R2	R3	Average
NaCl						
1 Control	pH	-	8.3	6.9	7.7	7.6 ± 0.70
	Dissolved oxygen	mg L^{-1}	7.07	6.14	6.14	6.45 ± 0.54
	Temperature	°C	28.2	27.4	27.1	27.5 ± 0.57
2	pH	-	7.8	8	7.9	8 ± 0.10
	Dissolved oxygen	mg L^{-1}	7.08	7.02	7.06	7.05 ± 0.03
	Temperature	°C	28.2	28.2	28.2	28.2 ± --
3	pH	-	7.5	7.8	7.8	7.7 ± 0.17
	Dissolved oxygen	mg L^{-1}	7.54	7.05	7.35	7.3 ± 0.25
	Temperature	°C	26.5	26.4	26.3	26.4 ± 0.10
4	pH	-	7.7	8.3	8.2	8.3 ± 0.32
	Dissolved oxygen	mg L^{-1}	6.14	6.63	6.76	6.8 ± 0.33
	Temperature	°C	27.1	25.9	25.8	25.7 ± 0.72
Formaldehyde						
1 Control	pH	-	7.8	6.8	7.7	7.4 ± 0.55
	Dissolved oxygen	mg L^{-1}	7.02	7.04	7.18	7.08 ± 0.09
	Temperature	°C	27.9	24.7	25.3	25.9 ± 1.70
2	pH	-	7.7	8.3	8.3	8.1 ± 0.35
	Dissolved oxygen	mg L^{-1}	7.03	7.09	7.03	7.1 ± 0.03
	Temperature	°C	26.9	26.7	26.7	26.8 ± 0.12
3	pH	-	6.5	6.6	6.7	6.6 ± 0.10
	Dissolved oxygen	mg L^{-1}	7.04	7.19	7.17	7.1 ± 0.08
	Temperature	°C	24.6	24.6	24.6	24.6 ± --
4	pH	-	6.5	6.6	6.8	6.6 ± 0.15
	Dissolved oxygen	mg L^{-1}	7.37	7.34	7.36	7 .4 ± 0.02
	Temperature	°C	24.6	24.5	24.6	24.6 ± --

outside the optimal ranges for any of the parameters recorded in the different treatments.

The reactions of the organisms manifested different behaviors in response to the doses of the treatments applied once exposed to the doses of the treatments applied. In NaCl, fish were initially lethargic in the bottom of the fish tank, becoming active after 1-2 min in the dose at 30 g L^{-1}. For 10 min, fish were lethargic, darkening skin, reducing swimming behavior. At the end of the treatments (around 20 to 30 min in the recovering tank with clean water), fish returned to their normal pigmentation and behavior back to normal as well.

Organisms exposed to formaldehyde treatment at a dose of 0.200 mL L^{-1} for 2.5 min at the beginning of treatment were initially lethargic and were active after 1.45 min. In the dose at 0.230 mL L^{-1} for 3 min, there was no significant change in their behavior displayed. In the dose at 0.250 mL L^{-1} for 10 min, fish were agitated immediately upon entering water treatment and despite there being no change in dissolved oxygen during the experiments. Fish were aggregated around the oxygen source at different times during the treatment. In this last dose, all the organisms changed from their initial coloration to a darker one during the whole treatment, a possible manifestation of the stress during the trials. During treatments at different doses, only two deaths were recorded during the sodium chloride treatment. Therefore a 99% survival was achieved.

The abundance of trichodinids varied significantly (KW, H (3, N = 120) = 16.54, $P < 0.01$) between formalin treatments. Through the multiple comparisons test, it was determined that the abundance of trichodinids in the concentrations of 0.250 mL L^{-1} 10 min^{-1} and 0.230 mL L^{-1} 3 min^{-1} were significantly ($P < 0.01$ and $P < 0.05$, respectively) lower than in the control.

Likewise, it was found that the abundance of trichodinids also varied significantly between the different treatments with NaCl (KW, H (3, n = 120) = 11.71 $P < 0.01$). In this case, the multiple comparison tests showed differences between the doses of 10 g L^{-1} 3 min^{-1} and that of 20 g L^{-1} 5 min^{-1}, being significantly lower ($P < 0.01$) the abundance of trichodinids in the first dose (10 g L^{-1} 3 min^{-1}). It should be noted that the

Figure 3. a) Trichodinids in a fresh-mounted smear from the gill arches, b) differential interference contrast microscope, c) an adhesive disc of *Trichodina* in silver nitrate impregnation, d) *T. pediculus*, e) *T. compacta*, f) *T. nigra*, and g) *T. centrostrigeata*. Scale bars: a,e,g = 50 µm; b,c,d,f = 20 µm.

abundance of trichodinids in the control group did not differ significantly with the abundance recorded in the individuals of the three NaCl doses. In this study, new reports of trichodinid specimens identified in tilapia cultivated in Tabasco, Mexico are described (Fig. 3).

DISCUSSION

Trichodiniasis is one of the major diseases that occur in aquaculture and some monogeneans. Recently, Aguilar-Aguilar *et al.* (2014) provided a wide helminth species inventory parasitizing these fish taxa. However, the protozoan parasites occurrence on these fish has not been documented. A few knowledges about the effects of this disease on farming or aquaculture conditions. According to Valladão *et al.* (2015, 2016) the trichodinid species that are most frequently reported as parasites of Nile tilapia are *Trichodina centrostrigeata* Basson, Van As & Paperna, 1983, *T. compacta* Van As & Basson, 1989, *T. magna* Van As & Basson, 1989 and *Paratrichodina africana* Kazubski & El-Tantawy, 1986; which have also been identified in South America (Valladão *et al.*, 2013). In this study, new reports of trichodinid specimens identified in tilapia cultivated in Tabasco, Mexico are described (Fig. 3).

The more susceptible organisms to trichodinids infection were fish (3-6 cm in total length). The larger fish are most affected because the infection can be cumulative and damage may be associated with other parasites, such as *Tilapia mossambica*, probably caused by a fungus described by Bunkley-Williams & Williams Jr. (1995).

The organism's behavior during the experiment showed different reactions once exposed to doses of treatments applied. When introduced to sodium chloride, some fish showed a lethargic swim behavior and moved towards the bottom of the tank; they were active after ~2 min. In the dose to 30 g L^{-1} for 10 min,

fish were lethargic with a slight change of skin color (darker), however, when fish were placed in clean filtered water, all fish returned to their natural skin coloration ~20 min after treatment. Throughout all treatments, at different doses, a survival rate of 99% was obtained.

During this study, the presence of other organisms, *i.e.*, protozoa (mixosporidis, ciliates, and flagellates), was noted. Also, the presence of amoebae was observed which, after being exposed to doses of NaCl 10 g L^{-1} for 3 min, were still active. Within the group of helminths, monogeneans were recorded in skin and gills. However, the identification was not considered for the aims of this research. It was observed that high doses of both substances evaluated in this study caused a decrease in activity, in all parasites, so further tests are recommended to evaluate other doses and to extend the exposure times in order to eliminate or reduce these pathogens.

Vargas *et al.* (2003) used formalin and sodium chloride as treatments in their study, these authors recorded a prevalence of 43% in the control group and using a dose of 3% sodium chloride for 10 min reduced the prevalence to 2%. For formalin, the doses of 50 and 250 ppm both for 60 min reduced the number to 32 and 9%, respectively, for both doses. These authors worked with fry sexually reversed fry (length ~2.77 cm) of *O. niloticus*.

In this study, the major intensity of infection occurred in the larger sizes (4-6 cm) with intensities of infection of 10-75 trichodinids on the skin and 10-20 trichodinids on gill arch. Control replicas of gill treatments did not show significant differences in the intensity of infection, however, in the values obtained from skin, significant differences in the intensity of control treatments were presented. These differences could be due to differences in sampling time, climatic changes, conditions, and variations of the culture system (management), environmet temperatures could contribute to the increase or decrease of trichodinids in the control groups (Valladão *et al.*, 2013, 2014).

Zanolo & Hissashi (2006) mentioned that monogeneans and ciliated protozoa as trichodinids are usually found in aquatic environments and should be kept under constant monitoring of environmental parameters. Regarding the quality parameters of water, in this study the main change observed was a slight water acidification (pH 6.6), however, trichodinids decreased, this could be due to the effect of increasing formalin dose of 0.230 mL L^{-1} for 3 min 0.250 mL L^{-1} for 10 min, both showed 100% effectiveness.

Apella & Araujo (2005) mentioned that all organisms have mechanisms of regulation of the cytoplasmic pH that allows them to maintain their homeostasis. Maintaining a constant pH in the cytoplasm is very important for the survival of microorganisms and acidification or alkalinization leads to a decrease in the function of vital cell components. In this work, it was observed that both sodium chloride and formaldehyde are effective for controlling trichodinids with both dose and exposure time.

The action mechanism of sodium chloride, which eliminates protozoa and bacteria (Kirschner, 2004), controlled by an osmotic pressure difference, and for formalin, is alkylation of chemical groups of proteins, and nucleic acids. Alkylating agents are generally attached to the methyl or ethyl group of proteins and DNA, translating these molecules as nonfunctional and cause the death of the microorganism (Lim, 1998). As for possible damage, observations of the gills in organisms exposed to formaldehyde treatments showed inflammation in the gill lamellae.

Gills are the main target organ for pollutants (Biagini *et al.*, 2009); thus, the gill epithelial tissue is an excellent parameter to evaluate the effects of environmental variables, toxics and water quality (Wong & Wong, 2000; Verján *et al.*, 2001; Mazon *et al.*, 2002). Formalin and sodium chloride are used as chemotherapeutic agents and may cause damage to branchial tissue because these are substances that have a toxic effect on the fish as observed by Mert *et al.* (2014). Color changes also occurred in fish exposed to doses of sodium chloride and formaldehyde treatments at different doses, observed as darkening of the skin in response to the treatments. Nouh & Selim (2013) presented similar results in a study by exposing to *O. niloticus* (25 mg L^{-1}) of formalin, with organisms presenting a darkening of the skin among other symptoms. The change in color according to Reichenbach-Klinke (1975) and other fish (Kalogianni *et al.*, 2011; Movahedini *et al.*, 2012; Clifford *et al.*, 2017; Correia *et al.*, 2017) can have various causes, including the presence of toxic substances in wastewater, such as chlorine.

The use of chemicals is regulated by official standards and management of its adequate disposal to each chemical protocol to prevent damage to the environment and can be helpful in controlling diseases of organisms in an aquaculture system. However, formaldehyde (Pahor-Filho *et al.*, 2012) caused mild to severe hyperplasia and detachment of respiratory epithelium in the gills of juvenile *Mugil liza* (Valenciennes, 1836). The tolerance to formaldehyde may be fish species-specific; it may also affect swimming performance at every developmental fish stage, *i.e.*, larvae or adult. Unfortunately, in this study, the possible effect of formaldehyde on the tilapia host

was not considered. To the best of our knowledge, no fish used in the experiments showed any erratic behavior or skin disorder. There is a lack of knowledge regarding parasitic disease treatments for fish worldwide. In consequence, the effectiveness of some treatments is not entirely understood. Nevertheless, further studies are still needed to determine and validate the use of chemicals to control ectoparasites on fish and to establish appropriate doses according to the tolerance of the species of organism to be treated, (*i.e.,* in regions with a tropical clime). In the present study, it was observed that the parasites (monogeneans) associated with trichodinids only decreased the intensity of infection within the higher doses of treatments applied (observational data).

Finally, the results in this study indicated that 10 g L^{-1} for 3 min, 20 g L^{-1} for 5 min, and 30 g L^{-1} for 10 min treatment with NaCl are 100% effective in eliminating the infection trichodinids in *O. niloticus*, and formalin 0.250 mL L^{-1} for 10 min was 100% effective in eliminating infection. Doses of 0.200 mL L^{-1} for 2.5 min and 0.230 mL L^{-1} for 3 min decreased only the trichodinids infection by 40% and 60% for these treatments. Taking into account that trichodiniasis presents pathogenicity causing death in fish and larval stages (Valladão e*t al*., 2015), it poses a significant threat to fish aquaculture as they have the potential to cause vast economic losses. Therefore, the implementation of preventive measures during early production fish stages strategies is highly recommended. Treatment with formaldehyde at a concentration of 10 g L^{-1} used for 3 min (sodium chloride) proved to be effective.

For this reason, the present study shows the effectiveness of this treatment in cultured Nile tilapia *O. niloticus* in aquaculture activities in Mexico. This study presented two effective treatments however chemical treatments commonly used (*e.g.,* formalin) may cause environmental hazards including antihelmintic resistance, the risk of residues and toxicity to fish. In this context, the anti-parasitic efficacy of "natural" or "environmentally friendly" alternatives to control fish diseases in this particular region (*i.e.*, sodium chloride), or further biodegradable treatments, will be evaluated for commonly occurring fish diseases. We may suggest using the implementation of the natural management system, which may cause less environmental impacts and thus further investigation is required in this context, the use of phytotherapy is a reliable option, Valladão *et al*. (2015) performed an extensal review on this topic. For instance, further natural treatments might be tested for its efficacy in suppressing ectoparasites, along with several other, potentially active metabolite extracts from natural sources. The findings regarding the parasite and host relationship suggest that fish under captivity are highly affected by trichodinids and hence should be the central area of focus for the prevention and control of this type of parasitism.

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