



## A Study on Trichodiniasis in Farmed Nile Tilapia and Gold Fish at Sebeta National Fishery and Other Aquatic Life Research Center, Central Ethiopia

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### Abstract

Ethiopia has large water resources with estimated surface area of 733 km<sup>2</sup> of major Lakes and dams, 275 km<sup>2</sup> of small water bodies and 7,285 km long rivers within the country. The country has home of highly diversified flora and fauna. More than 200 species of fish are present in lakes, rivers and dams of Ethiopia. A cross sectional study was conducted from November 2017 to April 2018 in Sebeta National Fishery and Other Aquatic Life Research Center, central Ethiopia to study clinical sign, prevalence and risk factors of in Trichodiniasis in fish. 384 Live fish were collected randomly after the fish were collected by fisher man using fish net. Wet smear technique was conducted from gill tissue biopsy and skin scraping. The diseased fish had signs of dullness, detachment of scales, excessive mucus accumulation, ulcerations, congested fin base and pale gill. Out of 384 fish, 233 (60.7%) were found to be positive for trichodiniasis. Analysis of possible risk factors has shown that, there was statistically significant association ( $P < 0.05$ ) between prevalence of disease and sex, parasites infestation, examined organs, body weight and total body length. However, the difference was not statistically significant ( $P > 0.05$ ) between disease occurrence with species and pond number in area. In conclusion, the present finding has demonstrated that trichodiniasis is one of the important parasites of fish in the study area. Therefore, further epidemiological investigations are required to determine the *trichodina* species and different risk factor on the occurrence of the disease.

**Keywords:** Fish; Prevalence; Sebeta Trichodina; Trichodiniasis

### Introduction

Ethiopia is a land-locked country which has approximately 7400 Km<sup>2</sup> surface area of water body and 7185 Km<sup>2</sup> long river network and endowed with sizable amount of lotic and lentic environments [1]. The numbers of constructed water dams are also in progress for hydroelectric power generation and irrigation purpose including the Great Renaissance Dam. As a result of these ecological variations, the country has been the home of highly diversified flora and fauna. More than 200 species of fish are known to present in lakes, rivers and dams of Ethiopia [2]. The annual fish production potential of the country was estimated to be 30.000 to 51.000 tons. Despite the availability of huge potential for fish production, the country has annual consumption of 240g per person, which is the lowest in Africa [3]. But the sector has different

challenges like lack of recognition, absence of universities dealing fisheries and aquaculture, lack of trained personnel, poor coordination among stakeholders, poor enforcement of decrees and high turnover of the fishery staffs into other sectors and weak extension services and linkage [4].

Fish are cold-blooded most diverse group of vertebrate animals occupying a variety of marine, fresh and other water habitats. Each species of fish has preferred ranges for the various parameters of water quality, such as temperature, dissolved oxygen and salinity [5]. Their diversity is reflected in the large number of living species with over 33,984 known species in world (Igor., et al. 2017).

Like humans and other animals, fishes may suffer from diseases which lead to severe economic losses and like other farming sec-

tors, disease problems increases as aquaculture activities are intensified and expanded. Bacteria, virus, fungal and parasitic disease are affect fish and among this parasitic diseases have great economic particularly in the tropics [6].

Fish diseases now are widely spread due to high water pollution which changes the water quality that reduces the immunity of fishes to diseases [7]. Most of fish diseases especially in warm water fishes might be occurred as a result of parasitic infections that are caused by ectoparasitic [8] and are typically present either on the surface of the fish, within the gills or both [9]. The intensification of fish in the farms creates disease problems that originate from overcrowding and deteriorating water quality. In such cases of stress, several species of parasites may become pathogenic interfering with feeding and respiration of fishes. The intensification of fish in farms deteriorating water quality such as unsuitable water temperature, pH, carbon dioxide and free ammonia concentrations create the disease problem. Also the water pollution accelerates the life cycle of the parasites and promotes their spread [10].

Trichodiniasis (slime disease) is protozoan diseases caused by ectoparasitic *Trichodina*. *Trichodina* species are the most identified pathogenic protozoan ectoparasites where they can easily spread among most of fish hosts causing serious pressures to fish, particularly under culture conditions [11]. It is frequent in freshwater and marine fishes that are stressed by harsh conditions, overcrowding and high water pollution. *Trichodina* present on the fins, skin and gill arches [12]. In the case of light infection they act as ecto-commensals and the infected tissues remain in a good health condition and when high number of *Trichodina* is present then they considered a pathogenic ciliates and cause severe injury. In general, fish organs differ from the other exposed vertebrate organs because of its watery environment, the living epidermal cells of fish skin are in direct contact with the environment and subjected to at least two types of stresses, osmotic pressure gradients between the cells and the water and physical forces originating from the water itself, the other environmental hazards as rocks and from the harming organisms such as fungi, bacteria, and water-born parasites to the skin [10].

In Ethiopia a few studies were conducted on fish production, parasite species [13]. Study on temporal variation of fish parasites [14]. Study on temporal variation of internal fish parasites [3]. Therefore the objectives of this thesis are aimed;

- To determine the prevalence of trichodiniasis in cultured fishes at National Fishery and Other Aquatic Life Research Center.
- To study the clinical signs fish infected by trichodiniasis
- To identify risk factors associated with prevalence trichodiniasis.

## Material and Methods

### Study area

A cross sectional study was conducted from November, 2017 to April, 2018 in central Ethiopia, Oromia region at Sebeta National Fishery and Other Aquatic Life Research Center which located in the town of Sebeta, 24kms south west of Addis Ababa city. Sebeta fish farm has established in 1977 under the ministry of agriculture now called National Fishery and Aquatic Life Research Centre. Now it is under Ethiopian Institute of Agricultural Research with a mandate of conducting research related to fish and other aquatic life resources, source of fish fingerlings and training of personnel's from different institutions of the country. The center has 32 fish rearing and experimental ponds constructed from earthen ponds, concrete ponds and lined ponds. The fish species in the center include *Oreochromis niloticus*, *Clarias garipinus*, *Cyprinus carpio*, and *Tilapia zilli* and *Carasius auratus*. It is situated at 2200m above sea level and an altitude of 8°55'N 38°37'E covering a total area of 16 hectare. The area is characterized by a moderately warm climate with annual mean temperature of about 21°C. The area gets annual rainfall of about 866–1200 millimeters. Physicochemical parameters of water (dissolved oxygen, PH, temperature and conductivity) in study area range are 4.5-8 mg/L, 6.5-9, 20-30°C and 30500µc/cm respectively [13].



**Figure 1:** Sebeta National Fishery and Other Aquatic Life Research Center ponds (NFALRC).

### Study design and sampling technique

Cross sectional type of study was conducted from November 2017 to April 2018 at Sebeta National Fishery and Other Aquatic

Life Research Center to study clinical sign, the prevalence of trichodiniasis and risk factor on cultured *N. tilapia* and Gold fish. Simple random sampling method was employed to select the study fishes after the fish were collected by fisher man using fish net. During sample collection necessary risk factors were properly taken (sex, species and pond number).

### Sample size determination

The sample size was determined according to Thursfield [15] by considering 50% expected prevalence whenever there was no information about the prevalence of the disease in area, 95% confidence level and 5% precision. The sample size was calculated as follows.

$$n = \frac{1.96^2 \times P \times \exp(1 - P \times \exp)}{d^2}$$

Where: n = required sample size

P exp = expected prevalence

d = desired absolute precision

Therefore, 384 fish were sampled to study the prevalence of trichodiniasis.

### Study animals

The study was conducted on Nile tilapia (*Oreochromis niloticus*) and Goldfish (*Carassius auratus*). *N. tilapia* was collected from pond number one and hatchery house aquarium (from number 29-38) and Goldfish from pond number seven.

### Study methodology

#### Sample collection

During the period of 14-11-2017 to 27-4-2018 a total number of 384 fish were collected from Sebeta National Fishery and Other Aquatic Life Research. Out of collected fish *N. tilapia* (*O. niloticus*) was 259 and 125 Goldfish (*C. auratus*). Out of 384 fishes 317 fishes are collected from pond and 67 fishes are collected from hatchery house aquarium. Then a live fish were transported to the parasitology laboratory of National Fishery and Other Aquatic Life Research Center by using plastic containers that partially filled with pond water which fish were collected. In laboratory the fish body weight and total body length was measured by using balance and long ruler respectively. The body weight of examine fishes range

from 6-225g with mean of 43.33g and total body length are range from 5-26cm with the mean of 13.96cm. These are according to Harpreet., *et al.* [16].

### Clinical examination

The external body surface of the investigated fishes was carried out on a live fishes in glass aquaria and plastic container. Skin, fins, gills and other external features were examined and gills were examined by lifting operculum [17]. Clinical signs and abnormalities appeared on the body surface of diseased fishes were registered.

### Parasitological examination

Scraps from skin were prepared by scraping the body surface from cranial to caudal and wet smears were made on dry clean slides with a drop of water from pond which fish was collected then covered by cover slip and examined under microscope (40X) lens. Gill tissue was prepared by cutting primary gills lamella, then put on microscope slide covered by cover slip and examined under microscope in order to detect the presence of *trichodina* parasites.

### Statistical analysis

The raw data were entered and managed in Microsoft excel worksheet and analyzed using SPSS-version 20 software. Descriptive statistics and chi-square test were employed to determine the prevalence of trichodiniasis and association of risk factors with the disease respectively. Level of significance was considered at  $p < 0.05$ . The prevalence (%) of the parasite was estimated between numbers of infected fish and the number of examined fish expressed in percentage.

## Results

### Clinical signs

Clinical signs appeared on both *N. tilapia* (*O. niloticus*) and Goldfish (*C. auratus*) showed dullness and detachment of scales. Excessive mucus accumulation and ulcerations were observed. Bases of some fins were congested. Gills of some fishes were pale white and congested in others with excessive accumulation mucus.

### Parasitological Isolation

In fresh wet mount preparation, the parasite is very motile and appears as a circular shaped ciliated organism which surrounded with several circular rows of cilia and supported with a circle of more centrally lying hook lets.



Figure 2: *N. tilapia* (*O. niloticus*) species in pond.

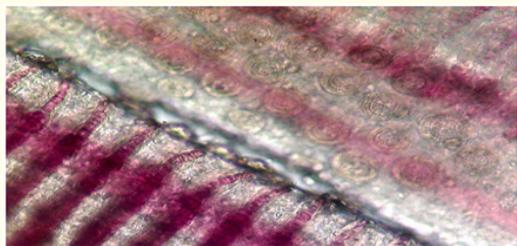


Figure 3: *Trichodina* isolated from gill tissue.



Figure 4: *Trichodina* isolated from skin

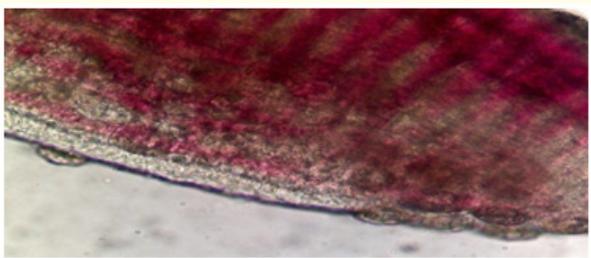


Figure 5: *Trichodina* with adhesive disc isolated from gill tissue.

### Overall prevalence of trichodiniasis

Out of 384 examined fish during the study period (December, 2017 to April, 2018) 233 were infected with trichodiniasis (Table 1).

Examined fishes	Positive for trichodiniasis	%
384	233	60.7%

Table 1: Overall prevalence trichodiniasis.

### Prevalence of trichodiniasis in relation to specie

The prevalence of trichodiniasis was high in Goldfish (*C. auratus*) species (64%) and the lowest prevalence has been recorded in *N. tilapia* (*O. niloticus*) species (59.1%). The Analysis shows there was no statically significance association between the prevalence of trichodiniasis and examined fish species ( $P = 0.509$ ) (Table 2).

Species	Examined fishes	Positive (%)	$\chi^2$	p-value
Gold fish	125	80(64%)	1.351	0.509
Nile tilapia	259	153(59.1%)		
Total	384	233(60.7%)		

Table 2: Prevalence of trichodiniasis in relation to species.

### Prevalence of trichodiniasis in relation examined organs

The prevalence of trichodiniasis on both skin and gill (37%) showed higher prevalence. There was a statistically significant association between prevalence of trichodiniasis and the infected organ ( $P = 0.000$ ) (Table 3).

Examined organ	Examined fish	Positive (%)	$\chi^2$	P-value
Skin	384	79(20.6%)	3.8	0.000
Gill	384	13(3.4%)		
Both	384	142(37%)		
Total	384	233(60.7%)		

Table 3: Prevalence of trichodiniasis in relation to infected organs.

### Prevalence of trichodiniasis in relation to sex category

The prevalence of trichodiniasis in female fish (68.1%) was higher that of male fish (58.94%) and relation of sex for the occurrence of trichodiniasis showed that there was statistically significant association ( $P < 0.05$ ) between sex and occurrence of disease (Table 4).

Sex	Examined fish	Positive (%)	$\chi^2$	p-value
Female	233	144(61.8%)	2.123	0.000
Male	151	89(58.94%)		
Total	384	233(60.7%)		

**Table 4:** Prevalence of trichodiniasis in relation to sex category.

In this study, sample was taken from fish that are belongs to different ponds and the highest prevalence was recorded in those fish taken from hatchery house N. tilapia (64.2%) followed by pond number seven or Goldfish (64%) and the lowest prevalence was observed on N. tilapia collected from pond (57.3%). Significant association was not observed between prevalence of the disease prevalence and fish collected area ( $p > 0.05$ ) (Table 5).

Pond	No examined	Positive (%)	$\chi^2$	p-value
No	192	110(57.3)	4.361	0.359
No	125	80(64)		
No	67	43(4.2)		
Total	384	233(60.7)		

**Table 5:** Prevalence of trichodiniasis in relation to ponds.

No= Number; HHA= Hatchery House Aquarium.

### Prevalence of trichodiniasis in relation to body weight and total body length

The prevalence of trichodiniasis was high in fish which has body weight greater than mean value (64%) and the lowest prevalence has been recorded in fish which has body weight less than mean value (59.1%). In case of body length high prevalence was observed in fish that has body length greater than mean value. Analysis of trichodiniasis with both body weight and total body length of the fish has revealed that there was significance association between the prevalence of trichodiniasis and body condition measure ( $P = 0.000$ ) (Table 6).

Body measures	No of examined fish	Positive (%)	$\chi^2$	P-value
BW			4.653	0.000
≤ mean	259	153(59.1%)		
> mean	125	80(64%)		
TBL			1.573	0.000
≤ mean	225	149(58.4%)		
> mean	159	84(65.1%)		
Total	384	233(60.7%)		

**Table 6:** Prevalence of trichodiniasis in relation to body weight and total body length.

### Degree of trichodina infestation

Depend on density of parasites under microscope, 22.7% fish are highly infested, 22.9% fish are moderate infested and 15.1% fish are low infested. There was statically significance association between *trichodina* infestation and occurrence of disease (Table 7).

Infestation	Positive (%)	$\chi^2$	p-value
HI	87(22.7%)	3.5	0.000
MI	88(22.9%)		
LI	58(15.1%)		
Total	233(15.1%)		

**Table 7:** Degree of trichodina infestation.

HI= High Infested; MI= Moderate Infested; LI= Low Infested.

### Discussion

The external fish protozoan parasites such as *Costia*, *Ichthyophthirus multifilius* and *Trichodina* are common in cultured fish as specific phenomena which may external infect fish and strong enough to cause miss shape and sometime be responsible for mortalities in fish population following the epidermal tissue damage caused by the protozoan, bacterial and fungal infection may recognized [18]. *Trichodina* is among one of the most common ectoparasites fish. This parasite lives normally in a few numbers in the mucous surface of skin and gills. When host, parasite and environment relationship is broken by nutritional deficiency, poor water quality and infectious or parasitic proliferates rapidly and become responsible for severe epidermal lesions and disease out breaks. It may cause serious damage to the epithelial or epidermal cells by their constant attachment and also by their movement [19].

Two types of fish species, Nile tilapia (*O. niloticus*) and Goldfish (*C. auratus*) collected and studied from different ponds in Sebeta National Fishery and Other Aquatic Life Research Center for study of protozoa disease. In this study clinical sign of trichodiniasis, and prevalence of trichodiniasis was studied and *Trichodina* parasite was isolated from both *O. niloticus* and *C. auratu*.

The recorded clinical signs were noticed in moderate infestation and highly infestation of parasites. The clinical signs of trichodiniasis in both *O. niloticus* and *C. auratu* were signs of dullness and detachment of scales. Excessive mucus accumulation and ulcerations was observed. Bases of some fins were congested. Gills of some fishes were pale white and congested in others with excessive accumulation mucus. The severities of clinical signs were in correlation with the infestation of *trichodina* parasites. The sign of detachment scale, ulceration, congestion of fin and gill are due to the mechanical action of the cilia of *trichodina* and continuous

movement of the parasite on the surface epithelium of the skin and gills but the massive production of mucus is due to defense mechanism to eliminate the parasite or dilute its irritating effects. In the case of light infection when few numbers of *trichodina* are present on skin and gills no clinical sign was observed. This could be due to they act as ectocommensal protozoans and the infested tissues remain in a good health condition. These findings agreed with study in Sudan by Soliman., *et al.* [20] who studied the clinical signs of trichodiniasis.

For parasitological examination wet mount was prepared, in wet mount preparations, the parasites appeared as circular ciliated organisms which were highly crowding and rapidly motile. Under 40X magnification, the parasite was shown to have several circular rows of cilia and a circle of more centrally lying hooklets (Figure 5). This finding agrees with study in Brazil by Carla., *et al.* [21]. The harmful effects of the parasite resulted from the adhesive disc of *Trichodina* and the sharp rim of the border membrane which bite into the surface of the host epithelial cells and strongly act as a sucker causing host irritation (Figure 6). These activities of the parasites, consequently, lead to severe loss of surface epithelium of skin giving a good chance for secondary pathogens as bacteria or fungi to invade.

Regarding the prevalence of trichodiniasis in examined fishes, the overall prevalence of *trichodina* based on wet smear examination in this study (60.7%) was lower than previous findings reported in Sudan by Samia., *et al.* [18] which is 80%. The lower prevalence of trichodiniasis recorded in this study as compare to the above mentioned areas with the higher prevalence areas could be due to the differences in agroecology, management system and water quality (chemical, physical and biological) content of water in different countries. However, the result of the present study nearly agrees with study in Lake Koftu (71%) and Wonchi farms (56%) by Marshet [13]. Tadesse [22] also reported prevalence trichodiniasis ponds of Yemlo and Wonji with a prevalence of 56.7 and 46.7% respectively. Moreover, this could also be due to the fact that the study has been undertaken mainly in different area in which water quality has different ranges of dissolved oxygen, PH, temperature and conductivity.

In this study, the prevalence trichodiniasis between species was 64% in *C. auratus* and 59.1% in *O. niloticus*. This is due to *O. niloticus* more resistant and has ability to adapt poor water quality

when compared with *C. auratu*. There was not statistically significant association ( $P > 0.05$ ) between fish species and trichodinosis disease. This finding agrees with the report in Sudan by Samia., *et al.* [18] who said that *trichodina* are have low host specificity and are therefore, widely most families of fresh water fish.

The stronger association ( $P < 0.05$ ) of the infection with trichodiniasis in relation to the infected organ has been demonstrated in this study. Higher prevalence was observed for both (37%) followed by skin (20.6%). Skin was more affected than gill (3.1%). This could imply that skin more exposed to parasites than gill. The finding agrees with report in Sudan by Samia., *et al.* [18].

The study has shown that the higher prevalence of the disease condition was observed in pond number seven (64.5%), followed by hatchery house aquarium (64%) and lower in pond number one (57.3%). This small difference was might be in relation to water quality which pond number one got fresh water daily than pond number seven and temperature difference between hatchery house and pond. There was not statistically significant association ( $P > 0.05$ ) between trichodiniasis and ponds. This may be due to *trichodina* parasites was worldwide distribution.

The prevalence in female fish (61.8%) was different to that of males (58.94%) in this study. Higher prevalence in female fish could be due to the physiological stress loaded on female animals due to hatch eggs in their mouth until eggs develop to larva as compared to male fish. Also there was statistically significant association ( $P < 0.05$ ) between sex and trichodiniasis disease. This finding was agrees with study in Lake Elan by Gebawo [14].

In the present study, there was an increase trichodiniasis disease in fish which has body weight greater than mean value (64%) as compared fish has body weight less than mean value (59.1%) and also prevalence of trichodiniasis higher in fish which has total body length greater than mean value (65%) and lower prevalence in fish has total body length less than mean value (58.4%). These is may be due surface area of large fishes are small which increases the chance for parasites to infested their host. There was statistically significant association ( $P < 0.05$ ) between total body length and body weight and trichodiniasis disease. The current study disagrees with Lue., *et al.* (1999) report that prevalence of infection decreased with increasing fish size and with study in Sudan by Samia., *et al.* [18].

Analysis of parasites infestation in the association of disease occurrence has revealed that, there was statistically significant association ( $P < 0.05$ ) in between degree of infestation and trichodiniasis prevalence. These indicate that number of parasites have influence on the occurrence of disease. This finding agrees with the study in Egypt by Mohamed *et al.* (2015).

### Conclusion and Recommendations

Trichodiniasis affects freshwater fishes causing serious damage to fish. The severity of disease depending on the density of *Trichodina* parasites and parasites appeared as circular ciliated organisms which were highly crowding and rapidly motile. Dullness, detachment of scales, excessive mucus accumulation, ulcerations, congested fin and pale gill were observed clinical sign in case of moderate and highly infested fish. The study showed that trichodiniasis is prevalent in Sebeta National Fishery and Other Aquatic Life Research Center central Ethiopia. The prevalence of trichodiniasis has no significant association with pond number and species of fish during the study period. However, the disease has a significant association with sex, examined organs, infestation of parasites, body weight and total body length. Fish higher body weight and body length than mean value were highly affected with the infection of trichodinosis. Skin of fish was more likely to be affected by trichodinosis than gills. In this study high prevalence of the disease was observed in Goldfish (*C. auratu*) than Nile tilapia (*O. niloticus*). In general, results from this study indicate that trichodinosis infection has a great significance for the fish culture [23-33].

Therefore, based on the findings the following recommendations are forwarded:

- Infected fish should get treatments to ensure their immune status.
- Managemental practice like overfeeding, overproduction and irregularly water change which initiates infestation of parasites should be avoided.
- Serious preventive mechanism should be set up in the center.
- Further epidemiological investigations are required to determine the *trichodinia* species and different risk factor on the occurrence of the disease.

### Declaration

This thesis paper entitled as "A Study on trichodinosis in Sebeta National Fishery and Aquatic Life research center, Central Ethiopia"

has been submitted by MILLION SHIFERAW for presentation with my approval as academic advisor.

### Signature

Advisors name: MESELU AHMED (DVM, MSc).

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