

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan



Research Article

Control of *Riboscyphidia* sp. (Ciliate) Infection in Asian Sea Bass (*Lates calcarifer*), Cultivated in the Red Sea

¹Hussien A.M. Osman, ²Abeer E. Mahmoud and ¹Amany M. Kenawy

¹Department of Hydrobiology, National Research Centre, Dokki, Giza, Egypt

²Department of fish diseases, Animal Health Research Institute, Assuit Laboratory, Agriculture Research Center, Dokki, Egypt

Abstract

Background and Objective: Sessile ciliates live as eco commensals (low numbers) and parasites (high numbers) on different hosts, like mollusks copepods, mysids and fish. *Riboscyphidia* ecto- protozoan is moderately pathogenic but high numbers of it on the gills can physically prevent gas exchange. The present study aimed to describe the epizoic ciliates *Riboscyphidia* found on the Red Sea cultured Asian sea bass and obtain more information on the Epidemiology of the parasite with special references to control and histopathological examination of naturally infected sea bass. **Materials and Methods:** The occurrence of epizoic ciliates on the adult Asian Sea bass. About 100 Asian sea bass were collected by the fishing net at a private marine fish farm at Ismailia governorate and transferred to the hydrobiology laboratory at National Research Centre. A parasitological and histopathological study of epizoic sessile ciliate species was done. ANOVA test was used for Statistical analysis. **Results:** *Riboscyphidia* sp. was found and isolated after parasitological examination of investigated adult's Asian sea bass. The prevalence of Riboscyphidiosis was 64%. Sessile ciliates were found on gills, skin and fins. The clinical signs of Riboscyphidiosis were respiratory distress, flashing and off food. Histopathological alterations in naturally infested Asian sea bass were investigated. **Conclusion:** The treatment of choice of Riboscyphidiosis was prolonged immersion by Copper citrate with a dose of 0.56 mg mL⁻¹ for 7 days.

Key words: *Riboscyphidia*, Asian sea bass, epizoic ciliates, histopathological, copper citrate, prolonged immersion

Citation: Osman, H.A.M., A.E. Mahmoud and A.M. Kenawy, 2021. Control of *Riboscyphidia* sp. (ciliate) infection in Asian sea bass (*Lates calcarifer*), cultivated in the red sea. Pak. J. Biol. Sci., 24: 19-24.

Corresponding Author: Hussien Abd El-Fattah Mohamed Osman, Department of Hydrobiology, National Research Centre, 33 Al Bohooth St., 12622, Dokki, Giza, Egypt Tel: 00201006554214

Copyright: © 2021 Hussien A.M. Osman *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The intensive fish breeding leads to the recognition of several serious infections of which ectoparasites are striking by their rapid development spread by direct dissemination among all living in close contact.

One of the largest and most widely distributed mobile ciliate genera is *Trichodina* present on aquatic animal's hosts¹. Sessiline ciliates live as commensals (low numbers) and parasites (high numbers) on different hosts, like mollusks², copepods, mysids^{3,4} and fish⁵. At high numbers, these ciliates have been reported as causing mortality in juvenile and adult cultured fish, causing severe economic losses in different localities of the world⁶.

The ectocommensal ciliates *Riboscyphidia* attach to the gills or skin with a holdfast⁷. Attachment causes superficial destruction to the cells⁸. Like the sessile, colonial commensals, reproduce by simple binary division and use the host primarily for nutrition and attachment. They derive little, if any, nutrition directly from the host. They feed also on bacteria and suspended organic debris, which is prevalent in food. Thus, they are a good indication of bad water quality⁹.

Riboscyphidia ciliates parasites for fish, when presented on the fish with low numbers but when the fish stressed (Infected with bacteria, parasites or protozoa or change in the water parameters) act as ectocommensal. Literature about *Riboscyphidiosis* was scanty and a lot of data was missed about non-motile (Sessiline) ciliates. Thus, the present study aimed to investigate the epizootic of ciliates infect the Red Sea cultured Asian sea bass and obtain more information on epidemiology of the parasite depending on host characteristics and environmental factors and searching for the method to control infection of *Riboscyphidia* in such fish.

MATERIALS AND METHODS

Studying area: The study was carried out at the Department of Hydrobiology, National Research Centre, Dokki, Giza, Egypt, from September-December, 2019.

Naturally infected fish: Total 100 (1-2 kg) the Red Sea cultured Asian sea bass was collected from private marine fish farm cultured in circular cement ponds at Ismailia province, collected in plastic bags with oxygen and transferred to the Department of Hydrobiology, National Research Centre, Dokki, Giza. Clinical, post mortem and parasitological examinations were done.

Parasitological examination: Scraping from skin, fins and gills were taken, spread on glass slides, dried, fixed with methanol and stained with potassium permanganate and covered with cover slides. Gill filaments were cut and placed in a Petri dish filled with filtered Seawater and examined with a compound microscope (X40)¹⁰.

Isolation and identification of the parasite: Wet mounts from skin or gills with parasites were prepared and examination was done through the light electric compound microscope using high power (X40)⁹. Prevalence of infection of cultured Asian sea bass with *Riboscyphidia* sp. was also determined.

Treatment design: Naturally infected 120 Asian sea bass were divided into four groups each group 10 with three replicates, 1st group subjected for treatment with formalin bath 150 ppm for 60 min and the 2nd group subjected for treatment with formalin bath with 250 ppm for 30 min. While, the 3rd group was subjected to treatment with chelated copper-like copper citrate (2.23 g+5 H₂O+1.5 g citric acid) 0.56 mg mL⁻¹ for 7 days (prolonged immersion), while 4th group was set as control group without treatment (Table 1).

Histopathological studies: Histopathological samples were taken from the skin, fins, gills and musculature of naturally infected investigated fish, Asian sea bass fixed in 10% formol saline for 24 hrs. Samples were prepared and examined according to Bancroft and Stevens¹¹.

Statistical analysis: Data were analyzed for significant differences using the ANOVA test at p<0.05 using SPSS program version 9.0.

Table 1: Treatment of *Riboscyphidia* infection in the Red Sea cultured Asian sea bass

Groups	Drug	Number of fish	Treatment type	Dosage of drug	Duration
1st	Formalin	10	Bath	150 ppm	60 min
2nd	Formalin	10	Bath	250 ppm	30 min
3rd	Copper citrate	10	Prolonged immersion	0.56 mg mL ⁻¹	7 days
4th	Control	10	No treatment	----	-----

RESULTS

Clinical signs and postmortem lesions: Infested Asian sea bass showed, lethargic, flashing, swimming upside, surfacing, gulping the atmospheric air and even jumped outside water, sloughed scales especially at the region of the caudal surface and caudal peduncle and tail fins also at the region of the dorsal musculature. Ulcers also may

found on the lesion or not with the growth of green algae on the exposed skin. Post mortem lesions were focused on gills that appeared severely congested stuck and slimed (Fig. 1a, b). *Riboscyphidia* sp. attached to primary gill filaments surface epithelium resulting in blocking of gas exchange leading to respiratory distress and suffocation of infected fishes (Fig. 2a-d).

Table 2: Efficacy of treatment of *Riboscyphidia* infection in the Red Sea cultured Asian sea bass

Groups	Drug	Number of fish	Treatment	Dosage of drug	Duration	Treated fish	Treatment (%)
1st	Formalin	10	Bath	150 ppm	60 min	9	90*
2nd	Formalin	10	Bath	250 ppm	30 min	7	70*
3rd	Copper citrate	10	Prolonged immersion	0.56 mg mL ⁻¹	7 days	10	100*
4th	Control	10	No treatment	-----	-----	----	-----

*Significance n = 10

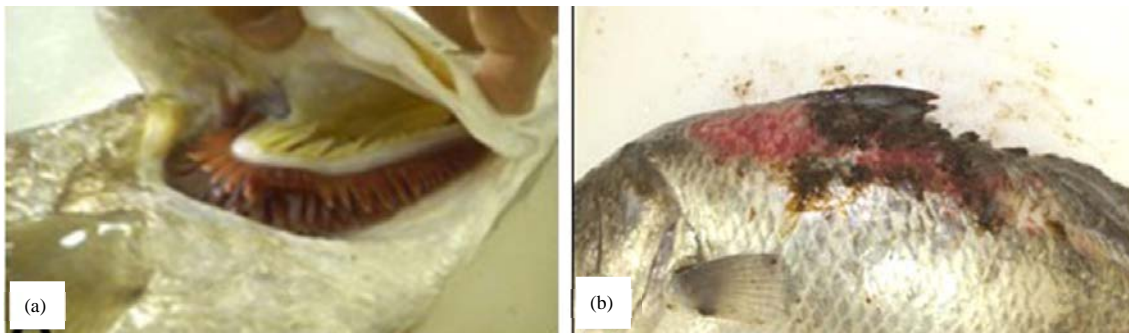


Fig. 1(a-b): Post mortem lesions of gills

(a) Infected Asian sea bass congested and stuck gills filaments and (b) Eroded dorsal fins with a shallow ulcer at the dorsal musculature of infected fishes

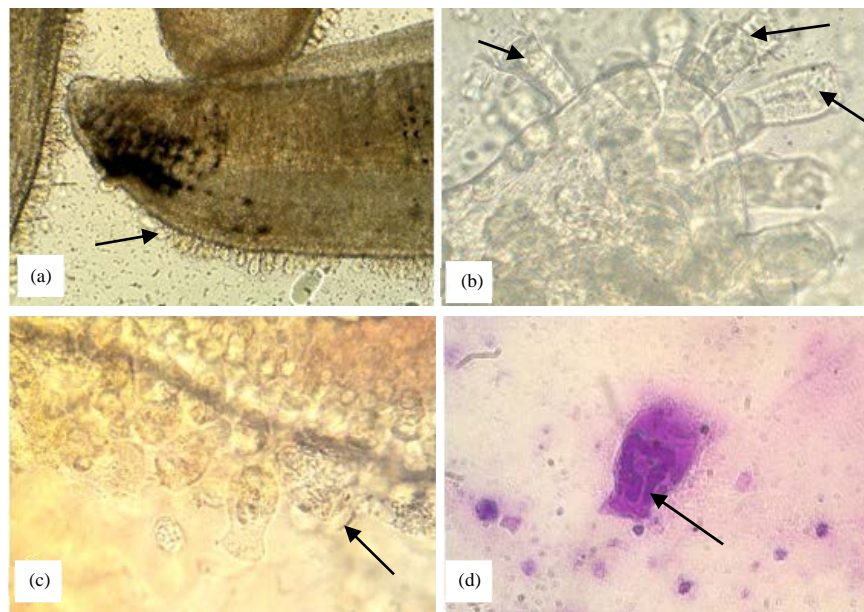


Fig. 2(a-d): *Riboscyphidia* sp. attached to surface epithelium of primary gill filaments

(a) Primary gill filaments with a high density of *Riboscyphidia* attached with the epithelial lining of the gill filament (arrow), (b-c) Magnified non-mobile ciliate *Riboscyphidia* (Arrows) and (d) Stained *Riboscyphidia* with potassium permanganate (arrow)

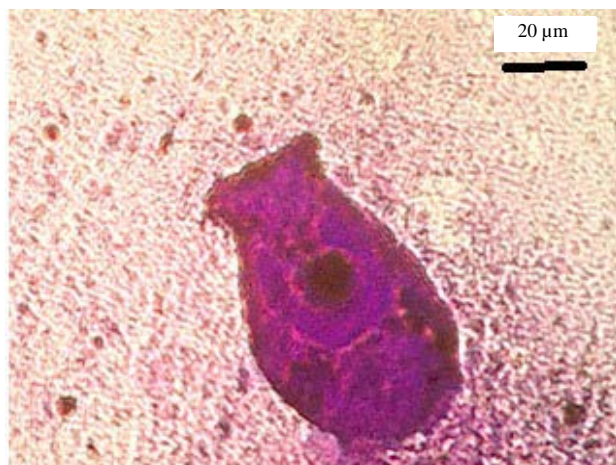


Fig. 3: *Riboscyphidia* parasite (ciliate) with horseshoe nucleus stained with potassium permanganate

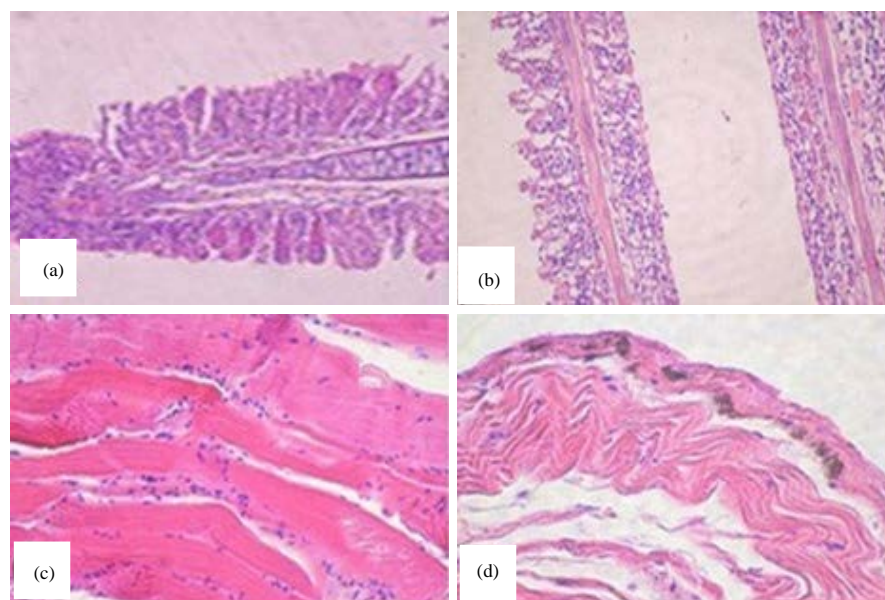


Fig. 4(a-d): Histopathological examination of gills, skin and musculature of Asian sea bass

Gill suffered from telangiectasis and severe hyperplasia and sticking of secondary gill filaments and infiltration of inflammatory cells (a-b), Skeletal muscle bundles suffered from Zenker's necrosis with infiltration of inflammatory cells, (c) Skin layer suffered from the focal deposition of melanin pigments (d)

Identification of the parasite: After microscopical examination and from morphological features of the isolated parasite it was identified as *Riboscyphidia* (Ciliate) sp. (Fig. 3).

Prevalence of *Riboscyphidia*: From 100 cultured Asian sea bass, about 64 fish was infected with *Riboscyphidia* sp. with a prevalence of about 64%.

Results of *Riboscyphidia* infection treatment: The present study displayed that the treatment of 1st group with a formalin bath 150 ppm for 60 min resulted in the treatment of

90% of fish, while the 2nd group displayed treatment of 70% only on the other hand 3rd group which was treated with chelated copper resulted that 100% of fish treated with no mortality of fish Table 2.

Histopathological studies: Samples from gills, skin and musculature were collected and subjected for histopathological examination and revealed that primary gill filament suffered from telangiectasia and severe hyperplasia and sticking of secondary gill filaments and infiltration of inflammatory cells, (Fig. 4a, b) Skeletal

musculature suffered from Zenker's necrosis and hyalinization with infiltration of inflammatory cells (Fig. 4c) and skin layer of infected fish suffered from focal melanin pigment deposition (Fig. 4d).

DISCUSSION

The present study revealed the epidemiology and pathogenesis and control of *Riboscyphidia* ciliate on cultured Asian sea bass. *Riboscyphidia* ectoparasite are moderately pathogenic but with high numbers of it on the gills can physically prevent gas exchange. They may also act as a portal for bacterial colonization¹². The goal of the study was to determine epizootic ciliates *Riboscyphidia* infecting the Red Sea cultured Asian sea bass and obtain more information on the epidemiology of the parasite depending on host characteristics and environmental factors and searching for a method to control infection of *Riboscyphidia* in such fish.

Regarding the clinical signs and post mortem, the present study revealed that infested Asian sea bass was showed, lethargic, flashing, swimming upside, surfacing and sloughed scales. Post mortem lesions were focused on gills that appeared severely congested and slimed, present results nearly agree with that obtained by Fernandez-Leborans *et al.*⁴ and Noga⁹ most infections were concentrated in gills thus all observed signs were respiratory distress like surfacing.

Concerning the identification of infected parasite present study displayed that from morphological features of the isolated parasite it was identified as *Riboscyphidia* (Ciliate) sp. according to Lom⁷.

Regarding the result of the treatment of infected fishes with *Riboscyphidia*, the present study revealed that the treatment of choice was chelated copper citrate, the result nearly agrees with the results recorded by Van and Basson¹ and Noga⁹.

Regarding the results of the histopathological examination of the present study revealed that gills, skin and musculature were subjected for histopathological examination and revealed that primary gill filament suffered from telangiectasis and severe hyperplasia and the skeletal musculature suffered from Zenker's necrosis and hyalinization. The results confirmed with the results obtained by Noga⁹, Bancroft and Stevens¹¹ and Lee *et al.*¹²

Riboscyphidia ciliate infection is ectocommensal when found on fishes with low numbers or as secondary infection accompanied by bacterial or mycotic infection, *Riboscyphidia* is easily treated but the treatment of choice was copper citrate bath treatment "prolonged immersion". It is moderately pathogenic for fishes causing death for young fish.

CONCLUSION

From the present study, it was concluded that *Riboscyphidia* infection mainly infects fishes as a secondary infection after bacterial or mycotic disease. And prolonged immersion of copper citrate at the dose 0.56 mg L⁻¹ was recorded as the best treatment for *Riboscyphidia* infection in the Red Sea cultured Asian sea bass.

SIGNIFICANCE STATEMENT

This study discovers the methods of control and treatment of *Riboscyphidia* when infecting Asian sea bass that can be beneficial for Veterinarians and people who work in fish industries. This study will help the researcher to uncover the critical areas of pathogenesis and epizootics of *Riboscyphidia* on infected the Asian sea bass with the determination of treatment of choice of *Riboscyphidia* that many researchers were not able to explore. Thus, a new theory on control of the *Riboscyphidia* may be arrived at.

REFERENCES

1. Van As, J.G. and L. Basson, 1989. A further contribution to the taxonomy of the trichodinidae (Ciliophora: Peritrichia) and a review of the taxonomic status of some fish ectoparasitic trichodinids. Syst. Parasitol., 14: 157-179.
2. Foissner, W., G.L. Hoffman and A.J. Mitchell, 1985. *Heteropolaria colisarum* Foissner & Schubert, 1977 (Protozoa: Epistylididae) of North American freshwater fishes. J. Fish Dis., 8: 145-160.
3. Jayasree, L., P. Janakiram and R. Madhavi, 2001. Epibionts and parasites of *Macrobrachium rosenbergii* and *Metapenaeus dobsoni* from Gosthani estuary. J. Nat. Hist., 35: 157-167.
4. Fernandez-Leborans, G., 2002. A New Suctorian, *Flectacineta isopodensis* (Protozoa: Ciliophora) Epibiont on Marine Isopods from Hokkaido (Northern Japan). Acta Protozoologica, 41: 79-84.
5. Kuperman, S., S.S. Schlosser, J.R. Kramer, K. Bucholz, V. Hasselbrock and T. Reich, 2001. Developmental sequence from disruptive behavior diagnosis to adolescent alcohol dependence. Addiction, 96: 629-636.
6. Van As, J.G. and L. Basson, 1989. A further contribution to the taxonomy of the trichodinidae (Ciliophora: Peritrichia) and a review of the taxonomic status of some fish ectoparasitic trichodinids. Syst. Parasitol., 14: 157-179.
7. Esch, G.W., T.C. Hazen, R.V. Dimock and J.W. Gibbons, 1976. Thermal effluent and the epizootiology of the ciliate epistylis and the bacterium aeromonas in association with centrarchid fish. Trans. Am. Microsc. Soc., 95: 687-693.

8. Lom, J. and J.O. Corliss, 1968. Observations on the fine structure of two species of the peritrich ciliate genus *Scyphidia* and on their mode of attachment to their host. *Trans. Am. Microsc. Soc.*, 87: 493-509.
9. Noga, E.J., 2010. *Fish Disease: Diagnosis and Treatment*. 2nd Edn., Wiley-Blackwell New Jersey, United States Pages: 536.
10. Lucky, Z., 1977. *Methods for the Diagnosis of Fish Diseases*. Amerind Publishing Co., New Delhi, Bombay, India, Pages: 140.
11. Bancroft, J.D. and A. Stevens, 1990. *Theory and Practice of Histological Techniques*. 3rd Edn., Churchill Livingstone, London, Philadelphia, ISBN: 9780443035593, Pages: 726.
12. Lee, J.J., S.H. Hutner and E.C. Bovee, 1985. *An Illustrated Guide to the Protozoa*. Society of Protozoologists, New Jersey, United States, Pages: 759.