Environment and Ecology 39 (2) : 390—395, April—June 2021
ISSN 0970-0420

Prevalence of a Protozoan Parasite *Tetrahymena rostrata* from Edible Oysters (Mollusca : Bivalvia) of Estuarine Environment of Sundarbans, West Bengal, India

Tanima Biswas

Received 30 November 2020, Accepted 11 January 2021, Published on 6 April 2021

ABSTRACT

Indian Sundarbans region, largest single continuous tract of diverse mangrove forest in India, covers an area of 9630 sq km, represents potential reservoir of very rich and diverse faunal and floral communities. *Crassostrea gryphoides* and *Saccostrea cucullata* are the edible oyster resources of Sundarbans region. Fisheries provide one of the main source of employment and production Sundarbans region. *Crassostrea gryphoides* and *Saccostrea cucullata* grows on hard substrate and generates three-dimensional structure in auatic environment. Such structure supports a diverse assemblage of organisms generally not found in surrounding soft-bottom habitats. Oysters can concentrate pathogenic microorganisms as a result of filtering large volumes of water. The protozoan parasite of the genus, *Tetrahymena rostrata* have been observed from the edible oyster, *Crassostrea gryphoides* and *Saccostrea cucullata* of estuarine aquatic environment of Sundarbans, West Bengal, India. *Tetrahymena rostrata*, with relatively slender body, narrowing anteriorly with slightly curved pointed end are isolated from the mantle, gill and labial palp of the oysters, *Crassostrea gryphoides* and *Saccostrea cucullata*. The infection observed only during monsoon and post-monsoon season and totally absent in pre-monsoon which reflects seasonal variation of the infection of *Tetrahymena rostrata*.

Keywords *Tetrahymena rostrata*, Prevalence, Protozoan parasite, Edible oyster, Estuarine environment.

Tanima Biswas
Department of Zoology, Ranaghat College, Ranaghat, Nadia 741201, West Bengal, India
Email : btanima19@gmail.com

INTRODUCTION

The estuarine aquatic environment of Sundarbans which was declared a biosphere reserve in 1989, is bestowed with a wide range of natural forest, terrestrial and aquatic resources-freshwater and marine communities, offering the largest mangrove concentration with a great biodiversity and the only mangrove tiger-land in the world. The edible oysters resources of this region are *Crassostrea gryphoides* and *Saccostrea cucullata* belong to phylum Mollusca and Class Bivalvia. They are capable of tolerating a wide range of salinity and heavy metal pollution (Biswas *et al.* 2013). The oyster habitats play an important role in the socio-economical interest of the coastal people as they can provide jobs in rural areas in agriculture, forestry, fisheries and the tourism industry. Although *Crassostrea gryphoides* and *Saccostrea cucullata* has been recognized as an important economic estuarine species due to its direct fisheries value, the diversity of oyster reef, ecological value of the habitat oysters create and the influence of oysters on estuarine function has been frequently overlooked. Oysters can concentrate pathogenic microorganisms as a result of filtering large volumes of water. Apart from the incidences of various

infectious protozoan parasites various associated organisms have been observed and identified by their characteristic features from edible oyster during the course of study (Biswas and Bandyopadhyay 2013, 2014a, 2014b, 2016, Biswas *et al.* 2011).

Parasites have evolved numerous ways to adapt to the dramatic changes in environment experienced throughout their life cycle. An alternation in habitat is communicated to the parasites by chemical or physical signals which trigger metabolic and ultra-structural changes. Parasite-induced alterations in host phenotype have been frequently reported in a wide range of protozoan and metazoan parasites with complex life cycles (Combes 1991, Poulin 1998). Parasitic diseases can pose major threats to animal populations and have serious economic impacts on host health, therefore, research into host parasitology ecology is very much essential.

The study of parasites and diseases affecting molluscs with economic interest is important both for the management of natural stock and for aquaculture. It could even be helpful for the sanitary evaluation for human consumption.

MATERIALS AND METHODS

A systematic monitoring practice was undertaken for a period of 3 years during 2009–2012. Samples of edible oysters, *Crassostrea gryphoides* and *Saccostrea cucullata,* were collected randomly during low tide from the two selected study sites namely, Frasergunj and Kaikhali of Sundarbans region, West Bengal during the study period. Oysters were collected seasonally, directly from the reefs of the specified sites during the study period. Stainless steel hammer and rod were used to separate oysters from their surrounding cliffs. Both *Crassostreagryphoides* and *Saccostrea cucullata* were collected from Frasergunj, *Crassostrea gryphoides* only were collected from Kaikhali. In the laboratory, the shell of the oyster were opened with a fine knife. One of the first stages of examination was to observe the size and color of the organs such as the adductor muscle, digestive gland, gill, mantle, labial palps, contents of stomach and intestines minutely. The smear materials were scrapped out from adductor muscle, digestive gland, gill, mantle, labial palps and other viscera tissues from oysters and placed on glass slides in 0.6 % saline and observed under the microscope for examination of parasites. Thin and uniform smears were drawn on slide without allowing them to dry. Semidried smears containing the protozoans were stained with Giemsa Photographs were taken with Olympus phase contrast microscope fitted with Olympus digital camera.

Prevalence of the infected parasites was also determined. The prevalence was calculated as number of infested oysters divided by number of observed oysters, multiplied by hundred.

$$\text{Prevalence} = \frac{\text{No. of hosts infested}}{\text{No. of hosts examined}} \times 100$$

RESULTS AND DISCUSSION

During the study period several protozoan parasites have been observed from the edible oyster, *Crassostrea gryphoides* and *Saccostrea cucullata* from Kaikhali and Frasergunj of South 24 Parganas of West Bengal, India. These include *Cristigera* sp., *Tetrahymena rostrata*, *Cryptosporidium* sp. and *Callimastixequi* infection (Biswas and Bandyopadhyay 2013, 2014a, 2014b, 2016, Biswas *et al*. 2011).

Systematic position of *Tetrahymena rostrata*

Kingdom	Animalia
Phylum	Ciliophora
Class	Ciliatea
Subclass	Rhabdophorina
Order	Hymenostomatida
Suborder	Tetrahymenina
Family	Tetrahymenidae
Genus	Tetrahymena

The protozoan parasite of the genus, *Tetrahymenarostrata* have been observed from the edible oyster, *Crassostrea gryphoides* and *Saccostrea cucullata* collected from Kaikhali and Frasergunj of South 24 Parganas of West Bengal during survey period. *Tetrahymena rostrata* are isolated from the

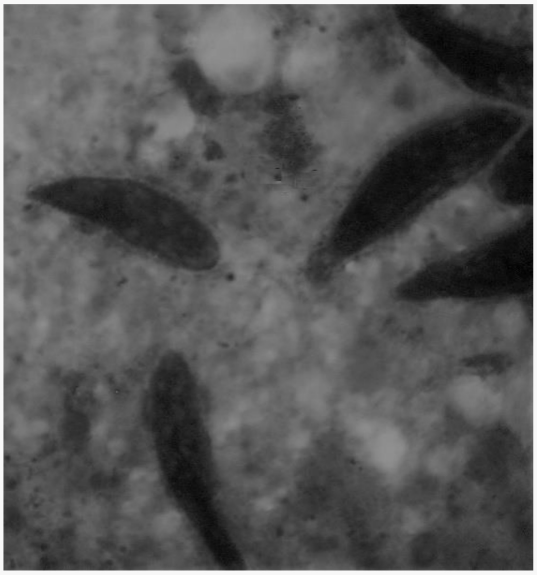

Fig. 1. *Tetrahymena rostrata.*

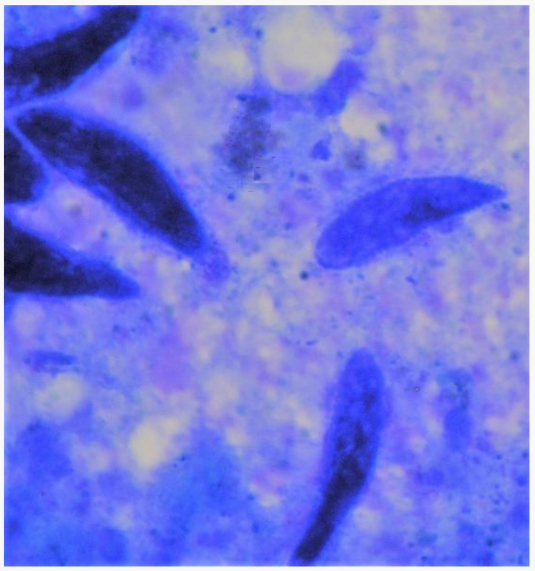

Fig. 2. *Tetrahymena rostrata* stained with Giemsa.

mantle, gill and labial palp of the oysters, *Crassostrea gryphoides* and *Saccostrea cucullata.*

Tetrahymena rostrata are with relatively slender body, narrowing anteriorly with slightly curved pointed end. The body contains food vacuoles, a terminal contractile vacuole, a spherical macronucleus placed more or less centrally and with ovoid micronucleus (Figs. 1 and 2). Infections of *Tetrahymena rostrata* are accompanied by yellow discoloration and extensive lesions in the gills and mantle. Gills of infested oysters are covered with cysts of different sizes and colors. Abscess-like lesions occur within the body wall, on the surface of the labial palps or mantle. A brown scar often occurs on the shell, adjacent to abscess on the mantle surface. Heavy

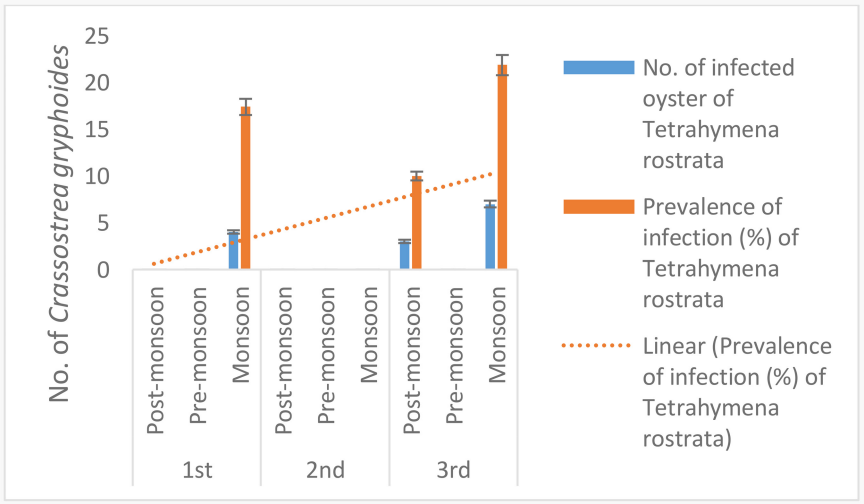

Fig. 3. Number of infected *Crassostrea gryphoides* and prevalence of infection (%) by *Tetrahymena rostrata* in Kaikhali.

Table 1. Data showing prevalence of *Tetrahymena rostrata* infection in edible oysters, *Crassostrea gryphoides* and *Saccostrea cucullata,* collected from Frasergunj and Kaikhali of Sundarbans region, West Bengal.

Sites	Host species	Year	Season	No. of total oyster	No. of infected oyster of *Tetra-hymena rostrata*	Preva-lence of infection (%) of *Tetra-hymena rostrata*
		1st	Post-monsoon	30	0	0
		1st	Pre-monsoon	26	0	0
		1st	Monsoon	23	4	17.391
	Crassostrea	2nd	Post-monsoon	30	0	0
	gryphoides	2nd	Pre-monsoon	30	0	0
Kaikhali		2nd	Monsoon	27	0	0
		3rd	Post-monsoon	30	3	10
		3rd	Pre-monsoon	28	0	0
		3rd	Monsoon	32	7	21.875
			SUM	256	14	49.266
		1st	Post-monsoon	25	2	8
		1st	Pre-monsoon	23	0	0
Frasergunj	*Crassostrea*	1st	Monsoon	26	0	0
	gryphoides	2nd	Post-monsoon	26	0	0
		2nd	Pre-monsoon	22	0	0
		2nd	Monsoon	26	3	11.538
		3rd	Post-monsoon	24	0	0
		3rd	Pre-monsoon	23	0	0
		3rd	Monsoon	25	2	8
			SUM	220	7	27.538
		1st	Post-monsoon	40	0	0
		1st	Pre-monsoon	36	0	0
Frasergunj	*Saccostrea*	1st	Monsoon	36	0	0
	cucullata	2nd	Post-monsoon	35	0	0
		2nd	Pre-monsoon	40	0	0
		2nd	Monsoon	43	4	9.302
		3rd	Post-monsoon	35	0	0
		3rd	Pre-monsoon	42	0	0
		3rd	Monsoon	37	3	8.108
			SUM	344	7	17.41

lesions appear as a swollen mantle edge or nodules on the mantle though these lesions are not unique to *Tetrahymena rostrata* infection.

A total of 256 *Crassostrea gryphoides* have been collected from Kaikhali out of which 14 are infected with this parasite. Similarly out of 220 *Crassostreagry phoides* and 344 *Saccostrea cucullata* from Frasergunj have been examined 7 and 7 are infected with this parasite respectively (Table 1).

The percentage of infection by *Tetrahymena rostrata* in edible oyster, in three seasons during study has been represented in Table 1. The percentage of infection by *Tetrahymena rostrata* are 17.391 and 21.875 in *Crassostrea gryphoides* collected from Kaikhali and 11.538 and 8 in *Crassostre gryphoides* from Frasergunj during monsoon. The percentage of infection by *Tetrahymena rostrata* are 9.302 and 8.108 in *Saccostrea cucullata* collected from Frasergunj during monsoon. Figs. 3 and 4 represents the number of infected *Crassostrea gryphoides* and prevalence of infection (%) by *Tetrahymena rostratain* Kaikhali and Frasergunj respectively. Fig. 5 reflects the number of infected *Saccostrea cucullata* and prevalence of infection (%) by *Tetrahymena rostrata* in Frasergunj. Though significant relationships have not been noticed of *Tetrahymena*

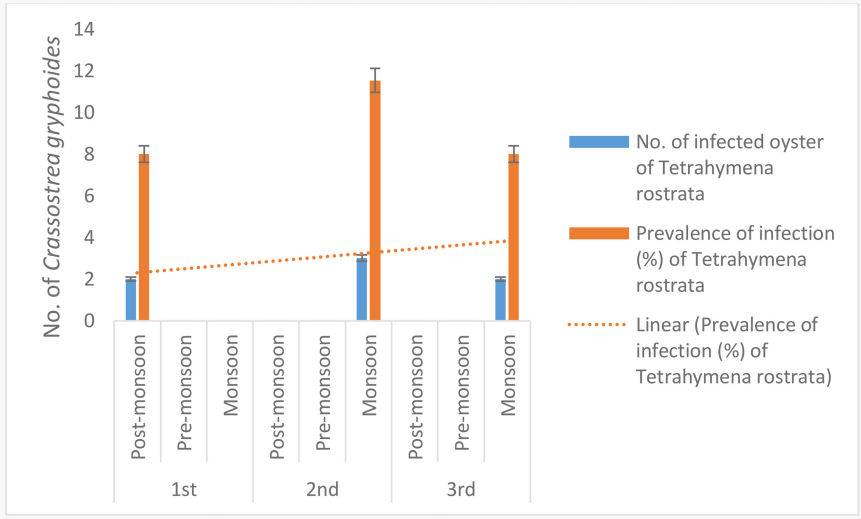

Fig. 4. Number of infected *Crassostrea gryphoides* and prevalence of infection (%) by *Tetrahymena rostrata* in Frasergunj.

rostrata parasites with environmental parameters while infecting the host, the infection observed only during monsoon and post-monsoon season and totally absent in pre-monsoon which reflects seasonal variation of the infection of *Tetrahymena rostrata*. During the present study, heterogenecity in *Tetrahymena rostrata* parasites has been observed as they are present in the edible oyster, *Crassostrea gryphoides* and *Saccostrea cuculata* collected from Kaikhali and Frasergunj of South 24 Parganas. Insignificant variation of prevalence is present between two selected edible oysters, *Crassostrea gryphoides* and *Saccostrea cucullata* and between the two selected sites i.e. Kaikhali and Frasergunj of South 24 Parganas of West Bengal during the course of study for the protozoan parasite *Tetrahymena rostrata.*

Tetrahymena (*Paraglaucoma*) *rostrata* (Kahl

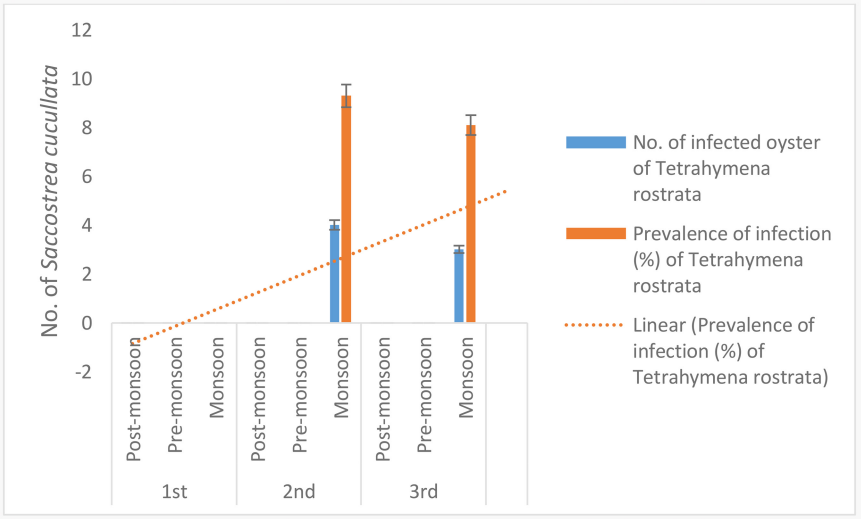

Fig. 5. Number of infected *Saccostrea cucullata* and prevalence of infection (%) by *Tetrahymena rostrata* in Frasergunj.

1926, Corliss 1952) occurs commonly in litter and occasionally in soil. It has previously been recorded from moss. In nature it feeds on cytolyzed or moribund tissue but in the presence of peptone it feeds on bacteria and flagellates. *Tetrahymena rostrata*, the ciliate is an obligate histophage and a facultative parasite of enchytraeid worms which it infects through degenerate setal follicles (Stout 1954). Ciliate infections in certain other invertebrates are also characterized by rapid pathogenicity (Stout 1954). It may also infect accidentally injured worms.

The protozoan *Tetrahymena rostrata* found in *Crassostrea gryphoides* and *Saccostrea cucullat* may be considered as transient and does not induce pathogenicity in epidemic scale. There appear to be ill-effects at either the organismic or tissue levels. It will be important to note whether the incidence of the parasite increases and whether pathogenicity is expressed. Whatever occurs will undoubtedly be linked to the oyster's resistance, the potential of parasite and physical conditions of the environment. The present study provides realistic and useful in for the risk assessment of food-borne infections related to the consumption of contaminated oyster. Although there is no doubt that *Crassostrea gryphoides* and *Saccostrea cucullata* can be contaminated with infectious agents that cause serious diseases to human being, people can consume such oysters after cooked well.

ACKNOWLEDGEMENT

The author is thankful to Prof. P. K. Bandyopadhyay, Department of Zoology, University of Kalyani, Kalyani, W. B. Sincere thanks are also due to Dr. Nelendu Jyoti Moitra and Avay Haldar, both of Ramkrishna Ashram Krishi Vigyan Kendra, Nimpith, South 24 Parganas, W. B. for their active cooperation.

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