Prevalence of a protozoan parasite *Cristigera* sp. (Ciliophora:Ciliatea) from edible oysters (Mollusca:Bivalvia) of Sundarbans, West Bengal, India

Tanima Biswas · Probir Kumar Bandyopadhyay

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Abstract The coastal region of West Bengal is bestowed with a wide range of natural forest and aquatic resources. The estuarine complex is a culmination of the interaction of land, sea and freshwater. It offers diverse specialised habitats such as mangroves, non vegetated mudflats, inter tidal zones and reclaimed areas. Such areas are home to a number of terrestrial, freshwater and marine communities. Edible oyster resources in these regions are *Crassostrea gryphoides* and *Saccostrea cucullata*, which are the key-stone species found in the intertidal zone and can tolerate huge variation of salinity. These are used as food by local people and marketed to earn cash. The population of this species has been declined due to parasitic infection and pollution. There are many protozoan parasites which infect these oysters causing diseases. During survey period, a protozoan parasite of the genus *Cristigera* have been observed only from the edible oyster *Crassostrea gryphoides* collected from Kaikhali and Frasergunj among three selected sites namely Kaikhali, Frasergunj and Digha of West Bengal mainly during monsoon and post-monsoon season. Considering such rare and specific prevalence of *Cristigera* sp, it may be considered as potential bio-indicator.

Keywords Edible oyster · *Cristigera* sp. · Protozoan parasites · Bio-indicator

Introduction

In Protozoa, comparatively highly complicated organisms are represented by numerous ciliates. Ciliates are a diverse and successful group of protozoan parasites. The genus *Cristigera* (Roux 1901) belonging to the phylum Ciliophora, class Ciliatea, order Hymenostomatida, family Cyclidiidae are common in invertebrates of marine and fresh water organisms. Most of the species of the genus *Cristigera* are free living ciliates excepting *Cristigera susmai* (Jamadar and Choudhury 1988) and *Cristigera crassostrae* (Biswas and Bandyopadhyay 2011), which are leading parasitic life. In India, the *Cristigera* parasites have so far been reported from *Crassostrea cucullata* collected from the meeting place of the Hooghly river with the Bay of Bengal, Sagar Island, West Bengal, India (Jamadar and Choudhury 1988) and from *Crassostrea gryphoides* from Kaikhali near Jaynagar-Majilpur block, 24 Parganas (South) West Bengal, India (Biswas and Bandyopadhyay 2011). Species of ciliates are capable of facultative parasitism (Corliss 1961). This paper deals with the incidence of the genus *Cristigera* from edible oyster *Crassostrea gryphoides* and *Saccostrea cucullata* of coastal region, West Bengal which have been bestowed with a wide range of natural forest and aquatic resources. The estuarine complex which is a culmination of the interaction of land, sea and freshwater offers diverse specialised habitats such as mangroves, non-vegetated mudflats, intertidal zones, reclaimed areas which are the home of a number of terrestrial, freshwater and marine communities. A number of rare and endangered species have been recorded in this region. Edible oyster recorded from these region are *Crassostrea gryphoides* and *Saccostrea cucullata* which are considered as one of the keystone species, found in the intertidal zone of this region and can tolerate huge

T. Biswas · P. K. Bandyopadhyay (✉)
Parasitology Laboratory, Department of Zoology,
University of Kalyani, Kalyani 741235, West Bengal, India
e-mail: prabir0432@hotmail.com

variation of salinity. These are used as food by local people and marketed to earn cash. Indeed, there are many protozoan parasites which infect these oysters for causing diseases. Thus population of this species has been declined due to parasitic infection and pollution.

Materials and methods

Host specimens, *Crassostrea gryphoides* and *Saccostrea cucullata* were randomly sampled from the Kaikhali, Frasersgunj of South 24 Parganas and Digha of East Midnapore district along the coastal region of West Bengal and brought alive or in moribund condition to the laboratory. Oysters were collected seasonally, directly from the reefs of the specified sites during the study period.

In the laboratory the shell of the oyster were opened with fine knife and smears from the body of oyster were scrapped out on glass slides in 0.6 % saline and observed under microscope for examination of parasites. For permanent preparations the smeared slides are semi air-dried, fixed in Schaudinn's fluid and stained in Heidenhain's iron alum haematoxylin for general staining. Silver impregnation techniques after Corliss (1953); Klein (1958) were employed to study the infra ciliature and kinetics of ciliates. The prevalence was calculated as number of infested oysters divided by number of observed oysters multiplied by hundred.

The temperature of the surface water was determined in situ during sampling using a mercury thermometer. pH is also measured in the field using a portable pH-meter. The bottle filled completely with the seawater sample was brought to the laboratory for measurement of salinity by electrical conductivity meter.

The prevalence of *Cristigera* infection to the edible oyster of each selected sites was compared to the hydrological parameters like temperature, salinity and pH by Pearson correlation test. Probability less than 0.05 ($P < 0.05$) was considered as statistically significant.

Results and discussion

During survey period, the protozoan parasite of the genus, *Cristigera* have been found from the edible oyster (*Crassostrea gryphoides*) collected from Kaikhali and Frasersgunj of South 24 Parganas among three selected sites, namely, Kaikhali, Frasersgunj and Digha along the coastal region of West Bengal mainly during monsoon and post-monsoon seasons. The genus *Cristigera* is characterized by a longitudinal, ventral groove in its body. Macronucleus spherical, usually located at the posterior half of the body accompanied by a small micronucleus. *Cristigera* sp. was isolated from the mantle, gill, and labial palp of the edible oysters, *Crassostrea gryphoides* only, which was collected from Kaikhali and Frasersgunj. No *Cristigera* sp. was found in

Table 1 Data showing the prevalence of *Cristigera* infections of the edible oysters, *Crassostrea gryphoides* and *Saccostrea cucullata*

Host species	Years	Season	Prevalence of infection (%) of <i>Cristigera</i> sp. in Kaikhali	Prevalence of infection (%) of <i>Cristigera</i> sp. in Frasersgunj	Prevalence of infection (%) of <i>Cristigera</i> sp. in Digha
<i>Crassostrea gryphoides</i>	2009	Post-monsoon	0	0	0
	2010	Pre-monsoon	0	0	0
	2010	Monsoon	8.696	0	0
	2010	Post-monsoon	10	7.692	0
	2011	Pre-monsoon	0	0	0
	2011	Monsoon	11.111	11.538	0
	2011	Post-monsoon	6.667	8.333	0
	2012	Pre-monsoon	0	0	0
	2012	Monsoon	12.5	12	0
<i>Saccostrea cucullata</i>	2009	Post-monsoon	0	0	0
	2010	Pre-monsoon	0	0	0
	2010	Monsoon	0	0	0
	2010	Post-monsoon	0	0	0
	2011	Pre-monsoon	0	0	0
	2011	Monsoon	0	0	0
	2011	Post-monsoon	0	0	0
	2012	Pre-monsoon	0	0	0
	2012	Monsoon	0	0	0

Table 2 Seasonal variation in water temperature, pH and salinity values of water during November 2009–August 2012

Sites	Years	Season	Temperature (°C)	pH	Salinity (PSU)
Kaikhali	2009	Post-monsoon	24.5	8.21	32
	2010	Pre-monsoon	35.2	8.3	34
	2010	Monsoon	32	7.96	27.5
	2010	Post-monsoon	23	8.14	29
	2011	Pre-monsoon	34.8	8.28	35.56
	2011	Monsoon	34.1	7.52	28.7
	2011	Post-monsoon	26	8.29	31.3
	2012	Pre-monsoon	36.6	8.34	34.8
	2012	Monsoon	33.2	8.1	27.6
	Average $\pm$ SD		31.044 $\pm$ 5.125	8.127 $\pm$ 0.257	31.162 $\pm$ 3.126
Frasergunj	2009	Post-monsoon	25	8.27	34
	2010	Pre-monsoon	34.7	8.35	35
	2010	Monsoon	31.5	7.92	32.2
	2010	Post-monsoon	22.8	8.3	33.23
	2011	Pre-monsoon	35	8.32	34.97
	2011	Monsoon	33.8	8.16	32.14
	2011	Post-monsoon	25.2	8.25	33.32
	2012	Pre-monsoon	35.7	8.36	35.6
	2012	Monsoon	32.9	8.17	32.35
	Average $\pm$ SD		30.733 $\pm$ 4.996	8.211 $\pm$ 0.137	33.645 $\pm$ 1.316
Digha	2009	Post-monsoon	27	8.25	29.44
	2010	Pre-monsoon	30.7	8.3	32
	2010	Monsoon	29.5	7.85	30.2
	2010	Post-monsoon	26	8.3	32.7
	2011	Pre-monsoon	32	8.32	32.19
	2011	Monsoon	31.8	8.1	30.7
	2011	Post-monsoon	27.8	8.25	32.5
	2012	Pre-monsoon	33.7	8.36	31.8
	2012	Monsoon	31.4	8.17	28.15
	Average $\pm$ SD		29.989 $\pm$ 2.585	8.193 $\pm$ 0.157	31.075 $\pm$ 1.558

Fig. 1 Relationship between prevalence of infection (%) of *Cristigera* sp. and temperature (°C) in Kaikhali **Fig. 2** Relationship between prevalence of infection (%) of *Cristigera* sp. and temperature (°C) in Frasergunj

Saccostrea cucullata collected from any of the three sites and in any seasons which reflects the species specific nature of *Cristigera* sp.

The percentages of infection by *Cristigera* sp. in edible oyster, in three seasons have been incorporated in Table 1. It is evident that prevalence of *Cristigera* sp. is dependent

on season. It has been observed in monsoon and post-monsoon season (June to February). It has also been noticed that *Cristigera* sp. are totally absent in particular pre-monsoon season, which leads to the conclusion that physiological factors of the host as well as the water quality are responsible for infestation. Though the parasitic infestation was noticed only in the adult oysters, the young oysters are more susceptible to infection; since they are more delicate. The water temperature, pH and salinity are the three important water quality parameters that are responsible for parasitic infestation as they fluctuate more rapidly. The data of these three parameters have been incorporated in Table 2. The correlation between surface water temperature ($^{\circ}\text{C}$) of three different seasons with the parasitic infestation in Kaikhali and Frasergunj has been depicted in Figs. 1 and 2 respectively. The correlation between mean pH value of three different seasons with the parasitic infestation in Kaikhali and Frasergunj has been presented in Figs. 3 and 4 respectively. The correlation between the salinity (PSU) in three different seasons and the parasitic infestation in the host at Kaikhali and Frasergunj has been incorporated in Figs. 5 and 6 respectively. The *Cristigera* infection is highest during monsoon when the

water quality deteriorates due to decrease of the temperature and Salinity level.

The graphs show that the prevalence of *Cristigera* sp. infection is more dependent on salinity than temperature and pH. Significant negative correlation is obtained between salinity of water and prevalence of *Cristigera* sp. in the edible oyster *Crassostrea gryphoides* collected from Kaikhali and Frasergunj ($P < 0.05$). The prevalence of *Cristigera* sp. infection shows no such significant correlation with temperature and pH ($P > 0.05$). Evans (1960) reported *Cristigera* sp. which was believed to be bacterial feeders from the Great Salt Lake, Utah. *Cristigera* sp. exhibited optimum growth in salt concentrations of 1–18 ‰; its growth completely declined at 23 ‰ salt concentration that is higher salinity. Cysts of *Cristigera*, however, could survive long periods in a saturated salt solution. Evans (1960) also concluded that *Cristigera* were specialized halophilic protozoa and that certain of the other species of protozoa may be salt-tolerant freshwater forms.

Among three selected sites namely, Kaikhali, Frasergunj and Digha the genus *Cristigera* have been found only from the edible oyster, *Crassostrea gryphoides* of Kaikhali and Frasergunj. No parasite infestation was recorded from the

Fig. 3 Relationship between prevalence of infection (%) of *Cristigera* sp. and pH in Kaikhali

Fig. 5 Relationships between prevalence of infection (%) of *Cristigera* sp. and salinity (psu) in Kaikhali

Fig. 4 Relationship between prevalence of infection (%) of *Cristigera* sp. and pH in Frasergunj

Fig. 6 Relationship between prevalence of infection (%) of *Cristigera* sp. and salinity (psu) in Frasergunj

oyster such as *Crassostrea gryphoides* and *Saccostrea cucullata* collected from Digba. It may be due to thick population of human being and more pollution as rapid alteration and development during recent times, has increased the pollutant discharge which led to the deterioration of the estuarine and related habitats quality. The waste water arising from anthropogenic and industrial activities also have been discharged into estuaries.

In the present study, a consistent general trend appeared that *Cristigera* infestation is not found throughout all the season. It is common in monsoon and pre-monsoon seasons. This is principally related to the salinity fluctuation of water. Prevalence of *Cristigera* sp. is negatively correlated with salinity of water. It can be concluded that the water quality has great impact on the abundance of pathogens and their ability to survive on host. The temporal and spatial variation trends of *Cristigera* sp. infestation in the body of oyster reflects the usefulness of using this species to identify temporal and spatial distribution patterns of salinity of aquatic bodies. Considering such rare and specific prevalence of the *Cristigera*, this species may be considered as a potential bio-indicator of environment.

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References

- Biswas T, Bandyopadhyay PK (2011) On the occurrence of a protozoan parasite from edible oysters of Sunderbans region of West Bengal. Proc 22nd Nat Cong Parasitol 315–318
- Corliss JO (1953) Silver impregnation of ciliated protozoa by the Chatton–Lwoff technique. Stain Thch 28:97–100
- Corliss JO (1961) The ciliated protozoa characterization classification and guide to the literature. Pergamon Press, New York, pp 89–118
- Evans FR (1960) Studies on growth of protozoa from the great salt lake with special reference to *Cristigera* sp. J Protozool 7:14–15
- Jamadar YA, Choudhury A (1988) Ciliates of some marine and estuarine molluscs from Indian coastal region: zoological survey of India. Biblio, Calcutta, pp 62–79
- Klein BM (1958) The dry silver method and its proper use. J protozool 5:99–103
- Roux J (1901) Faune infusorienne des eaux stagnantes des environs de Geneve. Mem fac sci l'Univ Geneve 1–148