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RESEARCH ARTICLE

Growth and health status of cultured edible oyster, *Crassostrea madrasensis* (Preston) in the Panadura estuary, Sri Lanka

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Abstract: To intensify the production of edible oyster in Sri Lanka in the future it is essential to analyse the status of the present culture practices. The present study was carried out to investigate the growth and health of cultured edible oyster, *Crassostrea madrasensis* in relation to the prevailing water quality parameters and external fouling in a small-scale, commercial farm located in the Panadura estuary, Sri Lanka.

The mean growth rate in shell length and shell width varied between 0.06 ± 0.01 mm and 2.19 ± 0.09 mm and between 0.02 ± 0.02 and 2.11 ± 0.39 mm per week, respectively. There were positive significant relationships between the growth rate in shell width and salinity, as well as the conductivity of culture water ($p < 0.05$). Mean total dissolved solids in culture water had a positive significant relationship to the growth rate of oysters in shell length ($p < 0.05$). External fouling organisms that grew on the outer shell surface caused significant reduction in growth rate as well as the weight gain of cultured *C. madrasensis* ($p < 0.05$).

Histological sections of the gills, mantle, digestive gland and digestive tract of oysters exhibited structures similar to plasmodia of a haplosporidian parasite, which reached the stage of rupturing during the period of low salinity. *C. madrasensis* could tolerate the low salinity, ranging from 0 gL^{-1} to 6.0 gL^{-1} with 83.0 ± 2.47 % survival; the highest mortality recorded during the period with low salinity in culture water was 15.00 ± 2.50 %.

Keywords: *Crassostrea madrasensis*, external fouling, haplosporidian parasite, oyster, water quality.

INTRODUCTION

Filter feeding bivalve molluscs are suggested as a good solution for the global "food security problem" (FAO, 2006). Bivalves efficiently convert phytoplankton and other nutrients in water into nutritious and high quality animal proteins, with minimal environmental impact. Absence of feed cost for on-growing, greater tolerance for crowding, abundance of natural seeds and relative ease of transport, contribute for the expansion of bivalve culture globally (Indrasena & Wanninayake, 1994a,b; FAO, 2002, 2006). Being an island with an extensive coastal area, there is a possibility for beneficial bivalve culture in Sri Lanka (Wanninayake *et al*., 1990). After successful preliminary studies (Indrasena, 1986), a commercial oyster farm was established in the Panadura estuary in the Western Province of Sri Lanka (Figure 1) in 1983, for culturing *Crassostrea madrasensis* (Ostreidae) as the principal species.

Environmental factors can significantly influence the physiology of bivalves, and therefore, modify the growth potential (Wong & Cheung, 2003). Though sedentary bivalves are able to withstand the wide variations in environmental conditions such as temperature and salinity [*Crassostrea* species is reported to grow well in salinities ranging from 24 gL^{-1} to 30 gL^{-1}(Tan & Wong, 1996)] that commonly occur in their natural habitats, their growth is highly affected by drastic fluctuations in water quality parameters, that could limit the available food. The success of bivalve culture mainly depends on the environmental variables that influence both bivalve physiology and food availability (Sasikumar *et al.*, 2007; Villa *et al*., 2009).

Apart from the environmental conditions, growth and survival of cultured bivalves are influenced by many other factors including stocking density, type of culture

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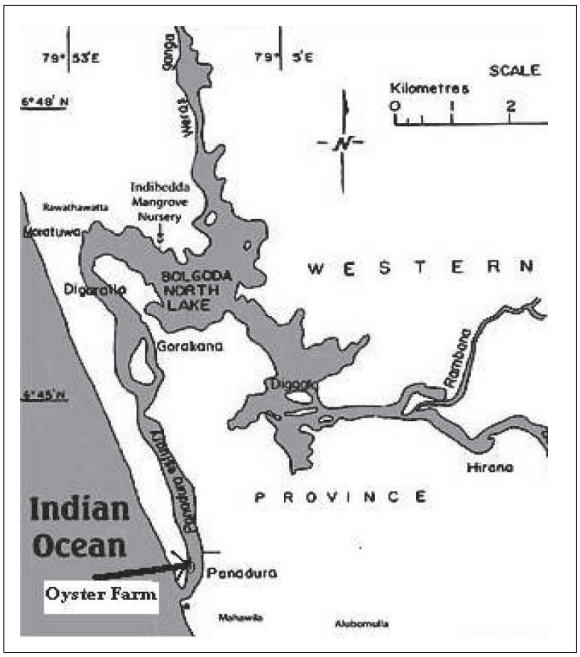

Figure 1: Location of the oyster farm at Panadura estuary, in the Western Province of Sri Lanka (Latitude: 6˚43'41.93" N – Longitude 79˚54'3.11"E)

unit, predation, fouling and cleaning regime (Bishop & Hooper, 2005). Decisions made by the farmers with regard to these factors are critical in influencing the production yield, operating cost and profitability (Josiah & Southgate, 2003).

As all the other aqua-cultured organisms, bivalve culture throughout the world has been plagued by epizootics, the most severe of which have been those caused by Protozoa (Lester, 1989). Sudden mass mortalities in bivalve populations have been witnessed; epidemics in commercially exploited species have repeatedly struck the industry, sometimes causing virtual extinction (Lauckner, 1983).

Growth retardations with mass mortalities in cultured *C. madrasensis* have been observed from time to time by Sri Lankan farmers. The exact reason/s for these conditions has/have not been investigated. The present study examines the effect of prevailing water quality parameters, external fouling and status of health on growth and mortality of oyster *C. madrasensis* cultured in a small-scale commercial farm, located in the Panadura estuary, Sri Lanka.

METHODS AND MATERIALS

Young specimens of *C. madrasensis*, measuring 20.00 ± 0.50 mm in length and 10.00 ± 0.50 mm in width were obtained from the spat collecters, cleaned using a metal brush to remove the epibionts (until all epibionts that could be seen with the naked eye are removed; shell surface of young *C. madrasensis* had some unidentified bivalve larvae as epibionts) and the initial shell length (the distance between the end of umbo and the ventral shell margin), shell width (length at the widest axis of the body) and total body weight, were recorded for each oyster. Six (1-6) groups of plastic culture baskets (30.00 × 25.50 × 10.20 cm), were arranged with four replicate baskets in each group. Twenty young oysters (best stocking density based on preliminary studies conducted at the same site) were placed in each basket. The baskets were covered with lids and suspended from bamboo rafts (at 1.0 m depth) by strong nylon ropes, at the oyster farm located in the Panadura estuary. The experimental culture baskets were randomly placed among commercial culture baskets. The experimental period was from June to December 2007.

Growth of oysters in relation to prevailing water quality parameters

Out of the six groups of culture baskets, group 1 was used for this experiment. Forty oysters from the selected group of baskets were randomly picked at biweekly intervals and the shell length and width of each oyster was measured to the nearest two decimal points. These data were used to calculate the increase in shell length (GR_L) and shell width (GR_W) per week, which was taken as the mean growth rate.

Transparency was measured using a secchi disc. The water samples were obtained at a depth of 1.0 m where the culture baskets were suspended, to measure other parameters. A mercury bulb thermometer, a refractrometer, a pH probe (WTW–pH–315i, Germany), a multi parameter probe (WTW-Cond-340i, Germany) and a DO meter (WTW-Oxi-315i, Germany) were used to record water temperature, salinity, water pH, conductivity, total dissolved solids (TDS) and dissolved oxygen (DO) respectively, at biweekly intervals.

Mortality of cultured oysters

The mortality of oysters in group 2 culture baskets was observed twice a month (biweekly), to obtain the percentage mortality.

Effect of external fouling on the growth of oysters

Culture baskets of group 3 and 4 were used for this experiment. The oysters in one group of baskets were maintained without removing the external fouling organisms growing on the outer shell surface throughout the experimental period, while the other group was cleaned at biweekly intervals to remove epibionts growing on the shell. However, culture baskets in both groups were cleaned to ensure the natural water exchange.

Forty oysters from each group of baskets were picked randomly and the shell length and shell width of all individuals were measured biweekly, to calculate the growth rate as increase in shell length (GR_L) and shell width (GR_W) per week. At the end of the experiment, final body weights (with shell) of each oyster in both groups were recorded after cleaning the shell surface thoroughly (in the group of oysters that were not cleaned at biweekly intervals, shell surface was fully covered with epibionts such as barnacles, polychaete worms, sponges, tunicates and bivalve larvae). The mean weight gain of the oysters that were cleaned at biweekly intervals and of the oysters that were allowed to grow with external fouling organisms on the shell surface was then calculated.

Health status of cultured C. madrasensis

Random samples of five individuals of *C. madrasensis* were obtained monthly from the fifth group of baskets and brought to the laboratory. Their shells were opened carefully and the exposed body mass was checked for abnormalities, i.e. being watery (an abnormal condition of water retention in body mass), presence of water blisters, necrotic areas, etc. (Reantaso *et al*., 2001). Wet mounts of the gill, mantle, digestive gland and digestive tract tissues were prepared using 0.7 % saline solution, and were observed under a light microscope for parasites and/or pathogens and for any other abnormal conditions.

Five oysters taken randomly from the last group of culture baskets at monthly intervals (sixth group) were used for histopathological studies. The gills, mantle, digestive gland and digestive tract of each oyster were fixed separately; sectioned and stained with haemotoxilin and eosin, using the standard procedure (Reantaso *et al*., 2001). The sections were observed under the light microscope for parasites and/or pathogens.

Initially, random samples of young *C. madrasensis* were observed for abnormalities and for parasites/ pathogens using the same procedure (visual observations, microscopic observations of wet mounts and observation of histological preparations).

Statistical analysis

Statistical analysis was performed with Minitab 14 software package. Linear regression analysis was used to check whether there were significant relationships between water quality parameters and growth rates of oysters (GR_L, GR_W), and also between salinity and percentage mortality (PM). The two sample t test was used to determine whether there was a significant difference between the growth rates of oysters that were allowed to grow with and without external fouling organisms on their shell surfaces.

RESULTS

Variations in the water quality parameters at culture site over the experimental period are presented in Table 1. Salinity, conductivity, total dissolved solids (TDS) and transparency significantly varied ($p < 0.05$) from time to time over the experimental period. The salinity showed a wide variation that ranged from 0.5 ± 0.5 to 28.00 ± 0.00 ppt while conductivity of water ranged between 0.37 ± 0.02 to 10.52 ± 0.02 ms cm^{-1}. The lowest TDS in the water at the culture site was 0.90 ± 0.00 and the highest value was 1730.50 ± 20.50 mg/L. The lowest transparency recorded was 47.20 ± 0.30 cm, which increased up to 178.75 ± 0.75 cm (Table 1).

Over the experimental period, slight fluctuations [not significant ($p > 0.05$)] were observed in water temperature (varied between 28.5 ± 0.5 and 30.5 ± 0.5 °C), pH (ranged from 7.13 ± 0.07 to 8.61 ± 0.05) and DO (fluctuated between 6.77 ± 0.73 and 14.85 ± 0.25 mg/L). There were no significant differences in growth rates (GR_L and GR_W) of cultured oysters with time, over the experimental period ($p > 0.05$). Mean GR_L and GR_W varied between 0.06 ± 0.01 and 2.19 ± 0.09 and between 0.02 ± 0.02 and 2.11 ± 0.39 mm/week.

Regression analysis revealed that there were no significant relationships ($p > 0.05$) between the GR_L or GR_W of cultured *C. madrasensis* and the temperature, pH and DO at the culture site over the experimental period. Though there was no significant relationship between the conductivity or salinity of culture water and GR_L ($p > 0.05$), there were significant relationships between the conductivity, transparency as well as the salinity of culture water and GR_W ($p < 0.05$). On the other hand, TDS of culture water significantly correlated to the GR_L of oysters ($p < 0.05$), while it did not show a significant relationship to the GR_W ($p > 0.05$; Figure 2).

Percentage mortality (PM) of cultured oysters varied between 0.00 ± 0.00 and 15.00 ± 2.50 % during the

Table 1: Mean temperature, transparency, conductivity, salinity, pH, DO and TDS of water at the culture site and GR_L, GR_W of cultured oysters

Time (weeks)	Temperature/ °C	Transparency/ cm	Conductivity/ ms cm^{-1}	Salinity/ ppt	pH	DO/ mg L^{-1}	TDS/ mg L^{-1}	GRL / mm week^{-1}	GRW / mm week^{-1}
Initial	(n = 5)	(n = 5)	(n = 5)	(n = 5)	(n = 5)	(n = 5)	(n = 5)	(n = 4)	(n = 4)
(0)	29.00 ± 0.25^a	123.45 ± 0.75^a	6.78 ± 0.03^a	25.50 ± 0.50^a	7.51 ± 0.03^a	7.33 ± 0.16^a	698.50 ± 1.50^a	-	-
2	29.00 ± 0.00^a	178.75 ± 0.75^a	8.06 ± 0.01^a	27.50 ± 0.50^a	8.03 ± 0.03^a	9.52 ± 0.06^a	819.50 ± 0.50^a	1.63 ± 0.48^a	1.90 ± 0.09^a
4	29.25 ± 0.25^a	96.10 ± 0.10^{bc}	10.34 ± 0.01^a	28.00 ± 1.00^a	8.03 ± 0.03^a	8.58 ± 0.18^a	730.50 ± 2.50^a	2.19 ± 0.09^a	2.11 ± 0.39^a
6	29.00 ± 0.50^a	107.70 ± 0.70^b	10.52 ± 0.02^a	28.00 ± 0.00^a	7.75 ± 0.15^a	6.77 ± 0.73^a	0.90 ± 0.00^b	0.06 ± 0.01^a	0.89 ± 0.04^a
8	30.50 ± 0.50^a	100.30 ± 0.10^b	1.78 ± 0.03^b	6.00 ± 0.00^b	7.96 ± 0.03^a	10.79 ± 0.09^a	545.50 ± 1.50^a	1.26 ± 0.21^a	0.22 ± 0.08^a
10	29.75 ± 0.25^a	66.00 ± 0.20^{bc}	0.37 ± 0.02^b	1.00 ± 0.00^c	8.33 ± 0.02^a	11.27 ± 0.27^a	334.00 ± 0.20^a	0.62 ± 0.01^a	0.53 ± 0.30^a
12	30.25 ± 0.25^a	47.20 ± 0.30^c	0.94 ± 0.02^b	2.50 ± 0.50^c	8.61 ± 0.05^a	13.08 ± 0.12^a	832.50 ± 1.50^a	1.99 ± 0.20^a	0.64 ± 0.03^a
14	29.25 ± 0.25^a	101.00 ± 0.10^b	1.85 ± 0.04^b	8.50 ± 0.50^b	8.14 ± 0.03^a	10.29 ± 0.01^a	625.00 ± 1.20^a	0.86 ± 0.05^a	0.02 ± 0.01^a
16	30.50 ± 0.50^a	79.10 ± 0.60^{bc}	0.63 ± 0.05^b	0.50 ± 0.50^c	7.21 ± 0.06^a	11.52 ± 0.12^a	522.50 ± 0.50^a	0.13 ± 0.10^a	0.12 ± 0.01^a
18	30.25 ± 0.25^a	83.55 ± 0.55^{bc}	6.18 ± 0.02^a	21.00 ± 0.00^a	7.13 ± 0.07^a	11.48 ± 0.22^a	0.90 ± 0.00^b	0.65 ± 0.05^a	0.54 ± 0.16^a
20	29.00 ± 0.00^a	105.80 ± 0.20^b	6.46 ± 0.03^a	21.50 ± 0.50^a	7.16 ± 0.31^a	14.85 ± 0.25^a	0.90 ± 0.01^b	0.49 ± 0.29^a	0.26 ± 0.01^a
22	28.50 ± 0.50^a	147.65 ± 0.65^a	5.78 ± 0.01^a	18.50 ± 0.50^a	8.06 ± 0.04^a	12.44 ± 0.08^a	0.90 ± 0.02^b	0.80 ± 0.20^a	0.25 ± 0.08^a

All data are represented as mean ± SEM. Values with different superscripts in a column are significantly different from each other ($p < 0.05$); n = number of samples to calculate the mean values

experimental period and there was a significant negative relationship between the PM and salinity of culture water ($p < 0.05$; Figure 3).

Figure 4 illustrates the GR_L and GR_W of two groups of oysters whose outer shells were cleaned at biweekly intervals to remove external fouling organisms, and of oysters that were not cleaned. At the beginning there was no significant difference in growth rates between the two groups, but later on a significantly higher GR_L and GR_W values ($p < 0.05$) were exhibited by the group whose outer shell surfaces were cleaned to remove external

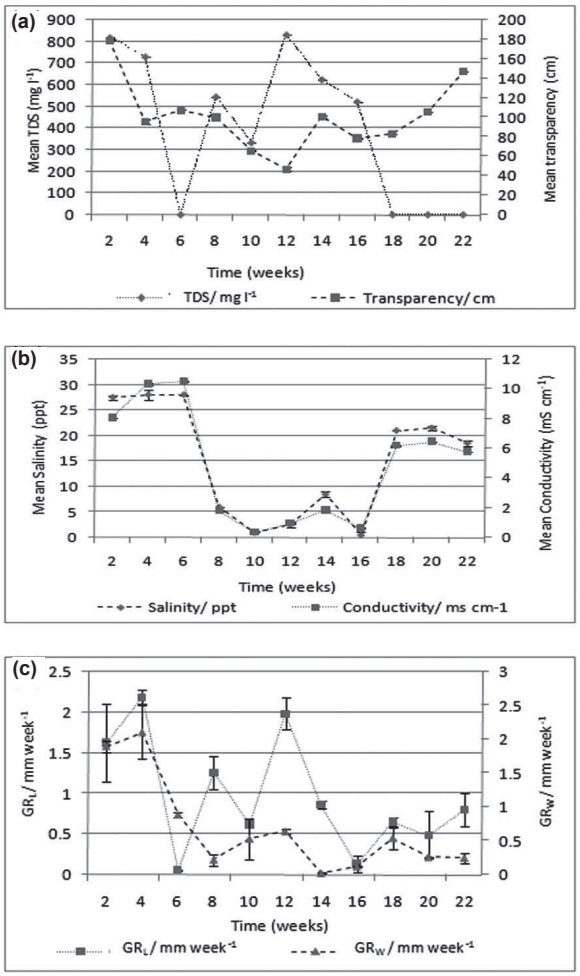

Figure 2: Fluctuations in mean total dissolved solids (TDS), transparency, salinity, conductivity of culture water [(a) & (b)] and the changes in mean growth rates of cultured *C. madrasensis* [(c); ± SEM] over the experimental period

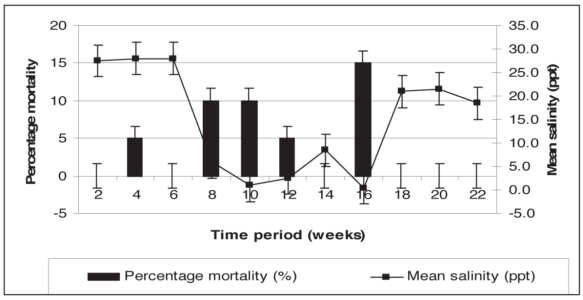

Figure 3: Percentage mortality (PM) of cultured *C. madrasensis* (±SEM) in relation to salinity of water at the culture site

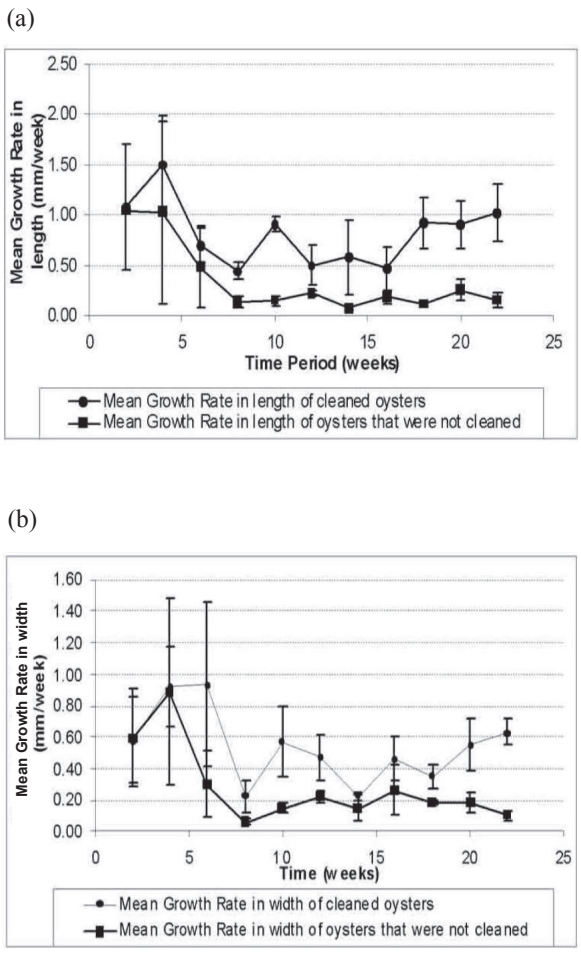

Figure 4: Changes of mean growth rate of oysters [in length, (a) and in width, (b) ± SEM] grown with external fouling and without external fouling on the shell surface

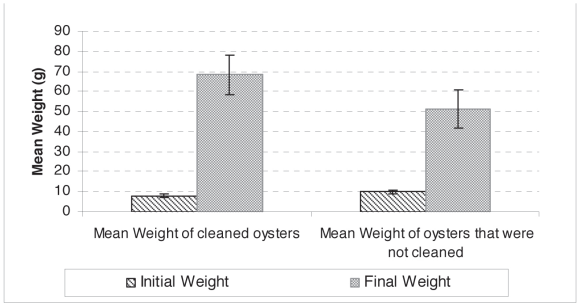

Figure 5: Mean initial and final weights (± SEM) of oysters grown with external fouling and without external fouling on the shell surface

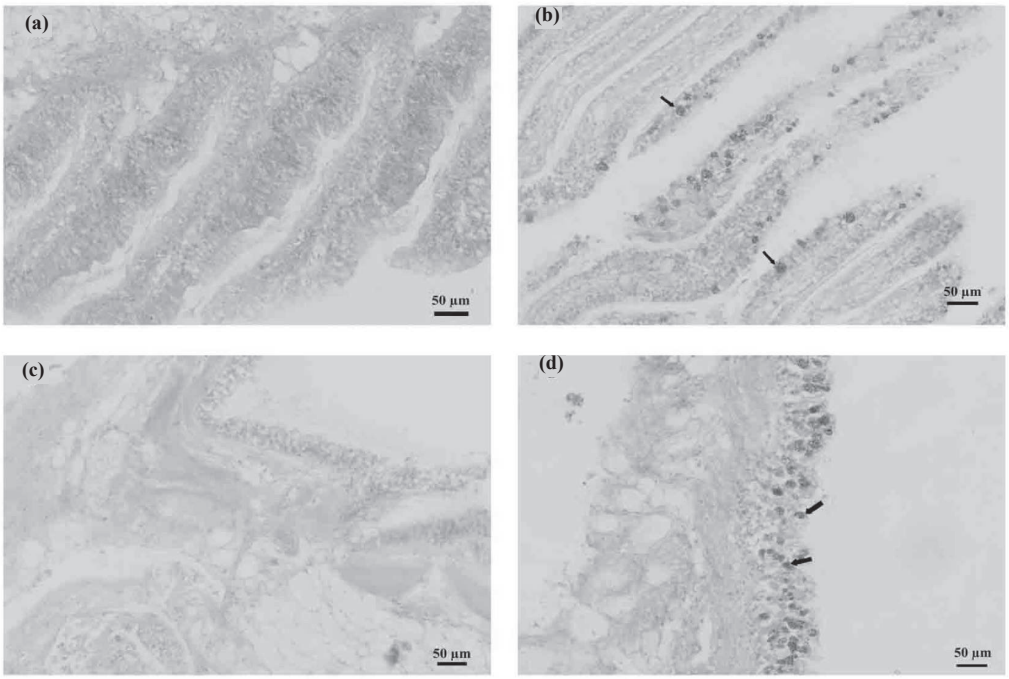

Figure 6: (a)- Gills of a healthy oyster; (b)- gills of an oyster showing structures similar to plasmodia (arrows); (c)- mantle tissue of a healthy oyster; (d)- mantle tissue of an oyster showing structures similar to plasmodia (arrows) of a haplosporidian parasite

fouling organisms settled on the shell. Despite the fact that the initial weights of oysters in both groups were not significantly different, the final weight of the group of oysters whose shell surfaces were regularly cleaned was significantly higher than that of oysters that were not cleaned of external fouling organisms ($p < 0.05$; Figure 5).

The young oysters that were observed initially did not have any abnormal condition and they were free from any parasite/pathogen. The body mass of oysters did not show water blisters, necrotic areas or any other abnormality at the end of the rearing period; there were no watery oysters. The wet mount preparations of different tissues of oysters did not reveal any abnormal condition. Histopathological sections of gills, mantle, digestive tract and digestive gland showed unusual structures, which were presumptively identified as plasmodia of a haplosporidian parasite (Figures 6 and 7). These structures in the oyster samples, which were collected during the low salinity period exhibited an appearance of rupturing and releasing spore-like small bodies (Figure 8).

DISCUSSION

During the present study, prevailing water quality parameters at the culture site of the Panadura estuary, Sri Lanka were recorded along with the growth rate of the oyster, *C. madrasensis* for a period of seven months. The results revealed that the growth rate, when measured as increase in width (GR_W), shows significant positive relationships to the salinity, transparency and conductivity of culture water ($p < 0.05$). Sasikumar *et al*. (2007) recorded cessation of growth in *C. madrasensis* cultured in Mulki estuary, Karnataka, India when salinity levels dropped below 1 gL^{-1}. According to these authors, the resumption in oyster growth rate occurred with increase in salinity of the estuarine water. Appukutan *et al*. (1998) reported similar trends in *C. madrasensis* cultured in Dharmadan estuary, India. According to Sasikumar *et al*. (2007), *C. madrasensis* could tolerate salinity changes from 0 – 41 gL^{-1} with cessation and resumption of growth, under unsuitable and suitable salinity, respectively. These authors have proposed that it could be cultured throughout the year in estuaries with seasonal changes in salinity as this species of oyster could adapt and survive.

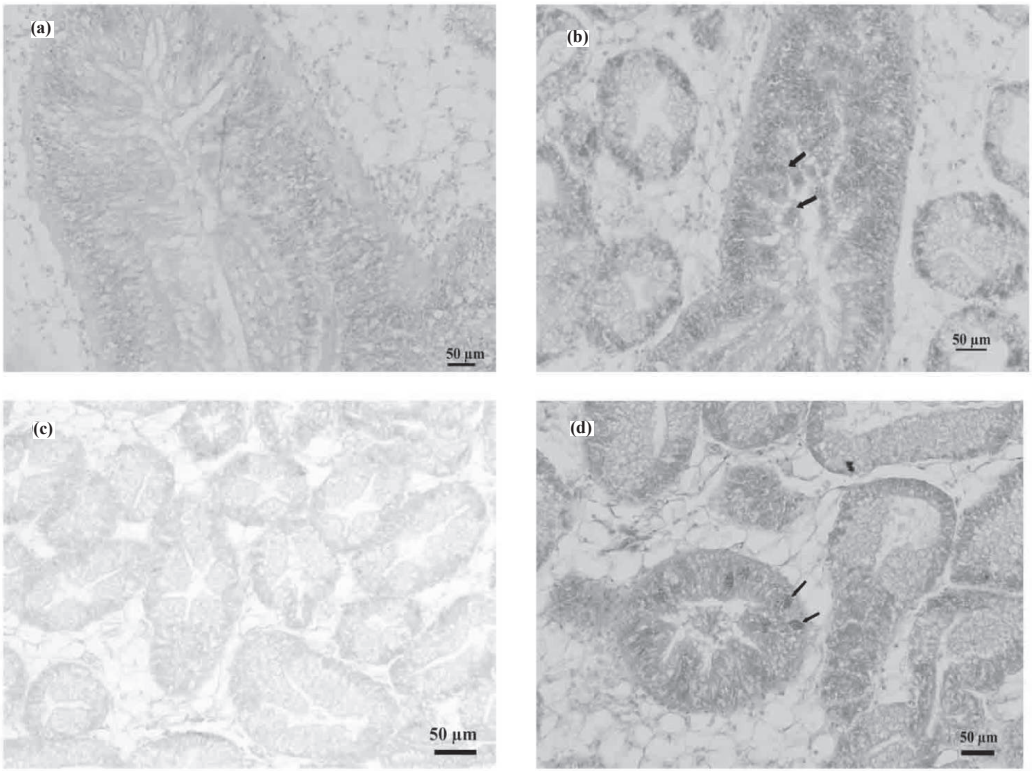

Figure 7: (a)- Digestive tract of a healthy oyster; (b)- digestive tract of an oyster showing structures similar to plasmodia (arrows); (c)- digestive gland of a healthy oyster and (d)- digestive gland of an oyster showing structures similar to plasmodia (arrows) of a haplosporidian parasite.

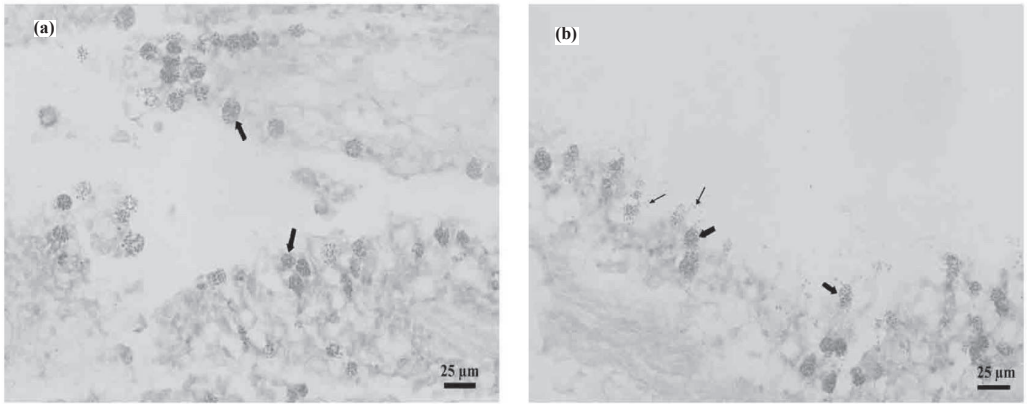

Figure 8: (a)-Structures similar to plasmodia of a haplosporidian parasite which is not in the state of rupturing (arrows); (b)-rupturing of the plasmodia like structures (thick arrows) dispersing small masses (thin arrows).

Results of the present study show that the growth rate of oyster *C. madrasensis*, when measured as the rate of increase in length (GR_L), has a significant positive relationship ($p < 0.05$) to the total dissolved solids (TDS) in culture water. Langdon and Newell (1990) have pointed out that oysters and mussels, apart from phytoplankton, also feed on detritus from sediment, dead phytoplankton and zooplankton and bacteria. The significant positive relationship between the growth rate of oyster and TDS of culture water observed during the present study could be due to the use of such items (that contribute for higher TDS) as food by *C. madrasensis* cultured in Panadura estuary, Sri Lanka.

However, during the present study, growth rates of oyster *C. madrasensis* did not show any significant relationship with temperature, transparency, pH and DO. The values of these parameters may be within the optimum ranges for the oyster due to good flow rate of the Panadura estuary that prevailed over the experimental period. According to Sasikumar *et al*. (2007) environmental changes, mainly temperature, appear to have much less influence on growth rate of oysters cultured in tropical areas.

According to available literature, in *Crassostrea* species, survival could exceed 75 % if the water salinity remains between 24 −30 ppt (Tan & Wong, 1996). Low salinity of culture water can depress shell and meat growth in oysters, through reduced mineral availability and stress-induced depression of metabolic activity (Rao & Nayar, 1956 cited by Sasikumar *et al*., 2007); these authors stated that *C. madrasensis* does not die due to low salinity. However, during the present study, mortality of oysters was recorded at low salinities in culture water indicating that there could be some other contributing factors, in addition to the low salinity, causing mortality of *C. madrasensis* of Panadura estuary.

The growth of fouling organisms on the shell surface often leads to decrease in the growth and survival of bivalves in suspended culture or reduces the value of the cultured species (Lodeiros *et al*., 2007). Lodeiros *et al.* (2002) observed a significant reduction of growth in different bivalve species and suggested that the limitation of valve opening in bivalves due to the weight of fouling organisms added to the shells of host, could be the reason for growth retardation. In addition, fouling organisms settled on the shell of bivalves such as barnacles, limpets, sponges and polychaetes can affect the health of oysters (Reantaso *et al*., 2001). During the present study, the mean growth rates (GR_L and GR_W) recorded for the oysters that were defouled at biweekly intervals were significantly higher ($p < 0.05$) than those of oysters, which were not cleaned. Mean final weight of regularly defouled oysters was also significantly higher ($p < 0.05$) than that of the oysters, which were not cleaned over the experimental period.

Some of the negative effects of external fouling are related to the reduction of water flow through culture enclosures that decrease the availability of food particles, dissolved oxygen in water and limit the dispersal of waste products. In addition, fouling filter feeding organisms could compete for food particles with the cultured bivalves or cause physical impairment (Lodeiros *et al*., 2002; Bishop & Hooper, 2005), while exerting strain on culture equipment due to excess weight of fouling organisms (Pit & Southgate, 2003). During the present study, culture baskets were defouled thoroughly allowing good water flow and only the external fouling organisms that were settled on the surface of shells were not removed to investigate the effect of them on the growth of oysters. The effect of interference by external fouling on opening and closing of valves seems to be greater on bivalves that lie on one side, such as oysters, as they have to use a greater force to lift the upper valve (Lodeiros *et al.,* 2007). Pit and Southgate (2003) reported that cleaning and removal of fouling organisms at every 2 or 4 weeks promoted the growth of *Pinctada maxima* juveniles in Indonesia and decreased the shell deformities compared to the oysters that were cleaned at every 8 or 16 weeks intervals. During the present study, defouling was done at two week intervals and it would be important to investigate the most suitable time intervals that give the maximum growth of *C. madrasensis* at the Panadura estuary, so that a cost effective cleaning programme could be adopted, as cleaning and removal of fouling organisms is a costly exercise (when man hours are assessed).

Protists in phylum Haplosporidia parasitize members of at least eight invertebrate phyla in freshwater and marine environments worldwide (Burreson & Ford, 2004), while haplosporidians in the genus *Bonamia* are known exclusively from oysters in euhaline to polyhaline coastal environments (Carnegie & Cochennec-Laureau, 2004; Paveena *et al*., 2008). Though the literature on diseases of cultured bivalves is extensive, there is no published work on diseases of cultured bivalves in Sri Lanka.

In the present study, parasites / pathogens or abnormalities were not observed in wet mount preparations of different tissues of cultured oysters. However, structures similar to plasmodia of a haplosporidian parasite (according to Lauckner, 1983; Balseiro *et al*., 2006; Carnegie *et al*., 2006; Abollo *et al*., 2008), were observed in histopathological preparations.

Rupturing of those plasmodia like structures and releasing spore-like small bodies were common in the oyster samples obtained during the period that the salinity of culture water became low. It would be interesting to perform definite diagnosis of those structures and to find out whether they contributed for the mortality of oysters recorded during the low salinity period of the present study. When the oyster production is expanded and intensified in the future, the diseases, which currently seem to be insignificant could become serious. Therefore, the present knowledge of health status is an essential pre-requisite for assessing whether disease profiles are changing as the industry expands/ intensifies.

CONCLUSION

Growth retardations of oyster, *C. madrasensis* was observed when the culture water was having low salinity, low transparency, low conductivity and low total dissolved solids. External fouling organisms that grew on the outer shell surface reduced the growth rate of cultured oysters significantly; regular defouling increased the growth rate. Cultured *C. madrasensis* seemed to be susceptible to a parasitic disease causing mortality at the time of salinity stress (during the period that the salinity of culture water became low).

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