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Effects of acidification on meroplanktonic oyster larval settlement in a tropical estuary

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Monthly variations in salinity, temperature and pH were studied in relation to settlement of meroplanktonic oyster larvae on cultches in Vembanad Lake, a tropical estuary along the southwest coast of India. A strong correlation between monthly pH values and oyster spat density (P=0.002) was evident. When the pH values in the estuary were low, the spat settlement density was poor (4.5/cultch), which was nearly half of the value (7.8/cultch) when the pH was alkaline. Experiments were conducted to evaluate the effects of extremes of temperature (20 to 35°C) and pH (6.5 to 8.5) on metamorphosis and survival of oyster larvae. In pH 6.5 there was 100% mortality and complete dissolution of dead shells in 24 h. Survival was highest (81%) in the control temperature of 27°C and less than 50% in 25 and 20°C. Treatment-wise differences in survival were significant (P<0.01). Study indicated that, if the pH drops below 7.0 during the meroplanktonic phase of oyster, recruitment will be affected and therefore oyster spat densities can be used as an indicator for acidification in tropical estuaries.

[**Keywords**: oyster culture, spat settlement, acidification, tropical estuary]

Introduction

Oceans absorb 24 million tonne of CO_2 each day and that the oceans have already absorbed more than 400 billion metric tons of carbon from the atmosphere due to human activities[1]. Carbon dioxide forms carbonic acid by dissolving in sea water thereby lowering the pH of the ocean water making it acidic.

Since 1751, the average pH of ocean surface waters has reduced from 8.25 to 8.14[2]. The Intergovernmental Panel on Climate Change's worst case scenario predicted that the pH of ocean waters could decrease to 7.85 by 2100[2,3]. This pH is much too low for most shelled plankton to survive and reproduce. Furthermore, with elevated levels of CO_2 lowering the pH, there would be less carbonate in the water, making it difficult for shelled organisms to form new structures[4]. Once the amount of carbonate drops below a critical level, animals can no longer absorb the nutrients they require, preventing them from forming shells[1,4]. Lower than normal pH levels can also lead to reduced egg size and a delay in hatching in some fish species also[5].There are indications that estuaries and coastal marine habitats are more susceptible to changes in pH than open oceans as they are shallower, less saline and have lower alkalinity and also because these regions are susceptible to substantial enrichment in CO_2 produced by the respiration of both natural and anthropogenic carbon[6].

As an adult, the oysters are benthic sedentary bivalves known to form shell reefs. The benthic structure caused by the horizontal and vertical expansion of oyster beds influences the particle transport, biological organization, nutrient trapping and sedimentation in the estuaries and coastal region[7]. Oysters spawn into the surrounding water, fertilization takes place in the seawater and the fertilized eggs develop into planktonic larvae.

The most common oyster in the estuaries of India is the Indian backwater oyster *Crassostrea madrasensis*. Fertilized egg of this oyster passes through a veliger, D-larvae, umbo, eyed larvae and settlesas spat after becoming the pedi-veliger and the entire larval cycle is usually completed in 15 to 20 days in tropical conditions[8]. During this phase,

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the oyster larvae are part of the micro and meso group of merozooplankton. They feed on phytoplankton, and in the food web, become a feed to other macro-plankters. Their survival and subsequent recruitment to a benthic ecosystem depends largely on the way the larvae is able to overcome the abiotic stress. In India oyster farming is carried out by women self-help groups as a livelihood activity[9] and the farmed oyster production in 2011 was 4058 tonnes[10].

While studying the hydrology of the marine zone of the northern region of Vembanad Lake, a tropical estuarine ecosystem, in relation to oyster spat settlement; significant inter-annual changes in the spat settlement pattern and density were observed. To investigate this, the pH and temperature of the oyster habitats were studied over a 3-year period and the decadal surface water temperatures were taken from global databases to look for occurrence of extreme values. Since low values of pH were observed in nature, the effects of extremes of these parameters on metamorphosis and survival of oyster larvae were studied experimentally in the hatchery.

Materials and Methods

The tropical estuary, Vembanad Lake opens to the Arabian Sea through two permanent openings, one of which is located in the northern region where oysters beds occur adjacent to the bar mouth (Fig.1). There is a vast permanent population of the Indian backwater oyster *Crassostrea madrasensis* which is also farmed in the estuary by the local villagers. The seed for farming is collected by placing spat collectors in the estuary during December-January which is the main spat-fall period. The monthly spat-fall records in number of oyster spat per cultch was used for correlating with monthly pH and temperature records.

Surface water temperature, salinity and pH in the oyster farming areas of north Vembanad Lake were recorded using digital meters (V2 multi-parameter probe, YSI, USA) every month during the years 2009-11. The three sampling locations with mean depth below 2m were the marine zone (ES -1) near the bar mouth, and two other stations to the interior in the order ES-2 and ES-3, the last being the upper reaches of the estuary and each separated by a distance of 5 to 8 km (see Fig.1 for latitude longitude positions).

To examine the long-term fluctuations of surface water temperatures, the records for Vembanad Lake from the ICOADS data (provided by the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA, from their Web site at http://www.esrl.noaa.gov/psd/database) were downloaded, decadal averages estimated and used for comparison with real-time values and with extreme values used for testing larvae in the hatchery experiment.

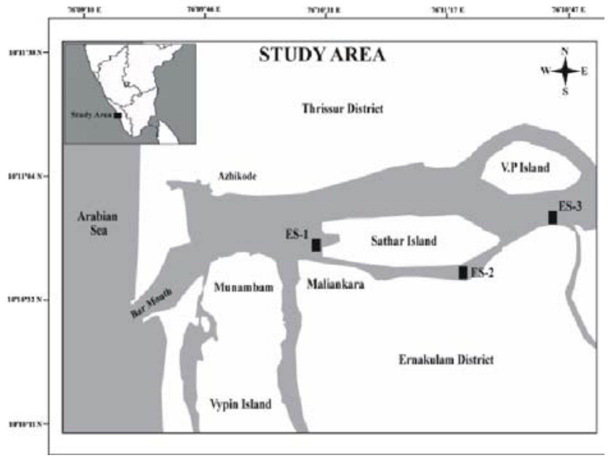

Fig. 1—Map of north Vembanad Lake showing bar-mouth opening into the Arabian Sea and the sampling stations (ES-1, ES-2 and ES3) near oyster beds and farming area.

Oyster spats are usually collected on shell clutches during the spawning period of the oyster. The clutches (5 shells in a string) were vertically hung from horizontal trestles erected in the estuary. Monthly visual counts of oyster spats on 50 random shell cultches were taken during 2009-2011 and represented as average number of spat per cultch for use as an index of successful reproduction events of the oysters.

Oyster broodstock were collected from the oyster reefs near Sathar Island in Vembanad Lake during September 2010 and induced to spawn by thermal stimulation in the oyster hatchery of Central Marine Fisheries Research Institute at Njarakkal in Vypin Island. The seawater for rearing larvae was pumped from the Arabian Sea for a couple of days, then passed through a series of cartridge filters (10 to 0.3μ) to remove physical impurities and finally passed through a UV filter to remove microorganisms. Active velar movements indicated the health status condition of the larvae.

The veliger larvae were exposed to treatment temperatures of 20, 25, 30 and 35ºC and the ambient temperature of 27^{o}C was taken as control. Temperatures were set and maintained in rearing containers using auto-thermostats. They were stocked in glass containers at the rate of 5000 larvae l^{-1} and thesalinity was maintained at 28. Larvae were fed with freshly cultured diatom *Isochrysis galbana*at the rate of 10,000 cells larvae^{-1}.Each treatment was maintained in triplicate and seawater was changed on every alternate day. Larvae were collected with specially designed sieves of mesh sizes 30 to 250 µm depending on the larval stage. Percentage survival was noted in each treatment by making counts using a haemocytometer. For assessing the influence of the particular experimental treatment on the metamorphosis of the oyster larvae, a grading system was used. Complete metamorphosis into spat from the initial veliger stage was considered as 100%. Development of the intermediate larval stages namely, D-larvae, umbo, eyed were graded as 25, 50 and 75% respectively and if most of the larvae metamorphosed into a particular stage, it was presumed that the development had progressed to the assigned percentage. In case two larval stages were observed in a particular treatment, then the average of the percentages assigned to the particular larval stage was taken.

Larvae were exposed to pH 6.5, 7.0, 7.5, 8.0, 8.2 and 8.5 with 8.2 taken as control pH. For pH manipulation the approach outlined in method 4 of Gattuso and Lavigne[11] was used with the modification of using weak acid (Citric Acid) and base (Tris buffer)[12]. Seawater with desired pH level was prepared just prior to water exchange, and invariably, pH could be maintained at ± 0.1. The useful buffer range for Tris (7-9) coincides with the physiological pH typical of most living organisms. The stocking density, feeding, water change and larval observations were the same as in the experiment on temperature.

The percentage survival of oyster larvae in various treatments was compared for significant differences using ANOVA (SPSS, ver.16.0). The monthly oyster spat density per cultch was also tested against pH, surface water temperature and salinity using Pearson Correlation.

Results

In general, in the lake system, average monthly temperature during the period 2009-11 ranged between 25 and 33.9°C with an average of 29.8°C (Fig. 2). A temperature peak was observed during April (pre-monsoon) and the minimum was observed in October (post-monsoon). The average salinity in the study area was 14.7 with a maximum of 33.3. The salinity was nil during the monsoon period (Fig2). The average pH of the estuarine region was 7.6 with a minimum of 6.2 and maximum of 8.7 (Fig. 2). Low values (less than 7.0) were observed in the upper reaches during August and September 2009 while at the same time in the marine regions the values were higher at 8.2 and 7.6 respectively. However, during the year 2011, pH values were consistently low from June to November in all the three stations.

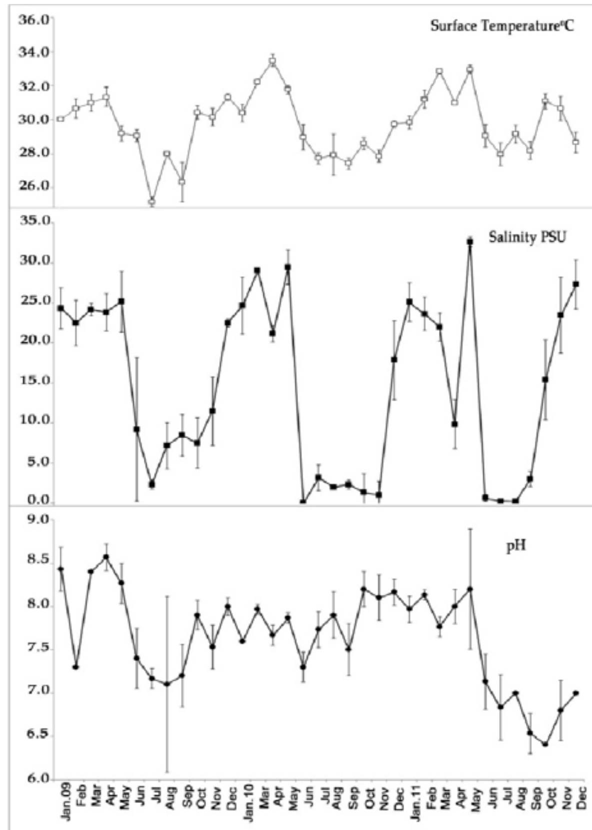

Fig. 2—Variations in surface water temperature, salinity and pH in the oyster farming area of north Vembanad Lake during 2009-2011. Values are averages from 3 sampling stations and vertical bars indicate standard deviations.

The monthly variations in surface water temperature during the period 1960-2009 were analyzed and it was observed that lowest temperature recorded was 25°C (June of 1990-99 decade) and maximum was 31.02°C (April of 1970-79 decade) (Fig. 3). The decadal average temperature during 1960-69 was 28.4°C which increased to 28.7 °C in the following decade. In the next two decades the average temperature declined to 28.5°C and 28.03°C respectively. However, during 2000-09, the average temperature was high at 29.03°C indicating a temperature difference (ΔT) of 0.79°C between 1960-69 and 2000-09. Real time observations of the surface water temperature during the three year periodshowed that the surface water values were slightly higher at 29.39, 29.65 and 30.23°C respectively.

In Vembanad Lake oyster spawning and spat occurred throughout the year with peaks in pre and post-monsoon. Mean annual pH, surface water temperature, salinity and oyster spat densities are shown in Table 1.

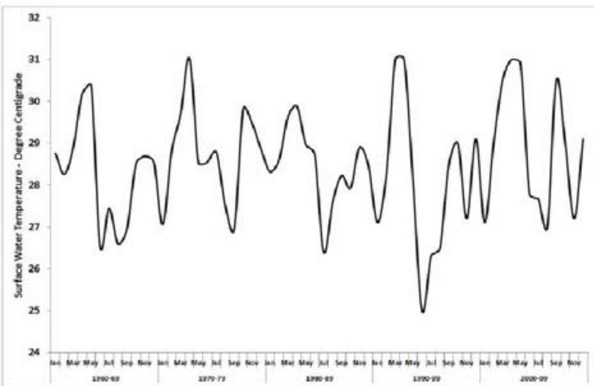

Fig. 3—Variations in mean monthly surface water temperatures on a decadal scale in northern Vembanad Lake. Data from ICOADS database.

While temperature and salinity values together showed an increasing trend from 2009 to 2011, the mean pH values increased in 2010 and then declined in 2011 to levels lower than in 2009. Monthly spat density was strongly correlated with the pH values (Pearson correlation coefficient=0.583; P=0.002). The spat settlement density was very poor (4.5/cultch) in 2011, being nearly half of the value (7.8/cultch) in 2009. In 2010, the monthly spat density was uniformly high (data not shown), when the monthly pH values did not show much fluctuation (Fig. 2).

Table 1: Annual mean values of pH, surface water temperature, salinity and annual mean oyster spat density in the oyster beds of northern Vembanad Lake during 2009-2011. SD=standard deviation.

Year	Annual Means			
	pH ± SD	Temperature °C ± SD	Salinity ‰ ± SD	Spat Density no/cultch ± SD
2009	7.49 ± 0.36	29.39 ± 1.98	11.67 ± 6.25	7.83 ± 2.25
2010	7.82 ± 0.29	29.65 ± 2.07	13.04 ± 12.31	10.90 ± 3.78
2011	7.31 ± 0.66	30.23 ± 1.69	15.29 ± 11.95	4.50 ± 3.18

Initially by Day-2survival of the veliger larvae in all treatment temperatures was 100% and thereafter there was progressive decrease in all the treatments (Fig.4). Survival was high and above 80% in the lower temperatures of 25 and 20°C upto Day-5 but decreased thereafter. None of the larvae survived in temperatures above 30°C after Day-5. Survival was highest (81%) in the control and less than 50% in 25 and 20°C. Treatment-wise differences in survival on different sampling days (except Day-2) were significant (P<0.01).

The larvae metamorphosed and completed all the intermediate stages before settling as spat on the Day-15 in the control (27 ºC) and 30 ºC treatment with a survival of 93 and 64% respectively (Fig.5). In lower temperatures of 25 and 20°C there was no proper development after Day-5. At 25°C the Umbo stage was prolonged with survival of 85% on Day-8 which was reduced to 32% on Day-20. However, even at the end of 20 days, the larvae did not metamorphose to the next stage though they were active.

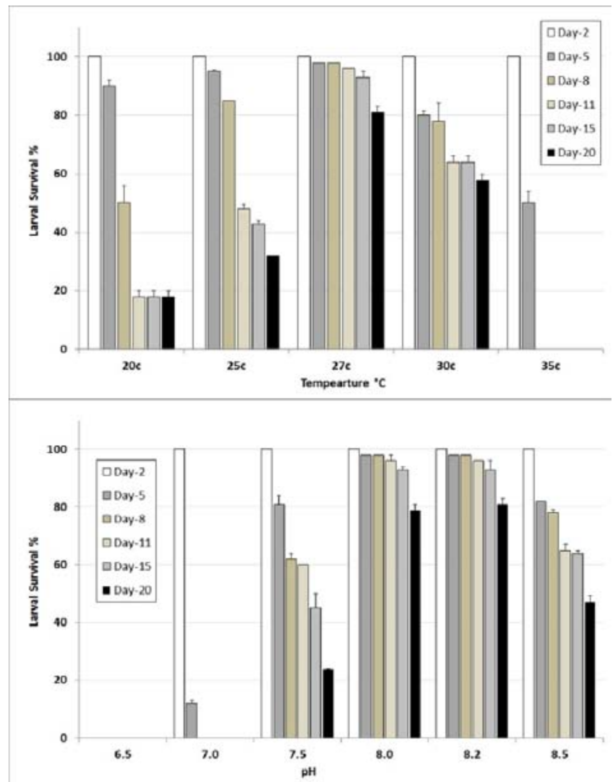

Fig. 4—Mean percentage survival of oyster larvae from Day-2 to Day-20 in experimental treatments for temperature and pH. Vertical bars indicate standard deviations.

In lower pH of 6.5, the larvae did not survive beyond the first day, and in pH 7.0, only 12% larvae developed to D-larvae. There was 100% mortality in this treatment on Day-5 (Fig. 4). There was a gradual decline in survival percentage in pH 7.5 reaching 45% by Day-15 when the larvae became spat. In this stage there was further mortality and only 24% survived by Day-20. All treatment-wise differences in survival were significant (P<0.01).

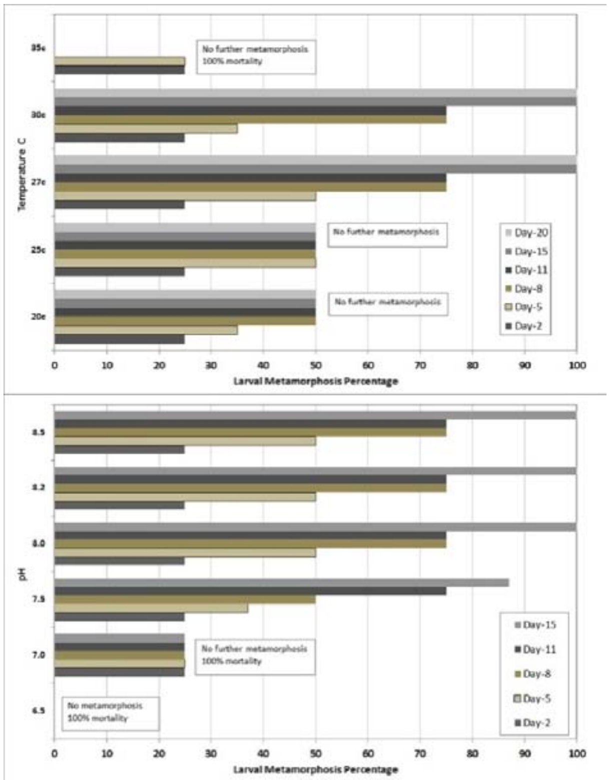

Fig. 5—Mean metamorphic stage of oyster larvae in percentage from Day-2 to Day-20 in experimental treatments for temperature and pH.

The larvae were active in pH 8.2 (control) and in treatments 7.5, 8.0 and 8.5. In all these treatments the metamorphosis of the veliger larvae to D-larvae and then to umbo and eyed stage progressed as in the control (Fig. 5). In pH 6.5 there was complete dissolution of dead shells, whereas, dead shells of larvae in all other treatments were intact. Dead shells of larvae in lower pH were also comparatively thin and fragile than the dead shells obtained in other treatments (Fig.6).

Discussion

The cyclic variations in surface water temperature, salinity and pH observed in the present study were typical of a tropical estuary influenced by seasonal monsoonal rains. The only variation from the normal was observed in the case of pH when low values were observed during the post monsoon period of 2011. Surface water temperature and salinity values showed a dynamic pattern influenced by the heavy seasonal rainfall (data not shown). Salinity reaches near seawater levels during the summer (March-May) and is close to fresh water condition during the monsoon. Normally, the pH is lowered due to the monsoon rainfall during June – August and this returns to low alkaline values during the post- monsoon. In 2010, the decline in pH values during post monsoon period was not substantial.Monthly mean oyster spat density was strongly correlated with the lowering of pH with low spat densities during occurrence of low pH as during the post-monsoon of 2011. The highest spat densities were observed during 2010 when the pH values did not show much fluctuation and were close to alkaline conditions. In the Ashtamudi Lake ecosystem, south of the Vembanad Lake, the oyster populations have peak spawning period during post-monsoon period withcorresponding increase in temperature, salinity and pH[13]. Success of the reproductive effort is indicated by the high spat density (24 numbers/cultch) during December. Quite clearly, this pattern has been affected in Vembanad Lake during 2011 when the pH values showed a rising trend only by December. Therefore, the low pH values were detrimental in the eventual spat settlement. In a recent study conducted to understand the impacts of elevated $p\mathrm{CO_2}$ and temperature on Sydney rock oysters, *Saccostrea glomerata*, it was observed that synergistically these had a negative impact on larvae causing reduction in growth, rate of development and abnormalities[14].

Fig. 6—Photomicrograph of healthy D-larvae in pH 8.2 (left pane) and dead shells in pH 6.5 (right pane). Arrow shows dissolution of shell.

It has been reported that organisms that incorporate calcium (Ca) and carbonate (CO_3) into their shells and tissues will be more affected by ocean acidification[3]. Normally, ocean waters are saturated with calcium and carbonate, allowing organisms to easily absorb calcium carbonate ($CaCO_3$) into their shells and bodily structures[1]making their shells hard and effective defense barriers. With elevated levels of CO_2 lowering the pH, there is less carbonate available in the water, making it difficult for organisms to form new structures[15]. Furthermore, at low levels of pH the carbonate in already formed shells starts to dissolve, causing shells to disintegrate. This phenomenon has already been observed in some plankton species[4] and in the oyster larvae in the present study.

The present experimental study also indicated that temperature between 27 and 30°C was congenial for the growth and survival of oyster larvae. Low and high temperatures, which were found to prolong the larval development and reduce the survival rate, were rarely observed (only in July 2009 when the temperature dropped to 25°C) in the natural bed indicating that the temperature variations in the estuarine region were well within the tolerable limits of the oyster larvae. Similarly, the range of temperatures observed during the analysis of decadal variations of surface water temperature in the study area indicated that the temperature fluctuations in the estuarine region were within the tolerable limits for the oyster larvae. In the temperate waters of Australia, 26°C was found to be the optimum temperature for fertilization of *S. glomerata*, and at 30°C there was no embryonic development. Whereas, in the tropical conditions of Vembanad Lake, 30°Cwas found to be congenial for growth and survival of *C.madrasensis* larvae, while at 35°C metamorphosis was affected and larvae did not survive beyond Day-5. Climate change impacts are known to affect seasons and it can be presumed that if the post- monsoon period in the tropics becomes cooler (<25°C) or if the pre-monsoon period becomes warmer (>30°C), crossing the limits for congenial larval growth, the oyster populations would be affected.

Based on the pH and salinity fluctuations observed in the oyster bed, we feel that the variations in pH in a tropical estuarine region where monsoon is strong, leading to drastic decline in salinities are detrimental to bivalve larvae. Though the pH values were normally above 7.5, there were instances when the pHs were low during the three year period. Very low pH values (less than 7) were observed during the monsoon and post-monsoon period of 2011. This can be attributed to the mixing of fresh rain water or land runoff from the coastal areas which are thickly populated.It has been indicated that seasonal discharges of acidic riverine water will further exacerbate acidification in estuaries, suggesting thepossibility of negative impacts to shellfish fisheries[16]. Similarly, it is reported that in estuaries, the saturation state of water (Ω) naturally decreases with decreasing salinity due to gradients in pH, calcium and carbonate ion concentration produced by the dilution of seawater with river water[17].

From the high mortality rates and dissolution of larval shell in low pH treatments, it can be inferred that, if the pH drops below 7.0 during the spawning and meroplanktonic phase of oyster, the recruitment to spat and adult stages will be affected. It has been indicated that bivalve larvae are more vulnerable than adults to increased CO_2 because larvae bio-mineralize aragonite, the more soluble form of $CaCO_3$ than calcite, the predominant material used in adult shell and that as atmospheric CO_2 concentrations increase, the proportion of estuarine habitat under-saturated for aragonite will increase[6]. In the present study it was observed that the larval shell completely disintegrates and the veliger larvae does not metamorphose in pH below 7.0 indicating that with acidification shelled larvae will be impacted. Moreover, under these stressed conditions, the metamorphosis of larvae was found to be prolonged. Such delay would increase the residence time of larvae in the plankton community as meroplankton, which can increase pre-settlement mortality especially due to predation and disease[18]. In a recent study on effects of ocean acidification on the metabolic rates of adult pearl oysters, mussels and scallops. It is reported that respiration and excretion rates were significantly lowered at lower pH. It is likely that the larvae are also similarly affected although it is difficult to gather similar proof for larvae[19]. In *S. glomerata*, adults exposed to elevated pCO_2 spawned larvae which were larger and had faster development[20]. This is an important adaptation of the oyster to acidification which however was not checked in the present study.

Conclusions

This study indicated that oyster larvae which form an important component of meroplankton and oyster spat densities might be used as an indicatorfor acidification and climate change in tropical estuaries. We show that the impact of acidification is substantial because as meroplankter the larvae of *C. madrasensis* are susceptible to changes in pH. If global acidification were to increase, oyster populations in tropical estuaries might be affected negatively leading to the loss of a critical habitat for many biota. Other tropical estuaries and oyster species need to be studied similarly in view of the serious threat of increasing acidification of waters.

Acknowledgements

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