Influence of Environmental Factors on Growth, Survival, and Heavy Metal Accumulation in Oyster, *Crassostrea madrasensis* (Preston, 1916) Cultivated in Negombo Estuary, Sri Lanka

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Abstract

The growth of marine bivalves is affected by the interactions of several environmental variables, particularly water salinity, temperature, and food supply. Influences of environmental parameters on daily weight gain (DWG), survival and heavy metal accumulation in 225 numbers of oysters, *Crassostrea madrasensis* (Preston, 1916), placed at five locations in the Negombo estuary, Sri Lanka, were investigated. One-way ANOVA indicated significantly higher ($P < 0.05$) DWGs (0.22 ± 0.01 and 0.16 ± 0.01 g.day^{-1}) in Pitipana and Munnakaraya, where highest mean salinity (20.9 ± 0.34 ppt) and chlorophyll-a (5.41 ± 1.49 µg.L^{-1}) were observed. Significantly lower growth rate (0.04 ± 0.02 g.day^{-1}) recorded in Thaladuwa, where lowest salinity (13.29 ± 1.13 ppt), highest turbidity (19.26 ± 0.99 NTU) and ammoniacal nitrogen (0.368 ± 0.078 mg.L^{-1}) were recorded. DWG showed a significant second-order polynomial relationships with chlorophyll-a ($R^2 = 0.44$, $P < 0.05$) and salinity ($R^2 = 0.28$, $P < 0.05$). Negative exponential relationships of DWG were evident with higher level of ammoniacal nitrogen ($R^2 = 0.24$, $P < 0.05$) and phosphate ($R^2 = 0.25$, $P < 0.05$). The high concentration of lead (1.883 mg.kg^{-1}) exceeded the EU permissible limit of 1.5 mg.kg^{-1} (wet weight) in oysters' tissue where urban wastewater is released to the lagoon. There appeared to be health concerns due to heavy metal accumulation in oyster tissues in polluted areas of the estuary. The findings of this study are useful for understanding the potential impacts of environmental changes on oyster resources and the long-term sustainability of oyster fisheries and aquaculture.

Keywords: chlorophyll-a, environmental effect, growth, resource management, heavy metals

Introduction

Oyster resources are potential sources of human food security and socio-economic sustainability due to their nutritious food value and economic and societal value through fishing and aquaculture. Oyster resources in the wild have declined worldwide as a result of overexploitation and habitat alteration (Kirby, 2004), and affected the ecosystem services they provide, including improvement of water quality through suspension-feeding activities, provision of feeding habitat and refuge for fish and other invertebrates, and commercial and recreational shellfish harvest by humans (Brumbaugh et al., 2006).

Oyster growth and mortality are multifactorial phenomena associated with several biotic and abiotic factors. Oysters can only survive in a relatively narrow range of the water quality conditions available in estuaries (Wasson et al., 2015). As marine sessile organisms, oysters are very sensitive to environmental changes due to their inability to exist in unfavourable conditions (Thomas et al., 2018). Studies elsewhere have shown that the formation and growth of marine bivalves were greatly affected by the interactions of several environmental variables in the habitat, particularly water temperature, food supply (Bayne and Newell, 1983; Brown, 1988; Dekshenieks et al., 1993; Tomaru et al., 2002; Carmichael et al., 2012; Freites et al., 2020), salinity (Ulanowicz et al., 1980; Horodesky et al., 2019; Sehlinger et al., 2019), turbidity (Huntington and Miller, 1989), type of microhabitat (Bartol et al., 1999)

and estuarine hydrodynamics (Dekshenieks et al., 2000; Wang et al., 2008; Huang 2010; Campbell and Steven, 2019).

Water quality characteristics in the estuaries critically depend on freshwater inflow, tides, and weather variables (Barron et al., 2002; Feng and Li, 2010). Salinity and temperature are the most critical factors affecting the oyster, *Crassostrea virginica* (Gmelin, 1791) growth and mortality (Rybovich, 2014; La Peyre et al., 2016). Estuary orientation, hydrodynamics, and lagoon management are critical in influencing local salinities (Dekshenieks et al., 2000; Palmer et al., 2011). Furthermore, changes in estuarine salinity regimes were found to impact oyster mortality due to changes in predation and diseases and their filtration and respiratory rate (Powell et al., 1995; Huang, 2010). Livingston et al. (2000) reported that the growth of oysters was positively influenced by salinity while other confounding factors, including river inflow, oyster density and current velocities, also affecting oyster growth.

Growth rates and productivity of oysters were influenced mainly by the supply and availability of food quality, measured as chlorophyll-a and percentage of particulate organic matter (Mitchell, 2001). Oyster filtration rates are increased with increasing water temperature, resulting in increased ingestion of food (Klinck et al., 1992). Powell et al. (1995) and Freites et al. (2017) also found that oyster growth increased with increasing food availability reflected by chlorophyll-a. Wasson (2010) found that the absence of oysters was associated with eutrophication, including elevated nutrient concentrations and turbidity and minimal tidal exchange of the Pacific coast estuary in California.

Snyder et al. (2017) noted that turbidity, an index of suspended particulate matter, negatively affects oyster *C. virginica* feeding/filtration at high concentrations due to diluting algal cells with predominantly inorganic matter. Many mechanisms have contributed to oyster decline globally, including overharvesting, water quality degradation, habitat losses due to urbanisation and agriculture, and diseases (Kirby, 2004; Brumbaugh et al., 2006).

The Negombo estuary is a shallow basin estuary of approximately 3,164 ha in extent, located between latitude 7°6′–7°12′N and 79°40′–79°53′E on the west coast of Sri Lanka. It is connected to the sea by a single narrow opening, the Negombo channel segment at its northern end, open year-round. The salinity of the estuary is strongly related to the monsoon rains and estuary salinity ranged spatially from 0–25 ppt (Gammanpila, 2010). Negombo estuary is a productive fishing ground and a sink for many anthropogenic effluents draining from its surrounding urban area. Oyster resource in the Negombo estuary system is important both ecologically and economically and as a source of food for the surrounding communities of the estuary. The native oyster, *Crassostrea madrasensis*

(Preston, 1916) of the Negombo estuary has been the subject of relatively few ecological investigations (Pinto and Wignarajah, 1980; Senadheera et al., 2000; Senadheera and Chandrika, 2005) and there are no studies to understand various biotic and abiotic characteristics pertaining to the oyster areas. The present study aimed to determine the environmental parameters (salinity, food availability, turbidity, and nutrients) on the growth of the oyster population and whether there is any contamination of heavy metals in oyster flesh. The findings of the study could provide a better understanding of the management and commercial use of oyster resources for long-term sustainability of oyster beds in the Negombo estuary.

Materials and Methods

Oyster growth and mortality data

Monthly field studies were conducted at five sampling sites, namely Thaladuwa, Munnakkaraya, Pitipana, Wedikanda and Dungalpitiya in the Negombo estuary (Fig. 1) from April to October 2018 to evaluate the seasonal changes of environmental parameters and growth of oysters.
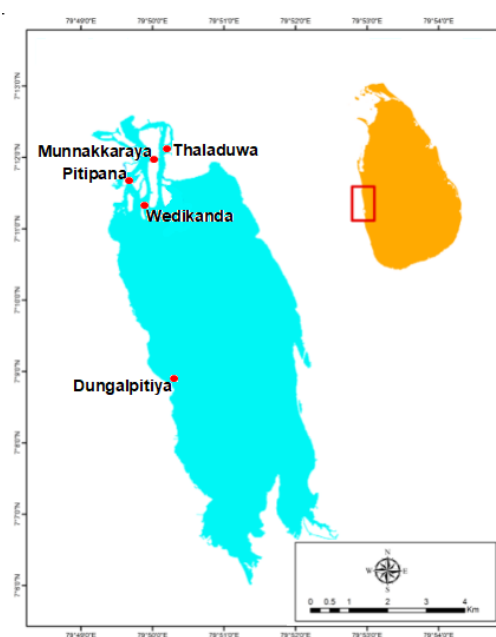

Fig. 1. Map of Negombo estuary depicting the cultivation sites of the oysters *Crassostrea madrasensis*.

The oysters *C. madrasensis* used in the experiments (n = 225) were collected from the Negombo estuary on the west coast of Sri Lanka (Location: 7°6′–7°12′N and 79°40′–79°53′E). After collection, oysters were washed to remove sediment and epibionts. Three plastic cages of 30 × 25 cm^2 size were installed at 30 cm above the bottom in each location and 15 oysters having a mean weight (± SD) of 26.07 ± 7.28 g, and length (± SD) of 50.3 ± 4.59 mm were stocked in each cage. The growth of each oyster in terms of length and weight were measured to the nearest millimetre and gram using Vernier calliper (± 0.5 mm) and digital balance (± 0.01 g),

respectively. After measurements, the oysters were returned to the cages. At each monthly sampling, oyster growth and mortality were recorded and, water quality adjacent to cages was determined. The experiment was completed when oysters reached marketable size.

Growth rates were calculated using the following equations;

Daily weight gain (DWG g.day^{-1}) = $(P_2 - P_1)/(t_2 - t_1)$

where P_1 and P_2 are the mean shell weight at time t_1 and t_2 (days), respectively.

Specific growth rate (SGR %) = (Ln Final weight – Ln Initial weight) / (Number of days)*100

Survival rate (%) = (Final number of surviving oyster / Initial number of oyster)*100

Plastic cages were cleaned or changed, and fouling organisms were removed with a knife every month to improve water circulation of cages.

Water samples were analysed as follows:
Water temperature, pH and salinity were measured using a thermometer, portable pH meter (HACH SensION 1, USA), and a refractometer, respectively. A portable turbidity meter (Eutech–TN 100, USA) was used to determine water turbidity. Dissolved oxygen concentration was determined by Winkler titration procedure (Mackereth et al., 1978). The chlorophyll-a concentration was determined spectro-photometrically after filtering samples through Whatman GF/C filters using the method described by Parsons et al. (1984). APHA (2012) ex-situ analysis was conducted for the determination of nitrate-N by 4500 E Cu/Cd reduction method, nitrite-N by 4500 B colorimetric method NED/sulphanilamide, phosphate by 4500 P E ascorbic acid method and ammoniacal nitrogen (NH$_3$-N) by 4500 NH$_3$ F phenate method. The amount of unionised ammonia was calculated according to the equations of Thurston, Khoo and Whitfield modified by Boueres (2001) based on salinity, water temperature and pH values in estuarine water. Three replicates were used for determining each water quality parameter. To determine water quality parameters in each location, duplicate field sampling was made fortnightly, and the mean value was determined.

For the analysis of heavy metals in oysters nine tissue samples obtained from oysters from each site were analysed using atomic absorption spectroscopy (AAS) (Bartram and Ballance, 2015).

For ethical clearance, permission for conducting research was obtained from the ethics review committee of University of Kelaniya (https://units.kln.ac.lk/ethics/).

Data analysis

The values of daily weight gain (DWG), specific growth rate (SGR) and survival rate of oysters and environmental parameters in each sampling location were compared using one-way ANOVA. The significance level for all analyses was set at $P < 0.05$. The data were ln+1 transformed to ensure compliance with the assumption of normality of data for ANOVA procedure. Tukey's multiple comparisons test was used to investigate the differences between the group means. Statistical correlation of oyster growth (DWG) with the variation in environmental parameters was tested by regression analysis. All statistical data analyses were performed using MINITAB software (version 16) and Microsoft Excel.

Results
Growth rate

Compared with Thaladuwa, significantly higher ($P < 0.05$) DWG (0.22 ± 0.01 and 0.16 ± 0.01 g.day^{-1}) were recorded in Pitipana and Munnakkaraya, where the highest salinity and chlorophyll-a were also recorded (Fig. 2A). Significantly lower DWG of oysters (0.04 ± 0.02 g.day^{-1}) was recorded in the Thaladuwa site with significantly lower salinity (13.29 ± 1.13 ppt), higher turbidity (19.26 ± 0.99 NTU) and ammoniacal nitrogen (0.368 ± 0.08 mg.L^{-1}). The SGR of oysters was highly variable between locations and ranged from 0.12 ± 0.06 % in Thaladuwa to 0.48 ± 0.01 % in Pitipana (Fig. 2B). The survival rate of oysters was not significantly different between sampling locations (Fig. 2C).
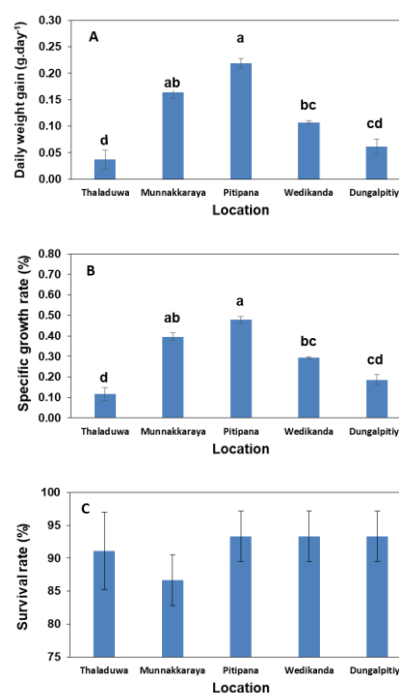

Fig. 2. Growth rate (± SD) (2A), specific growth rate (2B), and survival rate (2C) of oysters *Crassostrea madrasensis* at five different locations during the study period. Different superscript letters indicate significant differences ($P < 0.05$) among locations.

Environmental parameters

Analysis of variance (ANOVA) revealed that water quality differed between sites where oysters were placed for growth and survival studies (Table 1). Extremes of water temperature did not occur in the study areas (Fig. 3). Among the other physico-chemical parameters, mean salinity was significantly higher at Pitipana (20.9 ± 0.34 ppt), while the lowest salinity of 13.29 ± 1.13 ppt was recorded at Thaladuwa where there was an inflow of urban wastewater canal into the estuary. Turbidity at each sampling site was significantly different ($P < 0.05$) ranging from 8.54 ± 0.07 NTU in Dungalpitiya to 19.26 ± 0.99 NTU in Thaladuwa among five sampling locations. The mean concentration of ammoniacal nitrogen (NH_3-N), nitrate

and nitrite were highly variable ranging from 0.105 ± 0.003 to 0.368 ± 0.08 mg.L^{-1}, 0.003 ± 0.001 to 0.026 ± 0.002 and 0.003 ± 0.001 to 0.031 ± 0.008 mg.L^{-1} and significantly different among the sites (Table 1). The concentration of un-ionised ammonia was significantly different among sampling locations, and the maximum amount of 0.0242 ± 0.0022 mg.L^{-1} was recorded in Dungalpitiya. The highest mean value of phosphate was measured in Dungalpitiya, although this variation was not significant.

High contaminations of lead (1.883 mg.kg^{-1}) and mercury (0.015 mg.kg^{-1}) were recorded in oyster body tissues in Thaladuwa, where wastewater is discharged into the lagoon (Table 1).

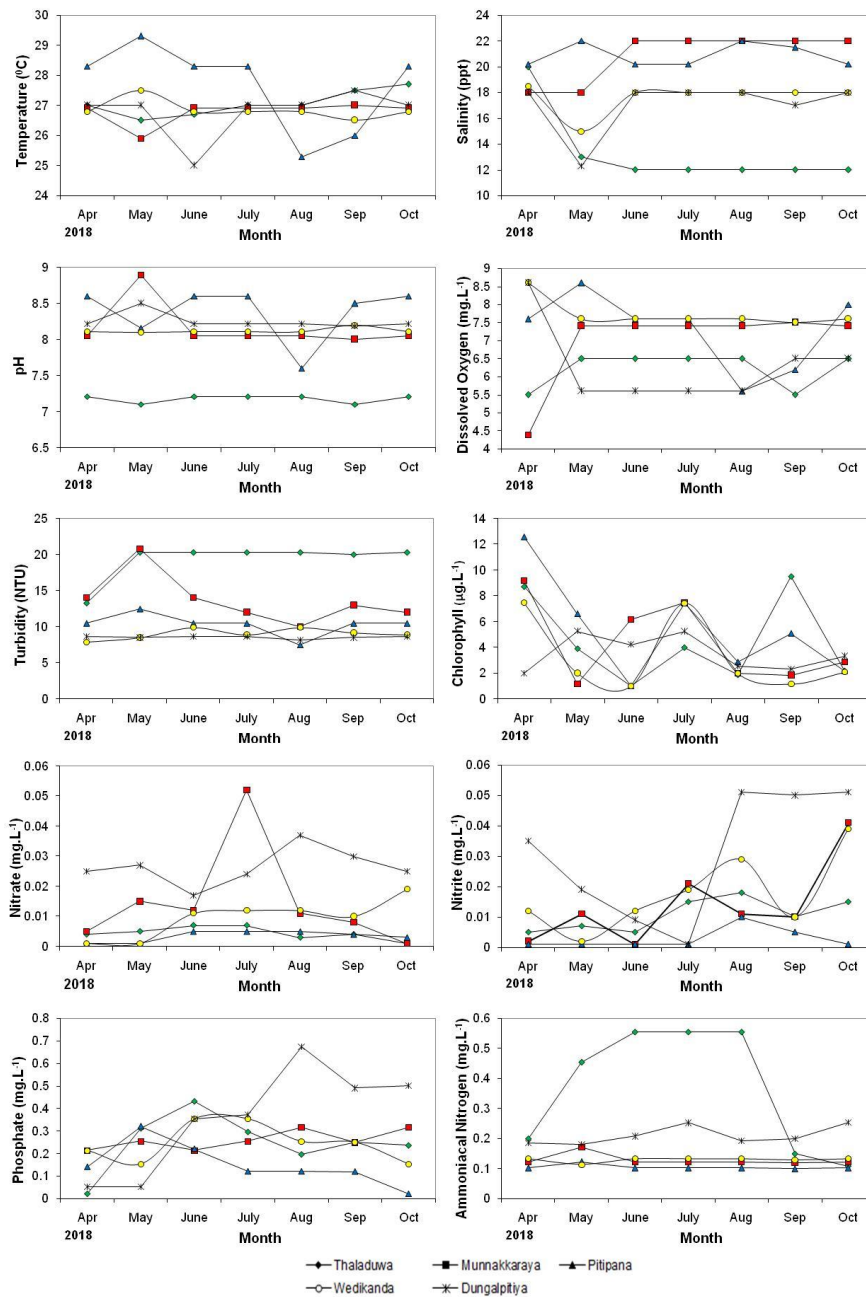

Fig. 3. Monthly variation of environmental parameters of five sampling sites of Negombo estuary during the entire sampling period. n = 15 for each parameter in each month.

Table 1. Physico-chemical parameters in sampling locations of Negombo estuary (April–Oct 2018) and heavy metal contamination in oyster *Crassostrea madrasensis* tissues.

| Parameter | Location | | | | |
	Thaladuwa	Munnakkaraya	Pitipana	Wedikanda	Dungalpitiya
Water temperature ($^{\circ}$C)	27.06 ± 0.16	26.77 ± 0.15	27.69 ± 0.55	26.86 ± 0.12	26.79 ± 0.31
Salinity ($^0/_{00}$)	13.29 ± 1.13[c]	20.86 ± 0.74[a]	20.9 ± 0.34[a]	17.64 ± 0.45[ab]	17.04 ± 0.8[b]
pH	7.18 ± 0.02[b]	8.16 ± 0.12[a]	8.38 ± 0.14[a]	8.12 ± 0.01[a]	8.26 ± 0.04[a]
Dissolved oxygen (mg.L^{-1})	5.89 ± 0.18[b]	6.99 ± 0.43[ab]	7.31 ± 0.39[ab]	7.73 ± 0.15[a]	6.29 ± 0.42[b]
Turbidity (NTU)	19.26 ± 0.99[a]	13.71 ± 1.29[b]	10.36 ± 0.55[c]	9.05 ± 0.27[c]	8.54 ± 0.07[c]
Chlorophyll (µg.L^{-1})	4.46 ± 1.27	4.38 ± 1.2	5.41 ± 1.49	3.32 ± 1.08	3.58 ± 0.51
Nitrite-N (mg.L^{-1})	0.011 ± 0.002[ab]	0.014 ± 0.005[ab]	0.003 ± 0.001[b]	0.018 ± 0.005[ab]	0.031 ± 0.008[a]
Nitrate-N (mg.L^{-1})	0.004 ± 0.001[b]	0.015 ± 0.006[ab]	0.003 ± 0.001[b]	0.009 ± 0.002[b]	0.026 ± 0.002[a]
Phosphate-P (mg.L^{-1})	0.249 ± 0.047	0.260 ± 0.016	0.152 ± 0.036	0.248 ± 0.031	0.356 ± 0.088
Ammoniacal-nitrogen (mg.L^{-1})	0.368 ± 0.078[a]	0.129 ± 0.007[b]	0.105 ± 0.003[b]	0.131 ± 0.003[b]	0.211 ± 0.011[b]
Un-ionised ammonia (mg.L^{-1})	0.0041 ± 0.0009[b]	0.0162 ± 0.0077[ab]	0.0188 ± 0.0033[ab]	0.0112 ± 0.0003[ab]	0.0242 ± 0.0022[a]
Heavy metal contamination in oyster tissues (mg.kg^{-1} wet weight basis)					
Mercury	0.015	0.003	0.009	0.004	0.011
Cadmium	0.211	0.858	1.084	0.565	0.667
Lead	1.883	0.673	0.353	0.694	0.461

Values expressed as mean ± SE. n = 15 for each parameter. Different superscript letters indicate significant differences ($P < 0.05$) among locations based upon ANOVA.

Relationship between growth and environmental parameters

There were significant positive second order relationships between DWG of oysters with chlorophyll–a ($R^2 = 0.44$, $P < 0.001$; Fig. 4A) and salinity ($R^2 = 0.28$, $P < 0.02$; Fig. 4B). However, turbidity (Fig. 4C) and nitrate (Fig. 4D) did not have a significant influence on the DWG of oysters ($P > 0.05$). Significant negative exponential relationships were observed between DWG of oysters and amount of ammoniacal nitrogen ($P < 0.01$; Fig. 4E), phosphate ($P < 0.01$; Fig. 4F) and nitrite ($P < 0.05$; Fig. 4G). Other environmental parameters including water temperature ($R^2 = 0.10$; $P = 0.27$), dissolved oxygen ($R^2 = 0.06$; $P = 0.43$) and pH ($R^2 = 0.06$; $P = 0.57$) had no significant effect on oyster growth.

Relationship between specific growth rate and chlorophyll–a showed significant positive second order relationship ($R^2 = 0.68$, $P < 0.001$; Fig. 5A) and salinity showed marginal nonsignificant relationship ($R^2 = 0.15$, $P = 0.055$; Fig. 5B). Nevertheless, turbidity (Fig. 5C) and nitrate (Fig. 5D) did not show significant effect on SGR of oysters ($P > 0.05$). There were significant exponential relationships between SGR of oysters and amount of ammoniacal nitrogen ($P < 0.05$; Fig. 5E), phosphate ($P < 0.01$; Fig. 5F), and nitrite ($P < 0.05$; Fig. 5G). However, exposure of oysters to a narrow range of un-ionised ammonia had no significant effect ($P > 0.05$) on oyster growth (Fig. 4H and 5H).

Discussion

Many studies in different regions have reported the influence of abiotic and biotic factors on oyster growth, reproduction, survival, distribution, and production (Powell et al., 1995; Livingston et al., 1999; Dekshenieks et al., 2000; Livingston et al., 2000; Apeti et al., 2005; Huang, 2010; Grizzle et al., 2018). Salinity is one of the most critical environmental factors affecting oyster populations (Livingston et al., 2000; Dame et al., 2002; Wang et al., 2008; Horodesky et al., 2019). The salinity range for optimal growth is reported between 20 and 25 ppt (Wang et al., 2008). Laboratory experiments (Horodesky et al., 2019) reported that *Crassostrea gasar* (Deshayes, 1830) could survive for long periods in waters with salinities ranging from 4 and 40 g.L^{-1}, even without access to food, but was intolerable when salinity was less than 2 g.L^{-1} and was greater than 50 g.L^{-1}. However, prolonged periods of low salinity can result in die-offs (Grosholz et al., 2008).

Temperature can play an essential role in reproduction (Baker, 1995). Salinity and temperature were found to critically control *C. virginica* growth and mortality in the Breton Sound Estuary, Louisiana, suggesting that seasonal changes to river discharge affecting water quality over the oyster grounds have profound impacts on oyster populations (La Peyre et al., 2016). Furthermore, according to Rybovich (2014) oysters (*C. virginica*) at the lowest salinity (annual mean = 4.8 ppt) experienced significantly higher mortality and lower
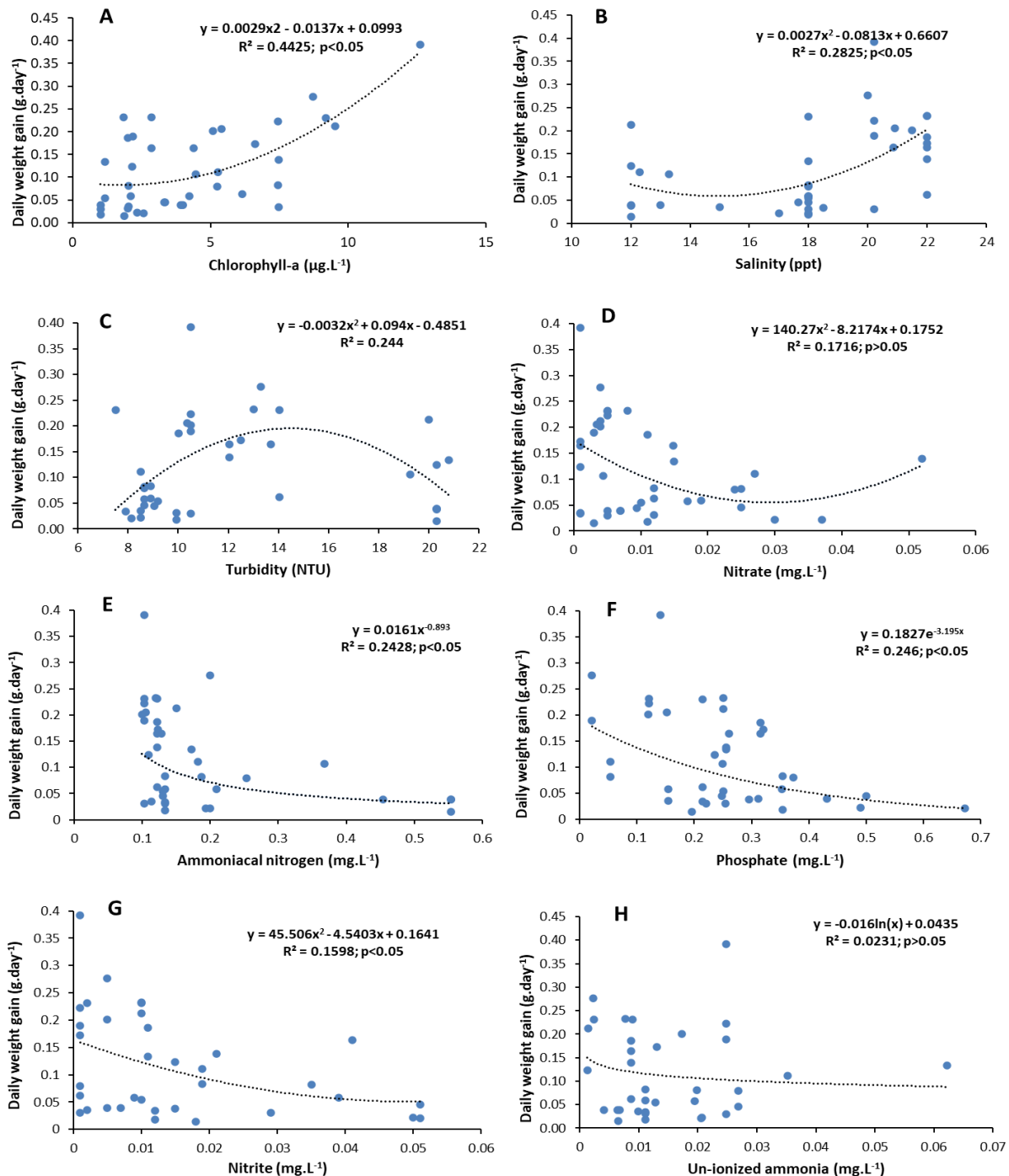
Fig. 4. Results of regression analysis of growth rates of the oysters *Crassostrea madrasensis* on environmental parameters during the study period. Significant level $P < 0.05$.

growth than oysters located in higher salinity (annual mean of 11.1 and 13 ppt). The author stated that oyster growth is best achieved at salinities 12–28 ppt.

Crassostrea virginica are able to survive in a wide range of unfavourable salinity conditions (Pollack et al., 2011; La Peyre et al., 2013) by closing their shells, minimising energetic demand, and relying on anaerobic metabolism (Michaelidis et al., 2005). However, it is only effective for a short period, and oysters are susceptible to prolonged seasonal and annual changes in salinity. In the present study, a weak correlation

between salinity and oyster growth indicated minimal influence on salinity because the high fluctuation of salinity did not occur among the study sites. Low correlation between oyster growth and other environmental variables such as chlorophyll, turbidity and nutrients may indicate that complex biological processes of multiple factors and their possible interactions govern oyster growth.

The increased freshwater influx from Dandugam Oya was associated with lower salinity patterns in the southern portion of the Negombo estuary. In contrast,
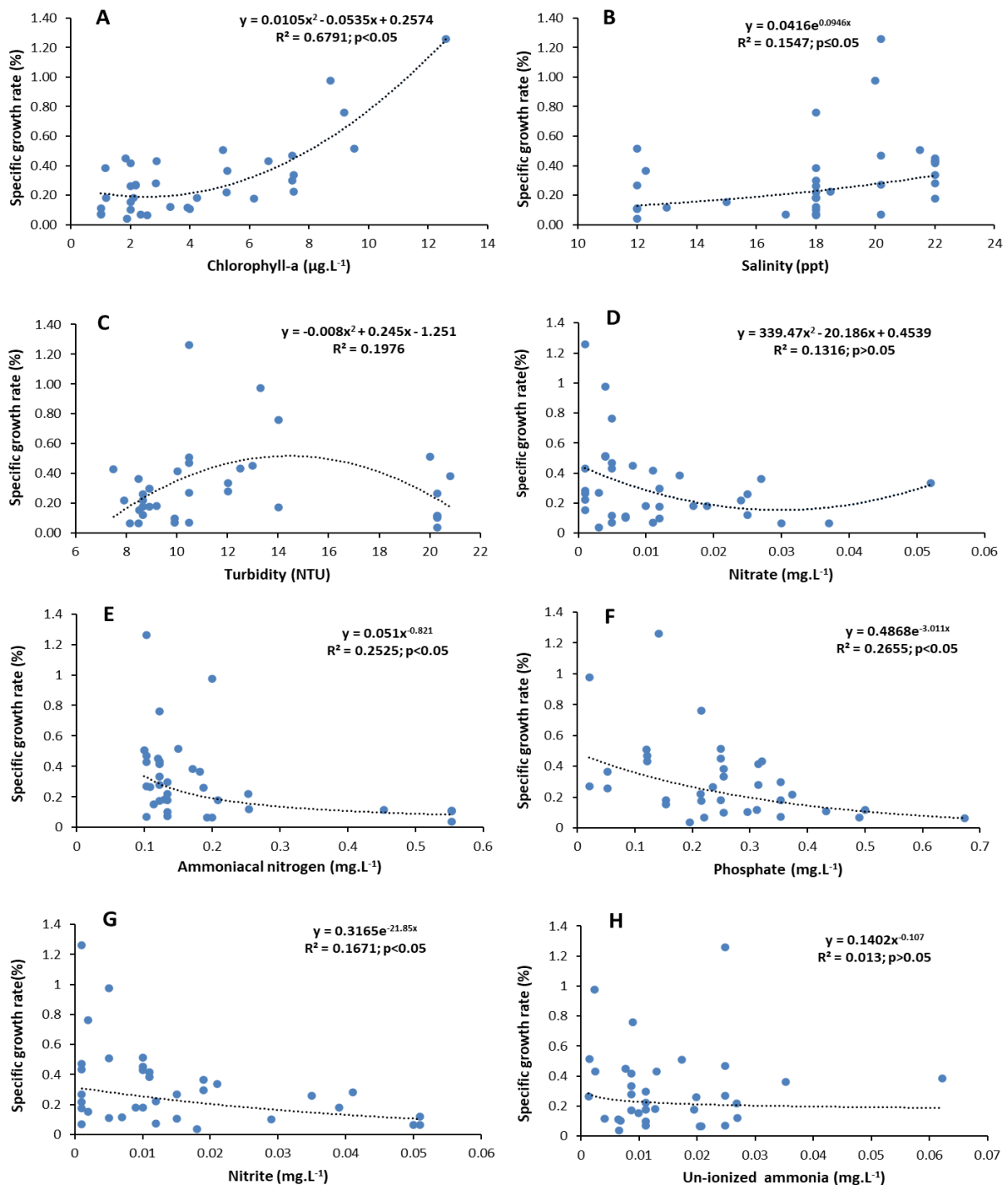
Fig. 5. Results of regression analysis of specific growth rates of the oysters *Crassostrea madrasensis* on environmental parameters during the study period. Significant level $P < 0.05$.

winds and tidal exchange from the northern part were associated with increased salinity. Bernard (1983) demonstrated that salinity changes greater than 10 % are particularly stressful and it may take several days for oysters to adapt to a new salinity regime. Hofmann et al. (1992) reported that a decrease in salinity (as long as salinities remain above 5 ppt) had considerably less effect on adult *C. virginica* populations than does a small change in temperature or food concentration. Nevertheless, Dekshenieks et al. (2000) suggested that variations of a few degrees in water temperature had less effect on the *C. virginica* population than

variations of a few parts per thousand in salinity. High water temperatures (>30 °C) and low salinities (<5 ppt) negatively impact *C. virginica* growth and survival, and high temperature alone may negatively impact on survival of market-sized oysters (Rybovich, 2014). Sehlinger (2019) has shown that even in the same salinity and temperature conditions, growth and mortality rates varied between estuaries because of differences in other environmental conditions (i.e., hydrology, food composition and quality) or localised genetic adaptations to environmental conditions.

In Negombo estuary, the release of urban wastewater had enormous changes in salinity regimes in Thaladuwa, which affected the health and growth of oyster population. Molluscs, including oysters, are osmoconformers, and therefore changes in environmental salinity directly translate into changes in intracellular osmolarity (Berger and Kharazova, 1997). Thus, changes in salinity, pH and their interaction can strongly affect metabolism and bio-mineralisation in these organisms (Dickinson et al., 2012). Overall salinity stress probably had a negative effect on the oyster's ventilation and osmoregulatory functions, leading to a reduction in tissue growth, possibly because of energy limitation in the stressed situation. However, biochemical mechanisms that control cellular osmolality following salinity stress may also differ geographically between conspecific oyster populations (Rybovich, 2014).

Microalgae are the basic diet for filter-feeding bivalves. Available food as estimated by the amount of chlorophyll-a, was of greater importance than water temperature (Brown, 1988). Growth is mainly influenced by the interaction between food availability and temperature (Rio-Portilla et al., 1992). Phytoplankton biomass which reflects primary production in estuarine systems exhibits considerable seasonal variation and phytoplankton biomass could have a significant impact on the survival of oyster, *Crassostrea gigas* (Thunberg, 1793) larvae, which depend on phytoplankton for food supply (Brown, 1988). Phytoplankton abundance (number of cells and chlorophyll-a) in the Golf de Cariaco, Venezuela, was a good predictor of growth for tropical scallops, *Euvola (=Pecten) ziczac*, and decreased growth and survival in high water temperature and low phytoplankton abundance, probably coincident with physiological stress (Lodeiros and Himmelman, 2000). Elvin and Gonor (1979) found that in *Mytilus californianus* Conrad, 1837 food levels explained 96 % of the variance in growth. The shell growth of *Pinctada fucata martensii* (Dunker, 1880) is influenced by temporal and spatial variation in water temperature and abundance of food (Tomaru et al., 2002).

The rapid urbanisation and local industrialisation of the Negombo area caused severe pollution problems in the estuary. Studies elsewhere have shown that increased nutrient levels may increase oyster recruitment (Minchinton and McKenzie, 2008) and moderate eutrophication may enhance oyster growth (Kirby and Miller, 2005). Nutrient enrichment may affect different species in different ways across locations, altering growth, survival or having no apparent effect (Carmichael et al., 2012). Further, elevated nutrient levels concurrently with an increase of microalgal production increase food supply for many bivalves and, in turn, increasing bivalve secondary production. However, the net effect of nutrient enrichment on bivalve production depends on the balance between positive and negative effects of eutrophication on the system (Carmichael et al., 2012).

Whereas further increase of nutrient loads and accumulation of organic matter may lead to low oxygen concentrations in near-bottom waters, affecting bivalves negatively (Cloern, 2001). The highest levels of nutrients in Dungalpitiya are most likely due to the input of organic matter from the surrounding shrimp farm. Aquaculture wastes containing higher concentrations of ammonia and nitrite which are toxic to the aquatic organism (Effendi et al., 2020).

In the aquatic environment, the amount of ammonia can be present in ionised (NH_4+) and or unionised (NH_3) form and concentration depend on temperature, pH and salinity condition in water (Jofre and Karasov, 1999; Figueroa-Lucero et al., 2012). Such nutrient discharge resulted from consequent release of a higher amount of un-ionised ammonia (0.242 mg.L^{-1}), which becomes toxic to aquatic animals due to its high lipid solubility and ability to diffuse across the cell membrane (Chen and Kou, 1993). Nevertheless, Figueroa-Lucero et al. (2012), further stated that both forms of ammonia are toxic and the effect of salinity at a given pH and temperature is negligible (Boyd, 2013). As such, ammonia toxicity might be the possible reason to retard growth of oysters in Dungalpitiya followed by Thaladuwa site. A general notable relationship was not observed between nitrate concentration and oyster growth in the present study, possibly due to a narrow range of variation of nitrate during the sampling period.

Widdows et al. (1979) stated that high particulate inorganic matter concentration could dilute the amount of food ingested, inhibiting growth of oyster, *Mytilus edulis* Linnaeus, 1758. The second-order polynomial relationship between water turbidity and DWG indicates that the growth rate had a greater initial increase and afterwards slowed down with an optimal turbidity level. Laboratory studies have shown that amount of suspended sediment greater than 0.1 g.L^{-1} reduced the growth rate of *Mercenaria mercenaria* (Linnaeus, 1758) larvae (Huntington and Miller, 1989) was a fractional decrease in larval growth at higher turbidity levels. A study on the growth of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) in a high-turbidity environment by Barillé et al. (2011) indicated that in the highest turbid waters, the growth was lower compared to intermediate turbidity waters, in spite of increased availability of food resources, including a large number of microorganisms. They found that oysters fed most efficiently in clear, non-turbid waters.

Turbidity has been strongly linked as a principal factor limiting phytoplankton growth rather than nutrient limitation (Cloern, 1987). The suspension of silt reflected by higher turbidity is frequently recorded in the water column in Thaladuwa. Further reduced oyster growth in Thaladuwa possibly resulted from elevated ammonia loading and water turbidity caused by sedimentation in the area. Elevated levels of total particulate matter as a result of urban wastewater and

wind-driven re-suspension of sediments at this shallow area could also have accounted for increased turbidity in the water.

Findings of the several studies (Apeti et al., 2005; Sobrino-Figueroa et al., 2007; Guzman-Garcia et al., 2009) suggested that metal levels in the lagoon sediment represented a potential pollution source for the benthic organisms and that bioaccumulation of heavy metals in their soft tissues are greater than in unpolluted environments (Apeti et al., 2005). However, heavy metal accumulation in water and sediments in sampling locations were not analysed in the present study. Nevertheless, contaminated heavy metals in sediments or water in the Thaladuwa area caused adverse effects on benthic organisms through bioaccumulation. The maximum lead concentration 1.883 mg.kg^{-1} was recorded in the oyster samples in Thaladuwa and exceeded the EU (EU, 2008) permissible limit of 1.5 mg.kg^{-1} (wet weight). Cadmium concentration in oyster mussels found in Pitipana was higher than the allowable limit of 1 mg.kg^{-1} (EC, 2001). High values of Pb and Cd may be the outcomes of many anthropogenic scenarios, including wastewater/waste discharge from Katunayake industrial processing and export zone where approximately 2 km away from the estuary, Katunayake international airport, few major fishing harbours in adjacent areas, and urban wastewater from the Negombo city where the population density is 50.87 people ha^{-1}. Die chemicals and metal disposed from various sources may be responsible for the output. However, the amount of mercury found in oysters from all sampling locations was lower than the acceptable level of 1.0 mg.kg^{-1}. Even though the accumulated mercury values are low, it is already present in the region. Depending on the frequency of consumption of shellfish, mercury can bioaccumulate in the human body. Since the accumulation of heavy metals and their effects on bivalves are risk indicators, the implementation of frequent monitoring programmes and the application of measures to improve the quality of the estuary are essential. In addition, other critical environmental factors such as bottom type, water depth and water velocity necessary for oyster growth, were not considered in the present study. Although the temperature is one of the important physical factors affecting marine and estuary organisms, the greatest variability of water temperature was not noted in Negombo estuary.

Conclusion

The present study demonstrated that growth rates of oysters, *C. madrasensis* (Preston, 1916) were highly site–specific and food availability and salinity positively impacted oyster growth while other environmental parameters such as turbidity, ammoniacal nitrogen and phosphate had a negative influence. Salinity influence by strong wave action in the estuary allowed brackish water as far as 7–8 km from the estuary mouth, probably causing the colonisation success of the oyster population within the Negombo estuary. A better understanding of the relative importance of environmental factors influencing estuarine oyster growth and mortality is critical for recognising suitable sites for sustainable exploitation of wild stocks of oysters and implementation of oyster aquaculture in the estuary.

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