The Bioscan 9(4): 1361-1366, 2014
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

# SALINITY INDUCED RESPIRATORY ALTERATIONS IN ESTUARINE CLAM *PAPHIA LATERISULCA* AT BHATYE ESTUARY, RATNAGIRI COAST, INDIA

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**KEYWORDS**
*P. laterisulca*
Oxygen consumption
Salinity
Size
Seasons

**Received on :**
11.12.2012

**Accepted on :**
10.10.2014

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**ABSTRACT**
Present study clearly indicated size as well as season dependant variation in oxygen consumption rate (OCR) in estuarine clam *P. laterisulca,* exposed to various salinity ranges (100% to 10%). During all three seasons (summer, post-monsoon and winter) smaller clams showed more sensitivity and tolerance undergone lower salinity stress condition than medium and larger size clam. Even, clams of all three sizes respond toward lower salinity ranges in similar fashion, their degree of tolerance and time taken by them to adjust in lower salinity varied with both size and seasons. Overall, all three size clams seasonal metabolic alteration indicates tolerance to 40% reduction in salinity from normal saline water.

## INTRODUCTION

Clams are sessile organisms and can not move from the adverse environmental conditions to the ambient environment. Respiratory rate and excretory rate of such organisms had been used as an activity index to understand the physiological processes. Earlier, Widdows (1985) suggested that the physiological responses of organisms to changing environmental conditions can be used as a tool for evaluation of quality of the environment. Any change in environmental conditions would affect the metabolic rate of an organism (Navarro and Gonzalez, 1998; Pedro *et al.,* 2004). During low tide, the intertidal zone of the estuary gets exposed to atmospheric air and clams which are buried in mud remain uncovered with water for certain period. Under such condition, the physiological and metabolic processes of clams are different than that of covered high tide water.

The growth of bivalves under particular conditions was calculated from measurement of physiological activities of bivalve such as assimilation, oxygen consumption and excretion rate used by Widdows and Johnson (1988).

Estuarine animals have better capability to acclimatize to variations in salinity during low and high tide; as these are exposed to varying environment than other habitat animals. Salinity is a key environmental factor for controlling the physiological processes of estuarine organisms, such as, feeding, growth, respiration and reproduction (Shumway, 1982).

Respiration rate is considered as an appropriate physiological indicator of catabolism representing the overall bio-energetic status and respiratory expenditure (i.e. costs of maintenance) of the shellfish (Smaal and Widdows, 1994; Trembley *et al.,* 1998; Goulletquer *et al.,* 1999). Metabolic rate is most often estimated as the rate of oxygen consumption and it represents a major loss of energy in bivalves. Lu *et al.* (1999) determined the energy loss of larvae and juveniles of the bay scallop *Argopecten irradians concentricus* (Say) by measuring their rates of oxygen consumption and ammonia excretion as well as the relationship between these physiological rates and body size.

*P. laterisulca* is one of the most important molluscs species from shellfish fishery point of view. It is harvested on large scale by fisherman at estuarine water because, this clam species mostly preferred as food by local fisher folks and coastal community. Study on the metabolic rate of such commercially important species is therefore very valuable to increase their fishery status, production and economics. Present investigation gives the detailed account on seasonal variations in metabolic rate by measuring oxygen consumption rate (OCR) of estuarine clam *P. laterisulca* depending on their size, exposed to both, various salinity ranges and time. This approach helps in monitoring the environmental quality and taking the appropriate remedial control measures, where the population of bivalves is affected beyond the critical level, as well as provide basic information to fishermen regarding development of shellfish culture practices along estuarine areas.

## MATERIALS AND METHODS

### Animal collection and maintenance

The estuarine clam, *P. laterisulca* was collected from Bhatye estuary (73°15′ east and 16°51′ north near Ratnagiri) during low tide from sub-tidal zone of estuary. The clams were cleaned and washed with the sea water and were acclimatized for 48 hours in laboratory conditions.

### Experimental set

After acclimatization only healthy animals having good valve movement as well as siphon activity were selected for experiment. Three size groups of clams (Small, 25-30 mm; medium, 35-40 mm; large 45-50 mm) were exposed to 10 lower salinity ranges (100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%) for eight days, where 100% saline water was normal estuarine water collected during high tide, therefore it was considered as control in all the seasons. These salinity ranges were maintained throughout experiment by adding distilled water. During experiment the water used for holding the bivalves was replaced with six hour interval.

Out of these salinity ranges, on the basis of 50% survival rate, salinity ranges from control to 40% were selected for estimation of OCR. OCR was determined by specially designed apparatus in the laboratory resembling the Galtsoff and Whipple apparatus (Galtsoff and Whipple, 1930). OCR was estimated by using glass respiratory jars having 1 liter capacity. Jars were wrapped with black papers to provide natural habitat to each individual clam. The selected clams of respective size were kept in respiratory jar and supplied with flow of filtered sea water, till the clams fully opened their siphon and extended the visceral organs. Water samples were collected before placing the animal in jar and after completion of one hour experiment. Dissolved oxygen concentration of collected water samples from respiratory jar were estimated by modified Winkler's method (APHA, 2005). The oxygen consumed by clam was calculated by difference between final and initial dissolved oxygen in water sample and expressed as the amount of oxygen used by single clam per liter per gram body weight per hour ($O_2$ $mll^{-1}g^{-1}hr^{-1}$). The values obtained were converted into $O_2$ $mgl^{-1}g^{-1}hr^{-1}$ by multiplying with the factor 1.428. For studying the effect of time interval on rate of oxygen consumption in both the species, the experiment conducted with 24 hour interval.

The results were obtained from mean of five separate determinants with standard deviations (Mean ± S.D.). Two way repeated measures ANOVA with Bonferroni post-test to compare replicate means by rows was used to study the significant effect of both salinity concentration and time of exposure on the rate of oxygen consumption by clams. The significance of test accepted at $P < 0.01$. All the statistical tests performed by using the software GraphPad Prism 5 software version 5.04.

## RESULTS AND DISCUSSION

All experimental sets were carried out during three seasons (summer, post-monsoon and winter) for estuarine clam *P. laterisulca* with respect to size groups (small, medium and large) which exposed to different salinity ranges *i.e.* from control (100%) to 40% sea water. In statistical analysis, OCR of clams exposed to salinity ranges from 90% to 40% salinity for all size groups and seasons were compared to OCR of control group clam with respect to exposure period (in hours). Results obtained during summer, post-monsoon and winter season were presented in Fig. 01 to 09. All the experiments were carried out under controlled environmental conditions in the laboratory.

The oxygen uptake in clams being inversely proportional to the size of organisms, when calculated on the basis of the wet weight of the clams (Mane, 1975a, b; Dhamne and Mane, 1976; Deshmukh, 1979). Similar results were noted in Psammobiid clam *Soletellina diphos* along Bhatye estuary at Ratnagiri (Taware *et al.,* 2012). In the present study, three size groups (small, medium and large) of clam *P. laterisulca* were exposed to various lower salinity ranges during three selected season *i.e.* summer, post-monsoon and winter. Oxygen consumption rate (OCR) increased with decrease in size and/ or weight in the clam species in all three seasons. Smaller clam showed higher value of OCR as compared to medium and larger clam (Fig. 01 to 09). In estuarine species, salinity induced physiological variation in species and environmental condition at their habitat alters from season to season. To

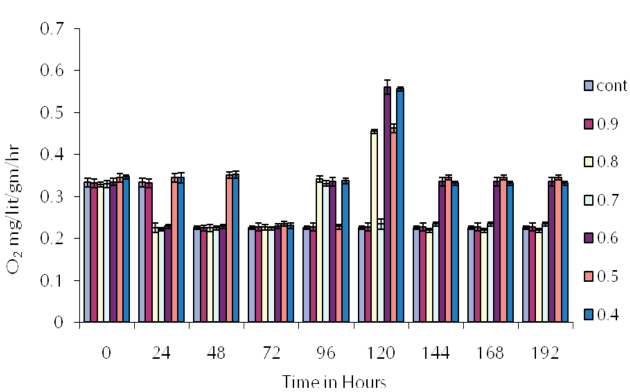


**Figure 01: Effect of salinity on the rate of oxygen consumption in small (25-30 mm in length) size *Paphia laterisulca* during summer season**

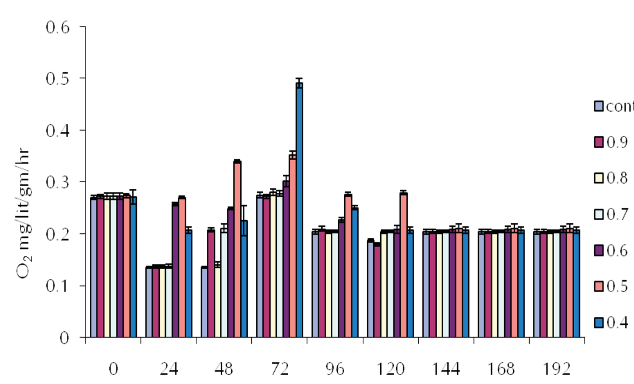


**Figure 02. Effect of salinity on the rate of oxygen consumption in medium (35-40 mm in length) size *Paphia laterisulca* during summer season**

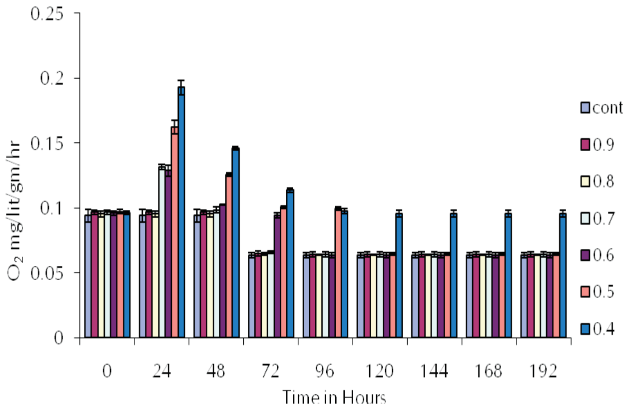


**Figure 03: Effect of salinity on the rate of oxygen consumption in large (45-50 mm in length) size *Paphia laterisulca* during summer season**

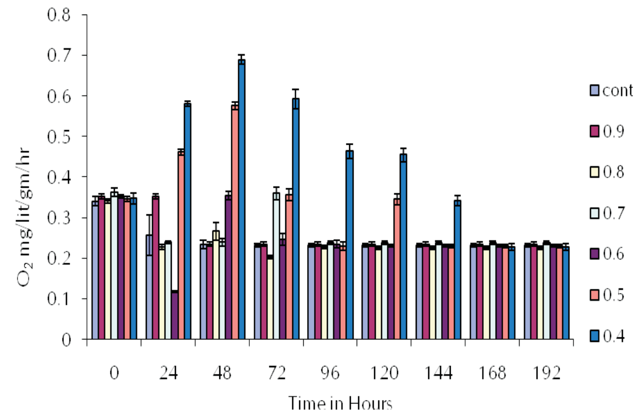


**Figure 04: Effect of salinity on the rate of oxygen consumption in small (25-30 mm in length) size *Paphia laterisulca* during post-monsoon season**

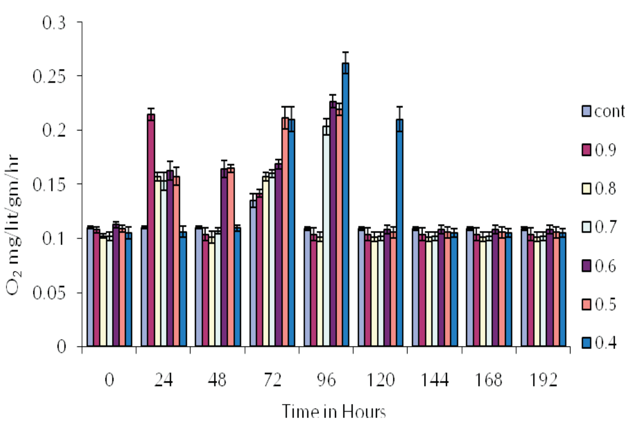


**Figure 05: Effect of salinity on the rate of oxygen consumption in medium (35-40 mm in length) size *Paphia laterisulca* during post-monsoon season**

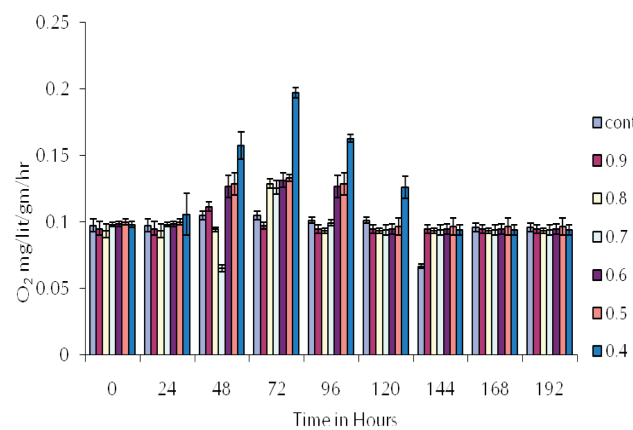


**Figure 06: Effect of salinity on the rate of oxygen consumption in large (45-50 mm in length) size *Paphia laterisulca* during post-monsoon season**

survive and prosper, it is essential for estuarine species to alter their physiology with estuarine conditions during its time of dwelling in the estuary. Therefore, each species requires definite range of salinity during specific times of the year with respect to season. Nelson *et al.* (1992) presented information on time of estuarine residence by life stage for 44 species common in Gulf of Mexico estuaries.

Previously, Gaudy *et al.* (2000) reported elevated OCR of *Acartia clausi* and *A. tonsa*, which was resulted from diverged salinity conditions of their habitat. Such elevated OCR in clams was related to the requirement of supplementary energy for osmoregulation. Highest respiration and excretion rates were found at lower salinity level (16.0) in *M. meretrix* (Tang *et al.,* 2005). In present study, clam *P. laterisulca* showed their normal OCR at different salinity ranges depending on season i.e. summer (38‰) (small, 0.332; medium, 0.271 and large, 0.094 $mgl^{-1}g^{-1}hr^{-1}$), post-monsoon (29‰) (small, 0.340; medium, 0.110 and large, 0.098 $mgl^{-1}g^{-1}hr^{-1}$) and winter (36‰) (small, 0.326; medium, 0.202 and large, 0.181 $mgl^{-1}g^{-1}hr^{-1}$). Whereas, during respective season normal salinity ranges of other seasons are stressful to clams, which alters their metabolic rate to overcome salinity stress.

The metabolic rates of estuarine animals are strongly dependant on the major environmental factor like salinity. As salinity changes during the tidal regime, the metabolic rate of animals is also changed. It has been observed by several authors that a decrease in salinity results in an increase in the metabolic rates of estuarine clams (Mane, 1975a, b; Dhamne and Mane, 1976). Deshmukh (1979) cited adaptation of *Meretrix meretrix* to lower salinity *i.e.* 70% salinity on the basis of his experimental results, which showed significant decline in oxygen consumption from 100% sea water to 70% seawater, whereas consumption rate increased with further decrease in salinity. In *K. opima* oxygen consumption rate was higher in summer season as compared to monsoon and winter season (Kamble and Muley, 2009).

OCR of clam *P. laterisulca* exposed to lower salinity ranges changes depending on size, seasons and period of exposure. In present study, during summer season, smaller clams showed late increment in OCR with higher significance ($P < 0.001$) in lower salinity ranges like 60%, 50% and 40% salinity compared to control group, which remain higher up to 8th day

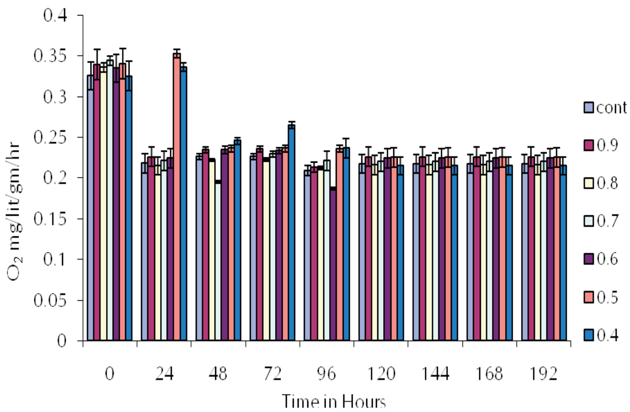


**Figure 07: Effect of salinity on the rate of oxygen consumption in small (25-30 mm in length) size *Paphia laterisulca* during winter season**

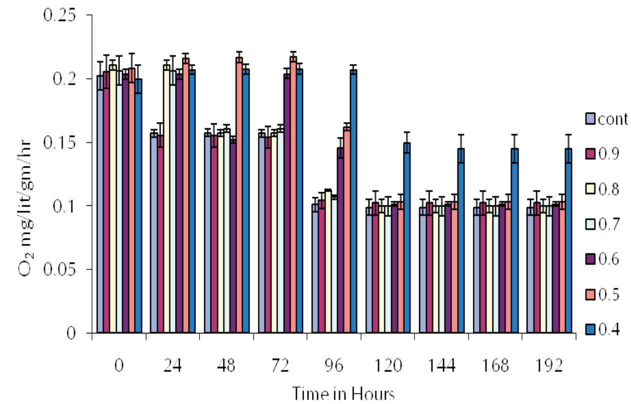


**Figure 08: Effect of salinity on the rate of oxygen consumption in medium (35-40 mm in length) size *Paphia laterisulca* during winter season**

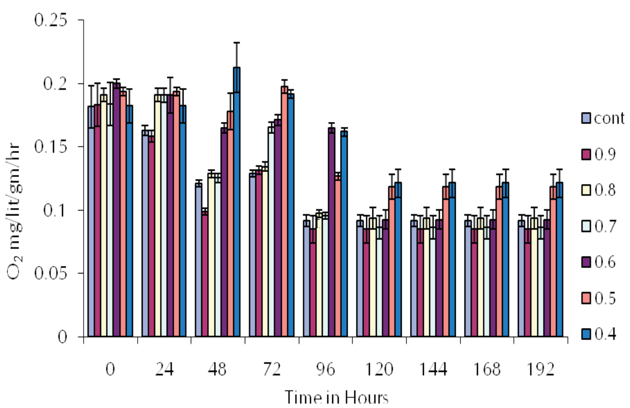


**Figure 09: Effect of salinity on the rate of oxygen consumption in large (45-50 mm in length) size *Paphia laterisulca* during winter season**

of exposure (Fig. 01). In medium size clams such significant increase in OCR ($P < 0.001$) was observed from 3rd to 5th day of exposure in 60%, 50% and 40% salinity range (Fig. 02). In larger clams OCR increment observed in 60% and 50% salinity range up to 3rd and 4th day of exposure but clams OCR in 40% salinity range remain higher throughout the 8 day period of exposure (Fig. 03). It indicates that smaller clams are more sensitive and undergo in stress condition in lower salinity ranges than medium and larger clam during summer season (Fig. 01, 02 and 03).

During post-monsoon season, in small size clams, OCR was significantly ($P < 0.001$) increased up to 3rd day of exposure in salinity ranges from 60% to 40%, while it was observed higher up to 6th day of exposure, only in clams from 40% salinity range (Fig. 04). In medium size clams, OCR was significantly increased ($P < 0.001$) up to 4th day of exposure in 60% to 40% salinity range, while clams in 40% salinity range continued to show increased OCR at 5th day of exposure (Fig. 05). Similar trend of OCR increment ($P < 0.001$) was observed in large size clams, but increment in OCR of clams in 40% salinity range was more prominent within 2nd to 5th day of exposure (Fig. 06). It indicates that, smaller clams can adjust their OCR and adjust more efficiently with lower salinity stress conditions than that of medium and large size clams during post-monsoon season (Fig. 04, 05 and 06).

During winter season, all three sized clams start responding with alteration in OCR from 24 hours of exposure. Smaller clams undergo in stress conditions with significant increase ($P < 0.001$) of OCR up to 4th day of exposure to 50% and 40% salinity ranges (Fig. 07). In medium size clams, significant ($P < 0.001$) increase in OCR was observed from 60% to 40% salinity range up to 4th day of exposure, in which clams from 40% salinity range showed increased ($P < 0.001$) OCR till the completion of 8 days period of exposure (Fig. 08). Similar pattern of OCR increment was observed in large size clams from 70% to 40% salinity range, but clams from 50% and 40% salinity range were observed with increased OCR till the 8th day of exposure (Fig. 09). It indicates that salinity induced OCR alterations were increased with increase in size of the clams during winter season (Fig. 07, 08 and 09).

Berger and Naumov (2001), observed in bivalve molluscs species *Portlandia arctica* and *Nuculana pernula* from White Sea, that OCR of animals exposed to lowered salinity suppresses initially and it recovered with acclimation of bivalves to new conditions. In his experiments bivalve species never reached their initial OCR even after 5 days exposure to lowered salinity ranges. They also stated that, it was possible that, the recovery of these clams might have been completed with further exposure period; however the experiments were not continued due to difficulties in maintaining these deep sea molluscs species under laboratory conditions. Generally, lowered OCR was not remaining constant or maintained more than 1 to 2 days (Berger and Kharazova, 1997). Time taken by bivalves for recovery may vary from species to species and depending on the range of degree of salinity variation at which animals were exposed. In *Littorina saxatilis* OCR increased after 1 day exposure, whereas in *Littorina obtusata* it takes period of 6 days to initial increment. Comparatively in some other species, OCR remains lower than normal level even after 16 to 22 days of acclimation to lowered salinity (Berger, 1986).

In present study, seasonal salinity variations in estuary were responsible for alterations in OCR increment pattern in clams, exposed to various salinity ranges, to adjust their metabolic activity to cope up with stress conditions. Overall in all three seasons (summer, post-monsoon and winter), smaller clams respond more readily to lower salinity stress by increasing their OCR than that of medium and larger clams. Moreover, with respect to seasonal stress response, winter season salinity stress was observed more stressful, as all three size groups of clams alter their OCR throughout 8 days period of exposure. Recently, Lagade *et al*. (2013) reported higher oxygen consumption rate in small size clams of *Soletellina diphos* than that of medium and large size clams, though out the year.

Declined OCR with respective to salinity fluctuation have been reported in molluscs species *Morula granulate* (Uma Devi *et al*., 1984). Rao *et al*. (1989) predicted lowering oxygen consumption mechanism as a part of adaptive variation by the animals which inhabit in intertidal zone; by observing decreased consumption rate in *Mytilopsis sallei* resulted from partial closure of valves exposed to both, lower and higher salinity ranges. Paganini *et al*. (2010) found increased metablic activities under higher salinity conditions in clam *Corbula amurensis*.

Salinity can increase the metabolic activity to compensate energy requirement to regulate internal body salt concentration (Lankford and Targett, 1994). Experiments with adult spotted sea trout, *Cynoscion arenarius*, red/black drum *Pogonias cromis*, sheepshead *Archosargus probatocephalus*, and Atlantic croaker show that metabolic activity can be reduced to critical levels when salinity deviates from optimum levels of 20-30‰ (Longley, 1994).

In conclusion, on the basis of normal OCR of clam *P. laterisulca* during summer, post-monsoon and winter season, it is clear that range of ambient salinity changes season to season. Under lower salinity stress conditions, smaller clam have higher sensitivity and tolerance capacity than medium and larger clam during all three seasons. Time taken by clams to adjust their metabolic rate to overcome from salinity stress differs season to season. Overall, all three size clams seasonal metabolic alterations indicate tolerance to 40% reduction in salinity from normal saline water. In natural conditions along Bhatye estuary, these clams distribution is limited to mouth of the estuary. But on the basis of these results, clam *P. laterisulca* can be successfully cultured in the deeper areas of estuary, where less than or equal to 40% reduction in salinity observed as compared to normal sea water.

## ACKNOWLEDGEMENT

The authors are very thankful to Prof. U. H. Mane, Director and Centre for Coastal and Marine Biodiversity, Bhatye, Ratnagiri, for his valuable suggestion and guidance throughout research work.

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