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Size-dependent seasonal variation in respiration of clam *Katelysia opima* (Gmelin) exposed to lower salinity ranges along Bhatye estuary, Ratnagiri, India.

Shital S. Taware* and Deepak V. Muley
Department of Zoology, Shivaji University, Kolhapur – 412 004 (M.S.) India..

*Correspondence e-mail: tawareshital_21@rediffmail.com

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Original Article

Abstract

In the present investigation, size dependent oxygen consumption rate (OCR) of estuarine clam *Katelysia opima* in relation to lowered salinity ranges (100% to 10%) was estimated seasonally. The sensitivity of clam *K. opima* increased with increase in size in summer season. During summer season, under low salinity stress conditions, increment in OCR was more prominent in medium (30-35 mm in length) and larger (40-45 mm in length) size clams as compared with smaller size (20-25 mm in length); though time taken by all three sized clams for OCR stabilization was same. During post-monsoon season, larger clam entered the stress condition earlier than that of medium and small sized clam exposed to lower salinity ranges. Smaller clam delayed their response to lower salinity of their habitat during post monsoon season. Time taken by clams to acclimatize in salinity stress conditions is prolonged in smaller clam compared to medium and larger clams in *K. opima* during winter season. These results indicate that time taken by the clam *K. opima* for recovery and acclimatization differs with size of clam, range of lower salinity and season.

Keywords: *Katelysia opima, oxygen consumption rate, salinity, size groups, exposure period, seasons.*

Introduction

Several environmental factors are known to influence the respiration of bivalves. The rate of oxygen uptake under various environmental conditions is well documented by many investigators in marine and estuarine bivalves. Environmental stress, resulted from intense temperatures as well as salinities with their variations may affect the number of species, with respect to their fitness, hence affecting biodiversity from molecular level to population level (Nevo, 2001; Parsons, 2005). Every organism from such conditions has capability to adapt depending on their regulatory processes. Once stress condition exceeds the threshold limit of tolerance, organisms falls under pathological state, which can lead to mass mortalities (Manduzio *et al*., 2005).

Estuarine environment is stressful, as river water brings in inorganic and organic nutrients as well as dilutes the seawater, which results in reduction in salinity. Estuarine animals typically experience conditions of lowered salinity during tidal cycle. However, during flood events they are likely to experience much lower salinities than normal for a longer period. Increased frequency and intensity of such flooded conditions is predicted from uncertainty in the impact of climate change circumstances in United Kingdom (Kay *et al*., 2006).

Temperature and salinity are two major environmental factors controlling marine species distribution and influencing physiological processes of marine and estuarine organisms, such

as feeding, respiration, growth and reproduction (Davenport, 1979; Newell and Branch, 1980; Shumway, 1982). The scope for growth under particular conditions, calculated from measurement of physiological activities such as respiration and excretion, can be used to estimate bivalve growth (Widdows and Johnson, 1988). Shumway (1981) has examined the combined effect of salinity and declined oxygen tension on respiration.

On several occasions the metabolism of individuals of a wide range of sizes has been measured, which showed variations in the metabolic activity depending on size and body weight with the influence of environmental or experimental conditions (Newell and Roy, 1973). For the study of energy production and its expenditure at organismal level, metabolism is one of the key factor of physiological activities in an organism (Brett and Groves, 1979). Respiration rate is considered as a correct physiological indicator of catabolism on behalf of the overall bio-energetic status and respiratory costs (i.e. costs of maintenance) of the shellfish (Smaal and Widdows, 1994; Trembley *et al*., 1998; Goulletquer *et al*., 1999). Although a study of the basic respiratory rates in hard clam was previously carried out (Cheng and Chan, 1998), little attention has been given to the effects of environmental parameters on their respiration rates. Resgalla Jr. *et al*. (2007) observed variations in physiological rates and in acclimatization capacity of mussel *Perna perna* by estimating the rates of respiration, excretion, clearance, and absorption efficiency at different temperature and salinity ranges in laboratory conditions; which enabled to understand its behavior in the environment.

K. opima is one of the most important molluscs species in shellfish fishery, as it is preferred as food by local fisher folks and coastal community. Study on the metabolic rate of such commercially important species is very valuable to understand its development and growth in estuarine conditions. Various authors worked on physiological processes under environmental conditions on commercially important molluscs along Ratnagiri coast (Mane, 1975 a, b; Dhamne and Mane, 1976; Deshmukh, 1979; Kamble and Muley, 2009).

Present investigation is aimed to understand the change in physiological status with variation in adaptive strength of clam *K. opima* in natural environment with respect to the size and seasonal variations in environmental conditions. Change in physiological activities like metabolism of clam *K. opima* and its ability to acclimatize in ever changing salinity conditions in an estuary were determined by taking oxygen consumption rate as a measure of variation in its metabolic activities.

Material and methods

Animal collection and maintenance

The estuarine clam, *K. opima* was collected from Bhatye estuary (73° 15′ E and 16° 51′ N near Ratnagiri) during low tide. 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 viz. small (20-25 mm in length), medium (30-35 mm in length) and Large (40-45 mm in length) of clams were exposed to 10 lower salinity ranges (100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%) for eight days, here 100% saline water was normal water of estuary collected during high tide, therefore it was considered as control in all the seasons (Table 1). These salinity ranges were maintained throughout experiment by adding fresh water. During experiment there were daily changes of double filtered estuarine water of respective salinity range with six hour interval.

The experiment for oxygen consumption rate (OCR) was performed in specially designed apparatus in the laboratory resembling with the Galtsoff and Whipple apparatus (Galtsoff and Whipple, 1930). OCR was estimated by using glass respiratory jars having one liter capacity fitted with rubber cork having inlet and outlet connected with rubber tube (Fig. 1). The selected clams of respective size were individually placed in respiratory jar and sealed with melted paraffin wax

Table 1. Physico-chemical parameters in experimental set.

Seasons	Parameters	Salinity ranges of experimental water in %									
		Control	90%	80%	70%	60%	50%	40%	30%	20%	10%
Summer	Salinity in ‰	38	35	33	30	27	23	19	14	10	06
	Temperature in °C	34 ± 0.5	34 ± 0.5	34 ± 0.5	34 ± 0.5	34 ± 0.5	34 ± 0.5	34 ± 0.5	34 ± 0.5	34 ± 0.5	34 ± 0.5
Post-monsoon	Salinity in ‰	29	26	23	20	17	14	11	08	06	03
	Temperature in °C	28 ± 1	28 ± 1	28 ± 1	28 ± 1	28 ± 1	28 ± 1	28 ± 1	28 ± 1	28 ± 1	28 ± 1
Winter	Salinity in ‰	36	33	29	26	22	19	16	12	08	04
	Temperature in °C	23 ± 0.5	23 ± 0.5	23 ± 0.5	23 ± 0.5	23 ± 0.5	23 ± 0.5	23 ± 0.5	23 ± 0.5	23 ± 0.5	23 ± 0.5

All the values of temperature are mean ± S.D.

Fig.1 Respiratory apparatus used during experiments

to provide air tight environment. Respiratory jar supplied with flow of filtered estuarine water, till the clams fully opened their siphon and extended the visceral organs. Once they opened their valves, the flow of water was stopped. A sample of estuarine water from respiratory jar was drawn carefully in stopper reagent bottle for determination of initial oxygen content. After one hour, sample of water from the experimental respiratory glass jar was drawn to find out the oxygen content. Dissolved oxygen concentration of collected water samples from respiratory jar were estimated by modified Winkler's method (APHA, AWWA and WPCF, 1992). The oxygen consumed 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 ml/lit/gm/h). The values obtained were converted into O_2 mg/lit/gm/h by multiplying with the factor 1.428.

Statistical analysis

The values presented in results are means of five observations with standard deviations (Mean ± SD). One way ANOVA was done for comparison. The significance of test was accepted at $P < 0.01$. Statistical analysis was performed by using GraphPad software.

Results

In all the experiments carried out during three seasons (summer, post-monsoon and winter) on estuarine clam *K. opima* of the three size groups (small, medium and large) were exposed to different salinity ranges i.e. from control (100%) to 40% sea water (Table 1). OCR of three size group of clams exposed to the salinity ranges from 90% to 40% salinity were compared with OCR of control group (100%) respective to exposure time (time in hours), size and season.

A) OCR of K. opima exposed to various salinity ranges during summer season

OCR of the three size groups (small, medium and large) of estuarine clam *K. opima* exposed to various salinity ranges were calculated for the summer season (Fig. 2.1, 2.2, 2.3). All the experiments carried out under controlled environmental conditions in the laboratory.

1. OCR of small sized clam *K. opima* exposed to various salinity ranges: In 90% (35‰) salinity set, OCR decreased significantly ($P < 0.001$) at 24 hour, whereas it increased significantly at 72 hour of exposure, beside these, OCR values obtained throughout experimental period of salinity

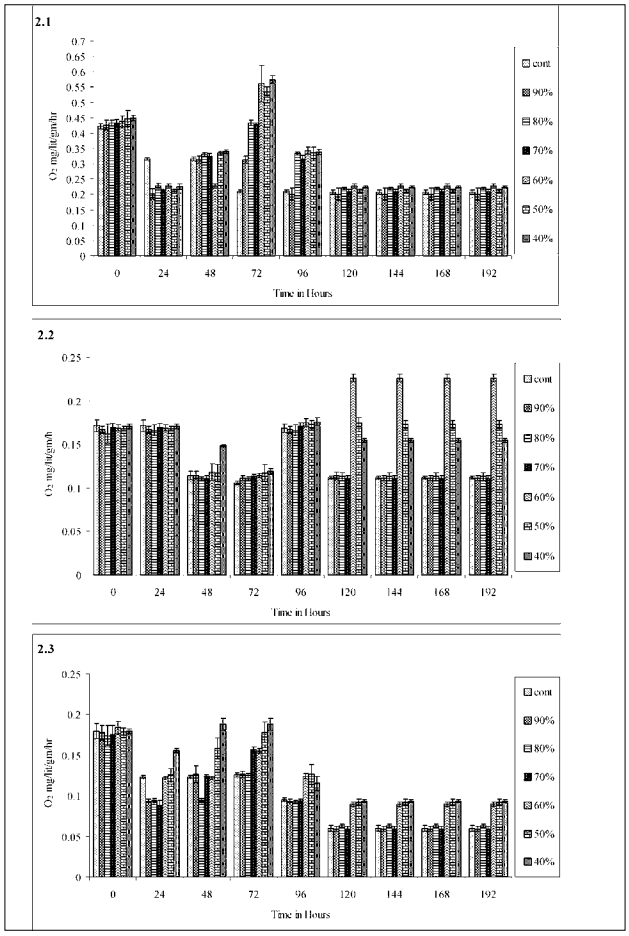

Fig.2 Effect of salinity on the rate of oxygen consumption in small (20-25 mm in length) (2.1), medium (30-35 mm in length) (2.2) and Large (40-45 mm in length) (2.3) *Katelysia opima* during summer season

exposure did not show any significant difference ($P > 0.05$) as compared to OCR of control group clams. In both 80% (33‰) and 70% (30‰) salinity set, OCR decreased significantly ($P < 0.001$) at 24 hour, whereas it increased significantly ($P < 0.001$) at 72, and 96 hour of exposure as compared to control group of clams. In 60% (27‰) salinity set OCR was decreased significantly ($P < 0.001$) simultaneously at 24 and 48 hour whereas it increased significantly at 72 and 96 hour of exposure as compared to OCR of control group. In 50% (23‰) and 40% (19‰) salinity set OCR decreased significantly ($P < 0.001$) at 24th hour of exposure, whereas it increased significantly ($P < 0.001$) from 48 to 96 hour of exposure as compared to control group clams, except clams from 50% (23‰) salinity set at 48 hour of exposure where increase in OCR was found significant ($P < 0.001$) (Fig. 2.1).

In all salinity ranges, OCR of clams decreased significantly ($P < 0.001$) at 24 hour, whereas it increased ($P < 0.001$) at 72 and 96 hour of exposure, from 80% (33‰) to 70% (30‰) salinity and from 60% (27‰) to 40% (19‰) salinity range it showed increment in OCR from 48 to 96 hours of exposure. OCR of clams exposed to all salinities showed stabilization with non significant change ($P > 0.05$) compared with control group clams. In clams from 90% (35‰) salinity set OCR stabilized at 96 hour; from 80% (33‰) and 70% (30‰) salinity set OCR stabilized at 120 hours, whereas at 60% (27‰) salinity, it stabilized at 144 hour of exposure. While at 50% (23‰) and 40% (19‰) salinity again it stabilized at 120 hour of exposure (Fig. 2.1).

2. OCR of medium sized clam *K. opima* exposed to various salinity ranges: In 90% (35‰), 80% (33‰) and 70% (30‰) salinity sets, there were non-significant ($P > 0.05$) change observed throughout the experimental period in OCR even though it stabilized within 120 to 196 hours of exposure. Comparatively, response of clams from 60% (27‰) salinity to 40% (19‰) was increased. Clams from 40% (19‰) salinity range showed highly significant increase in OCR ($P < 0.001$) from 48 hours to 192 hours of exposure except at 96 hours where change in OCR was non-significant ($P > 0.05$) as compared to control group of clams. Comparatively, clams from 50% (23‰) showed significant increase ($P < 0.01$) in OCR at 72 hours of exposure whereas, increase in OCR by clams from 60% (27‰) salinity set was observed with low significance ($P < 0.05$); even though clams from both the salinity ranges showed significant increase ($P < 0.001$) in OCR from 120 to 192 hours of exposure. All the clams exposed to various salinity ranges stabilized their OCR almost from 120 hours of exposure but, clams from 60% (27‰) to 40% (19‰) salinity set showed increased OCR ($P < 0.001$) even after stabilization from 120 to 192 hours of exposure (Figure 2.2).

3. OCR of large sized clam *K. opima* exposed to various salinity ranges: Clams from 90% (35‰) at 24 hours and from 80% (33‰) salinity at both 24 and 48 hours of exposure showed significant ($P < 0.01$) decrease in OCR of clams. Whereas clams from 70% (30‰) salinity showed highly significant ($P < 0.001$) decrease and increase at 24 and 72 hours of exposure respectively. Comparatively significant ($P < 0.01$) increase in OCR was observed from 72 to 192 hours of exposure at 60% (27‰) salinity range. Further salinity dilution resulted in to significant increase ($P < 0.001$) in OCR from 48 to 192 hours of exposure at 50% and 24 to 192 hours of exposure at 40% (19‰) salinity range. OCR of all clams stabilized from 120 hours of exposure, even when observed to increase from 60% (27‰) to 40% (19‰) salinity range (Fig. 2.3).

B) OCR of K. opima exposed to various salinity ranges during post-monsoon season

OCR of estuarine clam *K. opima* exposed to different salinity ranges in three size groups are presented in Fig. 3.1, 3.2, 3.3.

1. OCR of small sized clam *K. opima* exposed to various salinity ranges: In 90% (26‰) salinity set, OCR increased significantly ($P < 0.001$) only at 72 hour of exposure; whereas, clams from 80% (23‰), 70% (20‰) and 60% (17‰) salinity set had significant ($P < 0.001$) increase in OCR at both 48 and 72 hours of exposure, in addition to this clams from 80% (23‰) salinity range showed similar increase in OCR at 96 hours of exposure. Comparatively clams from lower salinity ranges like 50% (14‰) and 40% (11‰) salinity set showed significant ($P < 0.001$) increase in OCR from 24 to 72 and at 120 hours of exposure. Clams from control to 60% (16‰) salinity range stabilized their OCR from 120 to 192 hours of exposure, while clams from 50% (14‰) to 40% (11‰) salinity range stabilized from 144 to 192 hours of exposure (Fig. 3.1).

2. OCR of medium sized clam *K. opima* exposed to various salinity ranges: In 90% (26‰) salinity, clams showed significant increase ($P < 0.001$) in OCR at 72 hour of exposure as compared to control group clams. In both 80% (23‰) and 70% (20‰) salinity set OCR was increased significantly ($P < 0.001$) at 24 and 96 hour of exposure. Except clams exposed to 60% (17‰) salinity at 48 hours of exposure, both the 60% (17‰) and 50% (14‰) salinity set clams showed significant ($P < 0.001$) increase in OCR from 24 to 96 hours of exposure. Comparatively clams from 40% (11‰) salinity range showed significant increase ($P < 0.001$) in OCR from 48 to 120 hours of exposure. OCR of all the clams from control to 50% (14‰) salinity range were observed to be stabilized from 120 to 192

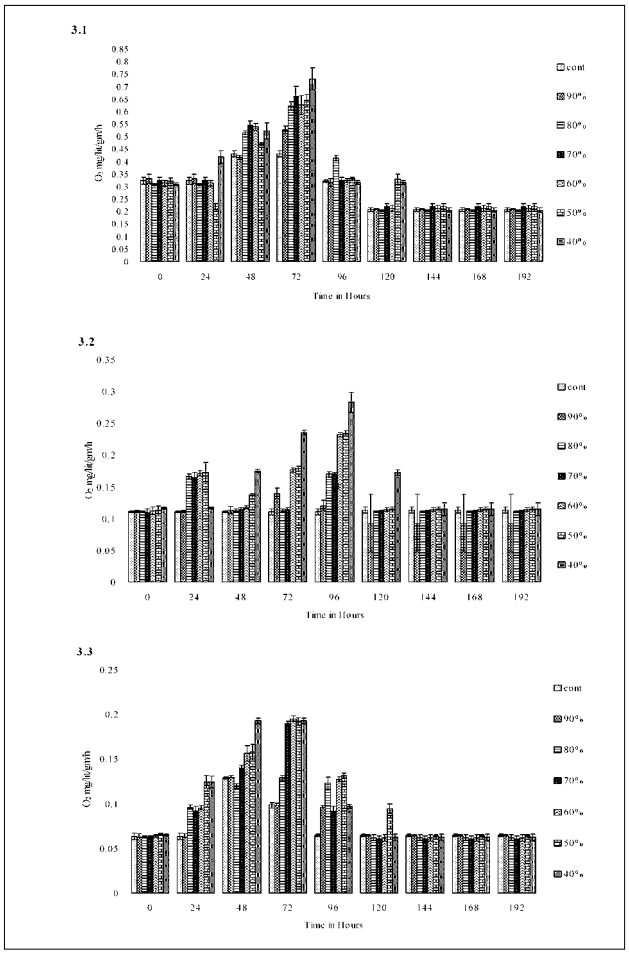

Fig.3 Effect of salinity on the rate of oxygen consumption in small (20-25 mm in length) (3.1), medium (30-35 mm in length) (3.2) and Large (40-45 mm in length) (3.3) *Katelysia opima* during post-monsoon season

hours of exposure, whereas clams from 40% salinity range stabilized their OCR from 144 to 192 hours of exposure (Fig. 3.2).

3. OCR of large sized clam *K. opima* exposed to various salinity ranges: In 90% (26‰) salinity set OCR was increased significantly ($P < 0.001$) at 96 hour of exposure. In 80% (23‰) salinity set OCR was increased significantly ($P < 0.001$) at 24 and 96 hour of exposure. In case of 70% (20‰) salinity set at 24, 72 and 96 hours of exposure significant increase ($P < 0.001$) in OCR was observed, whereas at 48 hour of exposure its increase was low ($P < 0.05$). Comparatively, clams from 60% (17‰) to 40% (11‰) salinity range observed with significant ($P < 0.001$) increase in OCR from 24 to 96 hour of exposure, while such increment continued up to 120 hours of exposure in clams from 50% (14‰) salinity range. Clams from 90% (26‰) to 40% (11‰) salinity stabilized their OCR from 120 to 192 hours of exposure, except clams from 50% (14‰) salinity which stabilized their OCR from 144 hours to 192 hours of exposure (Fig. 3.3).

C) OCR of Clam K. opima exposed to various salinity ranges during winter season

OCR of three size groups (small, medium and large) of clam *K. opima* exposed to different salinity ranges during winter season are presented in Fig. 4.1, 4.2, 4.3.

1. OCR of small sized clam *K. opima* exposed to various salinity ranges: In 90% (33‰) salinity set OCR of clams showed no significant change compared to control group, while clams from 80% (29‰) salinity set showed significant decrease ($P < 0.01$) in OCR at 120 hours of exposure. Clams from 70% (26‰) salinity set showed decrease in OCR from 48 to 120 hours of exposure but with low significance ($P < 0.05$). Clams from 60% (22‰) salinity showed significant ($P < 0.001$) decrease in OCR only at 24 and 72 hours of

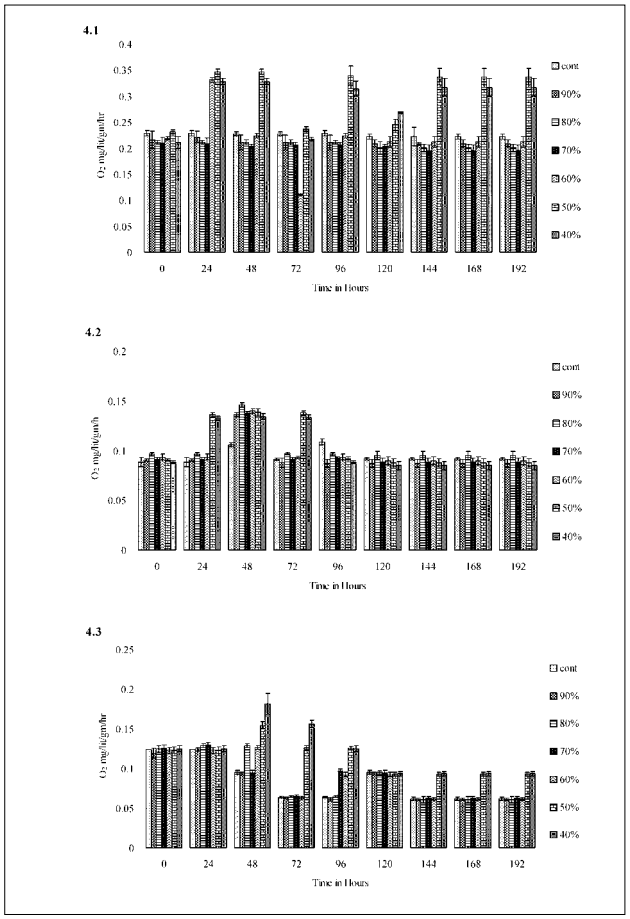

Fig.4 Effect of salinity on the rate of oxygen consumption in small (20-25 mm in length) (4.1), medium (30-35 mm in length) (4.2) and Large (40-45 mm in length) (4.3) *Katelysia opima* during winter season

exposure. Comparatively, clams from 50% (19‰) and 40% (16‰) salinity set showed increased OCR with significance ($P < 0.001$) throughout the experimental period from 24 to 192 hours of exposure, except at 72 hours of exposure. All the clams exposed to salinity ranges from control to 60% (22‰) salinity stabilized their OCR from 120 to 192 hours of exposure, except clams from 70% (26‰) salinity along with 50% (19‰) and 40% (16‰) salinity which stabilized from 144 to 192 hours of exposure (Fig. 4.1).

2. OCR of medium sized clam *K. opima* exposed to various salinity ranges: Clams exposed to 90% (33‰), 70% (26‰) and 60% (22‰) salinity range showed significance ($P < 0.001$) increase in OCR at 48 and 96 hours of exposure. In 80% (29‰) salinity set OCR increased ($P < 0.01$) at 24 hour and ($P < 0.001$) at 48 hours of exposure, whereas it significantly declined ($P < 0.001$) at 96 hours of exposure. Comparatively, clams from both 50% (19‰) and 40% (16‰) salinity range showed increased OCR ($P < 0.001$) from 24 to 96 hours of exposure, whereas clams from 40% (16‰) salinity range continued with significant increase ($P < 0.01$) in OCR from 120 to 192 hours of exposure. All the clams from 80% (29‰) to 40% (16‰) salinity range stabilized their OCR from 120 to 192 hours of exposure (Fig. 4.2).

3. OCR of large sized clam K. opima exposed to various salinity ranges: In 80% salinity set OCR was increased at 48 hours and in 70% (26‰) salinity it increased at 96 hours of exposure with high significance ($P < 0.001$). Whereas, in 60% (22‰) salinity set, it increased ($P < 0.001$) both at 48 and 96 hours of exposure. Comparatively, in both 50% (19‰) and 40% (16‰) salinity set OCR was increased ($P < 0.001$) from 48 to 192 hours of exposure, except at 120 hours of exposure. OCR stabilization was observed from 144 to 192 hours of exposure in clams exposed from control to 60% (22‰) salinity range, while it stabilized in 50% (19‰) and 40% (16‰) salinity range from 120 hours to 192 hours of exposure (Fig. 4.2)

Discussion

The metabolic processes of animals are considerably affected by their body size, as shown in the venerid species *K. opima*. Under both, normal and varying salinity exposure, smaller clams showed high respiratory rates than that of medium and larger clams (Mane, 1975a,b). The clams of lower weight have a greater consumption rate than those of greater weight - 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. Size and weight specific oxygen consumption in *Paphies donacina* for summer and winter season were described by Bayne *et al*. (1976). In present study, three size groups (small, medium and large) of clam species *K. opima* 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 all selected seasons. Smaller clam showed higher value of OCR as compared to medium and larger clam.

Salinity related changes in habitat requirement to species and actual habitat conditions at that period alter temporarily in estuaries depending on season. To survive, it is essential for estuarine species to adjust their salinity requirement with estuarine conditions during its time of residence in the estuary. That is, each species not only requires certain salinities, but it needs these salinities during specific times of the year. Nelson *et al*. (1992) present information on times of estuarine residence by life stage for 44 species common in Gulf of Mexico estuaries. Many benthic organisms depend on stable salinity regimes to survive and propagate. Typical estuarine benthic macro-invertebrates like Terebelid polychaetes and sipunculids were found to be adversely affected by salinity fluctuations (Ferraris *et al*., 1994). The growth and development of the marine polychaete *Arenicola cristata* and the gastropod *Ilyanassa obsolata* were both found to be adversely affected by interruption in ambient salinity (Richmond and Woodin, 1999). In this study, *K. opima* showed their normal OCR at different salinity ranges depending on season i.e. summer (38‰), post-monsoon (29‰) and winter (36‰).

Since bivalves are unable to osmoregulate extracellularly, their internal osmotic concentration changes rapidly with changes in external salinities. This is primarily due to high permeability of the body surface to seawater. Intracellular osmoregulation changes the intracellular ionic concentration, so that it equals to coelomic fluid osmotic pressure. These ionic concentration plays critical role in metabolic processes as they affect the enzymatic activities (Diehl, 1986).

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 and Dhamne & 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). Deshmukh (1979)

showed that as starvation progresses, oxygen consumption rate decreases in *M. meretrix*. Kim *et al*. (2001) reported that significant change in salinity like 35‰ to 20‰ have negligible effect on activity rhythm and oxygen consumption rate of the Manila clam, *Ruditapes philippinarum*.

In the present study, among three size groups, sensitivity and adaptability of clam *K. opima* decreased with increase in size of the clam during summer season. The observations on three size groups indicates that larger clam enters the stress condition earlier, when exposed to lower salinity ranges than that of medium and small sized clam, and sensitivity and adaptability of clams towards lower salinity ranges declines with increase in size of the clam during post-monsoon season as was observed during summer season. During winter season, smaller and larger clams enters in stress condition when exposed to lower salinity ranges like 50% (19‰) and 40% (16‰) salinity, whereas medium sized clam showed more resistance towards lower salinity ranges (up to 50% salinity) as compared to smaller and larger clams. As compared to summer and post-monsoon season, there is variation in size dependant response of *K. opima* during winter season, which is not clear.

Highest respiration and excretion rates were found at lower salinity level in *M. meretrix* (Tang *et al*., 2005). Respiration of many estuarine organisms such as *Acartia clausi* and *A. tonsa* (Gaudy *et al*., 2000) was enhanced when salinity conditions diverged from normal habitat to fulfill the need of supplementary energy for osmoregulation. Respiration rate of *Mytilus edulis* increased linearly with decreasing salinity (Stickle and Sabourin, 1979). In case of *K. opima*, all three size groups showed higher respiration rate under lower salinity exposure, overall up to 5th day of exposure during all three seasons (Summer, post-monsoon and winter).

Berger and Naumov (2001), observed in bivalve molluscs species *Portlandia arctica* and *Nuculana pernula* from White Sea, that oxygen consumption rate of animals exposed to lowered salinity suppresses initially and it recovered with acclimation of bivalves to new conditions. 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* consumption rate increased after one day exposure, whereas in *Littorina obtusata* it takes a period of six days for initial increment. Comparatively in some other species oxygen consumption rate remains lower than normal level even after 16 to 22 days of acclimation to lowered salinity (Berger, 1986). *P. perna* showed ample acclimatization to different salinities in the chronic tests, presenting inhibition of respiration rates only for salinities of 15‰. The amplitude of acclimation was observed to be higher (from 20 to 35 ‰); whereas the net growth rate being negative for 15 ‰ and reduced for 40 ‰ (Resgalla Jr. *et al*., 2007).

During summer season, medium and larger *K. opima* delayed their response as compared to smaller clam towards lower salinity stress conditions; even time taken by clams from all three size groups for OCR stabilization was same.

During post-monsoon season, clams from larger and medium group respond earlier to lower salinity ranges, whereas smaller clam delayed their response to lower salinity close to normal salinity of their habitat during low tide i.e. 60% (17‰).

Time taken by clams to acclimatize in salinity stress conditions is more extended in smaller clam compared to medium and larger clams in *K. opima* during winter season. From this study, it is evident that time taken by individual clam exposed to lower salinity ranges for recovery and acclimatization, differs with size of clam, range of lower salinity and season.

Declined oxygen consumption rate with respect to salinity fluctuation has been reported in *Morula granulate* (Uma Devi *et al*., 1984). Shumway and Youngsen (1979) observed valve closure mechanism in *Modiolus demissus* exposed to fluctuating salinities, and correlated it with oxygen consumption. Rao (1987) showed decreased consumption rate in *Mytilopsis sallei*, resulted from partial closure of valves exposed to both lower and higher salinity ranges. Lowering oxygen consumption is a part of adaptive mechanism by the animals which inhabit the intertidal zone.

Salinity can increase the metabolic activity to compensate energy requirement to regulate internal body salt concentration (Lankford and Targett, 1994; Longley, 1994). During periods of salinity stress, an increase in energy requirements not only alter metabolism but it may also affect activity and the endocrine system of organism, which may in turn influence their foraging activity, assimilation, growth, reproduction, and general health (Wohlschlag and Wakeman, 1978; Wakeman and Wohlschlag, 1983; Longley, 1994).

In the Bhatye estuary, degree of salinity varied from season to season. During summer season, salinity of estuarine water fluctuates from 38‰ to 35‰ with respect to tidal cycle i.e. 10% reduction as per experimental set carried out in laboratory conditions. During the post-monsoon season, clam naturally experiences 30 - 40% reduction from high tide water mark to low tide water mark. Comparatively, during the winter season 20% reduction in salinity from high tide to low tide was experienced by the clam species. Therefore, *K. opima* experience reduction in salinity from 10% to 40% depending on season. In present study, on the basis of respiratory alteration in *K.*

opima exposed to various lower salinity ranges, it is clear that, overall lower salinity tolerance limit is up to 70% salinity i.e. 30% reduction in salinity as compared to the normal salinity of estuary in respective season. But below their adaptive limit of the salinity range like at 50% and 40% salinity, critical physiological changes were marked in *K.opima*.

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