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Seasonal Biochemical Alterations in Estuarine Clam Katelysia Opima under Osmotic Stress Along Bhatye Estuary, Ratnagiri (M.S.) India

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Abstract : *The physiological response of estuarine clam Katelysia opima under osmotic stress was studied by exposing average sized clams (30 to 35 mm) to various lower salinity ranges (control to 10% salinity) for the period of 8 days. Level of biochemical constituents (protein, lipid and glycogen) in selected tissues (hepatopancreas, gonad, foot and gill) after completion of 8 days exposure period were studied and taken as a measure of physiological alterations in osmotic stress. Biochemical alteration in the clam after exposure to lower salinity ranges were distinctly observed below 70% salinity range, where biochemical constituents significantly reduced in both reproductive and somatic tissues but with variation in range of reduction.*

Keywords: Salinity, Katelysia opima, Physiological responses, Biochemical alterations.

Introduction

The distribution and abundance of the invertebrate fauna in marine environment is co-related with various important environmental parameters like temperature, salinity and oxygen, which are all directly related with the seasonal changes and inflow of water in the estuary. Water quality can be described in terms of physico-chemical and biological characteristics. Artificial or natural changes in the physical and chemical nature of freshwaters can produce diverse biological effects ranging from the severe to subtle. The responses of biological communities or individual organisms can be monitored in a variety of ways to indicate effects on the ecosystem. The reactions of individual organisms, such as behavioural, physiological or morphological changes, can also be studied as responses to stress or adverse stimuli. Some approaches are suitable for field use and some have been developed specifically for use in the laboratory (UNEP and WHO, 1996).

Alterations in environmental conditions adversely affect organisms in natural conditions, therefore studies on physiological mechanisms of organisms towards such conditions becomes important. Comparative biologist and ecologist have paid attention towards determination of physiological condition of organisms in a natural context (Wagner *et al.,* 1998). Among the marine environmental factors, temperature and salinity are the most important and relevant variables in the study of physiology. These variables determine the metabolism rate of the organisms and consequently, the extent of distribution of the species (Venberg and Vernberg, 1972).

As compared to marine environment, estuarine environment showed much variation in salinity, temperature, pH and other environmental parameters. There are great fluctuations in the salinity owing to the tidal oscillations and river discharge. Due to the differences in environmental conditions, animal inhabiting in marine conditions were not exposed to fluctuating environment as compared to estuarine animals. During monsoon, the salinity of water over the clam bed may remain low for considerably long period. Therefore, the clams in such areas have to adapt themselves in order to overcome these fluctuations. Growth,

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mortality and behaviour of early stage *Pecten maximus* is affected by rearing conditions of lower salinity and higher temperature in shallow coastal system (Christophersen and Strand, 2003). Larvae of *Paphia malabarica* shown higher survival and growth rate at higher salinity (25 - 33‰) and pH (8 - 8.5) (Gireesh and Gopinathan, 2004). Salinity is a key abiotic factor influencing small and large scale biotic interaction in intertidal ecosystems (Berger and Kharazova, 1997; Ingole and Parulekar, 1998). It determines the distribution (Crain *et al.,* 2004), physiological performance (Shock *et al.,* 2009) and reproductive success (Deschaseaux *et al.,* 2010) of wide range of organisms living on mudflats or rocky shores.

The general hypothesis is that biochemical/ physiological responses known as signal responses at higher levels of biological organization, and thus may provide early indication of environmental disturbance. In addition, individual-level indicators of stress may be used to detect fine sub-lethal effects, to which community-level measures may be insensitive (GESAMP, 1995). Biochemical indicators of condition and physiological stress may represent the organismal response to changing environmental conditions and their mechanism of physiological adaptations, therefore biochemical indicators are considered as an important one. Biochemical indicators of stress are characteristical components of the cellular stress response, which are up regulated as a result of exposure to environmental conditions, that stress induced by changing environmental conditions, resulting in alterations in metabolism, that impact on performance, growth, or reproductive output. Thus, measuring biochemical indicators of stress or metabolism can be used as a "photoprint" of the condition of the organism at the time, it was sampled (Dahlhoff, 2004).

The venerid clam *Marcia opima* is a largely exploited commercially important species. Information on the biochemical constituents of the meat would help to identify the best harvest season for the species coinciding with high nutritive value. The baby clam *M. opima,* has so far been indicated as *Katelysia opima* in Indian waters. Kamble and Muley, (2009) studied seasonal variation in the biochemical composition of same species from Kalbadevi estuary, Ratnagiri, West coast of Maharashtra.

Considering the deteriorating global environmental scenario and work done by previous workers from different parts of the world, present investigation is undertaken. In this study, alterations in the different physiological processes like behaviour and biochemical constituents of estuarine clam *K. opima* exposed to different ranges of low salinity (one of the most important environmental variable) is studied to understand the current status of their fitness.

Materials and Methods

Animal collection and maintenance

The estuarine clam, *Katelysia opima* was collected from Bhatye estuary during low tide by hand picking and digging with knife method. The clams were cleaned and washed with the sea water. After cleaning, the average size clams (30 - 35 mm) were selected and acclimatize for 48 hours under laboratory conditions. In all selected seasons viz. summer (March - May), post-monsoon (August - October) and winter (November - January), the same procedure was followed for animal collection and their maintenance in the laboratory. For experimental work only healthy clams were selected and tested.

Experimental design

For experiment 30 individual clams were exposed to ten lower salinity ranges (100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%) for 08 days. Here 100% saline water was normal water of estuary collected during high tide, therefore it was considered as control range in all the seasons (Table 1). These salinity

ranges were maintained throughout experiment by adding freshwater. During exposure period, double filtered experimental estuarine water of respective salinity range were changed with 6 hour interval.

Biochemical study

After completion of 8 days salinity exposure period, on the basis of 50% mortality, few clams from each salinity range (control to 40%) were sacrificed to separate selected organs namely hepatopancreas, gonad, gill and foot. These separated organs were blotted with filter paper to remove excess moisture. These selected organs were weighed and used to evaluate the effect of various salinity exposures on biochemical constituents like protein, lipid and glycogen.

Weighed wet tissues, separated from clams were used to estimate total protein content by using folin-phenol method (Lowry *et al.,* 1951). Total glycogen content was estimated by Anthrone method (De Zwaan and Zandee, 1972), while the total lipid content was estimated by the Vaniline method (Barnes and Black-Stock, 1973). All the estimations were repeated five times to calculate their mean value to get an accurate value. All the values are presented in milligrams per hundred milligrams of wet weight tissue (i.e. mg/100mg wet weight tissue). Total protein, lipid and glycogen content were estimated seasonally.

The data obtained was statistically analyzed by software Graphpad InStat using One-way Analysis of Variance (ANOVA) with Dunnett Multiple Comparisons Test, to confirm significant difference in biochemical content in both the clam species exposed to various salinity ranges. The significance of test was accepted at $P < 0.01$ and $P < 0.001$.

Results and Discussion

The effect of lower salinity (control, 90%, 80%, 70%, 60%, 50% and 40%) on changes in biochemical constituents like protein, lipid and glycogen content from selected organs (hepatopancreas, gonad, foot and gill) in different seasons were studied in experimental clam *K. opima.* The salinity ranges with lower than 50% mortality have been selected for biochemical changes in clams during all selected seasons (summer, post-monsoon and winter).

The ability of estuarine animals to bear rapid changes in the external environment is linked with the ability to regulate their internal environment. Therefore, it is necessary to become a successful estuarine dweller, that to attain a significant rate of acclimation to ever fluctuating estuarine salinity. The speed of salinity change rather than the magnitude was found to induce short-term stress responses in juvenile spat (Moser and Miller, 1994).

Baker and Hornbach (2001), found seasonal physiological and biochemical variations in two unionid mussel species *Actinonaias ligamentina* and *Ambelema plicata.* In these two species, *A. plicata* had great carbohydrate content than, that of *A. ligamentina*. Both of these species showed high carbohydrate and low protein content during the early summer season. They also noted drastic variation in protein content throughout the year as compared to other biochemical constituents, even though glycogen present in the bivalves considered as a major form of energy reserves.

In Kalbadevi estuary at Ratnagiri, *K. opima* spawn twice in a year (1st spawn during post-monsoon to early winter season and 2nd spawn during the first half to the second half of the summer season). Out of these two spawning season, major spawn was observed during post-monsoon season in the month of October and November; while minor spawn was observed during summer season in the month of March and April (Nagabhushanam and Mane, 1983). In this study, higher protein content in gonad of clam *K. opima* was recorded during post-monsoon (23.467 ± 0.133), while during summer season higher protein content has been observed in foot (20.333 ± 0.367) followed

by gonad (18.733 ± 0.107) (Table 2). Higher value of lipid was found in all the tissues during post-monsoon season followed by summer season, whereas lipid content in gonad was higher (4.215 ± 0.026) as compared to other somatic tissues (Table 3). Glycogen content has been observed with higher value in gonad (9.88 ± 0.08) during summer, while it decreased in post-monsoon season (Table 4). This seasonal study of clam *K. opima* from Bhatye estuary, showed that, clam *K. opima* displayed significant variation in biochemical composition (protein, lipid and glycogen) with respect to their reproductive cycle. Contribution of protein in the reproductive tissue was low as compared to glycogen and lipid stored in digestive gland, mantle and gonad. Ren *et al.,* (2003) observed somatic growth pattern which reflects protein concentration, which was maintained above 50% throughout the year with slightly seasonal or inter-annual variation. It suggests that somatic growth continued even after gametogenesis in bivalves.

Development of reproductive gland influenced seasonal variation in glycogen content in pen shell. In pen shell, glycogen content declined gradually up to the spawning season (Yurimoto *et al.,* 2003). Lomovasky *et al.,* (2004) correlated changes in biochemical constituents like protein, lipid and glycogen from three organ groups (foot and visceral mass, adductor muscle and siphon, mantle and gill) with reproductive cycle and season of maximum shell growth. They also observed low lipid content during November and high values during the summer season as well as indicating the energetic variation correlated with gamete emission in November and gamete maturation in summer season. Increase in protein content in all tissues of *Eurhomalea exalbida* during spawning season suggest that, protein may be acting as an alternative energy resource in adult bivalves.

An environmental stress, especially climatic stress is underestimated feature of organism's habitat in the wild. Resource depletion and inadequate nutrition becomes the norm during those circumstances, so that organisms often struggle to survive. Stress is an environmental probe that targets the predominant carrier of energy, the adenosine triphosphate (ATP). Therefore, stress reduces energetic efficiency, i.e. the organism's fitness, but increased energetic efficiency should evolve during adaptation of organisms to their habitats (Parsons, 2007). Maske *et al.,* (2005) noted decreased biochemical constituents like protein, lipid and glycogen in three estuarine clams *K. opima, M. meretrix* and *M. casta* exposed to lower salinity ranges. In the present study, clams of *K. opima* exposed to lower salinity ranges showed decline in all biochemical constituents (protein, lipid and glycogen) from selected tissues like hepatopancreas, gonad, foot and gill. More prominent decline in biochemical constituents was increased with decrease in salinity exposure from 70% salinity range and reach at maximum in lowermost range of salinity exposure (40% salinity).

Galap *et al.,* (1997) stated that, glycogen content of muscular tissues acts as a primary energy resource during gamete formation in bivalve *Glycymeris glycymeris* under starvation or food scarcity conditions. Reduction in glycogen content during starvation was correlated with increase in gonad lipid content (Fernandez-Reiriz *et al.,* 1996). In *Ensis arcuatus,* digestive gland appears to act as a vital reserve storage site for lipid, which was transferred to the gonad during gamete development. Similar situation was observed in case of glycogen present in adductor muscle, foot and digestive gland. Glycogen reserve from these tissues mobilized to gonad when gamete development started (Darriba *et al.,* 2005).

Clam *K. opima,* exposed to lower salinity ranges decreased more than 50% glycogen content specifically from foot, gill and hepatopancreas tissues of clam during all three seasons. Highest

decline in glycogen content (63% and 78%) was evident in foot of clams exposed to the lowest salinity range (40% salinity) during post-monsoon and summer season respectively. Such highest glycogen decrease in foot may be the result of glycogen mobilization to reproductive tissue for active gametogenesis and to fulfill the energy demand to cope up with salinity stress conditions. The decrease in glycogen content appears to be a sign of the adaptive response of an organism exposed to stress. Generally, the increased energy demand associated with the stress disturbs the carbohydrate metabolism and causes glycogen depletion which activates glycogenolysis. Next to carbohydrates, fats are the best energy source of the body. Therefore under conditions of stress, the bivalves utilize lipid and protein reserves to meet the increased energy demands and consequently entail a depletion of their lipid levels. The considerable decrease in total lipids might be a sequel to the efforts of the organism to replenish any glycogen deficiency caused by any extraneous reason (Sujatha *et al.,* 1996).

In the present investigation, during all seasons, salinity stress resulted in to higher utilization (ranges from 30.90 to 78.24%) of glycogen reserves in clam *K. opima* (Table 4). Therefore, lower salinity stress induced decrease in lipid content. It showed limited utilization (ranges from 21.12 to 51.83%) in both somatic and reproductive tissues (Table 3). On the basis of percent lipid reduction, in the reproductive tissues lowest lipid reduction (26.16%) was found during reproductive peak seasons like post-monsoon than summer (30.82%) and winter season (41.80%) respectively (Table 3). In severe/prolonged conditions of energy imbalance, lipid acts as energy source to fulfill the energy requirement, after utilization of a large proportion of carbohydrate reserve under such conditions (Beninger and Lucas, 1984). During gonad maturation, there is the transformation of these energy reserves from other body parts to the gonad. On the basis of this phenomenon, Taylor and Venn (1979) proposed the possibility of transformation of lipid from somatic tissues to the reproductive organs with an inverse relationship between lipid concentration of somatic and reproductive tissue.

Stress conditions also responsible for lowering of carbohydrate content to cope with high energy demand. Many authors agree that, the protein fraction used as a source of energy for maintenance only when carbohydrate resource greatly depleted (Camacho *et al.,* 2003). In clam *K. opima,* protein content of all the tissues of clams were highly reduced (20.87 to 58.32%) as a result of lower salinity exposure (Table 2). But the level of reduction of protein reserve from different tissues was lower than glycogen reduction (30.90 to 78.24%) (Table 4). Comparatively, the level of protein reduction in all tissues was lower during all three seasons (Table 2). Carrasco *et al.,* (2006) reported the decreased lipid and carbohydrate content in tissues with great proportion as compared to protein content of *Littorina littorina* and *Chorus gigantus* under 14 weeks starvation period. It denoted that, both the clams gave preference to carbohydrate and lipid for catabolism rather than protein.

Results of seasonal biochemical variations in different tissues, especially gonad and hepatopancreas of both the clam species were correlated with reproductive cycle. Biochemical alterations in the clam after 8 days period of exposure to lower salinity ranges were distinctly observed below 70% salinity range. The biochemical constituents like protein, lipid and glycogen were significantly reduced in both reproductive and somatic tissues but, with variation in range of reduction. In clam *K. opima,* biochemical constituents reduced to cope up with lowered salinity stress conditions, in which glycogen acts as a major source of energy followed by lipid and protein. Reduction in lipid content (21.12 to 51.83%) from different tissues confirms its use as energy reserve, after

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

Table 2 Salinity induced changes in protein content in different tissues of *Katelysia opima.*
(mg/100mg of wet tissue).

Salinity Ranges	Hepatopancreas			Gonad			Foot			Gill		
	Summer	Post-monsoon	Winter	Summer	Post-monsoon	Winter	Summer	Post-monsoon	Winter	Summer	Post-monsoon	Winter
100%	14.444 ± 0.183	14.222 ± 0.204	13.689 ± 0.234	18.733 ± 0.107	23.467 ± 0.133	17.911 ± 0.154	20.333 ± 0.367	14.022 ± 0.234	15.689 ± 0.336	10.222 ± 0.102	11.356 ± 0.168	8.4 ± 0.135
90%	13.555 ± 0.385 Ns (-6.15)	8.6 ± 0.133 *** (-39.53)	13.711 ± 0.193 Ns (+0.16)	15.467 ± 0.133 *** (-17.44)	21.622 ± 0.168 *** (-7.86)	17.444 ± 0.139 Ns (-2.61)	15.111 ± 0.177 *** (-35.24)	7.933 ± 0.240 *** (-43.42)	18.533 ± 0.133 *** (+18.13)	9.55 ± 0.267 ** (-21.74)	6.733 ± 0.636 *** (-40.70)	8.289 ± 0.269 Ns (-1.32)
80%	11.555 ± 0.385 *** (-20)	10.556 ± 0.779 *** (-25.78)	13.111 ± 0.138 Ns (-4.22)	15 ± 0.111 *** (-19.93)	20.222 ± 0.204 *** (-13.83)	16.956 ± 0.192 *** (-5.33)	14.444 ± 0.177 *** (-38.10)	9.378 ± 0.342 *** (-33.12)	15.489 ± 0.168 Ns (-1.27)	7.111 ± 0.385 *** (-30.43)	7.911 ± 0.102 *** (-30.33)	7.867 ± 0.105 Ns (-6.35)
70%	9.778 ± 0.385 *** (-32.31)	8.644 ± 0.301 *** (-39.22)	15 ± 0.223 *** (+9.58)	14.733 ± 0.211 *** (-21.35)	19.044 ± 0.139 *** (-18.84)	16.733 ± 0.115 *** (-6.58)	14.222 ± 0.238 *** (-39.05)	4.889 ± 0.555 *** (-65.13)	15.022 ± 0.336 Ns (-4.25)	7.111 ± 0.278 *** (-30.43)	5.111 ± 0.329 *** (-54.99)	8.822 ± 0.127 Ns (+5.03)
60%	8.444 ± 0.385 *** (-41.54)	9.044 ± 0.468 *** (-36.41)	9.511 ± 0.168 *** (-30.52)	14.333 ± 0.151 *** (-23.49)	17.489 ± 0.204 *** (-25.47)	15.8 ± 0.133 *** (-11.79)	12.444 ± 0.385 *** (-46.67)	6.578 ± 0.342 *** (-53.09)	14.178 ± 0.204 *** (-9.63)	7.995 ± 0.385 *** (-6.54)	5.511 ± 0.139 *** (-51.47)	5.556 ± 0.204 *** (-33.86)
50%	7.778 ± 0.077 *** (-46.15)	8.067 ± 0.115 *** (-43.28)	7.689 ± 0.154 *** (-43.83)	13.533 ± 0.115 *** (-27.76)	14.867 ± 0.115 *** (-36.65)	13.49 ± 0.168 *** (-24.69)	11.8 ± 0.133 *** (-49.43)	6.133 ± 0.115 *** (-56.26)	12.444 ± 0.138 *** (-20.68)	7.422 ± 0.102 *** (-17.61)	5.088 ± 0.077 *** (-55.19)	4.422 ± 0.177 *** (-47.35)
40%	7 ± 0.115 *** (-51.54)	7.356 ± 0.038 *** (-48.28)	7.156 ± 0.108 *** (-47.73)	12.222 ± 0.102 *** (-34.76)	13.044 ± 0.139 *** (-44.41)	12.422 ± 0.192 *** (-30.65)	10.844 ± 0.102 *** (-53.52)	5.844 ± 0.039 *** (-58.32)	11.511 ± 0.177 *** (-26.63)	7.089 ± 0.154 *** (-20.87)	5.022 ± 0.038 *** (-55.77)	4.044 ± 0.138 *** (-51.85)

All the values are mean of five observations ± Standard Deviation; P < 0.001 - *** (Highly Significant); P < 0.01 - ** (Significant);
P > 0.05 – NS (Non significant); Values in parenthesis indicates (-) % decrease and (+) % increase.

Table 3 Salinity induced changes in lipid content in different tissues of *Katelysia opima.* (mg/100mg of wet tissue).

Salinity Ranges	Hepatopancreas			Gonad			Foot			Gill		
	Summer	Post-monsoon	Winter	Summer	Post-monsoon	Winter	Summer	Post-monsoon	Winter	Summer	Post-monsoon	Winter
100%	1.74 ± 0.043	2.33 ± 0.075	1.807 ± 0.04	3.285 ± 0.075	4.215 ± 0.026	2.41 ± 0.017	1.095 ± 0.043	2.452 ± 0.020	1.392 ± 0.038	1.006 ± 0.024	1.883 ± 0.034	1.19 ± 0.037
90%	1.575 ± 0.075 Ns (-9.48)	1.613 ± 0.027 *** (-30.79)	1.745 ± 0.013 Ns (-3.46)	3.107 ± 0.087 Ns (-5.40)	4.005 ± 0.020 *** (-4.98)	2.372 ± 0.009 Ns (-1.56)	1.1 ± 0.043 Ns (+0.46)	1.467 ± 0.030 *** (-40.16)	1.938 ± 0.022 *** (+39.14)	0.977 ± 0.043 Ns (-2.86)	1.365 ± 0.049 *** (-27.49)	1.1455 ± 0.027 Ns (-4.04)
80%	1.677 ± 0.043 Ns (-3.59)	1.79 ± 0.019 *** (-23.18)	1.532 ± 0.068 *** (-15.24)	2.94 ± 0.015 *** (-10.50)	3.882 ± 0.011 *** (-7.89)	2.335 ± 0.017 ** (-3.11)	0.925 ± 0.043 ** (-15.53)	1.735 ± 0.041 *** (-29.26)	1.602 ± 0.024 *** (+15.08)	0.875 ± 0.024 ** (-13.04)	1.662 ± 0.016 *** (-11.69)	1.1595 ± 0.019 Ns (-2.87)
70%	1.575 ± 0.075 Ns (-9.48)	1.96 ± 0.026 *** (-15.88)	1.635 ± 0.020 ** (-9.54)	2.792 ± 0.087 *** (-14.99)	3.77 ± 0.017 *** (-10.56)	2.3 ± 0.019 *** (-4.56)	0.9 ± 0.075 ** (-17.81)	1.452 ± 0.026 *** (-40.77)	1.392 ± 0.087 Ns (0)	0.95 ± 0.034 Ns (-5.59)	1.742 ± 0.044 *** (-7.44)	1.0557 ± 0.019 *** (-11.56)
60%	1 ± 0.043 *** (-42.53)	2.109 ± 0.061 ** (-9.47)	1.59 ± 0.02 *** (-12.03)	2.772 ± 0.011 *** (-15.60)	3.557 ± 0.016 *** (-15.60)	2.222 ± 0.004 *** (-7.78)	0.7 ± 0.043 *** (-36.07)	1.415 ± 0.057 *** (-42.30)	0.995 ± 0.019 *** (-28.55)	0.8 ± 0.014 *** (-20.50)	1.762 ± 0.027 *** (-6.37)	1 ± 0.019 *** (-16.23)
50%	0.957 ± 0.011 *** (-44.97)	1.922 ± 0.011 *** (-17.49)	1.515 ± 0.013 *** (-16.18)	2.455 ± 0.011 *** (-25.27)	3.345 ± 0.022 *** (-20.64)	2.042 ± 0.016 *** (-15.25)	0.578 ± 0.02 *** (-47.17)	1.317 ± 0.024 *** (-46.28)	0.925 ± 0.043 *** (-33.57)	0.8 ± 0.043 *** (-20.50)	1.597 ± 0.015 *** (-15.14)	0.91 ± 0.017 *** (-23.77)
40%	0.852 ± 0.016 *** (-51.01)	1.6 ± 0.016 *** (-31.33)	1.308 ± 0.078 *** (-27.63)	2.272 ± 0.013 *** (-30.82)	3.112 ± 0.020 *** (-26.16)	1.402 ± 0.043 *** (-41.80)	0.527 ± 0.043 *** (-51.83)	1.282 ± 0.013 *** (-47.71)	0.91 ± 0.017 *** (-34.65)	0.692 ± 0.026 *** (-31.18)	1.485 ± 0.013 *** (-21.12)	0.68 ± 0.009 *** (-43.04)

All the values are mean of five observations ± Standard Deviation; P < 0.001 - *** (Highly Significant); P < 0.01 - ** (Significant); P > 0.05 – NS (Non significant); Values in parenthesis indicates (-) % decrease and (+) % increase.

Table 4 Salinity induced changes in glycogen content in different tissues of *Katelysia opima.* (mg/100mg of wet tissue).

Salinity Ranges	Hepatopancreas			Gonad			Foot			Gill		
	Summer	Post-monsoon	Winter	Summer	Post-monsoon	Winter	Summer	Post-monsoon	Winter	Summer	Post-monsoon	Winter
100%	5.13 ± 0.18	4.56 ± 0.052	8.427 ± 0.403	9.88 ± 0.08	7.413 ± 0.046	9.013 ± 0.231	8.333 ± 0.462	7.387 ± 0.660	6.827 ± 0.403	6.333 ± 0.346	5.013 ± 0.220	5.6 ± 0.4
90%	4.133 ± 0.146 *** (-19.43)	3.653 ± 0.024 *** (-19.88)	9.653 ± 0.323 *** (+14.56)	9.493 ± 0.046 Ns (-3.91)	7.173 ± 0.046 Ns (-3.24)	9.76 ± 0.139 Ns (+8.28)	8.267 ± 0.222 Ns (-0.80)	7.88 ± 0.08 Ns (+6.68)	6.853 ± 0.244 Ns (+0.39)	5.867 ± 0.335 Ns (-7.37)	3.84 ± 0.04 *** (-23.40)	6.213 ± 0.122 *** (+10.95)
80%	4.667 ± 0.246 Ns (-9.03)	3.733 ± 0.061 *** (-18.13)	6.427 ± 0.122 *** (-23.73)	10.827 ± 0.046 *** (+9.28)	6.453 ± 0.061 *** (-12.95)	8.267 ± 0.244 *** (-8.28)	5.867 ± 0.462 *** (-29.60)	7.747 ± 0.101 Ns (+4.87)	6.08 ± 0.139 *** (-10.94)	5.667 ± 0.146 Ns (-10.53)	3.787 ± 0.046 *** (-24.47)	5.733 ± 0.122 Ns (+2.38)
70%	4.533 ± 0.325 Ns (-11.63)	3.4 ± 0.04 *** (-25.44)	5.093 ± 0.257 *** (-39.56)	9.28 ± 0.08 *** (-6.07)	5.48 ± 0.08 *** (-26.08)	8.08 ± 0.139 *** (-10.36)	5.133 ± 0.462 *** (-38.40)	6.187 ± 0.227 ** (-16.25)	3.28 ± 0.08 *** (-51.95)	4.2 ± 0.28 *** (-33.68)	3.267 ± 0.061 *** (-34.84)	4.88 ± 0.139 *** (-12.86)
60%	3.733 ± 0.246 *** (-27.23)	3.333 ± 0.061 *** (-26.90)	4.267 ± 0.201 *** (-49.37)	9.12 ± 0.08 *** (-7.69)	5.387 ± 0.046 *** (-27.34)	7.493 ± 0.122 *** (-16.86)	2.133 ± 0.462 *** (-74.40)	3.773 ± 0.083 *** (-48.92)	3.093 ± 0.122 *** (-54.69)	4.667 ± 0.462 *** (-26.32)	3.387 ± 0.046 *** (-32.45)	3.333 ± 0.122 *** (-40.48)
50%	3.467 ± 0.092 *** (-32.42)	3.027 ± 0.046 *** (-33.63)	3.387 ± 0.092 *** (-59.81)	8.16 ± 0.212 *** (-17.41)	4.52 ± 0.12 *** (-39.03)	6.133 ± 0.092 *** (-31.95)	1.733 ± 0.046 *** (-79.20)	2.853 ± 0.061 *** (-61.37)	2.533 ± 0.046 *** (-62.89)	4.133 ± 0.122 *** (-34.74)	2.627 ± 0.092 *** (-47.61)	3.04 ± 0.139 *** (-45.71)
40%	2.587 ± 0.192 *** (-49.58)	2.853 ± 0.092 *** (-37.43)	3.227 ± 0.046 *** (-61.71)	6.827 ± 0.201 *** (-30.90)	4.16 ± 0.106 *** (-43.88)	5.707 ± 0.092 *** (-36.69)	1.8133 ± 0.369 *** (-78.24)	2.707 ± 0.023 *** (-63.36)	1.893 ± 0.185 *** (-72.27)	3.347 ± 0.257 *** (-47.16)	2.453 ± 0.046 *** (-51.06)	2.453 ± 0.046 *** (-56.19)

All the values are mean of five observations ± Standard Deviation; P < 0.001 - *** (Highly Significant); P < 0.01 - ** (Significant); P > 0.05 – NS (Non significant); Values in parenthesis indicates (-) % decrease and (+) % increase.

exhaustion of glycogen reserves (30.90 to 78.24%). As clam *K. opima* experienced overall 40% reduced salinity in natural estuarine conditions, same range of adaptability to lower salinity ranges was observed throughout the year, even though they naturally experience salinity fluctuation from 10% to 40% depending on season. But below their adaptive limit the salinity ranged from 50% and 40% salinity, critical physiological changes were marked in clam *K. opima*.

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