SOME BEHAVIOURAL ALTERATIONS IN ESTUARINE CLAM *KATELYSIA OPIMA* UNDER OSMOTIC STRESS ALONG BHATYE ESTUARY, RATNAGIRI (M.S.) INDIA

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ABSTRACT

The behavioural response of estuarine clam *Katelysia opima* under osmotic stress was studied by exposing average sized clams to various lower salinity ranges (control to 10% salinity) for the period of 8 days. Behaviour of clams like shell valve closure, siphon and mantle activity and mortality during 8 days exposure period were taken as a measures of behavioural alterations in osmotic stress. After exposure of different lower salinity, the behaviour patterns in clams have been affected significantly. From the results of shell valve closing behaviour of clams of *K. opima*, it was clear that initial period of lower salinity exposure during post-monsoon season is more stressful, as compared to winter and summer season. Mantle and siphon activity of clam *K. opima* showed positive correlation with salinity, but degree of change in response varies from season to season.

KEYWORDS: Salinity ranges, *Katelysia opima*, Behavioural patterns, etc.

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 the 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 affects an organisms in natural conditions, therefore studies on physiological mechanisms of organisms towards such conditions becomes important. Comparative biologist and ecologist paying attention towards determination of physiological condition of organisms in a natural context (Hofmann and Somero, 1995; Wagner *et al.,* 1998). Among the factors in the marine environment, 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 (Vernberg 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. The clams in such areas therefore have to adapt themselves in order to overcome these fluctuations. Growth, mortality and behaviour of early stage *Pecten maximus* 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 (Pequeux 1995; Shock *et al.,* 2009) and reproductive success (Deschaseaux *et al.,* 2010) of wide range of organisms living on mudflats or rocky shores.

The baby clam *Marcia opima, has* so far been indicated as *Katelysia opima* in Indian waters. 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 physiological processes like behaviour 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 medium 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. These salinity ranges were maintained throughout experiment by adding freshwater. Daily changes in double filtered sea water of the respective salinity range were made with 6 hour interval.

Table 1. Seasonal salinity variations in estuary and experimental set.

Salinity Ranges				**Salinity in %**									
Seasons		**L.T.**	**H. T.**	**Control/ 100**	**90**	**80**	**70**	**60**	**50**	**40**	**30**	**20**	**10**
Seasonal salinity in ‰	**Summer**	36 ± 0.55	38 ± 0.78	38± 1	35 ±1	33 ±1	30 ±1	27 ±1	23 ±1	19 ±1	14 ±1	10 ±1	06 ±1
	Post-monsoon	22 ± 4	29 ± 2	29± 1	26 ±1	23 ±1	20 ±1	17 ±1	14 ±1	11 ±1	08 ±1	06 ±1	03 ±1
	Winter	28 ± 0.36	36 ± 0.55	36±1	33 ±1	29 ±1	26 ±1	22 ±1	19 ±1	16 ±1	12 ±1	08 ±1	04 ±1

All the values are mean ± S.D.

Behavioural assessment of the clam was performed by selecting few behavioural patterns such as shell valve closure, mantle and siphon activity. All these behavioural patterns were observed for 08 days period of exposure to various salinity ranges during all three selected seasons. Along with these behavioural assessments, mortality of the clams during exposure period was also recorded for 08 days. Mortality observations were presented as % mortality of clam exposed to the respective salinity range in that experimental set.

RESULT AND DISCUSSION

In present study, Behaviour like shell valve closing/opening, mantle and siphon activities of clams were presented by different groups indicating percent individuals of clams i.e. A (more than 75% individuals), B (more than 50% individuals), C (more than 25% individuals) and D (up to 25% individuals). Salinity induced Behaviour and mortality pattern of clams during summer, post-monsoon and winter season were represented in table no. 2,4,6 and 3,5,7 respectively.

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). Clams have the ability to close their shell valves in response to unfavorable salinity conditions, to prevent internal soft organs from direct exposure to the surrounding seawater. During this time they respire anaerobically. Clams can keep their shell valves closed for several days in such environmental conditions (Baker *et al.,* 2007).

In *K. opima,* range of salinity to trigger valve closure and its extent of period were changes from season to season. During the summer season, clams from almost all salinity ranges (control to 10%) showed closed shell valves up to 3rd day of exposure, out of which clams exposed below 50% salinity showed higher number of individuals with closed shell valves (80 to 100%) (Table no. 02). During the post-monsoon season, clam of *K. opima* displayed closed shell valves around 60% individuals from 80% to 10% salinity range up to 8th day of exposure (192 hours) (Table no. 04). During winter season, clams of *K. opima* species from 80 to 10% salinity range displayed individuals having closed shell valves with 60% to 80% up to 5th day (120 hours) of exposure, which fluctuates with about 60% individuals up to 8th day of exposure (Table no. 06).

The blood cockle *Anadara granosa* is an osmoconformer species which closes its shell valve triggered by low salinity ranges below 19‰. Closure of the shell valves provides protection for relatively short periods of 3 days, therefore cockles are vulnerable to low environmental salinities which endure for longer time (Davenport and Wong, 1986). Asian clam *Corbicula fluminea* closed their valves naturally with extended periods up to 10 - 12 hours. The ability of clams to close the valve against any environmental stress, predation, pollution as well as any contamination with reduction in metabolic rate, saves an enormous amount of energy which can be beneficial during the starvation period (Ortmann and Grieshaber, 2003).

Clam *K. opima* in 50% to 30% salinity ranges showed increase siphon and mantle activity during first two days of exposure, which later reduced after the 3rd to 8th day of exposure during summer season (Table no. 02). During post-monsoon season, clam *K. opima* showed 60% siphon and mantle activity in clams from 80 to 50% salinity range till the 3rd day of exposure, further it was declined to 40% of individuals (Table no. 04). Comparatively, during winter season, clams exposed to 80 to 40% salinity showed increased siphon and mantle activity 5th day of exposure (Table no. 06). Stress conditions induced by lowering salinity changes from season to season. During the summer season due to salinity stress mantle and siphon activity reduced from 20% to 10% salinity, during post-monsoon season it starts from 40% salinity up to 10% salinity and during the winter season it was observed from 30% salinity to10% salinity.

The blue mussel response to a rapid drop in ambient salinity by closing its exhalent siphon to stop the ventilation of the mantle cavity, and then closes its shell, if the salinity change is large enough. Such change in salinity may be resulted from the ebb tide in estuaries with a large input of fresh water (Davenport, 1982). It can thus effectively isolate its tissue with half change in ambient osmotic concentration (Shumway, 1977). But according to Gilles (1972), animals cannot maintain shell closure position longer than about 96 hours, because during the shell closure period it must depend on stored nutrient reserves and anaerobic metabolism to sustain energy demands. Therefore, if those adverse salinity conditions remain for a longer period, then the animal opens its shell and adjust osmotically to the ambient conditions (Widdows, 1985 a,b).

Acute reduction in salinity may have a precise impact on clam survival. Reduction in salinity may also have sub-lethal effects on metabolic or feeding rates, resulting in reduced growth rate. For example, oyster spat subjected to low salinities for longer than 2 weeks did not immediately attain normal feeding levels to return to full salinities, and mortality continued to be high (Rodstrom and Jonsson, 2000).

During summer season, clams *K. opima* experienced 10% salinity reduction under natural estuarine condition, hence it is expected that they successfully acclimatized with such salinity fluctuation in natural conditions. But in laboratory experiment it was found that *K. opima* showed tolerance to lower salinity i.e. 40% salinity (19‰) with 87% survival rate (Table no. 03). As compared to the summer season, during post-monsoon season, due to the higher water influx from the river and adjacent lowland areas, estuarine water get diluted so estuarine animal experiences comparatively low salinity during post-monsoon season. During this season clam experience near about 40% low salinity than normal salinity of estuary during high tide (29‰) and low tide (17‰). So it is considered that in this period clams tolerance increased towards lower salinity as they continuously experienced nearly 40% lower salinity in natural environmental conditions. It was cleared during laboratory experiments, which showed 87% survival rate in clams exposed to 40% lower salinity range i.e. 60% lower salinity (11‰) in laboratory conditions compared to natural condition (Table no. 05). This indicates increase in clam's tolerance during post-monsoon season than expected range of tolerance in natural conditions. During winter season, clams experienced 20% salinity decrease from high tide to low tide water mark, which indicated that, clams can tolerate salinity from 100% to 80% during winter season (Table no. 07). Clams exposed to lower salinity ranges in laboratory condition showed their tolerance up to 40% salinity range (16‰). Clam *K. opima* from Bhatye estuary exposed directly to low salinity ranges from 30% to 10% salinity showed 77 to 100% mortality during summer, post-monsoon and winter season. But both, degree of salinity change and time taken by clams for 100% mortality was observed to be varied from season to season.

Sturmer, (2005) found that, Juvenile hard clams, *Mercenaria mercenaria*, having strength and rigidity towards sudden change in salinity reduction from 10 - 15‰ by experiencing less than 5% mortality. On the other hand, salinity declines with the extent of 20 - 24‰, resulted in significant mortality by 17%, when clams exposed step by step to these lower salinity; while 100% mortality observed in the clams which directly exposed to test salinity following 6 days of exposure. Kripa *et al.,* (2006) observed 100% mortality within 8 days in seed clams of *P. malabarica* (size 6 - 10 mm) exposed to 0, 5, and 10‰, whereas they found salinity greater than 25‰ is useful for clam farming as they observed 33 and 16% mortality in 10 days at 20 and 25‰ salinity. If low saline condition prevails, the seed must be removed within 10 days. They also observed that, the seed clams can tolerate exposure of low saline conditions up to 48 hrs at different percent survival. Such exposure will result in 12.5% mortality in 10‰ and 2.5% mortality in 15‰. In 20 and 25‰ salinity, survival of the clams will not affect up to 48 hrs of exposures.

During present study clam *K. opima* from Bhatye estuary showed overall tolerance towards lower salinity is up to 40% salinity range i.e. 19‰, 11‰, and 16‰ salinity for 8 days of exposure during summer, post-monsoon and winter season respectively. In brackish water areas of New South Wales, Australia, the clams *Katelysia rhytiphora* and *Tapes dorsatus*, were found to require salinities of 20 – 45‰ for survival, where these species can withstand longer than seven days at salinity ranges below 20‰. Therefore, they can only grow in the higher salinity range regions of the estuary (Nell, 1997). Similar observations were found with slight deviation in tolerance to salinity in the wedge clam, *Donax cuneatus*. It endures salinity dilution up to 22.05‰. Further dilution in salinity like 18.9‰ resulted in to erratic behavior with 100% mortality at 9.45‰ (Talikhedkar, 1981).

CONCLUSION

In the present investigation, the effect of environmental stress conditions like low salinity on estuarine clam *K. opima* under controlled laboratory conditions showed variation in physiological responses and tolerance with respect to the seasons. From the results of shell valve closing behaviour of clams of *K. opima*, it was clear that initial period of lower salinity exposure during post-monsoon season is more stressful, as compared to winter and summer season. Mantle and siphon activity of clam *K. opima* showed positive correlation with salinity, but degree of change in response varies from season to season. The range of lower salinity from which, prominent reduction in mantle and siphon activity observed during post-monsoon season (40%) was closer to control salinity range as compare to summer season (20%) and winter season (30%). Seasonal changes in salinity variation in natural environmental conditions can alter the tolerance capacity of clam.

During present study, clam *K. opima* from Bhatye estuary showed overall tolerance towards lower salinity up to 40% salinity range i.e. 19‰, 11‰, and 16‰ salinity for 8 days of exposure during summer, post-monsoon and winter season respectively. On the basis of behavioural response in clam *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 of normal estuarine water in that season. But below their adaptive limit of the salinity range like at 50% and 40% salinity, critical physiological changes were marked in this clam species.

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Table 02: Behaviour observations of Clam *K. opima* under exposure of various salinity ranges during summer season.

SVC = Shell Valve Closed, SVO = Shell Valve Open, M and S activity = Mantle and Siphon activity, A= (more than 75%), B= (more than 50%), C = (more than 25%), D= (up to 25%), - = absent

Hours	Ranges / Behaviour patterns	Control	90%	80%	70%	60%	50%	40%	30%	20%	10%
0 Hours	1. SVC	A	A	A	A	A	A	A	A	A	A
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	-	-	-	-	-	-	-	-	-	-
24 Hours	1. SVC	D	B	B	B	B	C	C	A	A	A
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	A	C	C	C	C	B	B	D	-	-
48 Hours	1. SVC	B	B	C	B	B	C	D	B	B	A
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	C	C	B	B	C	B	A	C	D	-
72 Hours	1. SVC	B	B	C	B	B	C	C	D	D	C
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	C	C	B	C	D	B	B	B	D	-
96 Hours	1. SVC	C	C	D	A	D	D	B	D	-	Nil
	2. SVO	-	-	D	-	-	-	-	-	-	-
	3. M and S activity	B	B	A	D	A	B	D	C	D	-
120 Hours	1. SVC	C	D	D	B	C	A	B	-	Nil	Nil
	2. SVO	-	D	-	-	-	-	D	-	-	-
	3. M and S activity	B	B	B	D	C	D	D	D	-	-

144 Hours	1. SVC	D	C	C	C	D	C	B	-	Nil	Nil
	2. SVO	-	D	-	-	-	-	-	-	-	-
	3. M and S activity	B	C	D	B	B	C	D	D	-	-
168 Hours	1. SVC	D	C	C	C	D	C	B	Nil	Nil	Nil
	2. SVO	-	D	-	-	-	-	-	-	-	-
	3. M and S activity	B	C	D	B	B	C	D	-	-	-
192 Hours	1. SVC	D	C	C	C	D	C	B	Nil	Nil	Nil
	2. SVO	-	D	-	-	-	-	-	-	-	-
	3. M and S activity	B	C	D	B	B	C	D	-	-	-

Table 03: Mortality of clam *Katelysia opima* under exposure to various salinity ranges during summer season.

Time in Hours	Control	90%	80%	70%	60%	50%	40%	30%	20%	10%
0 Hours	–	–	–	–	–	–	–	–	–	–
24 Hours	–	–	–	–	–	–	–	–	–	–
48 Hours	–	–	–	–	–	–	–	–	5	6
72 Hours	–	–	–	–	–	–	–	12	17	14
96 Hours	–	–	–	–	–	–	–	6	3	10
120Hours	–	–	3	–	–	–	–	7	5	–
144Hours	2	1	4	–	–	–	3	2	–	–
168Hours	–	–	5	–	–	–	1	3	–	–
192Hours	–	1	–	–	–	–	–	–	–	–
%Mortality	7	7	40	0	0	0	13	100	100	100

Table 04: Behaviour observations of Clam *K. opima* under exposure of various salinity ranges during Post-monsoon season.

SVC = Shell Valve Closed, SVO = Shell Valve Open, M and S activity = Mantle and Siphon activity, A= (more than 75%), B= (more than 50%), C = (more than 25%), D= (up to 25%), - = absent

Hours	**Ranges** / **Behaviour patterns**	**Control**	**90%**	**80%**	**70%**	**60%**	**50%**	**40%**	**30%**	**20%**	**10%**
0 Hours	1. SVC	A	A	A	A	A	A	A	A	A	A
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	-	-	-	-	-	-	-	-	-	-
24 Hours	1. SVC	A	B	A	A	A	B	C	A	B	B
	2. SVO	-	-	-	-	-	-	-	-	D	D
	3. M and S activity	D	C	D	D	D	C	C	D	D	D
48 Hours	1. SVC	B	B	B	B	B	C	C	A	B	D
	2. SVO	-	-	-	-	-	-	-	D	D	D
	3. M and S activity	C	C	C	C	C	B	B	D	D	D
72 Hours	1. SVC	B	C	C	B	B	C	C	C	D	Nil
	2. SVO	-	-	-	-	-	-	-	-	D	-
	3. M and S activity	C	B	B	C	C	B	C	C	D	D
96 Hours	1. SVC	B	C	B	C	C	B	C	D	Nil	Nil
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	D	C	D	C	C	D	C	A	-	-
120 Hours	1. SVC	B	B	B	B	C	C	C	D	Nil	Nil
	2. SVO	-	-	-	-	-	-	-	D	-	-

	3. M and S activity	D	D	D	C	C	C	C	C	-	-
144 Hours	1. SVC	B	B	B	B	B	B	B	D	Nil	Nil
	2. SVO	-	-	-	-	-	-	D	D	-	-
	3. M and S activity	C	D	D	-	D	D	D	D	-	-
168 Hours	1. SVC	C	B	B	B	B	B	B	D	Nil	Nil
	2. SVO	-	-	-	-	-	-	-	D	-	-
	3. M and S activity	C	D	D	C	D	D	D	C	-	-
192 Hours	1. SVC	C	B	B	B	B	B	B	D	Nil	Nil
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	C	D	D	C	D	D	D	D	-	-

Table 05: Mortality of clam *Katelysia opima* under exposure to various salinity ranges during Post-monsoon season.

Time in Hours	**Control**	**90%**	**80%**	**70%**	**60%**	**50%**	**40%**	**30%**	**20%**	**10%**
0 Hours	–	–	–	–	–	–	–	–	–	–
24 Hours	–	–	–	–	–	–	–	–	5	5
48 Hours	–	–	–	–	–	–	–	2	6	11
72 Hours	–	–	–	–	1	–	–	3	12	9
96 Hours	–	–	–	–	1	–	1	2	7	5
120Hours	–	–	–	–	1	–	2	8	–	–
144Hours	–	–	–	–	–	–	–	4	–	–
168Hours	–	–	–	–	–	–	1	–	–	–
192Hours	–	–	–	–	–	–	–	4	–	–
%Mortality	0	0	0	0	10	0	13	77	100	100

Table 06: Behaviour observations of Clam *K. opima* under exposure of various salinity ranges during winter season.

SVC = Shell Valve Closed, SVO = Shell Valve Open, M and S activity = Mantle and Siphon activity, A= (more than 75%), B= (more than 50%), C = (more than 25%), D= (up to 25%), - = absent

Hours	Ranges / Behaviour patterns	Control	90%	80%	70%	60%	50%	40%	30%	20%	10%
0 Hours	1. SVC	A	A	A	A	A	A	A	A	A	A
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	-	-	-	-	-	-	-	-	-	-
24 Hours	1. SVC	A	B	B	A	B	C	D	A	A	B
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	D	C	C	D	C	B	A	D	D	D
48 Hours	1. SVC	A	B	B	A	A	B	C	C	B	B
	2. SVO	-	-	-	-	-	-	-	-	-	D
	3. M and S activity	D	C	C	D	D	C	B	B	D	-
72 Hours	1. SVC	A	B	B	B	B	A	C	D	C	D
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	D	C	C	C	C	D	B	B	D	-
96 Hours	1. SVC	B	B	B	B	B	B	B	D	D	Nil
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	D	D	C	D	D	D	D	D	D	-
120 Hours	1. SVC	B	B	B	A	B	B	B	D	D	Nil
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	D	D	D	D	D	D	D	D	-	-

144 Hours	1. SVC	B	C	B	B	B	B	C	D	Nil	Nil
	2. SVO	-	-	-	-	-	-	-	-	-	-
	3. M and S activity	C	C	C	D	C	C	B	D	-	-
168 Hours	1. SVC	B	B	B	B	C	C	C	-	Nil	Nil
	2. SVO	-	-	-	-	-	-	D	-	-	-
	3. M and S activity	D	C	C	D	C	C	C	D	-	-
192 Hours	1. SVC	B	B	B	B	C	C	C	D	Nil	Nil
	2. SVO	-	-	-	-	-	-	D	-	-	-
	3. M and S activity	C	C	C	D	C	C	C	D	-	-

Table 07: Mortality of clam *Katelysia opima* under exposure to various salinity ranges during winter season.

Time in Hours	**Control**	**90%**	**80%**	**70%**	**60%**	**50%**	**40%**	**30%**	**20%**	**10%**
0 Hours	–	–	–	–	–	–	–	–	–	–
24 Hours	–	–	–	–	–	–	–	–	–	8
48 Hours	–	–	–	–	–	–	2	–	7	5
72 Hours	–	–	–	–	–	–	2	11	8	10
96 Hours	–	–	1	–	–	–	1	7	5	7
120Hours	–	–	–	–	–	–	–	5	6	–
144Hours	–	–	–	–	–	–	–	2	4	–
168Hours	–	–	–	–	–	–	1	1	–	–
192Hours	–	–	–	–	–	–	–	–	–	–
%Mortality	0	0	3	0	0	0	20	87	100	100

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