ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Environmental influences on the Mg/Ca ratios of an intertidal foraminifer *Rotalidium annectans* in the West coast of India

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Manuscript Info

Manuscript History:

Received: 18 August 2015
Final Accepted: 22 September 2015
Published Online: October 2015

Key words:

Foraminifera, trace elements, Mg/Ca ratio, environmental factors

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Abstract

Foraminifera (forams) are shell secreting marine protists. They incorporate calcium carbonate as well as trace elements into their shells. The trace elements incorporated in foraminiferal shells are commonly used as paleoproxies. The ratio of magnesium to calcium (Mg/Ca) in $CaCO_3$ shells of foraminifera is widely used to determine paleotemperatures. However, Mg/Ca is highly variable within and between species, suggesting a strong physiological influence on the incorporation of Mg^{2+} into the shells. The present work aims to study the influence of environmental variables on the incorporation of Mg into the shells of an intertidal foraminifer, *Rotalidium annectans*. The relationship between environmental factors (temperature, pH, salinity and Mg/Ca ratio of seawater) and elemental composition (Mg and Mg/Ca ratio of foraminifera) were analyzed for 17 months in shells of *R. annectans,* which were collected from coastal sediments of Payyannur (the west coast of India).

Results show that incorporation of Mg was mainly controlled by sea water temperature as Mg content of foraminifera was positively correlated with seawater temperature. An inverse correlation between seawater temperature and Mg/Ca ratio of *R. annectans* showed a strong physiological influence on the Mg partitioning in *R. annectans* as it is exposed to rapid temperature fluctuations in intertidal zone. An increase in the Mg/Ca ratio with a decrease in pH presumes a decrease in calcification rate, consistent with observations of ocean acidification due to higher atmospheric CO_2. The positive correlation between Mg/Ca ratio of seawater and Mg/Ca ratio of foraminifera showed a moderate significant Mg/Ca-seawater effect on Mg/Ca-foraminifera. Therefore variations in Mg/Ca ratios of seawater should be taken into account when evaluating the role of foraminifera in the past oceanic carbon cycle.

INTRODUCTION

Foraminifera are the most abundant, diverse and widely distributed shell secreting protists in the marine environment. They incorporate calcium carbonate as well as trace elements into their shells. The incorporation of these elements is controlled by environmental variables. Thus the trace element chemistry of foraminifera shell records an abundance of information about the environment in which the test grew. Hence they are commonly used as paleoproxies. The importance of elemental/ Ca ratio in palaeoclimatic studies has encouraged active research into the influence of biological and environmental factors into the incorporation of these elements into the shells.

Incorporation of trace elements into the shells is regulated by thermodynamic laws. Hence the trace elemental concentration in calcite shell helps to interpret the physical conditions present during the time of precipitation. Elemental partition coefficients (D) of trace elements between foraminiferal tests and seawater are found to be smaller than those predicted values from laboratory experiments (Elderfield *et al*., 1996). These suggest that the organism actively discriminates against uptake of these elements from seawater indicating a strong biological control on foraminiferal calcite precipitation (Bentov and Erez, 2006). However the relationship between environmental parameters and the chemical compositions of foraminiferal tests are cannot be fully understood. Studies also show the biological influence on test chemistry of foraminifera. Thus quantifying ecological and biological effects on trace element incorporation into foraminiferal test is vital to understand the reliability of species to record the ocean conditions. Therefore thermodynamic as well as kinetic (biological) factors have the potential to change the rate of calcification.

The mechanisms of trace element incorporation from seawater to foraminiferal shell include several elementary processes: for example, ingestion of food, digestion and assimilation of food by the digestive organs of foraminifera, and precipitation of shell materials mainly composed of $CaCO_3$ from body fluids. It is likely that the foods of foraminifera are consisting of a wide range of microorganisms, which also contain organic or inorganic particulate materials. Moreover, endocytosis of seawater, modification of vacuolized seawater within the cytoplasm, intracellular Ca-reservoir has been discussed for the foraminiferal biomineralization mechanism (de Nooijer *et al*., 2009a; Erez, 2003). The apparent foraminifera/seawater partition coefficients reflect all these effects, but in spite of such complexity, the obtained foraminifera/seawater ratios seem to have distinct characteristics. The ratio of conservative elements to Ca (Mg/Ca, Sr/Ca, B/Ca, etc) is nearly fixed in seawater. Thus variations of these ratios in foraminiferal shells reflect the impact of environmental parameters on calcification.

Mg/Ca in foraminiferal calcite is becoming an established paleotemperature proxy. Variations in the Mg/Ca ratio of foraminiferal shells reflect changes in environmental parameters, primarily temperature, which control Mg incorporation into calcite. Therefore, Mg/Ca calcite is frequently used to reconstruct past seawater temperatures. However, several studies revealed that application of Mg/Ca is limited by, amongst others, species-specific differences in Mg incorporation and the influence of Mg/Ca of the seawater. Several secondary factors may influence measured Mg/Ca ratios in foraminiferal calcite. Secondary factors include pH, salinity (Lea *et al*., 1999; Nu¨rnberg *et al*., 1996) and carbonate ion concentration [$CO3^{2-}$] (Russell *et al*., 2004). Field studies on trace elemental composition and the role of environmental variables in controlling foraminiferal elemental/ Ca ratios into the shells of *Rotalidium annectans* (which is the most dominant foraminiferal species in the West coast of India) is lacking (Fig 1). Hence the present work was designed to study the potential influence of environmental parameters on the incorporation of Mg and Mg/Ca ratio in *R. annectans* shells.

MATERIALS AND METHODS

Study area

The present study was carried out in the coastal regions of Payyannur (120 0' N, $75^0$12' E) (Fig 2) from the West coast of India. The rivers originating from Western Ghats provide large inputs of various micro and macro nutrients to the open sea. Monsoon alters the hydrobiologic profile of the Arabian Sea that triggers seasonal fluctuations in environmental variables. Thus South West coast of India provides an ideal environment to study the influence of environmental variables on the incorporation of trace elements and trace element/Ca ratios in foraminiferal shells.

Sample collection

Intertidal sediments were collected for a period of seventeen months from August 2011 to December 2012 at intervals of two months. For foraminiferal studies, top 1 cm of surface sediment was collected and preserved in Rose Bengal stain for identifying live organisms. The top 1-3 cm of sediment samples was placed in polyethylene plastic bag and immediately transported to the laboratory for further analysis. Live foraminifera (Rose Bengal stained) were picked in the same size fraction for trace elemental measurements to minimize sample heterogeneity.

About 10-20 intact, dried tests (≈1 mg) of Rose Bengal stained *R. annectans* (250 µm) were used for trace elemental analysis. *R. annectans* were identified by following Loeblich and Tappan (1987).

Trace Elemental Analyses

Foraminifera shells for trace element analyses were gently crushed, then processed according to the procedure of Barker *et al*. (2003). The major steps of this method involve several water and ethanol washings to remove clay, treatment with hydrogen peroxide in a boiling water bath to eliminate organic matter and leaching the samples with 0.001 M nitric acid to eliminate any adsorbed contaminants from the test. Prior to measurement, 350 ml of 0.075 M nitric acid was added to dissolve the samples and then centrifuged to separate insoluble residues and analyzed with a Flame Atomic Absorption Spectrophotometer (GBC932 Plus-3000 model).

Statistical Analysis

All statistical analyses were carried out by using Statistical Package for Social Science (SPSS) version 13.

RESULTS

Environmental variables

Temperature and pH of the seawater varied from 26.5^{0}C to 30.5^{0}C and 7.2 to 8.27 respectively (Table 1). The salinity in the seawater was ranged between 30‰ and 38 ‰.

Mg/Ca ratios of foraminifera shells and seawater

Mg/Ca ratios of foraminifera shells varied between 0.2 to 1.0 mmol/mol (Table 2). Overall the test carbonate of *R. annectans* has lower Mg/Ca ratios compared to *O. universa* , *Globigerinoides bulloides* , *Ammonia lobifera and Ammonia lessonii* (Lea *et al*., 1999; Elderfield and Ganssen, 2000; Russell *et al*., 2004; Segev and Erez, 2006). In the present study Fe/Ca ratios was found to be >0.1 mmol/mol, suggests samples were not contaminated with secondary diagenetic coating/detrital material. Biased Mg/Ca ratio values will obtain if the Fe/Ca ratio is <0.1 mmol/mol (Barker *et al*., 2003). The average Mg/Ca ratio of seawater is 5.07 ±0.17. The ratio ranged from 4.8 to 4.3 mmol/mol (Table 2).

Clear seasonal oscillations are evident in the Mg/Ca ratios in shells of *R. annectans* (Fig 3). Shell Mg/Ca ratios initially increase in magnitude until October 2011 then subsequently show a general decrease through to April 2012 and no consistent variation of Mg/Ca ratios was observed from April to June 2012. Shell Mg/Ca ratios increased, in the later part of the record, from June to August 2012 and shows a decline till December 2012.

Fig 1 Stereo microscopic image of *Rotalidium annectans* (Scale bar: 250µm)

Fig 2 Map of the study area
(Source: http//www.google.com/earth/index.html)

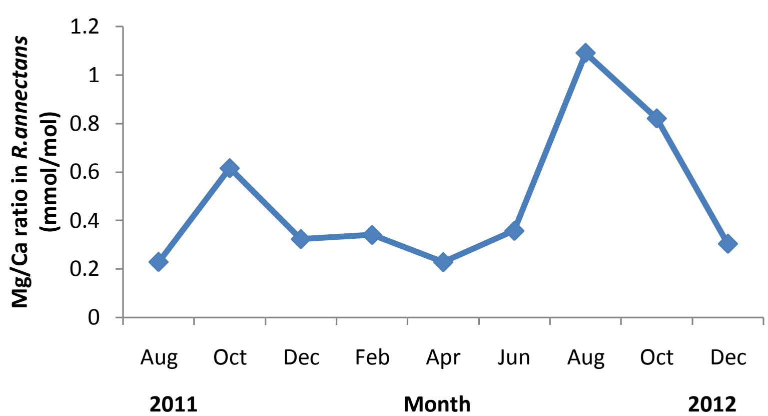

Fig 3 Monthly variations of Mg/Ca ratio in *R. annectans*

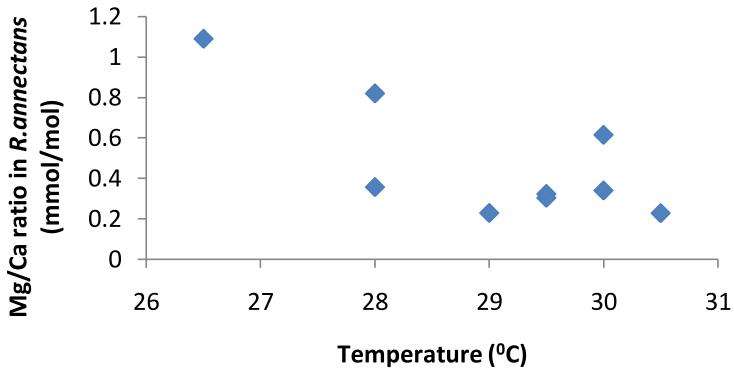

Fig 4 Mg/Ca ratio in *R.annectans* plotted verses Seawater temperature

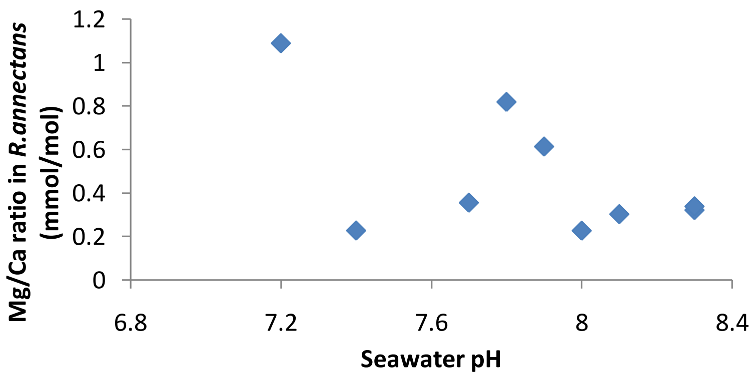

Fig 5 Mg/Ca ratio in *R.annectans* plotted verses Seawater pH

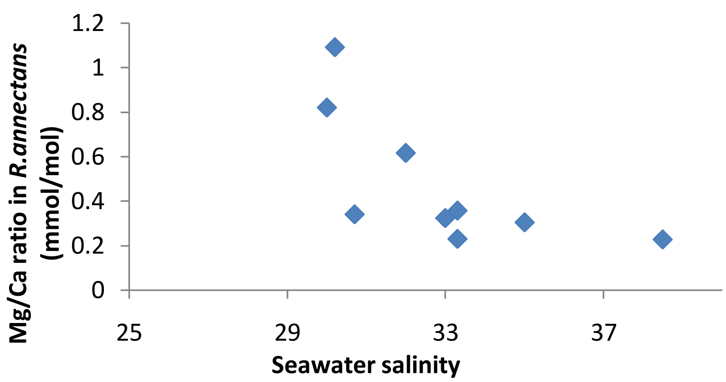

Fig 6 Mg/Ca ratio in *R.annectans* plotted verses Seawater salinity

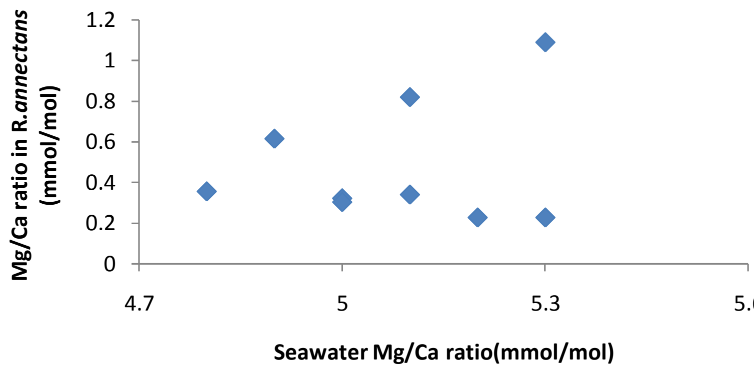

Fig 7 Mg/Ca ratio in *R.annectans* plotted verses Seawater Mg/Ca ratio

Table 1 Environmental variables at study site

Month	Temperature (^{0}C)	pH	Salinity (‰)
August 2011	29	7.4	33.3
October 2011	30	7.9	32
December 2011	29.5	8.3	33
February 2012	30	8.3	30.7
April 2012	30.5	8.0	38.5
June 2012	28	7.7	33.3
August 2012	26.5	7.2	30.2
October 2012	28	7.8	30
December 2012	29.5	8.1	35

Table 2 Magnesium (Mg), Calcium (Ca) concentrations and Mg/Ca ratio in seawater and foraminifera

Month	Mg		Ca		Mg/Ca ratio	
	Seawater(mg/L)	Foraminifera(mg/ml)	Seawater(mg/L)	Foraminifera(mg/ml)	Seawater(mM)	Foraminifera(mM)
August 2011	820	6.9	220.4	49.5	5.3	0.229
October 2011	1161	6.9	561	18.5	4.9	0.615
December 2011	977	6.5	360.7	33.4	5.0	0.323
February 2012	1364	13.4	441	64.7	5.1	0.34
April 2012	1387	26.6	440	192	5.2	0.228
June 2012	1031	4.5	320	20.8	4.8	0.357
August 2012	757	4.4	260	6.6	5.3	1.09
October 2012	1310	4.4	480	8.8	5.1	0.82
December 2012	827	6.3	354	28.3	5.0	0.304

Table 3 Correlation matrices of Mg, Mg/Ca ratio in foraminifera shells, Mg/Ca ratio in seawater, temperature, pH and salinity of seawater

	Mg	$Mg/Ca_{Foraminifera}$	$Mg/Ca_{Seawater}$	T (^{0}C)	pH	Salinity
Mg	1.00					
$Mg/Ca_{Foraminifera}$	-0.24	1.00				
$Mg/Ca_{Seawater}$	-0.15	0.32	1.00			
T (^{0}C)	0.13	-0.74	-0.20	1.00		
pH	-0.34	-0.55	-0.46	0.75	1.00	
Salinity	-0.08	-0.69	-0.16	0.57	0.27	1.00

DISCUSSION

The incorporation of Mg into inorganic as well as biogenic calcites precipitated from seawater exhibits strong temperature influence (Burton and Walter, 1987; Chilingar, 1962; Katz, 1973; Nurnberg, 1995; Lea *et al*., 1999; Elderfield and Ganssen, 2000). As a result, biogenic calcite Mg/Ca ratios are increasingly being used for temperature reconstructions. Several studies have demonstrated that factors other than temperature can influence the Mg content of both abiogenic and biogenic calcites. Mg/Ca ratio of the solution is thought to exert a strong control on inorganic calcite Mg/Ca ratios (Berner, 1975; Mucci and Morse, 1983). pH (Lea et al., 1999; Russell *et al*., 2004) and salinity (Lea *et al*., 1999 ; Nurnberg *et al*., 1996a, b) have been also shown to exert small, but significant, influences on Mg/Ca ratios in foraminiferal shells..

Relationship between Mg/Ca ratio of R.annectans and seawater temperature

Mg, being a divalent cation which substitutes Ca in the calcite skeleton. Over the past decade, the magnesium to calcium ratio (Mg/Ca) in foraminifera has been developed as a palaeoclimatic proxy for the temperature of the seawater in which the foraminifer calcified. Foraminiferal Mg/Ca has the potential to provide temperature reconstructions independently, which combined with shell $\delta^{18}O$ allow the reconstruction of seawater $\delta^{18}O$ *(Mashiotta et al.*, 1999*; Elderfield and Ganssen,* 2000). Mg incorporation in foraminiferal shells is temperature dependent through both thermodynamics and physiological processes (Lea *et al*., 1999; Rosenthal *et al*., 1997). Inorganic precipitation experiments suggested an increase in Mg/Ca ratio by ∼3% per °C increase in temperature (Lea *et al*., 1999; *Katz*, 1973). However, low Mg calcite incorporate less Mg than inorganic calcite and therefore greater response of Mg/Ca ratio in foraminifera to temperature (Rosenthal *et al.*, 1997; Lea *et al.*, 1999).

Most of the studies (core top, sediment trap and laboratory culture) have demonstrated a ∼9–10% exponential increase in Mg/Ca per degree Celsius in most species (*Nurnberg*, 1995; *Anand et al.*, 2003; Lea *et al*., 1999). The response of benthic Mg/Ca to temperature is less well constrained. Early studies of Mg/Ca in benthic foraminifera demonstrated a strong correlation with seawater temperature. *Rosenthal et al.* (1997) proposed an exponential temperature response of ∼11% per °C for *Cibicidoides pachyderma* from the Little Bahama Bank by using core tops. *Lear et al.* (2002) reanalyzed the samples of Rosenthal *et al.* (1997) and confirmed that ∼11% per °C response for *C. pachyderma* , and also observed that several other species such as *P. ariminensis* and *Uvigerina* spp. had lower temperature sensitivities (∼6% per °C). Marchitto *et al.* (2007) recently found that Mg/Ca values of *C. pachyderma* from warm water of the Florida Straits are much lower than those from the Little Bahama Bank, suggesting the authigenic contamination of some Bahamas measurements.

In the present study, Mg concentration in *R.annectans* shells showed a positive correlation with seawater temperature whereas a negative correlation was observed between Mg/Ca ratio and seawater temperature (Fig 4; Table 3) proves a strong physiological influence on the Mg partitioning in *R.annectans* as it is exposed to rapid temperature fluctuations in intertidal zone. As the strong biological control exerted on the biomineralization process by foraminifera has become increasingly evident (*Bentov and Erez*, 2006). But it is not clear that the response of Mg/Ca to temperature should be dominated by thermodynamics. However, these data also suggest that the sensitivity of Mg/Ca to temperature may actually decrease at higher temperatures rather than increase, as showed for *C. pachyderma* by Marchitto *et al.* (2007).

Relationship between Mg/Ca ratio of R. annectans and seawater pH

In aquatic environment, physical and chemical forms of metal and metal compounds are greatly influenced by the pH of water as it controls the solubility and concentrations of major metals. In foraminifera, pH has been described as a secondary control factor of Mg/Ca ratios, responsible for a ≈6% decrease of Mg/Ca per 0.1 pH unit increase (Lea *et al.*, 1999). Similarly there is a decrease in Mg content(r=-0.3) as well as Mg/Ca ratio (r=-0.55) of *R. annectans* shells with an increase in pH was observed in the present study (Table 3; Fig 5). The pH of seawater is a critical environmental factor in the marine environment. Ocean become more acidic as CO_2 dissolves in seawater. At present surface ocean is slightly alkaline, and it is saturated with calcium carbonate. As carbon dioxide in the atmosphere increases surface seawater become more acidic. This leads to the decrease in the calcium carbonate saturation state through the consequent reduction in [CO_3^{2-}], the carbonate ion concentration. These are well verified from models, time series data and hydrographic surveys (Feely *et al*., 2004; Caldeira and Wickett 2003, 2005). Since

Mg/Ca ratio increases with a decrease in pH, presumes a decrease in calcification rates of the benthic foraminifera in shallow water, resulting in the replacement of larger Ca ions by smaller Mg ions. Thus, our observations assume significance as ocean acidification is on the site due to higher atmospheric CO_2.

Relationship between Mg/Ca ratio of R. annectans and seawater salinity

Salinity also plays an important role in the Mg incorporation in *R.annectans*. There was a negative correlation (r=-0.69) with Mg/Ca ratio in *R.annectans* (Table 3; Fig 6). The incorporation of Mg into calcite is not well described inorganically. But Mg is known to inhibit the calcite precipitation (Davis *et al*., 2000). Trace elemental concentrations within the foraminifera tests are controlled by complex biological pathways and processes (Erez, 2003; Bentov and Erez, 2005). At the time of calcification, cytoplasm will surround pockets of seawater and then transported towards the calcification site. During the transport, foraminifera will modify the composition of the seawater within the pocket, or the vacuole. This may leads to an increase in pH and therefore an increase in total dissolved inorganic carbon (Erez, 2003) and this is carried out to facilitate calcite precipitation. Due to the cellular activities of the foraminifera, from the parent solution a significant amount of Mg^{2+} is removed, presumably from seawater leads to the reduction in Mg/Ca ratios of the foraminifer test. Environmental variables and foraminiferal Mg/Ca are related due to the influence of the variables on the Mg metabolism of the foraminifer (Bentov and Erez, 2006). However the real mechanisms by which foraminifera reduce and control the Mg concentration of their tests is still unknown (Erez, 2003; Bentov and Erez, 2006). It is difficult to suggest the possible mechanisms for the effect of environmental variables, including salinity, on Mg incorporation into foraminiferal shells, till the calcification processes of foraminifera are more fully understood.

Relationship between Mg/Ca ratio of R.annectans and seawater Mg/Ca ratio

Little is known about potential effects of seawater [Ca^{2+}] and [Mg^{2+}] oscillations on the trace metal and isotopic composition of calcareous foraminifers. Lacking other constraints, researchers have addressed differences in past seawater chemistry by scaling modern Mg/Ca verses temperature calibrations to past oceanic Mg/Ca. Mg content of biogenic calcite can be influenced by the physicochemical properties of ambient seawater (Chave,1954). Studies revealed that the Mg content of abiogenic calcite varies as a function of solution Mg/Ca (Chave,1954; Glover and Sippel, 1967; Kitano and Kanamori, 1966). Thus the Mg/ Ca ratio of aqueous fluids determines what kind of calcium or calcium magnesium carbonate going to be precipitate inorganically. Oscillations in the Mg/Ca ratio of seawater is due to the alternation between aragonite and calcite seas was identified by Hardie (1996). In nonskeletal calcite how the mole percent of Mg is substituting for Ca increases with the Mg/Ca ratio of ambient seawater was documented by Fu¨chtbauer and Hardie (1976, 1980).

In present-day seawater with Mg/Ca ratio of ≈5, the kinetically preferred mineral to be precipitated inorganically is aragonite because calcite growth is inhibited by Mg and possibly other ions (Davis *et al*., 2000; Morse and Bender, 1990). However, except for the family Robertinacea, all other foraminifera precipitate a calcium carbonate shell. The variations in Mg contents of co-existing species suggest that foraminifera have a strong physiological control on the Mg content of their shell. It has been postulated that foraminifera reduce the concentration of Mg in the solution from which they precipitate their shells. This process may require energy (Bentov and Erez, 2006; Erez, 2003). Because foraminifera use seawater vacuoles as their main source of ions for biomineralization (Erez, 2003; Bentov and Erez, 2001; Bentov and Erez, 2006), a lower ambient Mg/Ca ratio may result in higher calcification rates. Our results also showed a moderate significant seawater-Mg/Ca effect on shell Mg/Ca ratio as Mg/Ca ratio of shell was positively correlating with seawater-Mg/Ca ratio (r=0.32) (Fig 7; Table 3). Hence it may be suggested that the current Mg/Ca ratio in the oceans is not ideal for foraminiferal calcification. Therefore variations in Mg/Ca ratios of seawater should be consider when evaluating the role of foraminifera in the past oceanic carbon cycle.

CONCLUSION

Trace elemental compositions of foraminifera are becoming more commonly used as indicators of present and past conditions. This paper focused on the behaviour of trace elements (Mg/Ca) during $CaCO_3$ precipitation and the potential influence of environmental variables on elemental incorporation in the living shells of an intertidal foraminifer *Rotalidium annectans*. The result shows that temperature is the major controlling variable in the incorporation of Mg in *R. annectans*. Shell Mg/Ca-temperature relationship suggests a strong physiological influence on the Mg partitioning in *R.annectans* as it is exposed to rapid temperature fluctuations in intertidal zone. An increase in the Mg/Ca ratio with a decrease in pH presumes a decrease in calcification rate, consistent with observations of ocean acidification due to higher atmospheric CO_2. Significant Mg/Ca $_{seawater}$ effect on shell composition (Mg/Ca $_{foraminifera}$) of *R. annectans* showed the importance of Mg/Ca ratios of seawater for evaluating the role of foraminifera in the past oceanic carbon cycle.

ACKNOWLEDGEMENTS

First author (Amrutha) is thankful to Department of Science and Technology (DST), New Delhi for the financial grant to carry out the study under INSPIRE Programme. Author was used the research facility at Centre for Application of Radioisotopes and Radiation Technology (CARRT),University Science Instrumentation Centre (USIC), Mangalore University for the present study. We thank Centre for Water Resources Development and Management (CWRDM), Kozhikode for providing the Atomic Absorption Spectrophotometer (AAS) Instrumentation Facility.

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