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Unravelling the effects of elevated temperature on the physiological energetics of *Bellamya bengalensis*

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ABSTRACT

Temperature is one of the key environmental factors affecting the eco-physiological responses of living organisms and is considered one of the utmost crucial factors in shaping the fundamental niche of a species. The purpose of the present study is to delineate the physiological response and changes in energy allocation strategy of *Bellamya bengalensis*, a freshwater gastropod in the anticipated summer elevated temperature in the future by measuring the growth, body conditions (change in total weight, change in organ to flesh weight ratio), physiological energetics (ingestion rate, absorption rate, respiration rate, excretion rate and Scope for Growth) and thermal performance, Arrhenius breakpoint temperature (ABT), thermal critical maxima (CTmax), warming tolerance (WT) as well as thermal safety margin (TSM) through a mesocosm experiment. We exposed the animals to three different temperatures, 25 °C (average habitat temperature for this animal) and elevated temperatures 30 °C, 35 °C for 30 days and changes in energy budget were measured twice (on 15th and 30th day). Significant changes were observed in body conditions as well as physiological energetics. The total body weight as well as the organ/flesh weight ratio, ingestion followed by absorption rate decreased whereas, respiration and excretion rate increased with elevated temperature treatments resulting in a negative Scope for Growth in adverse conditions. Though no profound impact was found on ABT/CTmax, the peak of thermal curve was considerably declined for animals that were reared in higher temperature treatments. Our data reflects that thermal stress greatly impact the physiological functioning and growth patterns of *B. bengalensis* which might jeopardize the freshwater ecosystem functioning in future climate change scenario.

1. Introduction

Ever since the Industrial revolution, anthropogenic activities have tremendously increased green-house gases emission and caused global warming (Feely et al., 2004). It is now equivocally accepted that heat-waves are one of the pervasive consequences of global warming which are predicted to intensify in duration and frequency due to extreme climatic events, imposing elevated thermal stress (IPCC, 2013). Temperature elevation is a major concern in the scenario of global climate change which is a crucial environmental factor affecting the eco-physiology of aquatic ectotherms (Huey and Kingsolver, 1989; Hochachka and Somero, 2002). It has direct impact on metabolic rates, growth and reproduction and eventually on its survival (Van der Have and De Jong, 1996; Porter and Gates, 1969; Dallas and Rivers-Moore, 2012).

Invertebrates are broadly distributed throughout the ecosystems and can be used as excellent bio-monitors in ecosystem functioning studies (Lagadic and Caquet, 1998). In ectotherms, body temperatures are determined by interaction with environmental conditions. It is also a well-established fact that the tropical invertebrates are more vulnerable as they are already residing close to their optimal thermal edge (Deutsch et al., 2008). According to thermal tolerance paradigm, ectothermic species are highly regulated by subtle change in the environmental temperature for their survivability and performance attributes (Angilletta et al., 2002; Somero, 2010). The continuous physiological stress might limit the survival of such organisms and those possessing the ability to persist the stress with proper metabolic strategies would survive in future climate change scenario. Moreover, the acquired homeostatic capability would ultimately lead to decreased sensitivity due to prolonged thermal stress (Seebacher et al., 2015).

The potentiality to adjust with severe environmental changes is a key to survive and is brought about by comprising aerobic scope in aquatic ectotherms. Scope for Growth (SfG) is a sensitive physiological indicator which evaluates the instantaneous energy status of a particular animal

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and provides an insight into the energy allocation strategy it adopts in a particular environmental condition (Wang et al., 2015; Bayne and Newell, 1983). Usually, it is estimated by the surplus energy available for growth after completion of necessary metabolic functions (Genoni and Pahi-Wohstl, 1991). In spite of its significance, SfG has rarely been used to appraise the physiological status of any freshwater benthic animal. Thermal performance curves project the effect of temperature on animal fitness, which provides a physiological outline for explicating a major constituent of the influence of global climate change in a comprehensive manner (Huey and Stevenson, 1979). Furthermore, the Arrhenius breakpoint temperature (ABT), critical thermal maximum (CTmax), warming tolerance (WT) and thermal safety margin (TSM) can also be regarded as some of the most trustworthy parameter to carry out studies from an eco-physiological perspective in ectothermic animals and exhibit their vulnerability in case of elevated temperatures (Deutsch et al., 2008). The detailed study of critical thermal boundaries offers a significant insight about ecology, physiology and distribution in aquatic animals influenced by climate (Lutterschmidt and Hutchison, 1997). In depth knowledge on thermal traits and thermal window for growth can be a means to improve our understanding about the eco-physiological status of animals.

Gastropods are a large taxonomic group playing a pivotal role in ecosystem functioning and contribute significantly to the benthic biomass (Oehlmann et al., 2007). The gill breathing gastropod population are more susceptible to oxygen constraint with elevated thermal stress (Koopman et al., 2016). *Bellamya bengalensis* (viz, *Filopaludina bengalensis*) is the most extensively distributed freshwater snail in India which holds a major economic importance by serving as a food item for some aquatic species as well as for human consumption (Datta et al., 2016) and considered as a good bio-indicator species in bio-monitoring studies (Dutta et al., 2018).

Despite considerable research effort on marine invertebrates, the biological and physiological effects of thermal stress on freshwater snails have been neglected. The aim of this study was to decipher the changes in physiological responses of *Bellamya bengalensis* with respect to predicted future thermal elevation by studying the rate of body conditions and total growth. The sensitivity and resilience to elevated thermal treatments was investigated by measuring physiological energetics like measuring absorption, respiration and excretion rates to quantify the SfG. In addition, the thermal performance under stress as well as the Arrhenius breakpoint temperature (ABT) and critical thermal maximum (CTmax) were also studied. The present study would provide better insight to the physiological energetics of freshwater gastropods in the future global climate change mediated summer thermal elevation specifically in the tropical regions where organisms are already at the fringe of their thermal tolerance margin. Furthermore, it would be helpful in the perspective of sustainable aquaculture and freshwater ecosystem functioning.

2. Materials and methods

2.1. Collection and rearing of gastropods

Adult *Bellamya bengalensis* (26±2 mm) were collected in the month of June from a well-managed pollution free freshwater lake in Budge Budge, South 24-Parganas, West Bengal, India. This lake was monitored throughout the year as a part of the laboratory's ongoing programme for the assessment of water quality parameters. The surface water samples were collected using a Niskin water sampler (5 L) for the analysis of different hydrographic parameters every month throughout a year from multiple sampling sites at study area (Baag et al., unpublished data). The water temperature ranges from 19 °C to 33 °C throughout the year and during specimen collection the habitat temperature was 25 °C. Following collection, individuals were brought to the laboratory and kept temporarily in plastic aquarium filled with sufficient aerated water collected from the same lake. An ambient condition [Temp (25.79 °C±0.44), DO (7.88 ± 0.07), pH (7.65 ± 0.03)] was maintained as their natural habitat with constant aeration and acclimated for about two weeks prior further experimentation. Fresh iceberg lettuce was provided as daily ration for 6 h on every day to satiation. Unconsumed food materials were removed from the aquaria shortly after feeding. Freshly collected filtered water from lake was replaced weekly.

2.2. Experimental setup for exposure to different temperature treatment

Individuals were kept in covered plastic aquaria (40 × 28 × 20 cm) in three replicates (each holding five animals of 26±2 mm) per treatment with filtered fresh lake water. The water was replaced every week and solid metabolic wastes were removed regularly. The animals were set for three temperature treatments: 1) 25 °C, (T_{hab}) based on habitat's average thermal profile throughout the year and some other published literature from the same geographic belt (Chanda et al., 2019; Golder and Chattopadhyay, 2016) as control; 2) higher temperatures 30 °C, experienced by the animals in extreme summer months (Chanda et al., 2019; Golder and Chattopadhyay, 2016); 3) 35 °C as predicted elevated surface water temperature rise (2–4 °C) due to global warming in near future (IPCC, 2013). The desired temperatures were obtained by immersing a digitally controlled thermostat in water baths holding the experimental aquaria for the entire experimental period (Table 1). The desired temperature was obtained by gradual increment of the temperature at a rate of 1 °C/2 h. Food (Fresh Iceberg Lettuce) was provided every day and excess food was removed after 6 h. The experimental exposure continued for 32 days and physiological variables were measured twice on 15th and 30th day.

2.3. Body condition and survival of gastropods

The total weight (flesh weight + shell weight) of each animal was measured using an electronic balance (WENSER MAB 220) before and after the experiment to detect the alteration in total biomass. The animals were observed multiple times regularly to check mortality and dead animals were removed immediately from the aquaria. After the end of experiment, animals from each treatment were dissected to obtain the flesh weight and the foot muscle was removed to measure the changes in organ weight to get a clear indication of body conditions.

2.4. Total energy budget of gastropods

To evaluate the monthlong exposure of elevated temperature on the overall energy budget of the animals' ingestion rate, assimilation efficiency, absorption rate, respiration rate, excretion rate and Scope for Growth (SfG) were estimated by randomly selecting three animals (one from each three replicates) of the same treatment and considered its average value as one observation. Likewise, five observation data were generated for each treatment on both 15th and 30th days. The absorption rate was calculated from the ingestion rate and assimilation efficiency of the gastropods.

To calculate the ingestion rate individuals from replicate aquaria under same treatment were chosen and starved for 48 h before starting the feeding experiment. Each animal was provided with ad libitum preweighted fresh iceberg lettuce in individual feeding chamber and excess food was removed after 24 h. The ingestion rate was calculated as dry

Table 1
Water quality parameters (Temperature, Dissolved Oxygen, pH) maintained throughout the experimental period in the aquaria containing the animals. Values represent mean ± SD.

Treatments	Temp	DO	pH
25 °C	25.79 ± 0.44	7.88 ± 0.07	7.65 ± 0.03
30 °C	30.28 ± 0.31	7.33 ± 0.07	7.65 ± 0.02
35 °C	35.11 ± 0.31	6.77 ± 0.14	7.64 ± 0.03

weight (24 h at 60 °C) of food consumed per individual per hour. Subsequently, the faecal matter was collected after 24 h of excess food removal and dry weight obtained in a similar way mentioned above.

The assimilation efficiency was estimated using a formula-

$$AE(\%) = [F'-E'/(1-E')(F')]*100$$

where F′ and E′ are organic fraction of food and faeces respectively which is calculated by dry weight to ash free dry weight ratio (6 h at 450 °C) for both food and faeces (Conover, 1966).

Absorption rate was calculated multiplying the ingestion rate and assimilation efficiency. Ingestion rate was converted into energy equivalent by using its calorific value (620 J g^{-1}) (Hoefnagel and Verberk, 2017).

$$Ab = AE*I$$

Where Ab = absorption rate and I = ingestion rate.

To measure the respiration rate individuals were transferred into air tight containers of 200 ml each with fully oxygen saturated water and were kept enclosed for 60 min in respective treatment temperature water bath. Initial and final dissolved oxygen concentration was measured using a DO meter (Orion Star A223 dissolved oxygen/RDO portable meter kit) which was calibrated according to manufacturers' instruction at room temperature. The respiration rate was calculated as mg O_2 g^{-1}(total flesh weight) h^{-1}. To nullify the effect of specific dynamic action respiration rate was measured 24 h after the feeding experiment (Gaffney and Diehl, 1986; Secor, 2009; Seibel et al., 2012; Shin et al., 2014). The respiration rate was converted into its energy equivalent using a conversion factor 14.14 J mg O_2^- (Elliott and Davison, 1975). Blank samples without animals were used in every treatment for accurate measurements.

Finally, to measure the excretion rate, 25 ml water was collected from each feeding chamber and the phenol-hypochlorite method (Solorzano, 1969) was followed to evaluate the ammonia concentration in water. A value of 0.025 J μg^{-1} NH_4 was used as an energy equivalent to quantify the energy lost due to individual excretion (Elliott and Davison, 1975).

The Scope for Growth (SfG) was calculated using a formula -

$$SfG = Ab-(R + E)$$

Where SfG = Scope for Growth, Ab = absorption rate, R = respiration rate and E = ammonia excretion rate and expressed as J $ind^{-1}hour^{-1}$ (Widdows, 1985).

2.5. Physiological performance and thermal tolerance of gastropods

After maintaining the animals in above mentioned different temperature treatments (25 °C, 30 °C and 35 °C) for 31 days, on the 32nd day an increasing thermal ramp of 2 °C h^{-1} from 22 °C to 42 °C was used to assess the aerobic metabolic performance in gastropods by measuring the respiration rates at every temperature for all the treatment groups following Giomi et al. (2016). The methodology for calculation of respiration rate has been described above (2.4). The Arrhenius Breakpoint Temperature (ABT) was denoted as the temperature above which respiration drops drastically and was calculated following Parker et al. (2017). To evaluate the thermal tolerance indicated by CTmax the changes in locomotory behaviour of the gastropods were thoroughly observed throughout the ramping period. The temperature points from where the foot muscle contraction began to weaken, inward curling of lateral foot started taking place and the loss in motor coordination were noted (Lutterschmidt and Hutchison, 1997). Warming tolerance was calculated by the difference of CTmax and average habitat temperature (CTmax - T_{hab}). Thermal safety margin of the organism was evaluated by the difference between thermal optimum of the species and average habitat temperature (T_{opt} – T_{hab}) following Deutsch et al. (2008).

2.6. Statistical analysis

To investigate the effect of temperature and duration of stress (number of days) on total weight, body conditions, ingestion rate, assimilation efficiency, absorption rate, respiration rate, excretion rate, scope for growth and the thermal performance a two-way per mutational analysis of variance (PERMANOVA) was applied. The main test was done using temperature and duration as fixed factors and whenever significant interaction were found between these factors, a paired test was performed for each factor separately in each level from other factor for the energetics studies. For thermal performance analysis the factors taken into account were acclimation temperatures and ramping temperatures. All the analyses were performed in PRIMER 6 with PERMANOVA + add on.

3. Results

3.1. Body condition and survival of gastropods

No mortality was observed in 25 °C and 30 °C treatment groups but in case of 35 °C temperature treatment group one and four individuals were found dead on 3rd and 4th week of the experimental period, respectively.

The change in total weight of the gastropods differed significantly with temperature levels, duration and their interaction (Table 2). On both 15th and 30th days there was significant decrease in total weight for 30 °C and 35 °C treatment groups than 25 °C. On 30th day however a significant decrease was found in 35 °C group than the 30 °C group. A significant decrease was recorded on 30th day for the 30 °C and 35 °C groups compared to 15th day but no significant variation was observed for the 25 °C group (Fig. 1a). Furthermore, a significant statistically difference was also noticed in organ to flesh weight ratio between temperature levels and the value was decreased with increasing temperature levels with lowest at 35 °C and highest at 25 °C, howbeit there was no statistically significant difference between individual temperature groups (Fig. 1b).

3.2. Energy allocation strategy of gastropods

Ingestion rate followed by absorption rate differed significantly with temperature levels and the interaction between temperature levels and duration but not with the duration alone (Table 2). When results between different temperature levels were compared within experimental days, both ingestion rate as well as absorption rate on 15th and 30th day differed significantly with the value in 25 °C being much higher than that of 30 °C and 35 °C groups. Ingestion rate and absorption rates were also significantly high in 30 °C than 35 °C group on 15th day but not on 30th day (Fig. 2a and b).

In case of assimilation efficiency, the effect of temperature levels, duration and the interaction between them were all statistically insignificant. The assimilation efficiency ranged between 65% and 69%.

A statistically significant difference was recorded for respiration rate between temperature levels, duration and their interaction (Table 2). On both 15th and 30th day distinct differences were noted between all the temperature levels with respiration rate being the lowest in 25 °C and highest in 35 °C group. However, at each temperature level there was no significant difference between the durations but in case of the 35 °C group respiration rate was notably reduced on 30th day from 15th day (Fig. 2c).

Excretion rate significantly differed with the temperature levels, duration and also with the interaction between them (Table 2). On 15th day there was a significant increase in excretion rate for 30 °C groups than 25 °C groups whereas on 30th day there was a significant increase in excretion rate for 35 °C group than the remaining groups. At individual temperature level there was a significant variation for 25 °C and 30 °C groups and at both the temperatures excretion rate was found to be

Table 2
Summary of the main test of PERMANOVA on organ to flesh weight ratio measured on 30th day and six physiological variables in gastropods exposed to three levels of temperature treatments (25 °C, 30 °C and 35 °C) on 15th, 30th day of experiment.

Source of variation	DF	ERROR DF	Change in total weight			Organ/flesh weight ratio			Ingestion rate			Assimilation efficiency			Absorption rate			Respiration rate			Excretion rate			Scope for Growth		
			MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Temperature	2	8	0.0037356	983.41	**0.001**	52.237	33.175	**0.008**	4525.6	47.98	**0.001**	0.27287	2.2245	0.127	4068.2	39.767	**0.001**	6665.6	236.82	**0.001**	109.9	14.6	**0.001**	5.6445	785.79	**0.001**
Duration	1	4	5.2536E-05	13.83	**0.001**				154.2	1.6349	0.188	0.13827	1.1272	0.313	205.81	2.0118	0.128	138.57	4.9233	**0.012**	884.55	117.51	**0.001**	0.51566	71.787	**0.001**
Temperature X Duration	2	8	0.00010818	28.478	**0.001**				290.84	3.0834	**0.04**	0.036669	0.29894	0.718	293.95	2.8733	**0.039**	91.633	3.2557	**0.017**	135.7	18.026	**0.001**	0.38811	54.03	**0.001**

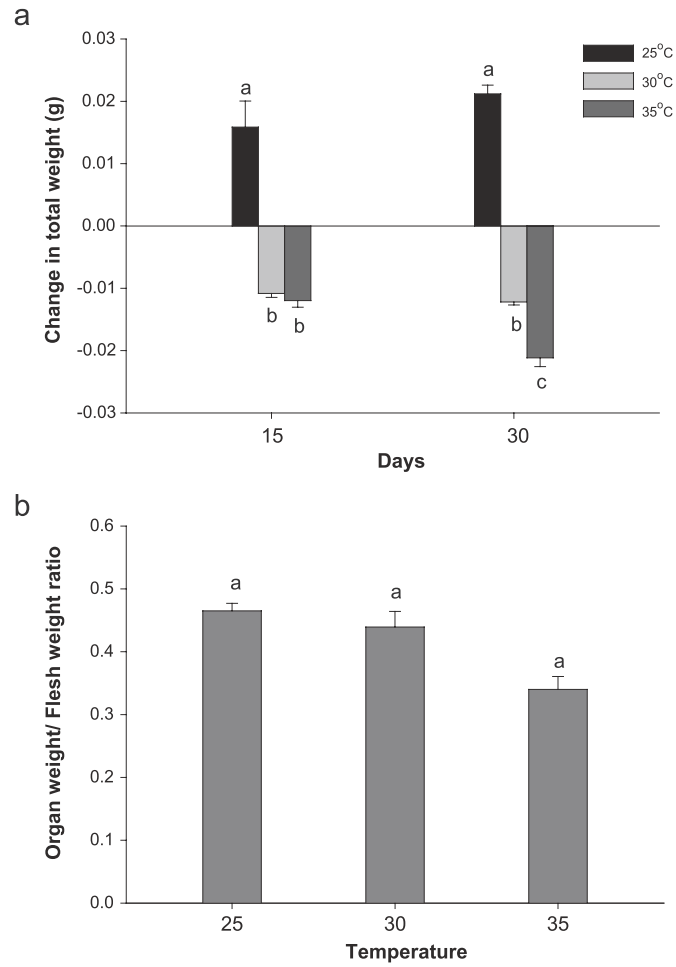

Fig. 1. Change in total weight (a) and change in organ to flesh weight ratio (b) of *Bellamya bengalensis* under different temperature treatments (25 °C, 30 °C and 35 °C) during the experimental period. Values represent mean ± SD (n = 5). Different letters indicate significant difference between treatment groups ($p < 0.05$).

reduced on 30th day from 15th day but in case of 35 °C the difference between the durations was statistically insignificant (Fig. 2d).

There was a significant effect of temperature levels, duration and their interaction on the SfG of *B. bengalensis* (Table 2). On both 15th and 30th day discernible differences were found between all the temperature levels with SfG being lowest at 35 °C group and highest at 25 °C group. However, at each temperature level there was no significant difference between the durations except in 35 °C group where SfG was found to increase at 30th day than on 15th day (Fig. 3).

3.3. Aerobic metabolism performance and thermal tolerance of gastropods

There was a significant effect of ramping temperature on the thermal performance of *B. bengalensis,* albeit the effect of acclimation temperatures (25 °C, 30 °C, 35 °C) and the interaction between them were insignificant. Though the main test had shown a significant difference for ramping temperatures but the pair wise test failed to show any statistically significant difference at individual levels. No profound impact was found on ABT/CTmax as well as on the thermal curve for animals reared in different temperature treatments for 30 days (Fig. 4). The respiration rate of *B. bengalensis* increased slightly from 22 °C to 26 °C and then rapidly till 34 °C, after which it gradually dropped from 36 °C to 42 °C. Based on the changes in respiration rate the ABT value was calculated as 36.25 °C, 36.12 °C and 36.07 °C for 25 °C, 30 °C, 35 °C

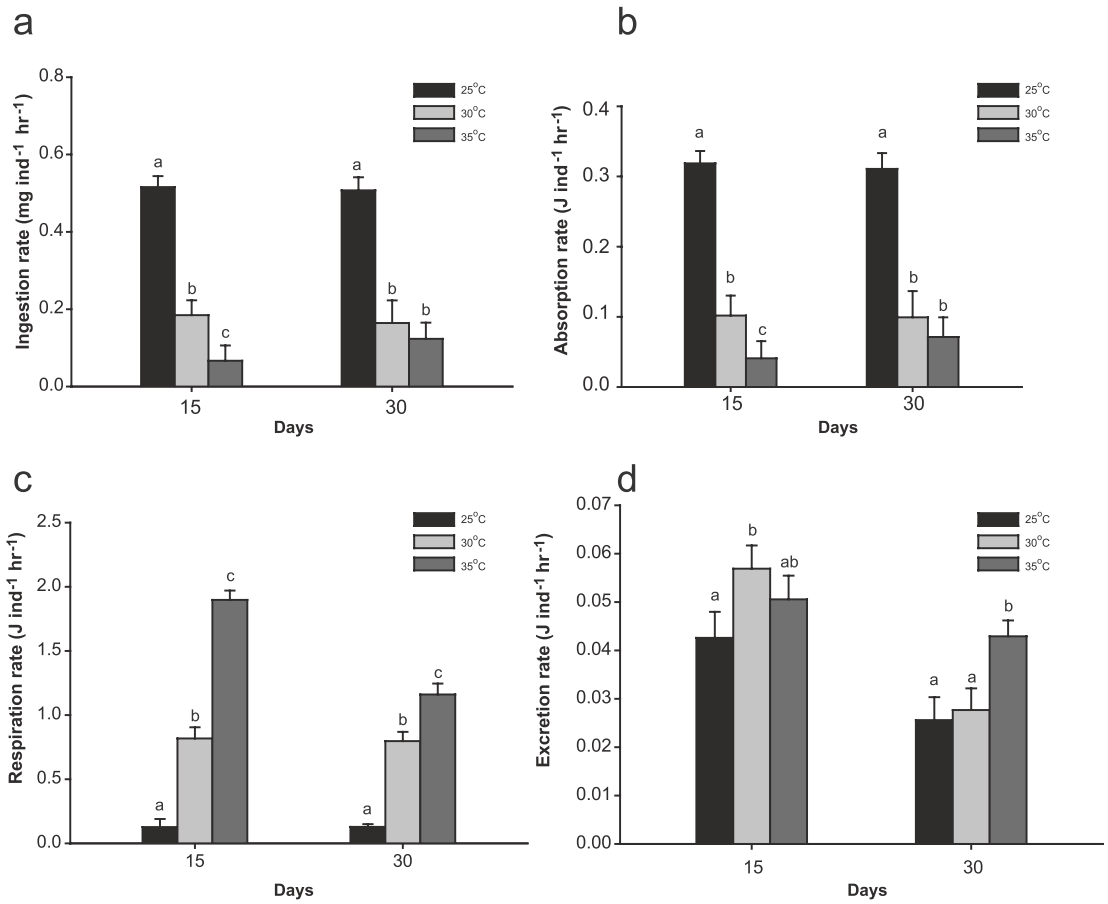

Fig. 2. Ingestion rate (a), Absorption rate (b), Respiration rate (c), Excretion rate (d) of *Bellamya bengalensis* under different temperature treatments (25 °C, 30 °C and 35 °C) on 15th and 30th day of the experiment. Values represent mean ± SD (n = 5). Different letters indicate significant difference between treatment groups ($p < 0.05$).

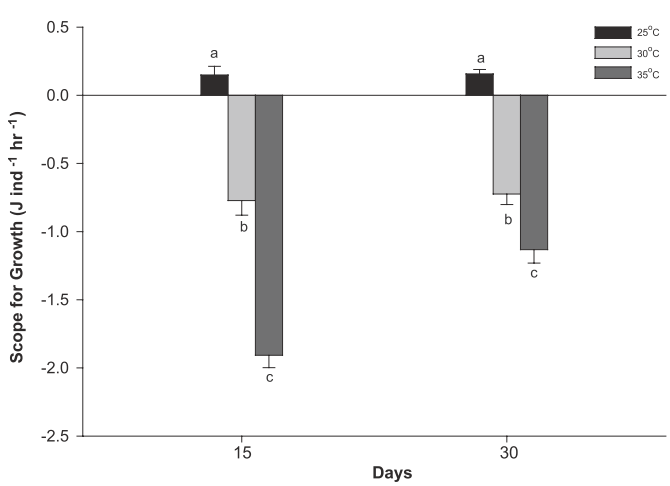

Fig. 3. Scope for Growth of *Bellamya bengalensis* under different temperature treatments (25 °C, 30 °C and 35 °C) measured on 15th and 30th day of the experiment. Values represent mean ± SD (n = 5). Different letters indicate significant difference between treatment groups ($p < 0.05$).

treatments respectively. In case of observed behaviour, gastropods were relatively locomotory inactive till 26 °C but a steady increased activity was observed with temperature elevation till 34 °C with a peak activity at 34 °C–35 °C. However, 36 °C onwards till 38 °C a noticeable weakening of foot muscle strength with loss of foot muscle contractility was observed. Inward curling of lateral foot was noticed between 40 °C to 42 °C. Thus, based on locomotory observations, CTmax was indicated to be at 36 °C for this animal. The warming tolerance and thermal safety margins were calculated 11 °C and 9 °C respectively (Fig. 4).

4. Discussion

The present investigation advocates a significant impact on the physiological energetics of aquatic poikilotherm *Bellamya bengalensis* in the impending climate change scenario mediated by the frequent summer heatwave events. Our results depict that the total weight of the organism reduced with both increase in temperature levels as well as prolonged duration of stress. According to Leung et al. (2017) the total weight of a marine gastropod *Thalotia conica* reduced with prolonged heat stress irrespective of changes in the CO_2 concentration which corroborates our findings. In their subsequent investigation on *Phasianella australis*, Leung et al. (2018) also stated that the total biomass of *P. australis* reduced after prolonged exposure to elevated temperature but not with increased CO_2 concentration which are in concordance with present results where total weight is reduced in business unusual scenarios. Working on a fresh water gastropod *Lymnaea stagnalis*, Hoefnagel and Verberk (2017) found that the body conditions and growth improved at higher rearing temperature, albeit it refutes our result but it has to be kept in mind that the rearing temperature for their experiment was set much below the CTmax which might be within the organisms' optimum range. Contrastingly in present experiment the rearing temperature was set close to the CTmax which might have contributed to the adverse results. In the present study, with increased thermal stress organ to flesh weight ratio was also reduced, which indicates insufficient energy supply for maintenance of the required

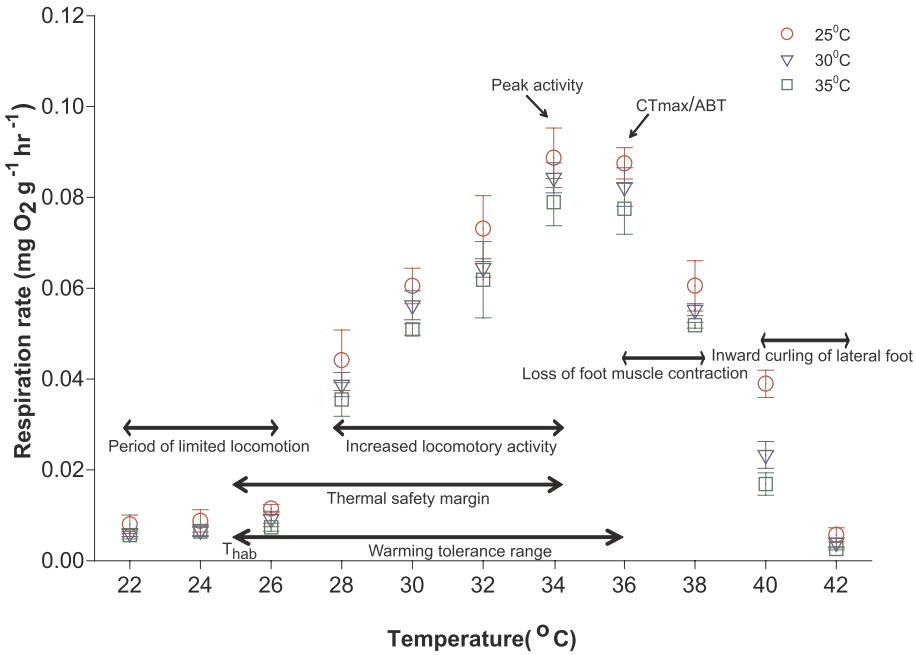

Fig. 4. The thermal performance curve of *Bellamya bengalensis* along an increasing thermal ramp (+2 °C hr^{-1}) from 22 °C to 42 °C after 31days of exposure to different temperature treatments (25 °C, 30 °C and 35 °C) indicating CTmax (thermal critical maxima), ABT (Arrhenius breakpoint temperature), T_{hab} (average habitat water temperature), WT (warming tolerance), TSM (thermal safety margin) values for all the treatment groups. Values represent mean ± SD (n = 5).

energy demand. Furthermore, this might cause depletion of stored energy reserves for providing temporary relief against the unfavourable situation. This observation is in agreement with the results shown by Leung et al. (2017) where they found a prominent decrease in organ to flesh weight ratio with increased temperature regardless the CO_2 concentration.

Physiological performances are best at an optimum range of environmental parameters (Pörtner et al., 2005) which can be affected beyond optimal tolerance range due to reduction in their capability of performance. In our study, ingestion rate followed by absorption rate decreased initially with elevated temperature on 15th day, might be due to prolonged sub-lethal stress conditions where physiological processes were hindered governed by supressed energy production (Brown et al., 2004). However, on 30th day in elevated temperature treatment group (35 °C), a slight increase in both ingestion and absorption rate was noticed, which can be regarded as a sign of acclimatization. A similar observation was noticed by Wang et al. (2015) in case of marine bivalve *Mytilus coruscus* where the clearance rate was lower in 30 °C than 25 °C and it increased with prolonged exposure on 7th and 14th day. In case of marine gastropod *T. conica,* Leung et al. (2017) had recorded reduced ingestion and absorption rate at elevated temperature irrespective of the CO_2 treatment. Newell and Kofoed (1977) also found a synonymous result for gastropod *Crepidula fomicata* where feeding rate gradually decreased with increase in temperature levels beyond the thermal tolerance margin.

Our results showed statistically indistinguishable difference in assimilation efficiency between the temperature treatments and duration of exposure. Absorption efficiency (AE) was not found to be a function of temperature in some previous studies (Laing et al., 1987; Wilbur and Hilbish, 1989; Albentosa et al., 1994). Similar results were obtained by Navarro et al. (2016) in case of mussels *Mytilus chilensis* where AE were not affected by temperature treatments. Zhang et al. (2015) found that AE was not significantly affected on Day 2 in various temperature treatments for subtidal gastropod *Nassarius conoidalis*. Working with another species of scavenging gastropod *Nassarius festivus*, Zhang et al. (2016) also noticed that AE was not significantly affected by temperature which is in accordance with our present findings.

The saturation level of oxygen in water gradually decreases with parallel increase in temperature levels which obstructs oxygen delivery to tissues and the oxygen uptake efficiency in the gastropods. Under elevated temperature, restricted ability to ventilate and circulate leads to a progressive discrepancy between oxygen requirement for maintenance and oxygen delivery to tissues which finally leads to hypoxia and anaerobic metabolism in aquatic organisms (Frederich and Pörtner, 2000). To cope with this kind of adverse situation respiration rate tends to increase with increasing temperature levels. The respiration rate ranged from 0.12 to 1.89 J ind^{-1} hr^{-1} and 0.12–1.16 J ind^{-1} hr^{-1} for 15 and 30 days respectively which are in accordance with respiration rates previously reported for this organism (Londhe and Kamble, 2013) and some related species of gastropods (Liu et al., 2011; Zhang et al. 2015, 2016; Leung et al., 2017). The statistically significant decrease in the respiration rate on 30th day for the 35 °C temperature groups might be a result of acclimatization as mirrored by increase in absorption rate simultaneously. According to Zhang et al. (2015) respiration rate was significantly low at all lower temperature levels irrespective of CO_2 concentration on Day 3 and 31 for a subtidal scavenger gastropod *N. conoidalis.* Working with another species of scavenging gastropod *N. festivus*, Zhang et al. (2016) showed an alike observation where energy expenditure due to respiration was significantly high at 24 °C and 30 °C than at 15 °C. Similar results were also reported by Guzmán-Agüero et al. (2013) for oyster *Crassostrea corteziensis* where respiration rate was shown to gradually increase with increase in temperature levels. In thick shell mussel *Mytilus coruscus,* Wang et al. (2015) showed a considerable drop in the respiration rate at 30 °C than 25 °C throughout the experimental period which is an unusual observation and different from our results. This causes a swift decline in rate of respiration and upsurge the utilization of anaerobic metabolic pathways (Pörtner, 2002). Respiration rate generally increases with temperature elevation up to a certain optimum frontier but beyond that it rapidly declines caused by the reduced capacity of oxygen delivery and/or diminished mitochondrial functions under longstanding thermal stress (Sokolova et al., 2012).

The rate of ammonia production reflects a measure in the rate of protein catabolism, which may differ with the nutritional status of the

animal (Griffiths and Griffiths, 1987). Thus, ammonia excretion rate has been used as a stress indicator for various organisms and it generally increases with temperature due to the metabolic energy demand (Anestis et al., 2010; Fernández-Reiriz et al., 2011; Guzmán-Agüero et al., 2013). This phenomenon was also observed in our results, where at higher stressful temperatures levels ammonia excretion rate were significantly higher than the control temperature treatment. At 15th day, ammonia excretion rate of 30 °C treatment was higher than 25 °C and subsequently on 30th day it was significantly higher in 35 °C treatment. Zhang et al. (2015) found a similar result in case of *N. conoidalis* where ammonia excretion rates were significantly high at higher temperature on Day 3 and Day 31, irrespective of CO_2 concentration. In another study on *N. festivus* by Zhang et al. (2016) ammonia excretion reduced significantly at low temperature on both Days 3 and Day 31 which supports our findings. Navarro et al. (2016) also found similar results in case of mussels *Mytilus chilensis* where ammonia excretion rate increased in mussels exposed to the highest temperature 16 °C in Chile. Guzmán-Agüero et al. (2013) found ammonia excretion rate increases with increasing temperature for oyster *Crassostrea corteziensis* up to 29 °C which is in accordance to our observation but then sequentially reduced at 32 °C might be because the temperature is beyond the animal's thermal window.

Physiological alterations are obligatory in response to stressed environmental condition as animals are incapable to respond to the new situation (Durrant et al., 2013). The ability to handle such drastic changes is essential for survival and might specify the tolerance level of organisms (Folkedal et al., 2012). The Scope for Growth (SfG) estimate provides an insight to the instantaneous energy status of any particular animal as well as the body conditions and fitness (Smaal and Widdows, 1994). Thus, it is essential to carry out detailed studies to delineate the SfG of commercially and ecologically significant species in the predicted future heatwave events. The model established by Gianguzza et al. (2014) postulated an elevated metabolism leads to an increased growth rate when energy is not restraining. Nevertheless, if the metabolism increases incessantly, it leads to metabolic depression and a reduction in scope for growth because the energy requirement for growth cannot be maintained by the supplied energy. In the present findings, the SfG was negative on 15th day for both the stressed temperature treatments (30 °C and 35 °C) albeit on 30th day the value of 35 °C treatment group improved slightly showing some signs of enhancement in the physiological conditions. In mussel *Mytilus coruscus*, Wang et al. (2015) reported lower SfG for the higher temperature 30 °C than that of 25 °C on Day 1, 7 and 14 at various pH conditions which corroborates our findings. The result reported by Leung et al. (2017) for gastropod *T. conica* also revealed an energy deficit condition indicated by a negative SfG at elevated temperature irrespective of the CO_2 concentration which also supports our results. Anestis et al. (2010) also highlighted negative SfG value for *M. galloprovincialis* at elevated temperature, concomitant with a noteworthy reduction in the clearance rate. The information gathered from present study depicts that during prolonged thermal stress the SfG tends to be negative indicating energy insufficiency. It can be inferred that in extreme stress conditions the animals' prior focus is on respiration and excretion rather than ingestion which indicates that elevated temperature might disrupt feeding activity resulting a negative SfG by catabolism of prestocked energy reserves. This might hinder essential cellular and physiological functions, which might disturb the population perseverance in future stressed environmental scenario.

Thermal performance is one of the paramount factors in determining the influence of elevated temperature on aerobic scope. Even though temperature induced constrained aerobic scope are of utmost importance to accurately delineate the metabolic physiology in aquatic animals, it has not received much attention compared to other phenomenon of the metabolic response to elevated temperature. We observed no significant difference between animals acclimated to different temperatures, advocates the fact that acclimation has inadequate ability to improve thermal tolerance levels (Van Maaren et al., 2000; Sandblom et al., 2016) suggesting the restricted capacity to alter thermal niches (Araujo et al., 2013). From our results the pejus (suboptimum) temperature ranged between 28 °C to 32 °C where respiration rate increased considerably, which indicates metabolic adjustments with changing temperature (Clarke, 1998). The Arrhenius breakpoint temperature (ABT) and thermal critical maxima (CTmax) reached around 36 °C and from there transition to partial anaerobic mitochondrial metabolism might commence caused by the progressively insufficient capacity of circulation and ventilation that disintegrates the oxygen delivery systems in the animal's body (Sokolova and Pörtner, 2002). It recognizes a physiological edge where oxygen resource and energy balance cannot be maintained further and specifies the upper threshold temperature for the long-term existence of stressed animals. The entire animal's aerobic scope depletes and gradually cease beyond the thermal optimum indicated by loss of foot muscle contraction and eventual inward curling of lateral foot muscle. Beyond the CTmax physiological performances like feeding and locomotion are impaired and triggers critical challenge for the survival of stressed animals. Although the warming tolerance and thermal safety margin values were comparatively large and not very near to the thermal edge of this animal, howbeit, this is not the scenario in all seasons. In extreme summer months the water temperature rises close to thermal optimum making the species susceptible to future projected global warming scenario.

5. Conclusions

The present study demonstrated elevated temperature induced alteration in the eco-physiological aspects of tropical aquatic gastropod *Bellamya bengalensis* exposed to climate change induced elevated water temperature below its thermal tolerance limit. Nevertheless, they are able to survive by compromising their energy budget and depleting their stored energy reserves. Temperature showed a significant effect on the ingestion, absorption, respiration, excretion rates as well as SfG in animals; however, a slower acclimation process was noticed which might need a considerably longer time. The ABT/CTmax values indicate that the animals are living at the edge of their thermal tolerance limit which might jeopardize their population structure making their survival time limited in future. An increase in the water temperature for extended period of time would deteriorate hypoxemia and worsen the systemic oxygen tensions. Moreover, it might alter the set points of acid-base regulation which can constrict the thermal space and would eventually drop the optimum performance of this animal. Further, long term studies on ill effect of thermal elevation on different geographic population of this species could be an interesting topic to explore.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

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