

## Effect of the oxygen on the thermal performance of terrestrial isopods

### Abstract

Climate change is obvious, however, an actual mechanistic system to calculate the impact of climatic change on the living organisms is still a questionable aspect. Climate changes on ectotherms and especially isopods can be highly drastic however still these aspects have not been scientifically studied accurately. It is because of this reason, the research work for present work focuses and has been selected as a specific area of the research study. It is predicted that human activities are leading to catastrophic changes in the whole environment. Pollution and changes in temperature too have been the sole factors due to human activities itself. In coming time we are actually putting lives of ourselves and other living things in danger. Strong scientific focus on the subject area has been seen across the world. Reason being at least to put on papers for the world the damages done by human actions and then come out with solutions so that world can be as beautiful as it was for the future generations also.

The present work is specifically focused to study the adaptations of Isopods to oxygen levels under different temperature conditions. This work will contribute to the research community over the understanding of the impact of oxygen levels which is a parameter of climatic change or conditions on the living organisms. This will help to develop the understanding of the behavioral aspects of Isopods to different oxygen levels (A change in climate) and will help us in understanding the impact of human activities on the living organism and ourselves in the whole world.

Results were, there exist a significant difference between the thermal performance curves of the Isopods at Hypoxia and Normoxia (the two conditions we studied). The data calculated showed near around linear curve in hypoxia conditions for Isopods as compared to the non-linear curve under normoxia conditions. This significantly showed the drastic changes in hypoxia conditions have over the Isopods, which results in lower performance of Isopods at lower concentrations of Oxygen.

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## Introduction:

Temperature can be considered as most influential of all the abiotic factors that influence the physiology & ecology of the ectotherms (Huey & Kingsolver 1993, van der Have & de Jong, 1996, Sibly & Atkinson, 1994, Stevenson, 1985). Ectotherms are the organisms which maintain their body temperature using the external sources and it's likely to increase organisms' risk of overheating (Gunderson & Stillman, 2015). They may be facing the risk of decline as an outcome of temperature change (Moritz & Agudo, 2013, Rohr & Palmer, 2013), what is especially important in the context of increasing global temperature\_(to be added before final revision request).

Similar to temperature, oxygen is also an essential factor required for the life to thrive (to be added before final revision request). Lower levels of oxygen result in the physiological or ecological changes in an organism's physiology.

Comment [U2]: What changes??

These changes are, behavioral changes, adjustment behaviors to the temperature changes, changes from aerobic metabolism to an anaerobic metabolism, and in extreme cases even death or extinction. Portner in his various publications has clearly mentioned the description of OCLTT, which is Oxygen and capacity dependent thermal tolerance theory. Under this theory it is assumed that oxygen does have wide impact over the changes in behavior of living organisms as, oxygen changes result due to climatic changes, and thus changes in oxygen availability and wide gap between the availability or demand and supply of oxygen results in subsequent adaptation behaviors in the Isopods, and other living organisms, Such a hypothesis is an important incurrence on the understanding of the impact of climatic changes, or the overall Global warming happening across the regions world over.

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-Such changes force the organisms to develop adaptations such as hyperventilation failing which the species face extinction (Huey, 2005). The change of oxygen level impacts the biology of the organisms such as body size (Yang et al., 2014) and the rate of development (Horváthová et al., 2015)., there hasn't been much evidence that supports the impacts on the part of behavioral changes. (Noble, 2012)—Still the predictions are difficult, as one researcher and many others have faced the difficulty to specifically calculate and quantify the impact of climatic changes over the living species at the lower or lowest level.

**Comment [U3]:** And here write about OCLTT

OCLTT which is Oxygen and Capacity dependent limited thermal tolerance, describes the changes in thermal performance and tolerance of the living organisms depicting strong hypothesis that Oxygen does impacts the performance of the organism or the living object. Portner, has in his various publications repeatedly mentioned the case of oxygen and possible impact over the thermal performance. Temperature related statistics, or strata have impact due to the widening gap in the supply and demand of the oxygen. The theory clearly postulates that with Rising temperature, the demand for the oxygen also rises. As the oxygen supply remains the same, the gap between the supply and demand increases with the rising temperature, and the metabolism shifts to anaerobic from aerobic. This further leads to shortening of thermal window. And greater risks towards extinction due to rise in temperature.

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We can take a cue from the past incidents that led to the evolution of many terrestrial species from the aquatic life as there were possible lower oxygen levels and high temperatures (Berner, VandenBrooks & Ward, 2007). They may also be responsible for the change in the species interaction, responses to various cues and change in the performance (Vasseur et al., 2014). According to temperature size rule (TSR), the increased temperature leads to the lower body size at maturity (Angilletta, Jr., & Dunham, 2003, (Atkinson, 1994, van der Have & de Jong, 1996). Huey suggested that since the physiological rates are relative to change in temperature, this may influence the behavioral and ecological performance of the ectotherms (Huey & Kingsolver 1993) |

~~Research work carried out by (Woods, H.A., et al. 2014) focused to scientifically study and state the calculations of Impact of climate change using Macroclimate and Microclimate terminologies as a system to be more specific in research and focused his work to study the change on the following broad factors, 1. Interaction of Macroclimate and Microclimate with Living and Nonliving subjects, 2. How ectotherms (mobile) filter the impact of changes in microclimatic systems, & 3. The changes in body temperatures of ectotherms either help them to evade the impact or to prevent the drastic weather conditions on the body as much as it is possible for the body. (Buckley et al., 2013; Deutsch and Tewksbury, 2008; Huey et al., 2012).~~

~~Among the studied ectotherms, marine animals have shown significant behavioral and physiological changes (Pörtner, Mark, Böck, 2004; Schurmann et al 2016; McBryan et al 2013) such as forcing fishes to leave their nocturnal shelters making them prone to predation (Nilsson, Östlund Nilsson, Munday 2010; Claireaux, Webber, Lagardère, Kerr 2000;) or resulting into reduced growth productivity (Fitzgibbon, Strawbridge, Seymour 2007). Similar to aquatic ectotherms, the thermal performance among reptiles and amphibians in their larval stage is reduced during normal hypoxic condition especially among amphibians because eggs and larvae thrive in aqueous environments (Gangloff and Telemeco, 2018). It can be assumed that ectotherms are susceptible to the change in temperature and level of oxygen (Angilletta, 2006; Horváthová et al., 2015). The physiological and reproductive changes that occur due to the effect of temperature include the decrease in body mass but increased abundance (Robinson, McLaughlin, Marteinsdóttir & O'Gorman, 2018).~~

Isopods have been selected as they are sensitive to changes and are quick to respond to changes among the living species. Thus the present work is highly effective to help in understanding the scientific reasons and cause and effect relationships between organisms and different oxygen levels on Isopods.

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The experiment will try to establish the relation of thermal performance on partner ~~OCLTT~~ Oxygen- and capacity limited thermal tolerance (OCLTT) theory. ~~OCLTT means a terminology, which describes the relationship between Oxygen and thermal performance. OCLTT has~~

**Comment [U4]:** In my opinion redundant, maybe to disussion

**Comment [U16]:** In my opinion redundant, maybe to disussion

**Comment [U5]:** citations

**Comment [U6]:** what says OCLTT??? It is the most important here, not behavior of fishes in climate change

~~attracted lot of attention among the scientific community. OCLTT means, Oxygen and capacity limited thermal tolerance~~, has shown possible way to learn about thermal performance among the ectotherms and in present case among the Isopods. The discussion part puts light on the concept using reference of a cited work already published in the journal.

OCLTT SAYS, that with rising temperature, the gap between the demand and supply of oxygen widens up, and the metabolism of the organism shifts from aerobic to an anaerobic metabolism. The resultant Anaerobic metabolism, leads, to shortening of Thermal Window, and greater sensitivity to changes in external temperature changes. Rising temperature will thus, either lead to lower performance, or in extreme cases even extinction from the nature. (Added by Devansh)

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Thermal performance curve(PC) are the key tool of determining the thermal sensitivity of the ectotherms. These curves help in confirming the performance of the animal on different body temperatures. The curves help in defining the optimal temperature at which the animal has optimal performance (Huey & Kingsolver, 1989). The TPC has three regions; the first phase is the rising phase which increases with the temperature rise. The second phase in the plateau phase which comprises of the thermal optimum of the factor and the third phase is the steep falling phase occurring at higher temperatures (Dell, Pawar and Savage, 2011,2013). We tried to establish the performance breadth of the animals at different body temperatures at hypoxia and normoxia (Huey & Kingsolver, 1989). (Dillon et al., 2012; Anderson et al., 2007). ~~The reason being Climatic changes are discussed, as the purpose of the present work is to study the impact of changes in oxygen level over the temperature condition of Isopods. Changes in Oxygen level is nothing but a climatic change which as a parameter has been selected to carry out the research work in present case.~~

Comment [U7]: not necessary

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Our experiment was performed on ~~the species of~~ terrestrial isopod common rough woodlouses Porcellio scaber, the same species used for the intramarsupial development. This would help in

comparing the results of the study with a former study on temperature and oxygen on the terrestrial isopods. As the previous study results suggested the negative correlation between the development and the low oxygen high-temperature levels, our study was to confirm whether we could obtain the similar results in thermal performance. Thus, we hypothesized there is a significant effect of temperature and oxygen levels on the thermal performance curve of *Porcellio scaber*. The results obtained from our study would be useful in further studies on the interaction of temperature and oxygen and their impact on the species.

Comment [U8]: what effect?

### **Materials and methods:**

#### **Animals:**

The subjects of the study were terrestrial isopods *Porcellio scaber* that were collected by hands in the late summer 2017 from their natural habitat in two old monasteries gardens (50.065104,19.931584 and 50.058948, 19.935965 respectively) from Old City in Krakow. After collection animals were put into plastic boxes of size.....containing a layer of wet sand and clay pot as a shelter. Once a week animals were fed with dry leaf mixture with the dominance of Adler and ash leaves sprayed with water. The boxes with animals were placed in the climatic chamber with the constant temperature set to 20°C and 12:12 photoperiod.

#### **Equipment & prerequisites:**

The experiment was performed on two thermal platforms (Biospekt, Poland) The dimensions of platforms were ..... And had a temperature range of..... The platforms were covered by Plexiglas (YETI – Agencja Reklamy, Poland) what allowed us to control the oxygen level during the experiment. The hypoxic conditions were achieved using an external gas control system with the use of ROXY-4 four channel gas regulator (Sable Systems Europe GmbH, Germany) which supplied nitrogen in order to lower oxygen level inside the chamber.

A glass beaker served as an arena for the running of the animals. The bottom of the beaker was layered with wet sand. One-cylindrical ring of diameter ..... was placed in the middle of the beaker and another ring of diameter ..... was placed that served as a track border. The actual temperature during trail was measured with ThermoChron iButton (Maxim/Dallas Semiconductor, USA) placed in the inner circle of the arena. We tested the temperatures in the range of 8-45 degree Celsius in the ascending order of 3 degrees, therefore, a total of 13

temperatures were tested. The order of animals and temperature testing was randomized, animals of both sexes were used in the experiment.

### **Procedure:**

Prior to trial, animals were weighed down using the automatic balance (Toledo XP26, Mettler, Switzerland) to the nearest 0.001 mg. Before the trial animal was acclimated inside the smaller circle (what minimized the possibility of movements before trial) to the experimental conditions for 15 minutes. After acclimation, the cylinder was tilted using the brush so that the animal could reach the main arena. The trial began from the start mark that was labeled by a permanent marker on the glass dish. One complete circular lap was recorded using the stopwatch (estopwatch.net). The maximum time for a single trial was 15 minutes however, the trial could be ceased in case of exhaustion (defined as any response of animal for three brush touches) or death. The experiment was run in two independent replications. Together 114 animals were tested. I

### **Statistical analysis:**

Data were analyzed with a nonlinear mixed model with the assumption of quadratic dependence of performance on temperature. The fixed part of the model was temperature, sex, and the logarithm of body mass. The random part was replication and contained also the random estimation of curve parameters. To test if the effect of replication is significant we run the similar model, but without random effect (with use of lm function) and then we compared the AIC of these models. Additionally, we analyzed with chi2 test the number of dead animals in temperatures above 37.59°C (the minimal temperature in which animal died during acclimation) between oxygen treatments.

The analysis were done with the use of with R 3.4.1 software (R Core Team 2017) with additional use of nlme (Pinheiro et al. 2018) and ggplot2 (Wickham 2009) packages.

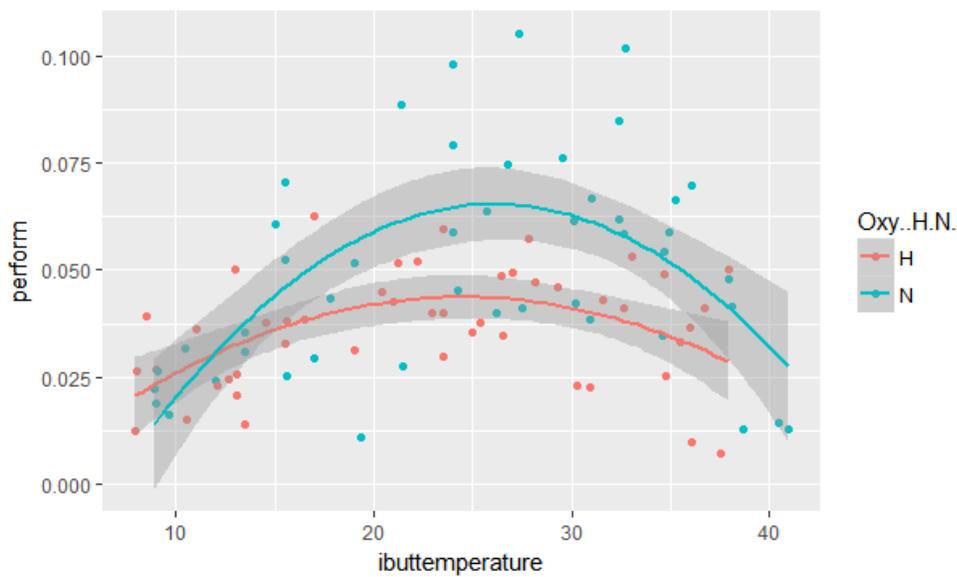
### **Result**

In the analysis of performance curve the models with and without random effect did not differed (likelihood ratio= 1.09, p=0.98) and AIC showed supported model without replication as random factor. We found significant difference between performance curve in hypoxia and normoxia. The curve formula in hypoxia:  $-0.005+0.004x-0.00009x^2$ ; in normoxia:  $-0.05+0.009x-0.0002x^2$ .

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All the parameters differed significantly (c:  $t=-2.06$ ,  $p= 0.04$ ; b:  $t=2.38$  ,  $p= 0.02$ ; a:  $t=-2.06$ ,  $p=0.04$ ) between two oxygen conditions. The effect of sex on intercept was not significant ( $t=-1.52$ ,  $p=0.13$ ).

We found a significant difference in a number of died animals in temperatures above  $37.59^{\circ}\text{C}$  between oxygen conditions ( $\chi^2=6.13$ ,  $p=0.01$ ) with more animals died in hypoxia.



The ~~graph here shows the~~ thermal performance curves of Isopods ~~at different temperature conditions~~ under two oxygen levels, i.e Hypoxia (red line) and Normoxia (blue line).

~~As seen from the results from this graph, it shows that the activity level and performance of Isopods at Hypoxic conditions reduces and get limited, as the oxygen levels are low. This shows that changes in the environment do have an impact on the living organisms and they tend to adapt to the changes as their body reacts and behaves accordingly. There does exist a direct relationship between behavioral aspects and changes in surroundings or environment.~~

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## Discussion (WRITTEN WHOLE BY & ADDED BY TRM WRITER'S® LLP)

**Comment [U9]:** In the beginning repeat what we have found and then built the story based on that

(Huey and Stevenson 1979) studied and published their work regarding the thermal biology of ectotherms. Their research brought changes in the research community and started the research focus on ectotherms and effect of changes in body and performance (Thermal performance). Their research checked the thermal sensitivity of ectotherms first time by any researcher in the world. 25 years since then many papers have been published in thermal biology and related to ectotherms, several laboratory studies have enumerated the thermal reaction norms of organismal traits in living organisms (Ji et al., 1993; Witz and Lawrence, 1993; Scribner and Weatherhead, 1995; Ji et al., 1996; Du et al., 2000; Angilletta et al., 2002a; Blouin-Demers et al., 2003; Chen et al., 2003; Elsworth et al., 2003; Zhang and Ji, 2004).

In research work discussed and published by McConnachie and Alexander (2004), it was found that relationship between temperature and performance is curvilinear.

The shape of thermal performance curve will always be curvilinear as the parameters changes under two broad levels, i.e. the lowest point and the highest point. No matter what the results are, the bell shape graph cannot be ruled out.

Thermal performance curves are the accurate estimation criteria to check the performance of living organisms studied in experimental cases.

Bulte´ and Blouin-Demers (2006) illustrated (TPC) Thermal- performance – curve's do have a characteristic size and shape. It's even known that many other thermal biologists have had mentioned a general form of parameters can easily describe these curves (Stevenson & Huey, 1979; Huey and Kingsolver, 1989; Angilletta et al., 2002b).

Performance curves are surrounded and restricted at extreme temperatures and possess a single intermediate mode. ~~There exists a linear or non-linear relationship between the parameters and performance of ectotherms. In the present study, two oxygen level parameters were examined and there relationship with the thermal biology of the Isopods was evaluated. During the results,~~

~~complete care of experimental conditions was taken care of. The curvilinear relationship between performance and changes in temperature and its effects on the performance were relative~~

~~As Bulte' and Blouin Demers suggest, we should avoid linear (or other) approximations of thermal performance curves that differ fundamentally from the characteristic shape. As an example, they compared the fit of linear and nonlinear functions describing the relationship between body temperature and food intake by lizards (data from McConnachie and Alexander, 2004). Although a linear function fits well over the range of 20–32 °C, a nonlinear function described the data better over a broader range of temperatures (20–35 °C). Bulte' and Blouin-Demers criticized McConnachie and Alexander (2004) for omitting the data recorded at 35 °C and using a linear model to describe a relationship.~~

~~During the experiment and after calculating the results, it showed and gave us learning in the right manner and helped our understanding of the adaptations of living organism to the changes.~~

~~Oxygen is very much and highly important for living organism as are other things important for life to exist in our nature. Any changes in any of these factors would have a direct impact on the living organisms, and this is what we observed during our experiment.~~

In a different research carried out by a researcher, the findings were, Thermal environmental conditions hold considerable significance for most levels of biological hierarchies (Pörtner et al., 2006). Because environmental temperature varies in time and space, organisms must deal with the thermal variation on a variety of ways and scales (Johnston and Bennett, 1996). At first, all organisms have evolved with thermal variability as a selection pressure (Osborn and Briffa, 2006). In this way, temperature variability would play an important selective role in the studies addressing its effect on life history variation (Loeschcke et al., 1997, 1999; Pétavy et al., 2001; 2004). In addition, the physiological responses range from immediate (in acute exposures) to acclimation (during chronic exposure ranging from several weeks to months; Glanville and Seebacher, 2006). Different studies have examined thermal acclimation at seasonal (Järvinen, 1989; Padilla-Gamiño and Carpenter, 2007; Angilletta, 2009), and daily scales (Sears and Angilletta, 2003; Tsuji, 1988; Angilletta, 2009), and all of them enlighten the importance and complexity of the thermal variation over the organism's performance. In this regard,

**Comment [U10]:** We use second order polynomial, we do not need to discuss it

experimental studies testing not only for the effect of mean temperature increases but also for the effect of environmental temperature variability on phenotypic traits emerges as important the effects of different environmental thermal conditions are directly related to the 'thermal safety margin' (TSM), defined as the difference between the organism's thermal optimum and its current climate temperature (Deutsch et al., 2008). Our analysis showed that higher values of thermal amplitude are correlated with lower values of performance. Therefore, a negative relationship between the population persistence time and the temporal variability was observed among the different taxonomic groups, trophic levels and habitat types (Inchausti and Halley, 2003). Indeed, the comparison between populations may indicate higher sensitivity to the thermal amplitude in lowland rather than in highland individuals, indicating that important effects over biodiversity may be expected in the context of the increasing thermal amplitude. First, Deutsch et al. (2008) predicted a general variability loss due to the increasing TSM magnitudes. Second, the expansion of a genetic variant with higher values of TSM may intensify the consequences of previous effects, increasing the variability loss. Taken together, our results and those of the previous studies indicate that thermal variability may produce important effects on biodiversity. Therefore, in order to develop more realistic scenarios of the effects of global climate change on biodiversity, the effects of the means as well as the variability need to be examined simultaneously.

**Comment [U11]:** What??!!!

(IPCC, 2014)The important thing to be considered is studying the nature and behavioral aspects of Isopods through an experiment can help us predict the way the living organism is changing in coming years and future. Even this can help us predict the future way before it is too late for us humans to react and act to prevent ourselves. Risk of extinction from the scientific study of present work can be found out easily and can tell us the rate and the pace many similar and other unidentified species are evicting from the world in present times. This is an indicator of restricting our activities which is basically hampering the world in which we all are living. (Potter et al., 2013)It is our responsibility to take actions in present to prevent the possible damages in future. In other words, our future lies only in our own hands.

**Comment [U12]:** Where we have variability??

~~Coming back to the topic, the climate changes on global level have highly complex nature of its impacts over the living organisms. Some adapt and some perish. Adaptions are not good, as they are changing the very nature of the nature itself, and perishing is the end in whole. (Codling et~~

**Comment [U13]:** To far conclusions!!! Write about how hypoxia shapes the performance curve, and than you can refer to global change

**Comment [U14]:** unnecessary

al., 2008). Even species who are adapting to changes in the climatic conditions is the warning signal that climate is hampering the living species. Either adapt or die. We are in present times are adapting to climatic changes as the small living species, world over. If we don't adapt we will also perish. It is thus important to restrict these climatic changes which is forcing the living species to adapt. We as humans can do many things, to Restrict these changes, so that the situation of doing adaptation and to do it or to perish can be ruled out.

Although based on much and many other earlier publications, the OCLTT hypothesis has been enthusiastically customary over the past decade, motivating a diverse spectrum of studies from ecology and behavior, to classic physiological investigations of the mechanisms dictating organismal function. Greatly of the intuitive appeal of the OCLTT hypothesis probably resides with the proposed linkage of a relatively simple physiological mechanism, the oxygen uptake, and transport systems, to a very complicated and often frustratingly difficult interplay between abiotic and biotic influences on fitness, growth and reproduction of animals in their native environment. Such integrative linkage at many levels of biological organization, i.e., from whole-organism fitness to mitochondrial ATP synthesis, is a desirable academic goal. However, much controversy remains as to whether the OCLTT hypothesis can be regarded as a unifying principle but both skeptics and supporters will agree that the OCLTT hypothesis must be carefully and critically evaluated. Such scrutiny should preferably involve experimental manipulation where oxygen transfer capacity or oxygen availability is altered to unravel the factors

**Comment [U15]:** please, write about OCLTT and how our results correspond to that, not about climat hange and what can we do.

Research work carried out by (Woods, H.A., et al. 2014) focused to scientifically study and state the calculations of Impact of climate change using Macroclimate and Microclimate terminologies as a system to be more specific in research and focused his work to study the change on the following broad factors, 1. Interaction of Macroclimate and Microclimate with Living and Nonliving subjects, 2. How ectotherms (mobile) filter the impact of changes in microclimatic systems, & 3. The changes in body temperatures of ectotherms either help them to evade the impact or to prevent the drastic weather conditions on the body as much as it is possible for the body. (Buckley et al., 2013; Deutsch and Tewksbury, 2008; Huey et al., 2012).

Among the studied ectotherms, marine animals have shown significant behavioral and physiological changes (Pörtner, Mark, Bock, 2004; Schurmann et al 2016; McBryan et al 2013) such as forcing fishes to leave their nocturnal shelters making them prone to predation (Nilsson, Östlund-Nilsson, Munday 2010; Claireaux, Webber, Lagardère, Kerr 2000;) or resulting into reduced growth productivity (Fitzgibbon, Strawbridge, Seymour 2007). Similar to aquatic ectotherms, the thermal performance among reptiles and amphibians in their larval stage is reduced during normal hypoxic condition especially among amphibians because eggs and larvae thrive in aqueous environments (Gangloff and Telemeco, 2018). It can be assumed that ectotherms are susceptible to the change in temperature and level of oxygen (Angilletta, 2006; Horváthová et al., 2015). The physiological and reproductive changes that occur due to the effect of temperature include the decrease in body mass but increased abundance (Robinson, McLaughlin, Marteinsdóttir & O'Gorman, 2018). (Added by Devansh)

(Added by Devansh)

Thermal performance of the Isopods from our observation of the research carried out in the laboratory, and after taking reference from the assumed OCLTT theory widely published by the Portner, it has been incurred that Hypoxia which is lower level of oxygen does impacts, the thermal performance of the Isopods. As it can be seen from the graph that Hypoxia condition graph is at lower curve level compared to the Normoxia level. From this it has been shown that Isopods do show changes in their adaptability to the temperature changes, under different levels of Oxygen which is 1. Hypoxia and , 2. Normoxia. We noticed that at hypoxia, many Isopods died during the experiment, which is pure signal of extinction from the nature, under hypoxia condition when external temperature changes occur, and in this case, extreme warm temperatures. (Added by Devansh).

Isopods have wider performance capability because of which they are extremely attractive experimental subjects. This is why, for the present work, Isopods have been selected to carry out the scope of research in the present research work. Isopods do show wide thermal window, and have greater adaptability window also.

From our experiment, we tested the adaptability and performance of the Isopods, under various temperature conditions, Cold (CTmin) and Hot, (CTmax), and changing the variability of oxygen

supply. From the beginning of the research, we wanted to test the hypothesis of OCLTT theory, so we choose Hypoxia and Normoxia conditions for the present work.

As mentioned the graph and its extrapolation, the curves were plotted and result was driven out under different experimental environment conditions. We found that Isopods, under Hypoxia conditions showed restricted performance or limited performance, and in extreme cases even we noticed lot of deaths of Isopods. From this we can easily understand that our research work is in alliance with the postulates of OCLTT theory. From the experiement we only found out the experiemental evidence of OCLTT theory, and thus we hereby suggest that more and more confirmation studies shall be carried out to confirm the reliability of OCLTT theory.

(Added by Devansh)

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