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Research Article

Sudden and Steep Harsh Environment Results in Over-Compensation in Digital Organisms

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Abstract

Adaptation to external environment to produce viable offspring is an important aspect of evolution. Although experimental studies adapting bacteria to various ecological niches had been carried out, they are usually time-consuming, labour-intensive, and represents one instance of life - biological life; hence, unable to generalize to all forms of life. Digital organisms (DOs), which are computer-simulated organisms, presents alternative life forms. In this study, DOs were used to evaluate ecological niche adaptation by measuring the fitness of the DOs in two varying external parameters (resembling oxygen and carbon). Two oxygen adaptation schemes (gradual and sudden) were tested in the context of two carbon environments (high and no carbon) while fitness is determined as the energy availability as the amount of metabolite E. Our results suggest that external oxygen decline impacts on energy production but sudden oxygen decline increases energy production regardless of carbon environment. Hence, sudden harsh oxygen deprivation may result in over-compensation in adaptation compared to gradual oxygen deprivation in both carbon environments.

Keywords: *Environmental Adaptation; Digital Organisms; Alternative Metabolism; Adaptive Over-Compensation*

Introduction

An organism's capability to survive, use the environment's resources and to produce viable offspring is defined as Darwinian fitness [1]. While spontaneous mutations [2-4] as a result of imperfect DNA replication [5], environmental stressors can provide pressure for selection [6-8]. Although there are subtle differences between studies to which fitness is defined, there is always the key point of reference back to environmental pressures driving the need for an organism to adapt and survive [6]. Natural selection provides a bias towards organisms that undergo mutations that are relevant for it to survive given environmental stressors [9]. This selective pressure may result in organisms well-adapted to survive a specific environment, known as ecological niche [10]; for example, desert plants evolve specific adaptations to arid environments [11].

Several studies had adapted bacteria to various ecological niches in experimental settings; such as, increased temperature [12], reduced temperature [13], ionizing radiation [14], reduced oxygen [15], food preservatives [16,17] and high salt [18]. However, such experiments are usually time-consuming and labour-intensive [19] giving rise to studies on improving efficiency [20]. Yet, these studies are performed on existing biological organisms or Earth-based life-forms. Fundamentally, biological organisms on Earth it is one of many possibilities or instances of life [21]; of which, many other possibilities may be considered "alive" [22]. Digital organisms (DOs), which are

computer-simulated organisms [23,24] and had been used to explore various evolutionary scenarios [25-32] with alternative metabolisms [33,34], is considered as instances of life rather than simulations of life [35] as DOs can be considered alive [36].

In this study, we used DOs in an alternative metabolic environment [33,34] to evaluate ecological niche adaptation, where two external parameters (resembling oxygen and carbon) are varied, by measuring the fitness of the DOs. Two oxygen adaptation schemes (gradual and sudden) were tested in the context of two carbon environments (high and no carbon). Fitness is determined as the energy availability from metabolism given the environmental conditions. Our results suggest that sudden harsh oxygen deprivation results in over-compensation in adaptation compared to gradual oxygen deprivation in both carbon environments.

Materials and Methods

Simulation system: Digital Organism Simulation Environment (DOSE) [37,38] was used as the simulation platform and a new genome interpreter, Dennis Interpreter, was defined based on DOSE's native genome interpreter, Ragaraja [39]. A total of 24 intracellular metabolites (Metabolite A to X) and 37 reactions (R1 to R37) were defined (Figure 1). Of the 24 intracellular metabolites; metabolites C, E, and O were defined as carbon, energy, and oxygen; respectively. Each of the first 35 reactions (R1 to R35, Appendix) were enzymatic-like two-reactants to two-products reactions. The only difference to biological enzymatic reactions was that reactants were not used in the production of products, which are similar to add operation in Avida instruction set [40]. R36 and R37 were defined as active transporters [41] of extracellular carbon (eC) and extracellular oxygen (eO) into Metabolite C (intracellular carbon) and Metabolite O (intracellular oxygen) respectively. Each DO was initiated with a chromosome of 10 repeats from R1 to R37, resulting in 370 enzymatic genes, which did not change in numbers during the simulation. The purpose of each DO was to evolve to optimize the amount of energy (E) produced within the given environment (eC and eO), was calculated as log(E) and used as the fitness score, through reactions with other metabolites. Metabolite E behaved as a metabolic endpoint with only production and not usage.

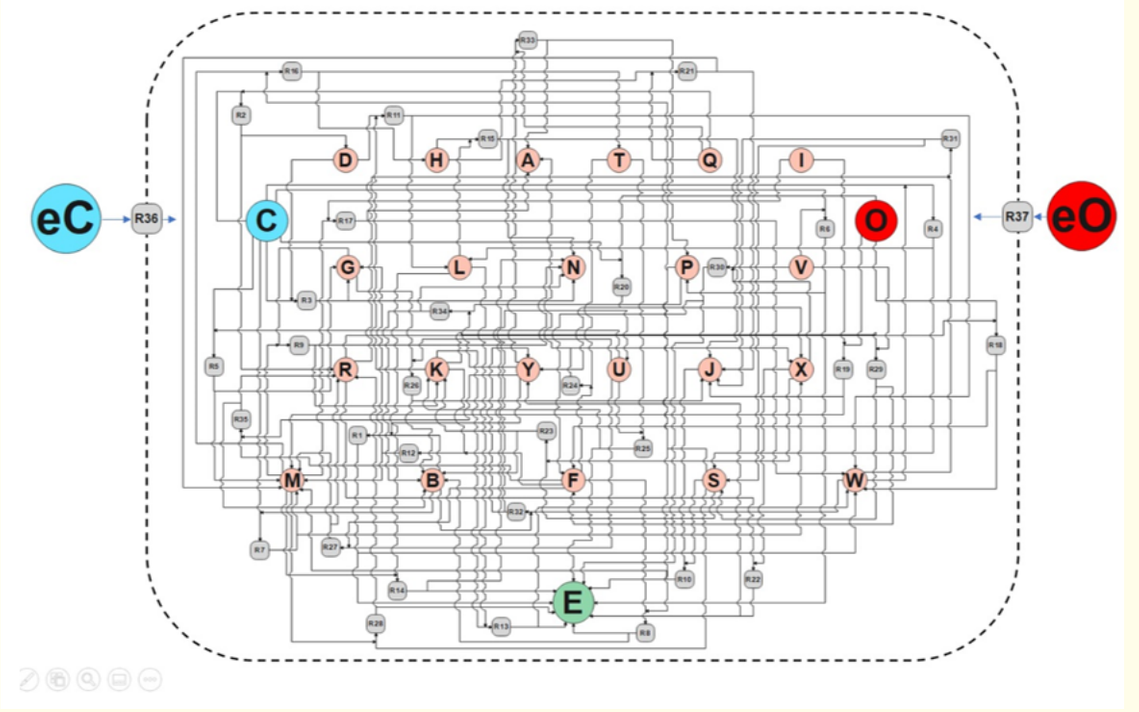

Figure 1: *Reaction map of Dennis interpreter. Extracellular carbon (eC) and extracellular oxygen (eO) were transported into the DO by R36 and R37 into intracellular carbon (C) and oxygen (O) respectively, which were used to produce energy (E). The amount of log(E) was then used as fitness score for the DO.*

Simulation setup: Each simulation consisted of 100 DOs for 900 generations. 20 replicated were performed for each trial. Mutation rate was set at 1% (about 3 to 4 base mutation per DO per generation). The selection process from one generation to the next is based on previous study [42-44]. Briefly, the lowest decile of the organisms by fitness were removed at each generation. However, in event where more

than 50% of the population were removed, a random selection of 10 organisms were removed instead. A random selection of remaining organisms after removal were replicated to top up the population to 100 organisms for the next generation. Each DO is given an empty cytoplasm (all intracellular metabolites were set to zero) before executing the metabolism coded by its genome.

Simulation trials: Two sets of trials were conducted - high, and no carbon - each consisting of one control and two treatments. In high carbon trial, the environment in control consisted of a constant 10 and 21 molecules extracellular carbon (eC) and extracellular oxygen (eO), respectively. In high carbon treatment A (gradual decline in extracellular oxygen), DOs were adapted for the first 100 generations to control environment (constant 10 molecules of eC and 21 molecules of eO), followed by a reduction of 3 molecules of eO per 100 generations to no extracellular oxygen from 100th to 799th generation, before reverting to control environment after 800th generation for another 100 generations. In high carbon treatment B (sudden decline in extracellular oxygen), DOs were adapted for the first 100 generations to control environment (constant 10 molecules of eC and 21 molecules of eO), followed by no extracellular oxygen from 100th to 799th generation, before reverting to control environment after 800th generation for another 100 generations. In no carbon trial, the environment in control consisted of a constant 21 molecules extracellular oxygen (eO). In no carbon treatment A (gradual decline in extracellular oxygen), DOs were adapted for the first 100 generations to control environment (constant 21 molecules of eO), followed by a reduction of 3 molecules of eO per 100 generations to no extracellular oxygen from 100th to 799th generation, before reverting to control environment after 800th generation for another 100 generations. In no carbon treatment B (sudden decline in extracellular oxygen), DOs were adapted for the first 100 generations to control environment (constant 21 molecules of eO), followed by no extracellular oxygen from 100th to 799th generation, before reverting to control environment after 800th generation for another 100 generations.

Statistical analysis: The mean fitness score was calculated as the average of fitness score from individual DO for each simulation and generation. The grand mean of fitness score for each generation was calculated as the mean fitness score across 20 replicated simulations. Paired t-test was used to compare between two grand means of fitness scores.

Results and Discussion

Significant difference in energy production between high and no carbon: Our simulation results demonstrate significant differences ($t = 3.498$, p-value = 0.000492) in energy production between high and no carbon controls. In biological systems, energy production in the form of adenosine triphosphate (ATP) molecules is largely dependent on carbon metabolism [45,46], which relies on the availability of carbon. Hence, this result is expected and suggests that the DO's metabolism bears resemblance to biological systems. However, the mean of energy production difference in no carbon against high energy production is 0.60624, suggesting that no carbon results in higher energy production compared to high carbon. This may be plausible as studies on *Escherichia coli* suggest that aerobic environments resulted in reduce carbon intake, increased growth rate and ATP production in compared to anaerobic conditions [47,48], indicating an interaction between carbon and oxygen availability on energy production. Yet, this also suggests that oxygen may be a limiting factor over carbon in terms of energy production, which is supported by our results. Importantly, our results suggest that the DO's metabolism may be suitable to study the effects of oxygen adaptation in the context of carbon availability, which is the focus of this study.

Extracellular oxygen decline impacted on energy production: In high carbon environment (Figure 2), there is significant decline in energy production from control to gradual extracellular oxygen decline (Figure 2A, $t = 3.514$, p-value = 0.000464) but non-significant increase from control to sudden extracellular oxygen decline (Figure 2B, $t = 0.813$, p-value = 0.406). However, in no carbon environment (Figure 3), the trend is reversed - there is no significant increase in energy production from control to gradual extracellular oxygen decline (Figure 3A, $t = 1.666$, p-value = 0.0961) but significant increase from control to sudden extracellular oxygen decline (Figure 3B, $t = 5.513$, p-value = 4.62E-08). Compared to control, sudden extracellular oxygen decline resulted in increase in energy production regardless of carbon environment whereas gradual extracellular oxygen decline resulted in decreased energy production in high carbon environment but increased energy production in low carbon environment. This suggests a non-linear relationship between carbon and oxygen avail-

ability to energy production, which had been suggested in several species, such as *Staphylococcus aureus* [49], *E. coli* [50] and *Aspergillus niger* [51].

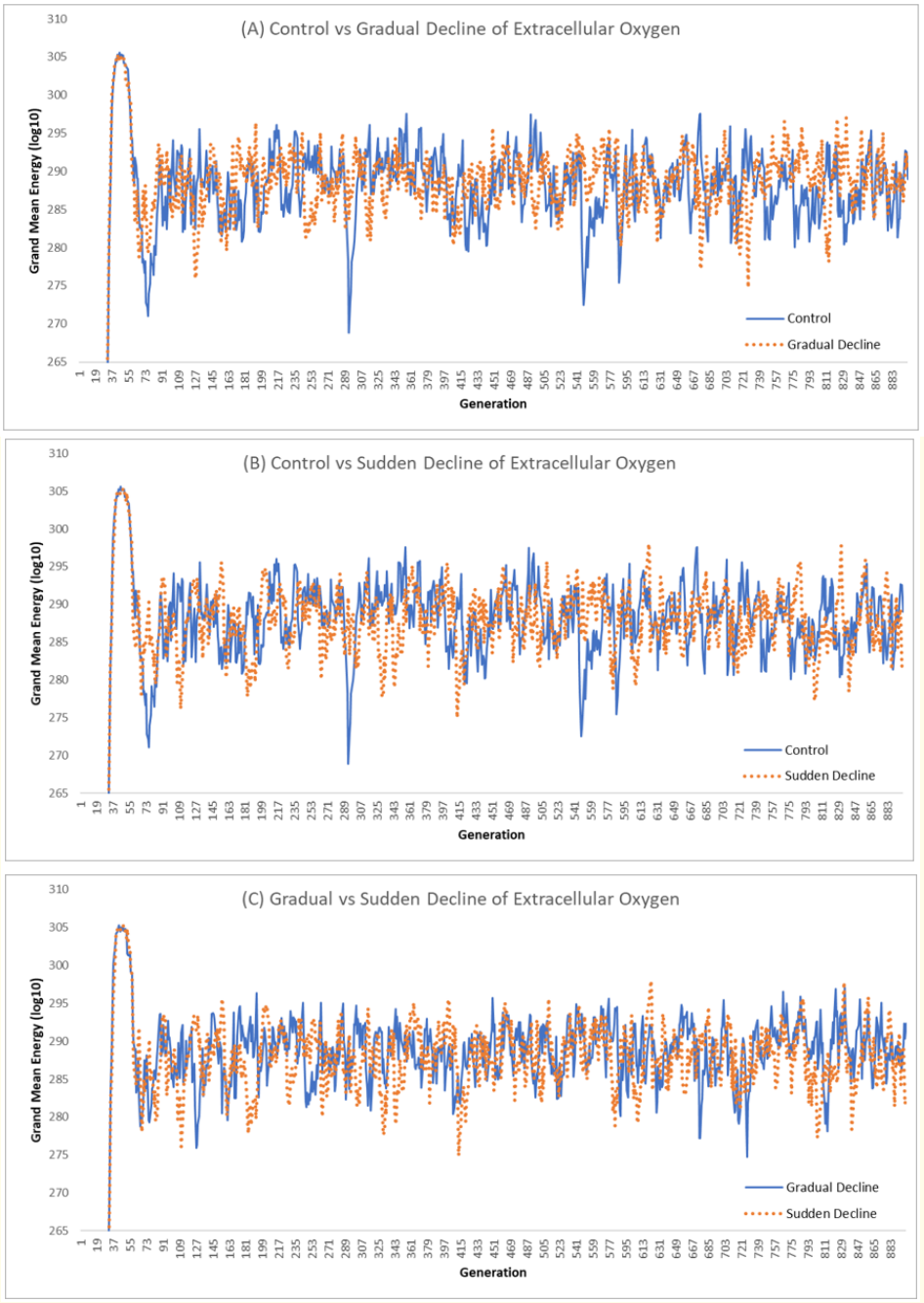

Figure 2: *High carbon environment.*

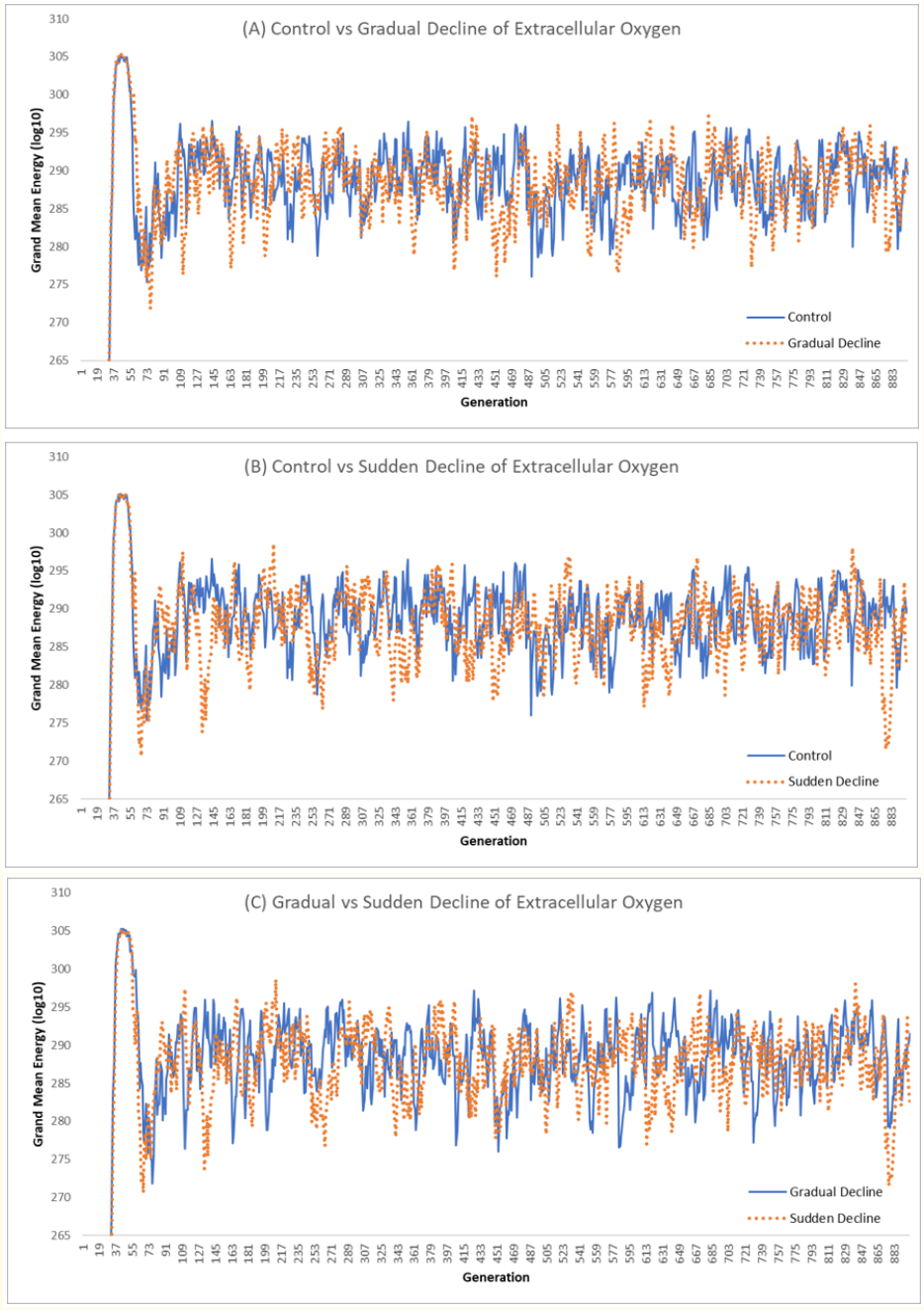

Figure 3: *No carbon environment.*

Sudden extracellular oxygen decline significantly increased energy production: While gradual decline in extracellular oxygen results in decline in energy production in high carbon environment, significant increases in energy production are seen in sudden decline in extracellular oxygen in both high (Figure 2C, t = 4.595, p-value = 4.93E-6) and no (Figure 3C, t = 3.744, p-value = 0.000192) carbon

environment compared to gradual decline in extracellular oxygen. This suggests that the evolutionary history (high vs no carbon) of DOs may have an impact on its adaptation to subsequent stress, which is supported by a similar study in *Pseudomonas fluorescens* [52]. Sudden extracellular oxygen decline resulted in increase in energy production regardless of carbon environment compared to control and is significant in no carbon environment (Figure 3B, t = 5.513, p-value = 4.62E-08). This suggests rapid adaptation of DOs to sudden harsh environment, which had been suggested by studies in biological organisms [53-58]. Our results may also suggest an adaptative over-compensation in sudden and steep harsh environments.

Conclusion

DOs in an alternative metabolic environment [33,34] were used to evaluate ecological niche adaptation. Our results suggest that sudden and steep harsh environments may result in over-compensation in adaptation compared to gradually increasing harsh environments.

Appendix: Reaction Definitions

Reaction	Definition
R1	Metabolite E and Metabolite W are equal to (Metabolite B + Metabolite Y)
R2	Metabolite R and Metabolite D are equal to (Metabolite C + Metabolite Q)
R3	Metabolite N and Metabolite G are equal to (Metabolite C + Metabolite D)
R4	Metabolite S and Metabolite L are equal to (Metabolite C + Metabolite W)
R5	Metabolite G and Metabolite M are equal to (Metabolite C + Metabolite U)
R6	Metabolite E and Metabolite P are equal to (Metabolite C + Metabolite V)
R7	Metabolite M and Metabolite X are equal to (Metabolite C + Metabolite B)
R8	Metabolite E and Metabolite B are equal to (Metabolite F + Metabolite P)
R9	Metabolite N and Metabolite K are equal to (Metabolite G + Metabolite M)
R10	Metabolite E and Metabolite M are equal to (Metabolite J + Metabolite S)
R11	Metabolite W and Metabolite L are equal to (Metabolite K + Metabolite D)
R12	Metabolite G and Metabolite M are equal to (Metabolite K + Metabolite F)
R13	Metabolite E and Metabolite W are equal to (Metabolite K + Metabolite L)
R14	Metabolite E and Metabolite N are equal to (Metabolite L + Metabolite M)
R15	Metabolite J and Metabolite N are equal to (Metabolite L + Metabolite H)
R16	Metabolite H and Metabolite T are equal to (Metabolite M + Metabolite S)
R17	Metabolite A and Metabolite W are equal to (Metabolite M + Metabolite I)
R18	Metabolite W and Metabolite F are equal to (Metabolite O + Metabolite R)
R19	Metabolite J and Metabolite M are equal to (Metabolite O + Metabolite I)
R20	Metabolite X and Metabolite Y are equal to (Metabolite O + Metabolite C)
R21	Metabolite J and Metabolite M are equal to (Metabolite Q + Metabolite H)
R22	Metabolite E and Metabolite Y are equal to (Metabolite R + Metabolite X)
R23	Metabolite K and Metabolite B are equal to (Metabolite S + Metabolite X)
R24	Metabolite X and Metabolite A are equal to (Metabolite T + Metabolite F)
R25	Metabolite E and Metabolite K are equal to (Metabolite T + Metabolite U)
R26	Metabolite J and Metabolite B are equal to (Metabolite U + Metabolite G)
R27	Metabolite R and Metabolite M are equal to (Metabolite U + Metabolite F)

R28	Metabolite E and Metabolite R are equal to (Metabolite M + Metabolite S)
R29	Metabolite S and Metabolite F are equal to (Metabolite V + Metabolite K)
R30	Metabolite E and Metabolite F are equal to (Metabolite V + Metabolite X)
R31	Metabolite J and Metabolite S are equal to (Metabolite W + Metabolite R)
R32	Metabolite Y and Metabolite U are equal to (Metabolite W + Metabolite B)
R33	Metabolite P and Metabolite A are equal to (Metabolite Y + Metabolite Q)
R34	Metabolite B and Metabolite N are equal to (Metabolite Y + Metabolite P)
R35	Metabolite B and Metabolite R are equal to (Metabolite Y + Metabolite F)
R36	Metabolite O is equal to (eO + Metabolite O)
R37	Metabolite C is equal to (eC + Metabolite C)

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