Title :

Mechanistic feedback and non-mechanistic whole systematic autonomy of living organisms : what is the difference ?

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1)   Homeostasis of the physiological parameter : as a mechanistic control

Because some physiological parameters are essential to maintain life, and its deviation may sometimes cause fatal impact, homeostasis of these parameters are indispensable to the living organism. Feedback is a kind of self-control mechanism, to detect the change of some crucial parameters as 'signal' and to adjust the physiological parameters as 'response' in order to cancel the initial deviation of the parameter.

Feedback may sometimes formulate the protocol to revise the procedure to realize the designed goal, which is induced by genome strongly or weakly (for example, procedure of morphogenesis by the apoptosis).   It may consist of complex multistage feedback loops, which induced to follow the one way chain-reactions of cause-and-effect relationships.   Essentially, the feedback system may be constructed by a sum of connected Signal/Response relationships, which are mechanistic and inductive.

But the living organisms are more than the sum of these mechanistic procedures.   They formulate the structure by their autonomy, which may be a source of diversity and fitness of living organisms.   There may be a general rule to explain how the structures of the living organisms are emerged as a whole system.   DEB model may suggest the general simple rule of metabolism , which is applicable to all the living organisms.   But it also must be noted that Organ may be more than the sum of the component cell.

General saying 'Whole is more than the sum of its parts.' could be rephrased as 'The cell is more than the sum of its molecule.' and 'The organ is more than the sum of its cell.' and 'The individual life is more than the sum of

its organ' and 'The population is more than the sum of its individual life'. When the equilibrium of the whole system is established, DEB model could draw the general rule of living materials, that leads the system to the homeostasis. But in the transition of the system, DEB model might not be applicable, strictly. Homeostasis is realized by the physiological given parameters at their equilibrium conditions and combinations.

2) Homeostasis of the Microcosm : as a sustainability of the system

Microbial ecosystem (Microcosm) consists of environmental stochasticity, demographic stochasticity of microbes and their random combinations, which cause the instability of the whole ecological system. This is a nature of non-linear complex system, of which dynamics are heuristic in general. As the reference experimental ecosystem, the aquatic microbial ecosystem of autotroph protozoa, heterotroph protozoa and saprotroph bacteria (Kawabata-microcosm) has been scrutinized. Outline of Kawabata microcosm is illustrated in **Appendix A**.

Population is a sum of each individual, but population dynamics as a whole may not be the sum of each individual dynamics. It must be emphasized again that for any living organisms, the whole is more than the sum of its parts. Population dynamics of each species should overshoot and undershoot before they reach the equilibrium balances of their own. When the dynamics were lost by some impacts (for examle, radiation exposure, chemical toxicants, etc.), a present equilibrium would be lost and it may shift to the another equilibrium balances possibly, or extinct possibly. In the shift of equilibrium, the ecosystem is transient, and drastic selections are forwarded naturally to increase the fitness of the ecosystem as a whole (to increase the sustainability of the ecosystem).

Homeostasis of the microbial ecosystem depends on the structure formed by the connected interactions between the lives and environment, Even if the primary conditions are different, Kawabata-microcosm reaches the equilibrium with same structure, eventually. The equilibrium of the ecosystem is self-organized, by not only the physiological control but also the relative interactions between each microbe and environment.

The effect of acute irradiation on this self-maintaining microbial ecosystem demonstrate the non-lethal response in one component species could trigger a lethal response in another. Heuristic and systematic approach is required to find out the general rule to organize the system structure and dynamics. I expect DEB model will be useful to find out some sets of physiological parameters, which makes it possible to reach the sustainable equilibrium. And to study about the stochasticity and randomness of the ecosystem in the transient conditions, computer simulation based on the particle-based computational ecology that built-in the DEB model will reveal the mechanism of ecological robustness against the environmental impact and its dose-response relationship. Our simulation model is named SIM-COSM, of which procedures related to allometry are illustrated in **Appendix B**.

3. DEB model : a possible rule for the general relationship between the size of body and metabolic process

At first appearance, DEB model is provocative, because it suggests the 'objective' observation that includes the implicit subjective interpretation from irregular point of view. We generally plot the time dependent change of population, and find a linear population dynamics. But if we think about the complex relations included in the data, the linear regression must be regarded no more than the most simple model that only showed the good agreement with data. If the linear model is not based on the theoretical general thinking of basic mechanisms, it can not reveal any secret of the system. To fit the data is not so important for the model. Theoretical consistency that make sense between each component basic physiological model, like DEB model, is most important.

It is difficult to accept the too simple relationship between body size and metabolism (allometry) as the general rule of microbes in the actual microcosm. Some empirical observations suggest that the dynamics of the ecosystem depend on the time after inoculation (integration or the history of ecosystem). DEB model suggests the relations of body size and metabolism at the equilibrium conditions, where time is defined infinite or stopped. To

split the observation of ecosystem by time, the interactions and state variables shows the dynamic budget of the system.   DEB model may produce the effective analytical method to understand the interactive mass-energy budget of the system at the time of interest.

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Appendix A　　　　Aquatic microbial ecosystem (Kawabata microcosm)

　　　　The Kawabata microcosm consists of :
- Heterotroph ciliate protozoa, Tetrahymena thermophila B, as a consumer, of which body length is 40 micro-m in steady-state (7) and eat bacteria with oral structure of 4-5 mm in diameter.
- Autotroph flagellate algae, Euglena gracilis Z, as a primary producer that absorb CO_2 (H_2CO_3) gas and synthesize organic materials with light energy, of which body length is 40 micro-m in steady-state.   And,
- Saprotroph bacteria, Escherichia coli DH5 a, as a decomposer, of which length is about 1mm, that dissolves metabolic wastes or corpses (detritus) into humus or biogenic salts and CO_2 (H_2CO_3) which can be recycled by protozoa.
- The culture medium was 10 ml of #36 Taub and Dollar's salt solution containing proteose peptone of 500 mg / L in the flask tube.   The tube is firmly isolated from the outer environment to prevent gas and materials exchange.

　　In the above concept, material-cycle must be organized as a complex of symbiotic metabolisms.   The flask was batch-cultured with fluorescent 2,500 lx lamps under 12 hour light-dark cycle to make a circadian rhythm of Euglena

in an incubator at 25 °C (energy flow). Microbes maintain themselves and realize symbioses with combined interactions of resource use competition, predation, mutualism or protocooperation.

Microcosm is a experimental model that pro-vides biotic or abiotic simplicity, controllability, repeatability to evaluate the ecological effects of radiation exposure and other environmental toxic agents.

Single culture :

- When Tetrahymena was cultured alone in the microcosm medium, it terminated within 20 days after inoculation.

- When Euglena was cultured alone, it could not reach population levels as it does in the microcosm (three species).

- When E.coli was cultured alone, it could not reach population levels as it does in the microcosm (three species).

Two species culture :

- Tetrahymena and E.coli :   Tetrahymena can not live without E.coli, because it grazes E.coli as its main resources and feeds it up.

- Euglena and E.coli :   In the early stage of culture, proteose peptone supports the population growth of Euglena and E. coli, and they reach their plateau at 20 - 30 days after inoculation when proteose peptone may exhaust. After the exhaustion of proteose peptone, the two species-environment system is maintained with energy fixed by photosynthesis of Euglena, and supported with organic materials dissolved by E. coli from metabolites or the corpses of the species.

In the 3 species microcosm :

- Tetrahymena, Euglena and E.coli can coexist for 1 year and more.

Appendix B   SIM-COSM   –   a particle-based model of the microcosm

Population orders :

Tetrahymena (predator) is 10^4 cells/ml, Euglena is 10^5 cells/ml and E.coli is 10^8 (at first days) to 10^6 cells/ml (prey).   Particle-based identification is done for Teterahymena and Euglena.   E.coli is not identified as a cell, but a population density at each patch of environment (10201 patches

for 0.1 ml of water), which diffuse by swimming and growth by proliferation. So, the SIM-COSM model generates around 10,000 - 12,000 particles of Euglena and 5,000 - 3,000 particles of Tetrahymena. Populations changed drastically by the time after inoculation.

The substrate is protease peptone + taub medium (inorganic required for the growth). Single batch culture of E.coli showed a typical logistic growth pattern (up to 10^8 cells/ml), and after several days, it started to decrease constantly but slowly (1 year later, small population is still survived). If their population was limited by peptone, they could not show such a slow decrease in the SIM-COSM model, because E.coli run out their reserves by no intake, and no reserve caused the decrease of structural mass. When the structural biomass is less than the 1/8 of the max biomass with enough substrate, E.coli dies in the SIM-COSM model. 1/8 is from the observation of the E.coli lengths of alive and dead at each day after inoculation, but the value has a large uncertainty (deviations from the average) indeed.

Single batch culture of Tetrahymena showed an exponential growth (doubling time about 4 hours) in first 10 days, and died out by the following sudden extermination which occurred within 10 more days. They can utilize protease peptone, but they does not form phagosomes in the body (cell), because there is no particle food, like E.coli.

In the SIM-COSM model, it is assumed that Tetrahymena can only use the solute part of Protease peptone, and its intake is done by the diffusion through the membrane, which is proportional to the body surface.

Solute peptone may be less than 1 % of total peptone, by the mass and energy budgets assumption (just by the empirical metabolic parameters of Tetrahymena).

Single batch culture of Euglena increased exponentially (doubling time about 12 hrs) but the carrying capacity is less than the coupled (Euglena + Tetrahymena, Euglena + E.coli) or tripled culture (Euglena + Tetrahymena + E.coli) . In the SIM-COSM model, Euglena can utilize the part of peptone

(not solute peptone) , and its intake is done by the diffusion through the membrane, which is proportional to the body surface.   When Euglena is cultured with another microbes, Euglena ca utilize another microbe's metabolic materials and/or detritus, which is originated from the peptone not usable by Euglena.

Protease peptone consists of many kinds of organic (proteins, fats, amino acids, etc.), and actually, the food web is much more complicated, if the multi compounds of the peptone are taken into account.   In this context, I think it necessary to review the DEB book 2nd ed. section 5 (several substrates, etc.).   In the SIM-COSM model, Protease peptone is divided into solute part (for Tetrahymena and E.coli), diffusive part (for Euglena and E.coli) and rest (for E.coli only, most of the peptone is included in this class).

In the latest SIM-COSM model, accumulation of nitrogen is not covered suffiently, which is left for future assignment.   One reason is that release and intake of nitrogen (NH4) is not sometimes balanced in the aquatic environment, empirically.   NH4 in the water was balanced at low level (closed to zero) in the first phase of triple culture of Euglena, Tetrahymena and E.coli, and shifted to the elevated level.   Uptake of nitrogen is an important part of Euglena, which makes photosynthesis, and following biosynthesis (nitrogen is required in this process). Second reason is the extraordinary high pH values in the water of aquatic microcosm.   Repeated experimental results of pH showed that the pH 8 - 9 in 50 days after inoculation as the results of experiment. For Euglena, proper pH for growth is 4 to 5.   pH value is one of the most puzzled issues, which is not yet answered clearly.

Chemical forms of Inorganic carbon, released into environment as CO_2 , is mostly changed to $CO_3(2-)$, and $HCO_3(-)$.   It is not yet clear but, Euglena may have the path to intake $HCO_2(-)$ and change it to CO_2 by the biosynthesis process (paying energy for the process) or not.   Some kinds of sea algae have this kind of dual input processes (CO_2 direct, HCO_3- to CO_2 conversion process) in front of the main photosynthesis process.   Some articles say that

Euglena also can use HCO3-, but the experimental results were not included in them.   Under these severe conditions of pH 8-9, it is truly skeptical if photosynthesis of Euglena is done normally.   Radioactive carbon (C-14) tracer experiment, and stable isotope ratio (delta-value) of Euglena studies are ongoing by my colleagues to clarify the photosynthesis process of Euglena.

Related to the pH, we have one interesting result.   Single culture of Euglena in the different substrate (NH4Cl as nitrogen source + taub medium) , pH of the water is 7 at the inoculation and 4-5 after 50 days inoculation. Maybe, this is because Euglena utilized NH4 and absorbed CO2 in the water by their photosynthesis and biosynthesis,   the resulted balance of NH4   and CO2 in the water is made low pH.   Another mechanism is included in the taub medium components, which is a mixture of several inorganic chemicals. Details are not yet explained totally, but some reasons of the elevated pH 8-9 in the peptone culture are proposed in the SIM-COSM model.

In the dark, Euglena acts as heterotroph protozoa.   They intake organic materials and assimilate them to form the reserves, which allocate (maintenance + growth) and (development + reproduction) by k-rule. Euglena reproduces itself by dividing lengthwise, sharing materials and energy, equally.   Cost for Mitosis (M-phase) is development cost (or maturity maintenance cost) and cost for the synthesis (S-phase) is reproduction cost in the SIM-COSM model.   Each particle (cell) of Euglena (and Tetrahymena) has their own cell cycle indicator, related to G1 (G0) - S-G2-M phase cycles. There is the checkpoint between the G1 and S by size (mass) and environmental condition,   and there is another checkpoint between G2 and M by the size (mass), environmental condition, and verify the copy of genetic information. Based on the survival curve of each microbe, the impact to each cell is estimated. Each particle (cell)   eats, respirates, wastes, stock, pay maintenance and growth, and develop and reproduce in the computer by interaction between particles and environment (E.coli is a part of environment for Tetrahymena and Euglena).

In the SIM-COSM model, E.coli is the only ones that decompose wastes. But experimental results of gamma radiation exposure (250 Gy) to the Microcosm at 50 days after inoculation, E.coli (lethal-50%-dose, LD50 = 50Gy) was died out, and Euglena (LD50 = 330-170 Gy) and Tetrahymena (LD50=4000Gy) were survived.   When Tetrahymena was cultured alone (without E.coli) with peptone, they extinct within 20 days after inoculation. But, in the above exposure experiment, Tetrahymena population was reduced down significantly in comparison with control (no gamma radiation case), but survived for 150 days and more (eventually extinct within a year).   There was no E.coli and no peptone left in the environment after exposure of radiation. By this experimental results may suggest that Tetrahymena can utilize the wastes (detritus) if they starved severely.   It must be considered if metabolic parameters of survived Tetrahymena (Euglena) are not changed by such a high dose of radiation exposure.   This is not yet included in the SIM-COSM model, left as my future assignment.

There is a predator - prey relationship only between Tetrahymena and E.coli.   (Tetrahymena eat on E.coli.)   There are several kinds of inorganic ions and organic materials (substrate peptone, waste material, dissolved dead body, etc).   SIM-COSM model partitioned 10 ml of water formally into 100 homogeneous cells of 0.1 ml.   Actually, dead body and detritus is settled at the bottom of the tube, which may be a niche of E.coli.   Tetrahymena move by swimming generally against gravity, and swim down to the bottom of the tube as a foraging behavior.   Euglena swim generally toward the light (and against the too strong light).   They move to quest for CO_2, and when the tube is open to the outside air, they swim up to the surface of water in the light, and swim downwards in the dark.   These spatial (vertical) structures due to gravity are not included in the SIM-COSM model.   We may need a help of super computer and make parallel computing to take this vertical structure of the environment, and 3 dimensional (vertical) behaviors of Tetrahymena and Euglena into account.

The web of interactions causes the non-linear response of the total

system, of which behavior is heuristic.   At first glance, the heuristic behavior and formation of structure may be self-organized.   Emergence is a surprise and guide us to the fundamental questions.   But, once, we understand the rules and mechanisms, the emergence is no more emerged, because we already know what it is.

At first, Microcosm is a kind of black box, of which behaviors (input/output relations) were inconsistent each other logically.   Most of the experimental results were emergence for me, but now, some of them have already not been emerged in my recognition.

Microcosm that consists of only 3 species microbes and simple environment , is complex enough, which includes the hidden mechanism of homeostasis as a whole system.   SIM-COSM is no more than a model, which is not real but simplified theoretical thinking tool.   I hope this tool will be beneficial for the experimental scientists to understand their target ecosystem, and the experimental results may correct and improve the model, in return.   Fitting to the results of Microcosm experiment is not so much important for the SIM-COSM model.   Consistency of the biological mechanisms in the model is most important for the future development.   I believe if I can understand what the Microcosm is, I can get one of the key to understand what the ecosystem is.

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