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Ecological Impacts of Environmental Toxicants and Radiation on the Microbial Ecosystem : A Model Simulation of Computational Microbiology

Masahiro Doi[1], Tetsuya Sakashita[1], Nobuyoshi Ishii,[1] Shoichi Fuma[1], Hiroshi Takeda[1],
Kiriko Miyamoto[1], K. Yanagisawa[1], Yuji Nakamura[1] and Zen'ichiro Kawabata[2]

[1] National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage, Chiba 263-8555, Japan
[2] Center for Ecological Research, Kyoto University, Kamitanakami, Hirano-cho, Otsu, Shiga, 520-2113, Japan

INTRODUCTION

From the view point of General System Thinking (1,2), ecology is a nonlinear system of complexity structured by interactions among components and environment. A mathematical system analysis is a model "which is an imperfect and shorthand illustration of the real ecosystem (3)", which is very difficult or impossible to solve numerically. Since very powerful mathematics and information tools are now available in terms of heuristic mathematical theory (e.g. cellular automata, genetic algorithm, etc.) and recent computer systems are highly sophisticated, it may be possible to describe how ecological systems and their components are likely to interact using computer simulation techniques by providing a simplified model on the basis of principles in the ecological systems, i.e., self-organization, feedback in response to disturbance, emergent behavior, diversity and so on (4).

The purpose of this study was: first to illustrate symbioses in the aquatic microcosm to test ecological principles (5) ; second to develop the computer simulation of the microbial closed-ecosystem as a self-sustainable system of complexity with connected feedback control between microbes and patched environment ; and third to investigate the dose-response of the microcosm exposed to γ-rays (6) for elucidation of the mechanisms that account for ecological impacts of radiation.

MATERIALS AND METHODS

1. EXPERIMENTAL MODEL ECOSYSTEM (MICROCOSM)

This study explores a microorganic closed-ecosystem (5) by computer simulation to illustrate symbiosis among populations in the microcosm. The ecosystem consists of :

(1) Heterotroph ciliate protozoa, *Tetrahymena thermophila B*, as a consumer, of which body length is 40 µm in steady-state (7) and eat bacteria with oral structure of 4-5 µm in diameter (8, 9) .

(2) 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 µm in steady-state (9, 10).

(3) Saprotroph bacteria, *Escherichia coli* DH5 α, as a decomposer, of which length is about 1µm, that dissolves metabolic wastes or corpses (detritus) into humus or biogenic salts and CO_2 (H_2CO_3) which can be recycled by protozoa (9).

The culture medium was 10 ml of #36 Taub and Dollar's salt solution (11) containing proteose peptone of 500 mg L^{-1} in the flask without exchanges of gas nor materials (material-cycle). 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 (5). Microcosm is a experimental model that provides biotic or abiotic simplicity, controllability, repeatability to evaluate the ecological effects of radiation exposure and other environmental toxic agents (4, 5). **Figure 1** shows the illustration of the principal interactions among protozoa, bacteria and patched- environment in the Microcosm.

Figure 2 shows the successional change of each organism's population cultured in the microcosm (5,6, 12). When Tetrahymena was cultured alone in the microcosm medium, it terminated within 20 days after inoculation as shown in Figure 2A. When Euglena and E. coli were cultured alone, they could not reach population levels as they do in the microcosm as shown in Figures 2B, 2C (5,6, 12). Tetrahymena can not live without E.coli as shown in Figure 2A and 2D, because it grazes E.coli as its main resources (12). In the early stage of culture, proteose peptone contributes to the population growth of Euglena and E. coli, and they reach their plateau at 20 - 30 days after inoculation when proteose peptone may exhaust as suggested in Figures 2E, 2F and 2G. After the exhaustion of proteose peptone, the microcosm is maintained with energy fixed by photosynthesis of Euglena, and supported

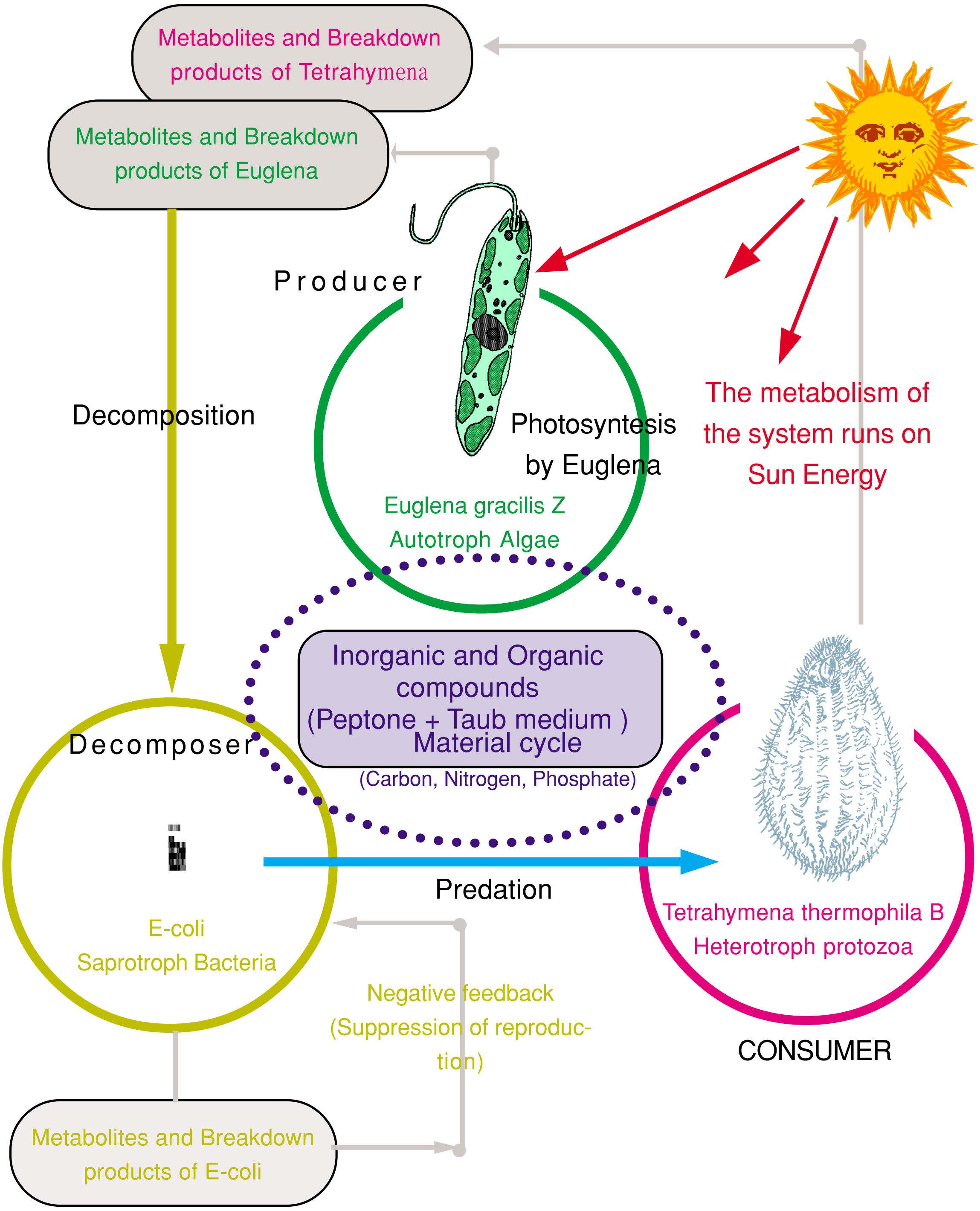


Figure 1 Principal interactions among protozoa, bacteria and environment in the Microcosm (5).

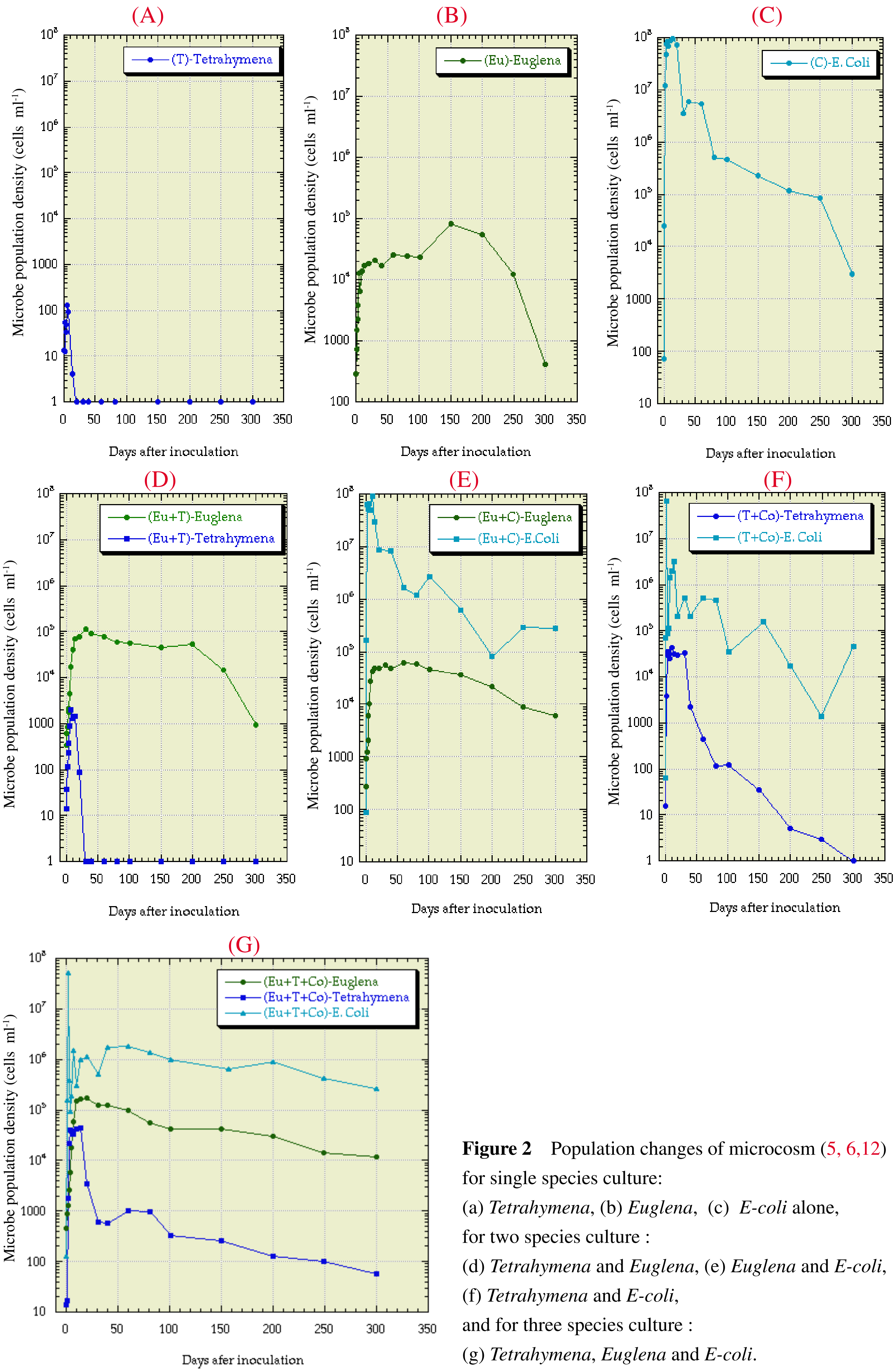


Figure 2 Population changes of microcosm (5, 6,12)
for single species culture:
(a) *Tetrahymena*, (b) *Euglena*, (c) *E-coli* alone,
for two species culture :
(d) *Tetrahymena* and *Euglena*, (e) *Euglena* and *E-coli*,
(f) *Tetrahymena* and *E-coli*,
and for three species culture :
(g) *Tetrahymena*, *Euglena* and *E-coli*.

with organic materials dissolved by E. coli from metabolites or the corpses of the species as shown in Figures 2E, 2F, 2G. In the microcosm, three species can coexist for 1 year and more as illustrated in Figure 2G.

2. PARTICLE-BASED COMPUTATIONAL MODEL ECOSYSTEM (SIM-COSM)

Since the ecosystem is self-organized by connected interactions among microbes and environment, their behaviors are written as a set of nonlinear differential equations, which can not be solved arithmetically. To analyze the behavior of self-organized nonlinear system of complexity, particle-based computer modeling is useful to replicate the real microcosm as the computational model ecosystem, SIM-COSM. SIM-COSM is written as a program of StarLogoT, a LISP-based computer programming language developed by the Center for connected learning and Computer-based modeling, Tufts University, Boston, U.S.A. (http://www.ccl.tufts.edu/cm/) as a superset of Starlogo developed by the Epistemology and Learning Group, Media Laboratory, Massachusetts Institute of Technology, Boston, U.S.A. (http://el.www.media.mit.edu/).

Environment is defined as 0.1 ml of water with organic and inorganic compounds of proteose peptone of 500 mg/L, which is divided into 10,201 (= 101 × 101) square lattice patches, of which side is 3,131 µm and height is 1 µm. **Table 1** shows a set of environmental parameters which is given to individual patch as an environmental attributes. For each environmental parameters and time step, each value of patch-parameters is shared with its neighboring patches by diffusion coefficient and calibration factor, which is scaled according to the time step (1 hour is regarded as 1 step) and lattice size (3,131 µm square). **Figure 3** illustrates basic concept of the particle-based modeling of SIM-COSM in this study which is constructed on the basis of MICROCOSM studies (5, 6).

Individual protozoa is regarded as a particle, which is defined as a set of demographic parameters to regulate individual substrate uptake efficiency, anabolism yield, maintenance rate and growth rate, based on the Dynamic Energy Budgets Theory in Biochemical Systems (13, 14). These demographic parameters are given to each protozoa by assuming them to fit the normal distribution within its standard deviation, 10 % of the mean. **Table 2** shows the list of demographic parameters and sample values derived from physiological and biochemical data of Tetrahymena (7,8,9) and Euglena (9, 10). When the biomass exceed the breeding threshold at the starting point of the cell cycle (G1-phase), Tetrahymena and Euglena step forward to synthesis (S-phase), and reproduce themselves by cell-division or cloning. Genetic floats in the population of Tetrahymena and Euglena can be observed by following up the clone generation. Each Microbes die when their biomass fell short of their lethal level. In the exponential growth phase, average cell cycle of Tetrahymena, Euglena and E. coli are 4 hours, 13 hours and 20 minutes (7, 9, 10).

2.1 OPTIMAL FORAGING STRATEGY OF TETRAHYMENA

In the model, it is assumed that E.coli forms an aggregation which cause a kind of contact inhibition to suppress the exponential growth of population. It is also assumed that maintenance rate and respiration rate of each protozoa is in proportion to its biomass. Tetrahymena is assumed to follow the foraging strategy by moving with cilia to the heading direction toward the maximum E. coli metabolites in the eight patches surrounded. Whether to stay or move from the present patch is decided by the conditional expectation as a function of its biomass. Since the biomass is regarded as a record of feeding in the past several hours, the foraging strategy is optimum if E. coli in the patch environment shows negative binomial (clustered) distribution (15) .

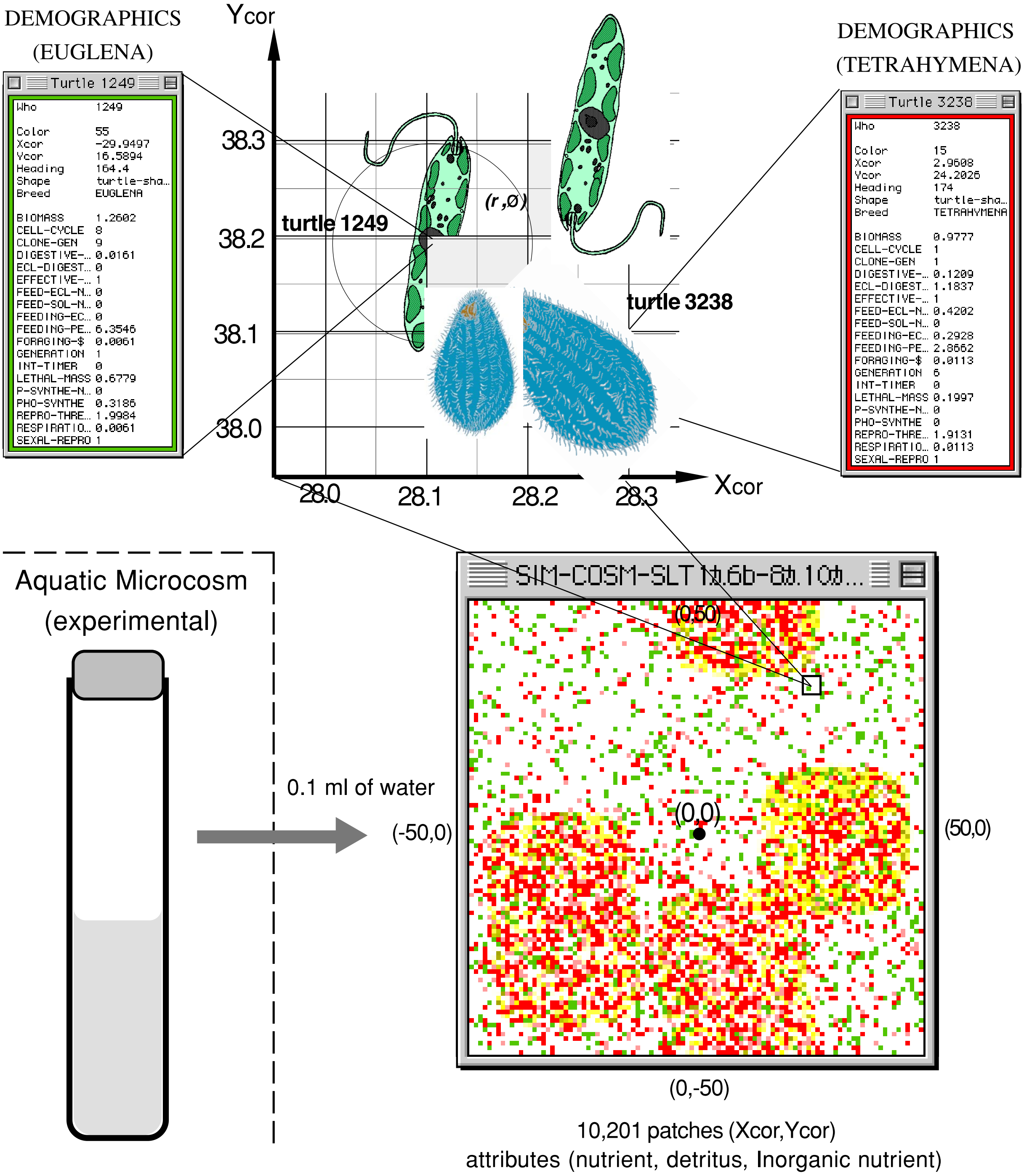
2.2 POTENTIAL OF HYDROGEN, CO_2 AND NH_3 CONCENTRATIONS IN THE LOCAL PATCHES

pH of culture medium is approximately 6.8 - 7.0, and it increases generally to 8.0 - 9.0 when microcosm reaches to the steady-state condition (16). Absorption of CO_2 (H_2CO_3) due to the photosynthesis of Euglena, and accumulation of NH_3 (NH_4OH) included in the waste materials and dead bodies may explain this partially. Taking the equilibrium of $[H_3O^+] \times [HCO_3^-] = k_\alpha [H_2CO_3]$ ($k_\alpha = 3.04 \times 10^{-7}$ mol / L) and $[NH_4^+] \times k_w = k_\beta [NH_4OH] \times [H_3O^+]$ ($k_\beta = 1.70 \times 10^{-5}$ mol / L) into account, H_2CO_3 content in each environmental patch can be estimated as $[H_2CO_3] : [HCO_3^-] = 0.033 : 1.0$, when pH is 8.0 at 25 ˚C. Equilibria of $[H_2CO_3]$, $[HCO_3^-]$, $[NH_4OH]$ and $[NH_4^+]$

in each local patch are adjusted in the computer simulation of SIM-COSM.

2.3 RADIATION EXPOSURE TO THE MICROCOSM

Tetrahymena is known to have great resistance to X-ray exposure, of which LD_{50} is 1,200 - 1,500 Gy (7, 17), and as well, Euglena resists X-ray, α, β and γ-ray exposure strongly (9, 10). The exact opposite of these protozoa, E. coli is sensitive to γ-ray exposure, which extinct immediately after irradiation of 500 Gy (6, 18). In the SIM-COSM, γ-ray exposure is simulated to hit environmental patches randomly in proportion to the exposure rate, and to kill E. coli cells included in the irradiated patch.



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Who           1249

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Ycor          16.5894
Heading       164.4
Shape         turtle-sha…
Breed         EUGLENA

BIOMASS       1.2602
CELL-CYCLE    8
CLONE-GEN     9
DIGESTIVE-…   0.0161
ECL-DIGEST…   0
EFFECTIVE-…   1
FEED-ECL-N…   0
FEED-SOL-N…   0
FEEDING-EC…   0
FEEDING-PE…   6.3546
FORAGING-$    0.0061
GENERATION    1
INT-TIMER     0
LETHAL-MASS   0.6779
P-SYNTHE-N…   0
PHO-SYNTHE    0.3186
REPRO-THRE…   1.9984
RESPIRATIO…   0.0061
SEXAL-REPRO   1
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Who           3238

Color         15
Xcor          2.9608
Ycor          24.2026
Heading       174
Shape         turtle-sha…
Breed         TETRAHYMENA

BIOMASS       0.9777
CELL-CYCLE    1
CLONE-GEN     1
DIGESTIVE-…   0.1209
ECL-DIGEST…   1.1837
EFFECTIVE-…   1
FEED-ECL-N…   0.4202
FEED-SOL-N…   0
FEEDING-EC…   0.2928
FEEDING-PE…   2.8662
FORAGING-$    0.0113
GENERATION    6
INT-TIMER     0
LETHAL-MASS   0.1997
P-SYNTHE-N…   0
PHO-SYNTHE    0
REPRO-THRE…   1.9131
RESPIRATIO…   0.0113
SEXAL-REPRO   1
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Figure 3 Concept of the particle-based modeling of SIM-COSM based on the MICROCOSM studies (5, 6, 17)

E. coli concentration	(cells patch^{-1})
Biomass of alive E. coli	(ng patch^{-1})
Proteose Peptone	(ng patch^{-1})
Metabolites of Tetrahymena	(ng patch^{-1})
Dead body of Tetrahymena	(ng patch^{-1})
Metabolites of Euglena	(ng patch^{-1})
Dead body of Euglena	(ng patch^{-1})
Metabolites of E. coli	(ng patch^{-1})
Dead body of E. coli	(ng patch^{-1})
H_2CO_3 (CO_2)	(mol patch^{-1})
HCO_3^-	(mol patch^{-1})
NH4OH	(mol patch^{-1})
NH4$^+$	(mol patch^{-1})

Table 1 Environmental attributes of individual patch.

Biomass (ng cell^{-1})
ATP reserves (ng)
Threshold biomass to reproduction (ng cell^{-1})
Threshold biomass to die (ng cell^{-1})
Absorbed culture medium (ng hr^{-1})
Grazed biomass of E. coli (ng hr^{-1})
Maximum Photosynthesis rate (ng hr^{-1} biomass^{-1})
Achieved photosynthesis products (ng hr^{-1})
Cell cycle (G0, G1, Mitotic-phase, Synthesis-phase)
Age of the cell (hr)
Time of birth (-)
Identification number of original cell (-)
Horizontal generations by cloning (-)
Vertical generations by mutation (-)
Assimilation rate of culture medium (%)
Assimilation rate of E. coli biomass (%)
Growth rate (%)
Respiration rate (%) in proportion to biomass (ng cell^{-1})
ATP reserve rate (%) in proportion to biomass (ng cell^{-1})

Table 2

List of demographic attributes of individual protozoa

RESULTS AND DISCUSSION

Computer experiments using SIM-COSM suggested an emergence, which explain probabilistic behaviors of protozoa and bacteria in the microcosm. **Figure 4a** shows the population changes of each organism from 0 to 10 days after inoculation, and the grazing behavior of Tetrahymena is illustrated in **Figure 5**.

Tetrahymena created in SIM-COSM was programmed to leave a patch when E.coli capture rate falls below the marginal energy requirement to pay for the physiological maintenance cost. The marginal energy requirement increases in proportion to the biomass (19).

0 - 12 hrs after inoculation ; Tetrahymena moved randomly in the environment, but their E. coli capture rate could not reach the marginal energy requirement.

24 - 48 hrs after inoculation ; E. coli proliferated exponentially by using proteose peptone and formed a cluster. In the core region of cluster, the shortage of proteose peptone and the contact inhibition may cause the ceiling of E.coli population in the patch. E.coli cells at the outer surface of cluster diffused to the neighboring patches and proliferated. Now, Tetrahymena encountered by chance with one of the E.coli clusters, and stayed in the patch because E.coli capture rate exceeded its own marginal energy requirement.

48 - 72 hrs after inoculation ; Tetrahymena had proliferated to the carrying capacity of the patch, as a overshoot, and gave up the patch because of the shortage of E.coli, its main food. Give-up-time (GUT) of the given patch was short in this case, because the marginal energy requirement was relatively high reflecting the large biomass of Tetrahymena which was carried by the rich proteose peptone in the overall environment.

72 - 120 hrs after inoculation ; Diffusion and proliferation of E. coli was carried by the proteose peptone in the virgin patches, and large population of Tetrahymena were fed. In this case, it was difficult for E. coli to form the cluster because of the strong predation by the large numbers of Tetrahymena. **Figure 4b** shows the population changes of each organism up to 40 days after inoculation. Population of E.coli decreased gradually around 20 days after inoculation and the decrease of Tetrahymena followed the trend with delay. This might be caused by the running out of proteose peptone in the overall environment, and the SIM-COSM simulation supported this hypothesis as shown in **Figure 6**. Sudden decrease of Tetrahymena population has a risk of extinction and there are some mathematical theories to predict it by the finiteness of population size (demographic stochasticity) and environmental stochasticity (20,21), which is left as

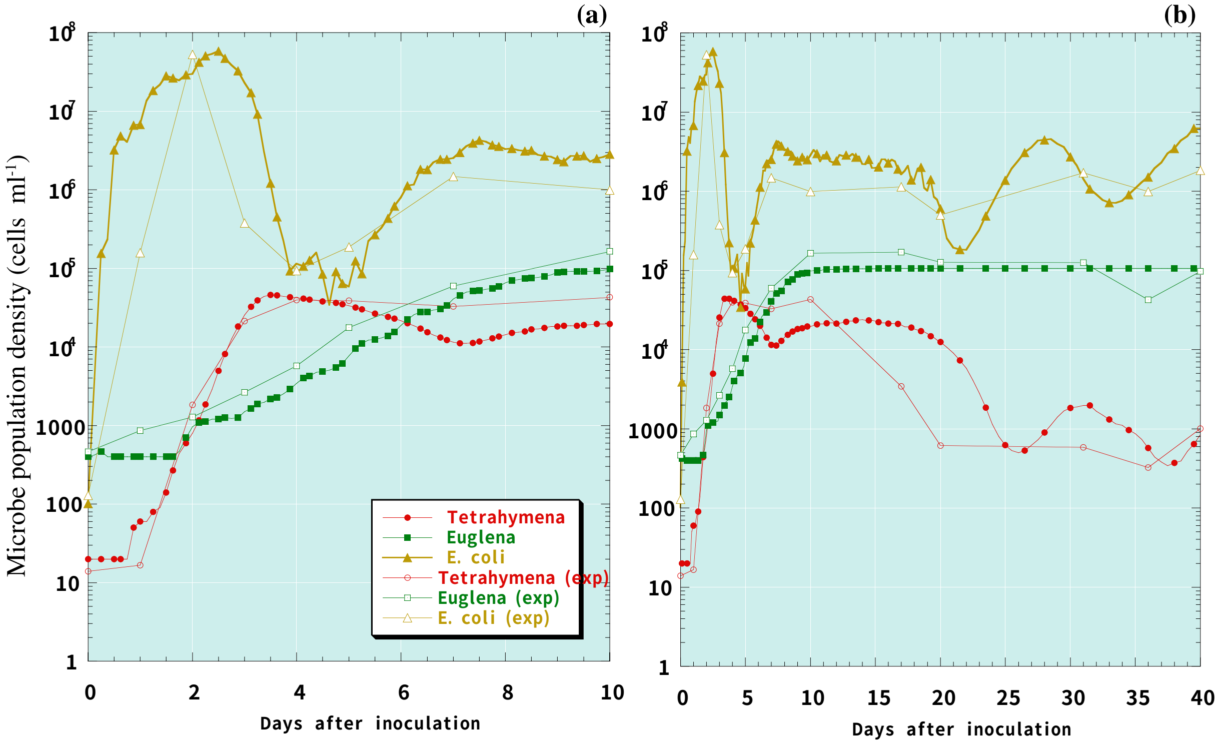
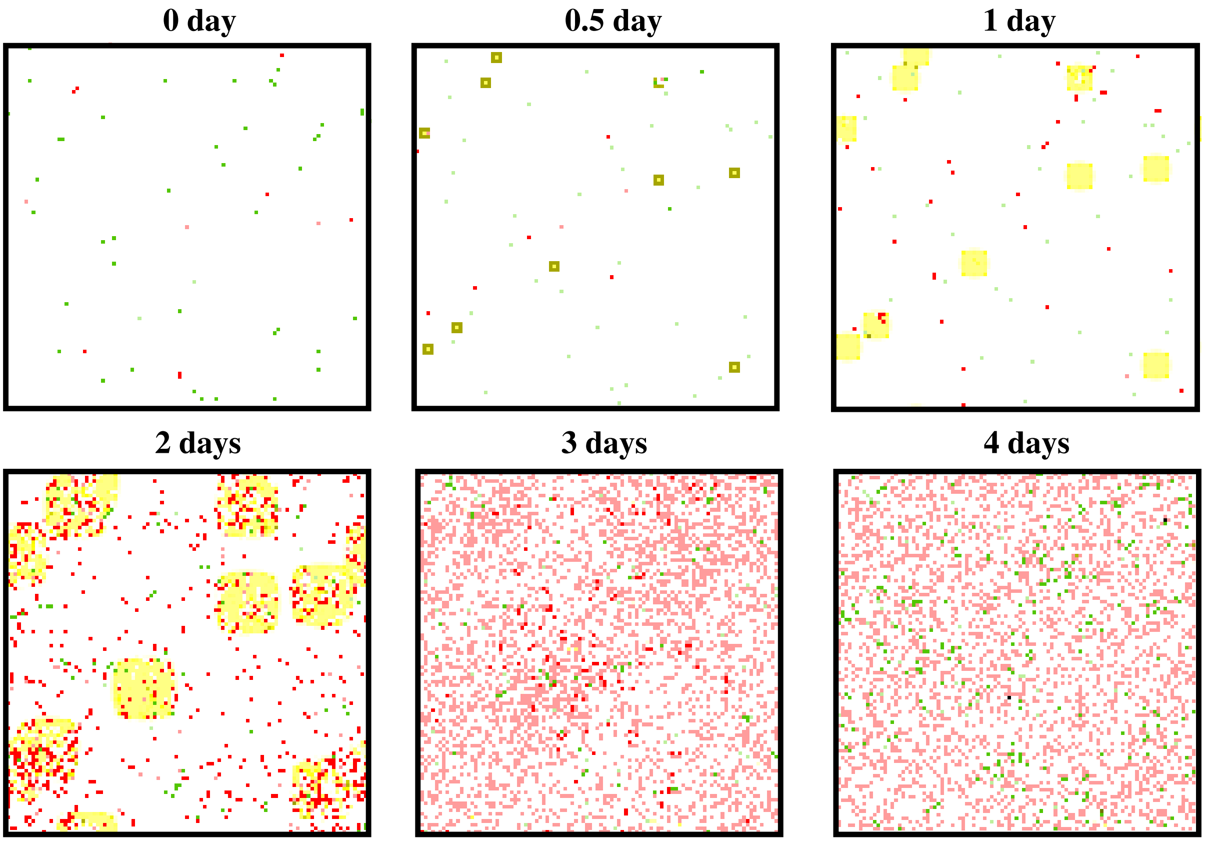


Figure 4 Population changes of each organism in the SIM-COSM : Dotted lines are experimental data from Kawabata et al. and Fuma et al. (5,6)


0 day

0.5 day

1 day

2 days

3 days

4 days

Figure 5 Grazing behavior of Tetrahymena : each red dot is a cell of Tetrahymena, green dot is a Euglena cell and background gradient of the patch environment shows the spatial distribution of E. coli.

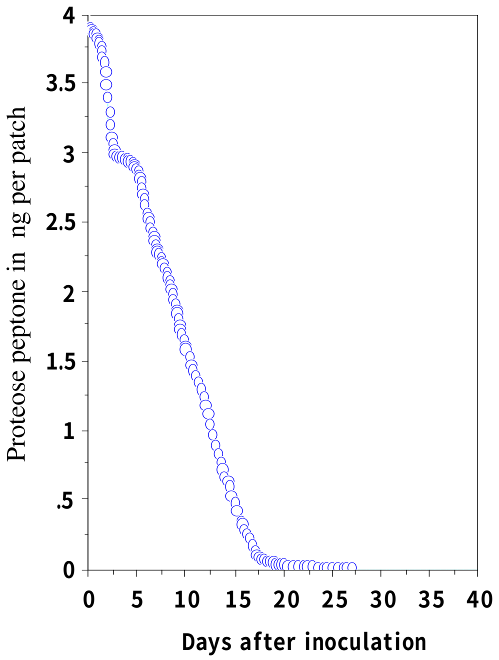


Figure 6

Proteose peptone in the environment of SIM-COSM

the future assignment. **Figure 7** shows the simulated changes of pH, CO_2 and NH_3 concentrations in the environment until 50 days after inoculation. Since Euglena can not synthesis CO_2 from HCO_3^- (10), high pH of the environment may inhibit the photosynthesis of Euglena which may cause slight decrease of population as shown in **Figure 4a** and **4b**.

Figure 8 shows population changes in the SIM-COSM after irradiation with X-rays in four cases : (a) control, (b) 25% lethal doses, (c) 46% lethal doses, and (d) 98% lethal doses, which was programmed to kill only E. coli cells in the irradiated patch. When SIM-COSM was stressed by radiation, direct hazard was observed as the sudden decrease of E. coli population. Because of its short cell cycle of 20 minutes (9), E.coli population recovered to control levels eventually as shown in the cases of (b), (c) and (d). Temporal reduction of E. coli population in these cases caused a starvation of Tetrahymena, and when the impact to the E. coli exceeded the tolerable level, Tetrahymena died out as shown in case (d).

In cases (b) and (c) , lethality against E.coli was tolerable and populations of three microbes were resumed to the normal level from macroscopic point of view. But, diversity of the Tetrahymena population may be lost during the recovery, because survived Tetrahymena was the winner of the heavy selection pressure. Importance of diversity must be discussed from the view points of evolution biology and conservation biology. Results shown in **Figure 8** can be regarded as a kind of systematic adaptive response to a certain level of X-ray exposure. When the impacts exceeded the tolerable level, extinction risk must be evaluated as a endpoint to compare the risk of chemicals, ultra violet, acid, rare materials and radiation exposure to the ecosystem (20, 21).

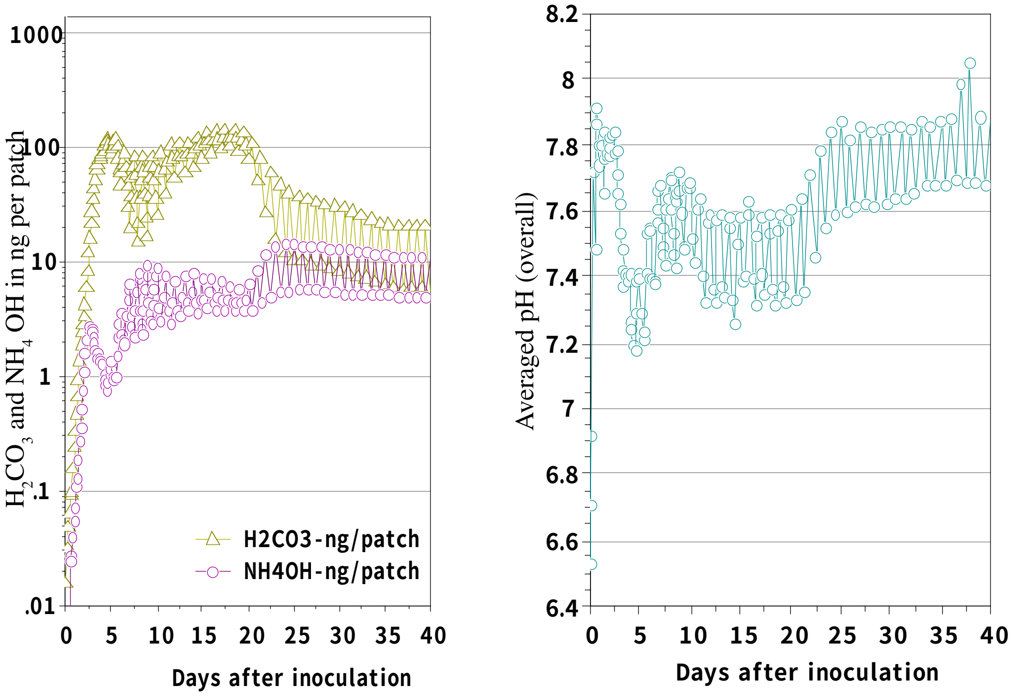


Figure 7 Changes of pH, CO_2 and NH_3 concentrations in the environment until 50 days after inoculation

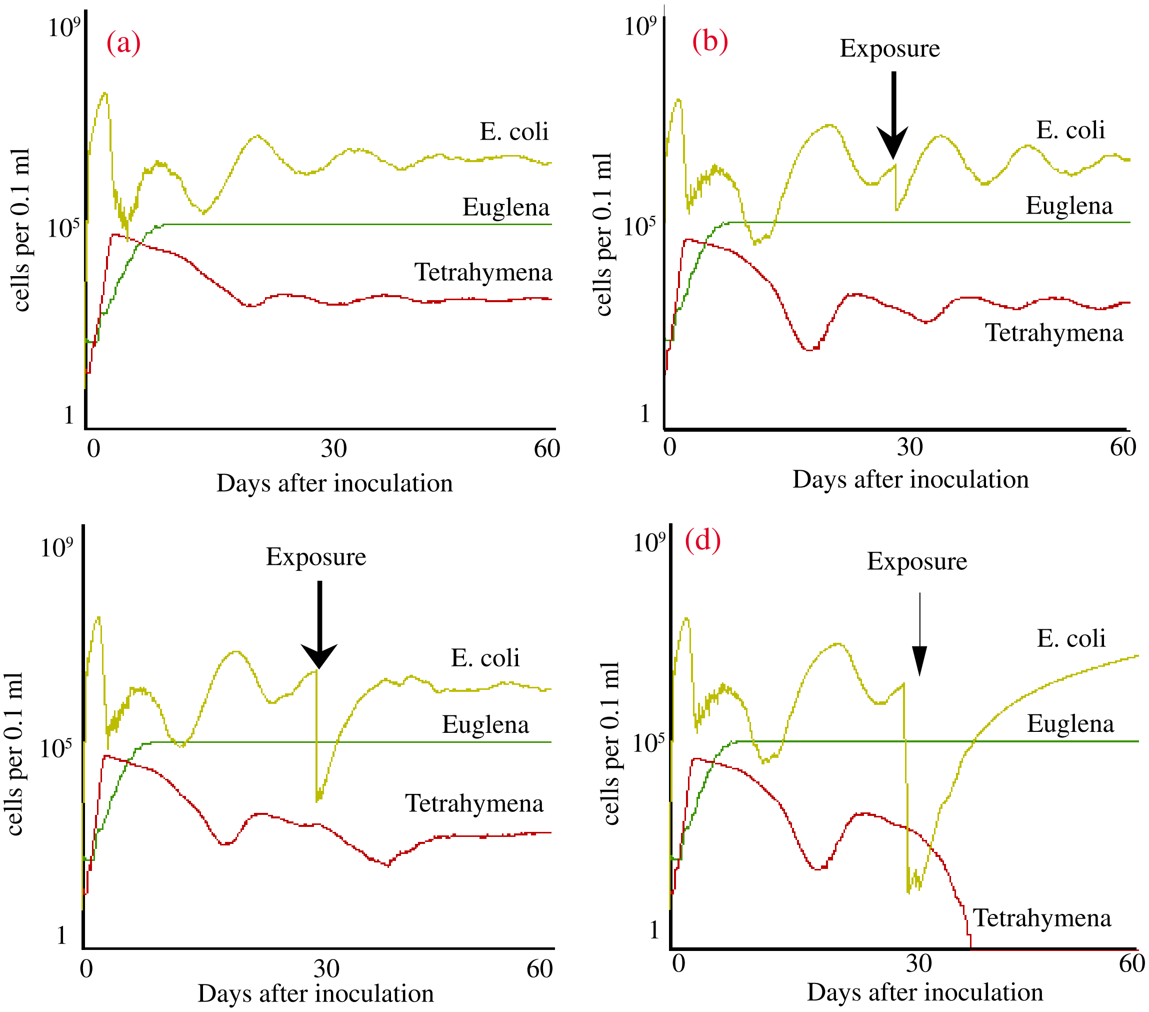


Figure 8 Population changes in the SIM-COSM after irradiation with X-rays :
(a) control, (b) 25% lethal doses , (c) 46% lethal doses, (d) 98% lethal doses against E. coli.

FUTURE SCOPE

In the simulation, the results of the population balance showed a probabilistic fluctuation and instability, since some demographic parameters of Tetrahymena and Euglena were assigned to realize the stochasticity. One of the future research directions is to investigate the relationship between ecosystem's instability and diversity to think about the self-conjugation of Tetrahymena, which is a kind of sexual reproduction that is suggested to introduce genetic renovations and diversity of the population. This future approach aims to find out the strategy of conservation and how diversity and similarity appeared in a chain of generations in a real ecosystem. To evaluate the extinction probability of the ecosystem as an endpoint of harm due to environmental stress (radiation exposure, chemical toxic exposure, etc.) is the ultimate scope of our SIM-COSM project, which is open to our future assignment.

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REFERENCES

1. Bertalanffy, L.V. General System Theory, Foundations, Development, Applications, Chapter 6, Open System Model in Biology) , George Braziller, New York (1968) .
2. G.M. Weinberg, *An Introduction to General Systems Thinking,* John Wiley & Sons, Inc. New York (1975).
3. C.J.Walters, *Systems ecology: the systems approach and mathematical models in ecology*. Fundamentals of Ecology 3rd.edition, Chapter 10, 276-292, W.B. Saunders Company, Philadelphia (1971).
4. E.P. Odum. *Fundamentals of Ecology 3rd.edition*, 211-213, W.B. Saunders Company, Philadelphia (1971).
5. Z. Kawabata, K. Matsui, K. Okazaki, M. Nasu, N. Nakano, T. Sugai, *Synthesis of a Species-Defined Microcosm with Protozoa*, J. Protozool. Research, 5, 23-26 (1995).
6. S. Fuma, H. Takeda, K. Miyamoto, K. Yanagisawa, Y. Inoue, N. sato, M. Hirano, Z. Kawabata, *Effects of γ–rays on the populations of the steady-state ecological microcosm*, Int. J. Radiat. Biol. 74(1), 145-150 (1998).
7. D.L. Hill, The Biochemistry and Physiology of Tetrahymena, Academic Press, Inc., New York (1972).
8. J.R. Nilsson, *Phagotorophy in Tetrahymena*, Biochemistry and Physiology of Protozoa, Academic Press Inc., ISBN0-12-444602-7, 339-379 (1979).
9. R.Y. Stanier, E.A.Adelberg, J.L. Ingraham, M.L. Wheelis, Introduction to the Microbial World, Prentice-hall, Inc., New Jersey (1979).
10. S. Kitaoka (ed.), Euglena -physiology and biochemistry-, Academic Press (in Japanese), Tokyo, ISBN4-7622-3601-2, 4-11 (1989).
11. F.B. Taub, A.M. Dollar, *The Nutritional Inadequacy of Chlorella and Chlamydomonas as food for Daphnia pulex*, Limnology and Oceanography, 13, 607-617 (1968).
12. H. Nakajima, Z. Kawabata, *Sensitivity Analysis in Microbial communities*, In Microbial Diversity in Time and Space (ed. Colwell), Plenum Press, New York, 85-91 (1996).
13. P.P.F. hanegraaf, *Mass and Energy Fluxes in Microorganisms according to the Dynamic Energy Budget Theory for Filaments*, Ph.D. Thesis, Vrije Universiteit, Amsterdam, ISBN90-9010847-5 (1997).
14. S.A.L.M. Kooijman, *Dynamic Energy Budgets in Biological Systems : Theory and Applications in Ecotoxicology*, Cambridge University Press, Cambridge, U.K. ISBN0.521.45223.6 (1993).
15. Y. Iwasa, M. Higashi, N. Yamamura. *Prey Distribution as a factor Determining the choice of Optimal Foraging Strategy*, The American Naturalist, 117 (5) , 710-723 (1981).
16. K. Miyamoto, S. Fuma, H. Takeda, K. Yanagisawa, Y. Inoue, N. sato, M. Hirano, Z. Kawabata, *Effect of Acidification on the Population of Growth Stage Aquatic Microcosm*, Comparative Evaluation of Health Effects of Environmental Toxicants Derived from Advanced Technologies, Kodansha-Scientific Ltd. ISBN4-906464-05, Chiba, Japan (1998).
17. J.S. Roth, G. Buccico, *Biochemical Studies on Irradiated Protozoa 3, Catalase activity in tetrahymena pyriformis W*, Journal of Protozoology, 12 (3), 432-438 (1965).
18. S. Fuma, K. Miyamoto, H. Takeda, K. Yanagisawa, Y. Inoue, N. sato, M. Hirano, Z. Kawabata, *Ecological effects of radiation and other environmental stresses on aquatic microcosm*, Comparative Evaluation of Health Effects of Environmental Toxicants Derived from Advanced Technologies, Kodansha-Scientific Ltd. ISBN4-906464-05, Chiba, Japan (1998).
19. E.L. Charnov, *Optimal foraging, the marginal value theorem*, Theoretical population Biology, 9, 129-136 (1976).
20. P. Foley, *Predicting Extinction Times from Environmental Stochasticity and Carrying Capacity*, Conservation Biology, 8, 124-137 (1992).
21. Y. Iwasa, H. Mochizuki, *Probability of Population Extinction Accompanying A Temporary Decrease of Population Size*, Res. Popul. Ecol., 30, 145-164 (1988).