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Key ecological challenges for closed systems facilities

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Abstract

Closed ecological systems are desirable for a number of purposes. In space life support systems, material closure allows precious life-supporting resources to be kept inside and recycled. Closure in small biospheric systems facilitates detailed measurement of global ecological processes and biogeochemical cycles. Closed testbeds facilitate research topics which require isolation from the outside (e.g. genetically modified organisms; radioisotopes) so their ecological interactions and fluxes can be studied separate from interactions with the outside environment. But to achieve and maintain closure entails solving complex ecological challenges. These challenges include being able to handle faster cycling rates and accentuated daily and seasonal fluxes of critical life elements such as carbon dioxide, oxygen, water, macro- and micro-nutrients. The problems of achieving sustainability in closed systems for life support include how to handle atmospheric dynamics including trace gases, producing a complete human diet, recycling nutrients and maintaining soil fertility, the maintenance of healthy air and water and preventing the loss of critical elements from active circulation. In biospheric facilities, the challenge is also to produce analogues to natural biomes and ecosystems, studying processes of self-organization and adaptation in systems that allow specification or determination of state variables and cycles which may be followed through all interactions from atmosphere to soils. Other challenges include the dynamics and genetics of small populations, the psychological challenges for small isolated human groups and backup technologies and strategic options which may be necessary to ensure long-term operation of closed ecological systems.

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1. Introduction: the meaning of closure

The concept of closure in life systems covers a wide range of meanings. Some ecological studies treat a watershed, a pond, a cave, a forest as entities with meaningful boundaries in spite of organisms and non-living material crossing those boundaries. In contrast, planet Earth as a whole supports an extremely tightly sealed ecological system, “the biosphere”, but still acquires relatively small amounts of meteoritic material from and loses light gases such as hydrogen to outer space (Morowitz et al.,

2005). Perfect and permanent material closure is virtually impossible for human-made systems, but significant closure can be achieved easily enough for most practical investigations or applications.

Material closure refers to material exchanges between the system in question and the surrounding environment. These materials include atmospheric gases, water, soil and living organisms. The degree of material closure can be measured as a percentage or weight/mass passage of materials from inside to out or outside to inside. Though ecologists talk about certain types of biomes as being more tightly closed than others – e.g. the high degree of nutrient retention and recycling in coral reefs or rainforests – they do not include water or air closure and so all natural ecosystems are inherently far more open than man-made facilities. All ecosystems receive inputs of rain and lose water

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through evaporation/transpiration and subsurface and surface runoff; lose/receive from ocean currents; have inputs from migratory birds and the movements of animals; receive and lose materials through the winds, which also impact plants and trees e.g. through creation of stress wood. Humans, aided by our powerful technology, are the largest movers of material on Earth, so much so that Vernadsky called our species “a geological force” (Vernadsky, 1997) though many animals also move matter. Material inputs/outputs are asymptotic to absent in closed ecological systems.

Energy closure refers to just the passage of energy. The Earth's biosphere is not energetically-closed, since it receives solar energy from our Sun and heat from the interior of the planet. A percentage of this energy, mainly in the form of heat, is “lost” from Earth, exchanged to our surrounding space environment. Energy closure would prohibit the discharge of waste heat, thus imposing very difficult thermodynamic requirements. It is not only unnecessary for a bioregenerative life support to be energetically-closed, but impossible because an infinite energy sink would be required. Waste and excess heat needs to be dissipated outside of any closed system facility. In addition, inclusion of energy generating devices within the materially-closed facility increases the amount of pollutants which have to be absorbed and mitigated by the living system inside.

Information can also be considered as circulating within the system or exchanged with the surroundings. We receive information from our solar system, galaxy and elsewhere in the universe, and also send out information, deliberately or inadvertently, as radiowaves and other electromagnetic spectra. Information closure would be self-defeating since any real-time analysis and help needed from outside would be unavailable; and communications from the inside would be prevented. Scientists or technicians on the outside can be informed real-time about changing conditions. For example, space missions without access to a Mission Control would be far more dangerous and difficult. This also applies to ground-based research facilities and prototypes. Information closure is ill-advised since the potential for generating knowledge through study of the functioning of the closed ecological system would be curtailed.

A successfully operating closed ecological facility includes significant autonomous control on interspecies interactions, which should be largely allowed to occur without human intervention except when species diversity is threatened. Control of material flows inside a closed system is largely done by human and mechanical control (air movement, water movement, humidity control etc.) though advice from outside specialists can augment decisions made by the crew and give another viewpoint re evaluating the health and functioning of the closed system, sometimes leading to a change in control strategies and parameters.

Material closure is the type of closure most important for facilities housing life support systems and other types of ecological systems used for research. So, henceforth

when we refer to closure, we exclusively mean material-closure, while energetic and informational openness will be assumed. For life support closed systems, loss of atmosphere or water must be replaced or regenerated from in situ space resources, and prevention of contamination from outside may be critical for preventing damage to crew or crop plants. The tighter the material closure, the more intensively the system inside may be studied without the complications of loss or inputs of materials.

To sustain material closure requires replicating the kinds of complex biogeochemical cycles and food web relationships which have developed naturally in our biosphere. Recognition of some of the reciprocal relationships, of competition, food chain linkages and symbiosis, between life forms dates back many centuries, but this understanding has deepened greatly in the last several decades. We continue to learn more and more about the intricate webs and pathways by which all life forms obtain essential supplies of material and energy. Living systems are also inextricably linked with their environment, e.g. its climatic ranges, soil and underlying geology and mineral resources.

Closed ecological systems emerge as powerful tools to study both how life systems do work and how they could work, opening the door not only to investigation of what already exists, but also to experimentation with variants. Recognition that the biosphere supported plants and animals by virtue of their reciprocal functions for millions of years without any outside supply whatsoever except for sunlight and Earth's interior heat has given rise to the idea that humans can leave the biosphere for long periods if they could properly configure and take along the essential functional features. Thus, we arrive at three intertwined reasons to build closed ecological systems: (1) for basic research to further understand how the biosphere works; (2) to experiment with variants to also understand more generally what biospheres are; and (3) to create portable and/or transferable life support systems and mini-biospheres so that humans could travel away from planet Earth, perhaps even eventually beyond the solar system, without need for resupply from Earth. Biosphere 2 was aimed at all three objectives.

For terrestrial applications, it is important to understand that an engineered closed ecological system will have dynamics which are strongly influenced by boundary and initial state conditions. Any artificial system will be unable to reproduce some important vectors on Earth's biosphere: e.g. deep ocean or lithosphere; glaciers and biomes which can't be included, so there are inevitable limitations on how well a man-made system can be used as a representation of our global biosphere (e.g. since Biosphere 2 was a model of the tropical biomes, there were no temperate or boreal ecosystems included). In addition, there needs to be serious consideration about the implications for scientific and statistical interpretation of results since financial constraints preclude making a series of replicate facilities. In addition, even the simplest of ecological systems is complex, perhaps non-linear, challenging researchers to use

more than just analytic, reductionist approaches to extract meaning from its operation and development. These challenges are comparable to those faced by Earth-system scientists who have no other replicates on which to conduct “controlled experiments”. These issues also mirror those of ecological microcosms and mesocosms, but these have proved very valuable in ecological research (Beyers and Odum, 1993).

In this paper, we take the Biosphere 2 project (Allen and Nelson, 1999; Nelson et al., 1993) and several other closed systems such as the Bios-3 facility in Krasnoyarsk, Siberia (Gitelson et al., 2003; Salisbury et al., 1997; Nelson et al., 2010), the Closed Ecological Experimental Facility (CEEF) in Japan (Nitta et al., 2000), the Biosphere 2 Test Module (Nelson et al., 1992) and the Laboratory Biosphere facility (Dempster et al., 2004) as case studies relevant to many of the issues that arise pursuant to designing, operating and researching closed ecological systems. It was a premise of all those projects that more could be learned and more rapidly learned by actually building and operating a closed ecological systems and mini-biospheres than an academic exercise to study these concepts abstractly just with computer simulation. That is not to suggest that planning of all these facilities did not proceed from an in-depth base of understanding past research – it did so by drawing on expertise of dozens of established scientists and employing established engineering practices for design and construction of the facility. Nevertheless, these initial closed ecological systems are only first approximations to what can be a decades long, if not centuries long, science of Biospherics and closed ecological systems to pursue the three aims identified above. The scale and scope of the Biosphere 2 facility differed from these other closed systems in both its overall size and its diversity of internal ecosystems. The other systems were focused on human bioregenerative life support and the study of related ecological processes.

On Earth, basic human life support usually means little more than provision of food and water and protection from extreme temperatures. Breathable air is generally taken for granted unless something happens that deprives a human from having it. Providing food can draw on knowledge developed from centuries of farming practice and refined by techniques to produce ever more food per unit area. Providing air on a sustainable basis with sufficient carbon dioxide for plants and sufficient oxygen and minimal potentially hazardous trace gases for humans introduces great difficulties and requires entirely different understandings including the balance of respiration of animals, plants and soils, plant photosynthesis, dealing with biogenic and technogenic trace gases and accommodating seasonal light and cropping variations.

If we contemplate remote life support without resupply from Earth, atmospheric closure becomes absolutely essential. It has distinctly different aspects: engineering challenges include prevention of leakage and contamination (Dempster, 1994a), using technologies to supplement or replace ones done with natural mechanisms (e.g. water cir-

ulation and climate) in the global biosphere. The ecological challenge is to achieve balanced recycling and sustainable healthy operation.

2. Ecological challenges of closure

2.1. *Reduced reservoir sizes and accelerated cycles*

Once a bioregenerative life support system achieves engineering closure with little atmospheric or other losses from the system, daunting ecological challenges remain to make the system operate in an acceptable fashion.

First, the system must succeed in supplying needed elements of life support: supply of oxygen and carbon dioxide within healthy ranges and control of buildup of any toxic trace gases; supply of water of good quality for drinking and human hygiene and irrigation/nutrients for the food crops; provision of complete nutrition for the crew; and recycling of all wastes, from inedible crop residues to human metabolic wastes. Within a closed system, a balanced and totally recycling ecological system is essential if the system is to persist.

Not only must interactions between elemental cycles be balanced, but smaller reservoirs and greater concentration of living biomass result in highly accelerated cycling rates. There can be nothing comparable to the vast atmosphere, oceans and soils of planet Earth. The potential for concentrating toxic elements in air and water, or for sequestering essential elements in soils, sediments, or biomass, becomes far greater in man-made ecologies.

Cycling times will greatly accelerate and fluxes are far greater than Earth's biosphere. The challenge: ensure these fluxes stay within acceptable limits for health and the cycles are completed. The primary cause of this challenge are the smaller reservoirs and altered ratios of active elements in man-made systems. For example, even in Biosphere 2 which as a global ecology laboratory included a range of natural biomes (coral reef ocean, marsh/wetlands, desert, savannah and rainforest) in addition to its agriculture and human residences/laboratories, the ratios of carbon dramatically differed from Earth's since the ocean contained only around four million liters (1 million gallons) and the atmosphere contained under spaceframe roofs was at highest a mere 25 meters instead of the 100 km of the Earth's atmosphere. With Biosphere 2's volume of six million cubic feet (180,000 cu m), a concentration of 1500 ppm CO₂ in its atmosphere is equal to about 70 kg of carbon (at 350 ppm, less than 20 kg). Also a closed system needs to have resources of nutrients and carbon to ensure good production of agricultural crops and development of other plant communities which necessarily start as young/small plants. To allow both for rapid growth and ecological self-organization a strategy of both species-packing and provision of adequate nutrients, such as N and P is required. This leads to drastically different ratios of soil, atmosphere, biomass, and ocean reservoirs (if an oceanic ecology is included) that govern the rate of change

and cycling of essential biogeochemical elements, such as carbon, nitrogen, and oxygen.

In the global biosphere there is roughly a 1:1 ratio between carbon in the living biomass and in the atmosphere (with CO₂ at 350 ppm). In Biosphere 2 the ratio was 100:1 and in the Laboratory Biosphere the ratio can be 240–700:1 depending on the size of crop grown. The ratio of soil organic carbon: atmospheric carbon is around 2:1 in the global biosphere, 5000:1 in Biosphere 2 and 1500:1 in the Laboratory Biosphere. As a consequence, the residence time for CO₂ in Earth's atmosphere is estimated at three years, in Biosphere 2 during its closure experiments, 1991–1994, around four days, and a half-day in the Laboratory Biosphere (Table 1) (Nelson et al., 2003; Schlesinger, 1991; Nelson et al., 1993; Bolin and Cooke, 1983; Dempster, 2002).

The Biosphere 2 “ocean” – with tropical coral reef – was a much larger reservoir/buffer for CO₂ than the atmosphere. But it also required human management since the large amounts of CO₂ absorbed (as HCO₃) tended to increase acidification of the ocean water. Salts were added to mitigate this acidification. Even with that chemical buffering, Biosphere 2's ocean pH fell to as low as 7.8 whereas “natural” coral reefs are in waters with a pH of 8.2–8.4 (Nelson and Dempster, 1996).

Since each closed ecological system facility has unique characteristics, it will be interesting to compare these residence times/cycling rates with, for example, the Bios-3 facility in Krasnoyarsk, Russia, the CEEF (Closed Ecological Experimental Facility) in Japan and the MELISSA project of the European Space Agency at the University of Barcelona, Spain.

2.2. Atmospheric dynamics

Without a balance of part of the biosphere in daylight or artificial light with active plant photosynthesis and a part in night-time or dark periods when respiration dominates, there are far larger diurnal fluxes in small closed ecological systems. Fig. 1 presents these fluxes in three systems: a/Biosphere 2 Test Module, a 480m³ facility where daily flux was as large as 300 ppm, b/Biosphere 2 where CO₂ varied by up to 600 ppm daily, and c/the far-smaller Laboratory Biosphere where CO₂ was input during crop growth periods and uptake could reduce CO₂ by several thousand ppm

(Nelson et al., 1992; Nelson et al., 1994; Dempster et al., 2009).

Another strategy eliminates soil from the bioregenerative system, relying on hydroponics for production of the food crops. In this situation CO₂ is more likely a limiting resource; a solution is to oxidize inedible crop residues, a strategy followed by the Russian Bios-3 facility where CO₂ was generally kept between 350 and 1700 ppm CO₂ (Terskov et al., 1979). However, eliminating soil takes away the possibility of amending in situ space soils (moon, Mars etc.) for the agricultural medium as well as simpler, less energy demanding methods of recycling waste and nutrients, as well as the benefits of soil for air biofiltration (Nelson and Bohn, 2011). The need for consumables supplying hydroponic nutrients reduces closure. Whether hydroponic systems can be kept going indefinitely has not been established. However, hydroponic systems are far lighter weight and have obvious advantages for space station and initial planetary life support systems until local regolith can be amended/modified to make soils.

Biosphere 2 exhibited a strong seasonal variance in CO₂ levels. During the month of December, 1991 when ambient light fell to its lowest levels (the day length for 21 December in southern Arizona is about 9.5 h), CO₂ averaged 2466 ppm. By contrast, during June 1992 when days were significantly longer (14.5 h on 21 June) and total light input greatest, CO₂ in the Biosphere 2 atmosphere averaged 1060 ppm (Fig. 1(b)). Outside ambient PPF (Photosynthetic Photon Flux) averaged 16.8 mol/m²/day during December 1991, and 53.7 mol/m²/day during June 1992. On average, 40–50 % of this is received inside Biosphere 2 because of structural shading and glass interception of sunlight. CO₂ dynamics are so responsive to incident light, that one can see reflected in daily CO₂ graphs the exact time cloud cover reduced incident sunlight. Tight coupling of atmospheric CO₂ to plant growth and short residence time of CO₂ in the atmosphere are likely to be even more pronounced in smaller space life-support systems, where the presence of crew members in small volumes increases the impact of human respiration on atmospheric cycling. The converse problem of lowered plant growth if CO₂ is deficient was revealed in several unmanned experiments conducted in the Biosphere 2 Test Module. There CO₂ levels were sometimes drawn down to between 200–300 ppm during peak daylight hours (Fig. 1(a)). The smaller Labo-

Table 1

Estimates of carbon ratios in biomass, soil and atmosphere in the Earth's biosphere, Biosphere 2 and the Laboratory Biosphere facility and an estimate of carbon cycling time as a consequence (after Nelson et al., 2003, data from Schlesinger, 1991; Nelson et al., 1993; Bolin and Cooke, 1983; Dempster et al., 2004).

	Earth	Biosphere 2	Laboratory Biosphere
Ratio of biomass C: atmospheric C	1:1 (at 350 ppm CO ₂)	100:1 (at 1500 ppm CO ₂)	240–700:1 (mature crop to atmosphere at 1500 ppm CO ₂)
Ratio of soil C: atmospheric C	2:1	5000:1	1500:1 (atmosphere at 1500 ppm CO ₂)
Estimated carbon cycling time (residence in atmosphere)	3 years	1–4 days	0.5–2 days

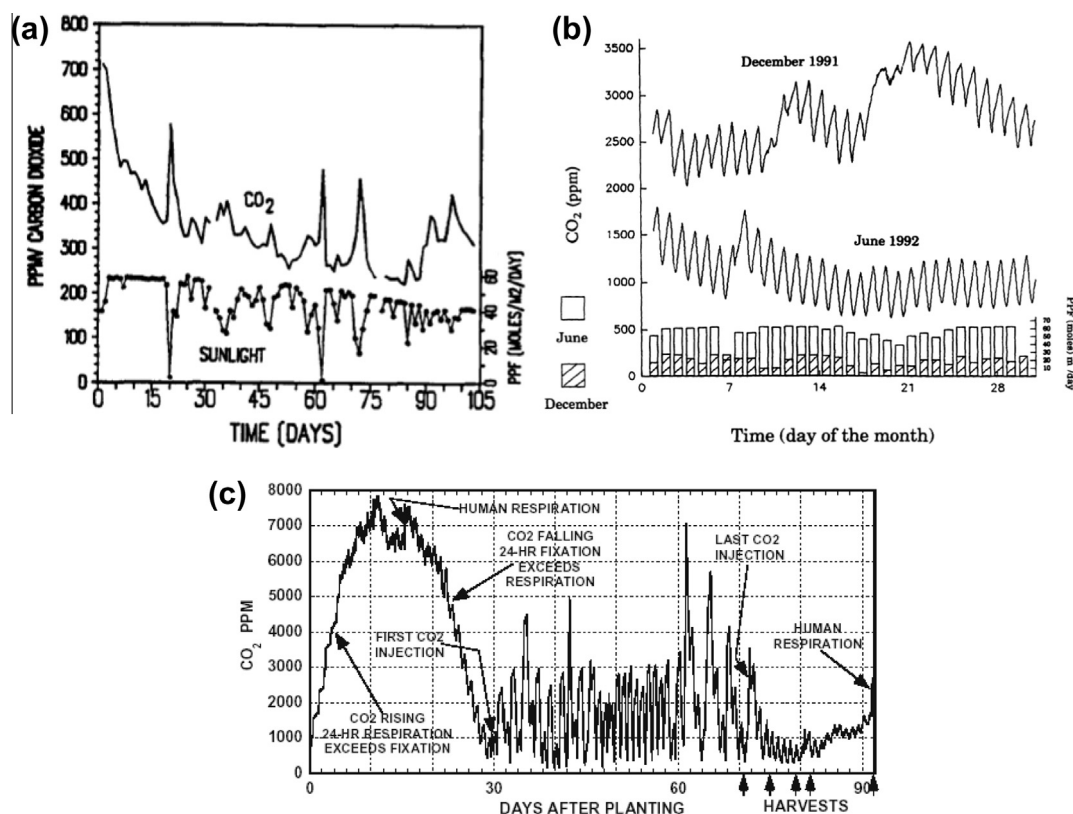


Fig. 1. Carbon dioxide dynamics in three closed ecological systems: (a) Biosphere 2 Test Module showing CO₂ and photosynthetic light flux from 25 June – 5 Oct 1987; (b) Biosphere 2 carbon dioxide levels during a low light month (Dec 1991) and a high light month (June 1992). Increases of CO₂ are seen on cloudy days in both periods, and variations of 500–600 ppm between day and night are typical; (c) CO₂ concentration in the Laboratory Biosphere with combined crops of wheat, cowpea and pinto bean grown in soil. Initially, while plants have not yet sprouted or are very small, CO₂ rises due to soil respiration (to day 10). As they grow and develop, increasing fixation rapidly draws CO₂ down (to day 29). It becomes necessary to inject CO₂ to supply feedstock (27 times from days 29 to 71). Occasionally humans enter the chamber and an increase is seen due to their respiration as noted on the graphs (Nelson et al., 1992, 1994; Dempster et al., 2009).

ratory Biosphere showed even greater fluxes of CO₂ (Fig. 1(c)).

Buffering these impacts requires development of techniques to store CO₂ for release when it may be required as a plant nutrient: either through oxidation of biomass or flushing of soil biofiltration units.

2.3. Trace gas challenges in closed systems

Potential buildup of trace gases has been a concern since the advent of manned spaceflight. Tightly sealed spaces permit the buildup of gases from technogenic, biogenic and anthropogenic sources (Dempster, 2008). These pose additional risks of secondary reactions. The problem becomes more acute with degree of closure and length of operation of the closed system and length of time humans are exposed. The extreme number of gases (e.g. hundreds in spacecraft and close to 2000 detected in office buildings (Wolverton, 1997)) and the paucity of research on what constitutes safe levels for extended human exposure, argues for robust preventive and controlling technologies to keep them at low levels.

Technologies to control trace gases include incineration, physical filtration e.g. charcoal filters, chemical scrubbing

and soil biofiltration. Bios-3 used a catalytic converter to control trace gases, while Biosphere 2 conducted extensive research on soil biofiltration as a lower-energy and more natural method. Soil biofiltration uses the vast numbers and wide diversity of soil microbes as the method to metabolize trace gases by active pumping air through the soil. In the Biosphere 2 research, for the first time, it was demonstrated that soil biofilters could also grow plants, including food crops. The Biosphere 2 research demonstrated both control of a number of potentially toxic trace gases but also demonstrated no loss of crop production; in fact minor improvements perhaps because the forced air passage prevented any part of the soil becoming anaerobic (Frye and Hodges, 1990; Nelson and Bohn, 2011).

2.4. Agricultural production and recycling/maintenance of high productivity

These challenges of an agriculture/food producing system inside a closed ecological system include producing a balanced, tasty and complete diet for space life support applications; and healthy integration of an agricultural unit in biospheric research facilities. Studies which show that one crop (e.g. rice or wheat) can produce the total caloric

Table 2

Food production from different crops and domestic animals for the nutrition of the eight-person biospherian crew during the first two year closure experiment in Biosphere 2, 1991–1993 (Silverstone and Nelson, 1996).

Crop	Total 2 yr yield kg	Grams per person per day	Protein (g.) person	Fat (g.)/ person	Kcal/ person
Grains: Rice	277	47	4	0.9	168
Sorghum	190	32	4	0.6	107
Wheat	192	32	4	0.7	108
Starchy vegetables: Potato	240	41	1	0.6	31
Sweet potato	2765	468	7	1.3	494
Malanga,yam	2	20	12	0	22
Legumes: Peanut, soybean, lab lab, pea, pinto bean	208	60	13	13.2	269
Vegetables: Beet greens, sweet potato greens, chard	637	108	1	0.2	22
Beet roots	760	129	2	0.4	57
Bell pepper, green beans, chili, cucumber, kale, pak choi, pea	331	57	1	1	15
Carrots	225	38	0	0.1	17
Cabbage	153	26	0	0	6
Eggplant	245	41	0	0	11
Lettuce, onion	289	49	0	0.1	11
Summer squash	513	87	1	0.1	17
Tomato	353	60	1	0.1	12
Winter squash	343	58	1	0.2	37
Fruits: banana	2171	367	2	10.5	220
Papaya	1216	206	1	0.2	53
Fig, guava, kumquat, lemon lime orange	133	23	0	0.1	11
Animal products: goat milk	842	142	5	5.6	99
Goat, pork, fish, chicken meat, eggs	94	108	3	3.1	38
Total produced	12432	2107	53	39	1823

input for humans or even diets based on just several crops ignore basic human psychology and nutritional needs. In Biosphere 2 some 80 plants were used, through the most important crops were grains (wheat, rice, millet), beans (lablab and cowpea), starches (sweet potato, taro), fruits (banana, papaya) and vegetables, especially squash, beets, tomatoes and salad greens. Table 2 presents crop production during the first 2-year closure experiment and the levels of nutrition supplied from the agricultural system in Biosphere 2. During the second closure experiment of six months in 1994, improvements such as better suited cultivars and improvement of farming techniques led to the complete supply of food and needed nutrients for the crew (Marino et al., 1999).

But achieving total nutritional supply does not solve other issues that closure raises, such as (1) control of potential pests and diseases without use of toxic chemicals (2) treatment (if necessary) and recycling of agricultural irrigation water (3) recycling of inedible crop residues and (4) recycling of human wastes. As Richard Harwood, Mott Professor (Emeritus) of Sustainable Agriculture at Michigan State University and a consultant to the agricultural system inside, noted: “There is no away in Biosphere 2”. In other words, the usual methods prevalent in our Earth’s biosphere: throw it away, i.e. send it “away” to “somewhere else” or the “solution to pollution is dilution”, become obviously untenable when “away” and “elsewhere” are still within the system, which is not only tightly sealed but very rapidly recycling. Furthermore, what is tied up in

refractory form (“dead-lock substances”) does not stay in circulation. These have the potential, if they are life elements, to limit the productivity and even longevity of the closed ecological system/or must be imported.

Options for control of pests/diseases include sterilization and strict quarantine to exclude unwanted vectors. Even if technically possible for small space life support systems, this approach will not work at the scale of even small biospheric laboratories. Other strategies include choosing varieties of crops resistant to pests and adoption of Integrated Pest Management techniques (beneficial insects, non-toxic sprays such as *Bacillus Thuringensis* and soaps), rotation of crops and using high diversity so problems with one particular crop will not devastate food production. But problems may arise, nevertheless. For example, an outbreak of broad mite in Biosphere 2, not seen in the two years of research in greenhouses and in Biosphere 2 itself in the two years of growing prior to closure. The mite decimated soybean and white potato plots and necessitated changing to resistant beans (lablab, cowpea) and starches (sweet potato, taro). Like all farming endeavors, a diversity of crops and alternative cultivars is critical should the unexpected occur.

2.5. Residence time and recycling of water/nutrients

Acceleration of the water cycle presents similar challenges. Closed ecological systems have far smaller water reservoirs than Earth. Even in biospheric systems like Bio-

sphere 2 with an “ocean biome”, residence times for all phases of the water cycle are far shorter because of these small buffers and the concentration of life.

We take Biosphere 2 as a case study of some options which might be followed. For example, potable water in the facility was produced through a condensate collection system followed by sterilization. Recycling of agricultural irrigation water involved a collection system in the basement for the water leaching through the soils (Dempster, 1994b). This “leachate water” mixed with varying percentage of fairly pure condensate water reduced total dissolved solid (TDS) levels and met operational guidelines. To this water supply was added the treated wastewater from the domestic animals, human residences, kitchens, laundry, workshops and laboratories. This wastewater was processed through sedimentation tanks and a recirculating constructed wetland with emergent and floating plants. Wastewater was held 3–5 days in the constructed wetland then could be disinfected with UV lights (which wasn't used during the closure experiments since there were no new infectious disease agents). The constructed wetland produced plant biomass used for feeding domestic animals and for composting. Remaining nutrients in the wastewater were returned to the agricultural soil in the irrigation water (Nelson, 1997, 1998; Nelson et al., 1999). The rapidity of water cycling in Earth's biosphere and in closed ecological systems is presented in Table 3.

Inedible crop residues were fed to domestic animals or composted in a traditional, passive fashion in a compost heap which heated up initially with fresh animal manure and then allowed to mature through the variety of natural, aerobic microbial reactions which convert organic matter to a rich organic soil (Nelson, 1998).

2.6. Dynamics and genetics of small ecosystem populations

In biospheric systems or special-purpose closed ecological systems not totally geared towards human life support, either analogues to natural biomes (such as the rainforest, savannah, thornscrub, desert, mangrove and coral reef ocean of Biosphere 2; or the geosphere/hydrosphere chambers of the Closed Ecological Experimental Facility at Rokkasho, Japan) can be created.

In these systems, ecological strategies include species-packing with more species than will probably be supported

long-term to allow for the reduction which will result from natural processes like ecological self-organization and selective adaptation to the environmental conditions of the facility. The hope is that enough of the multi-species selected for particular roles in food webs or to fill microhabitats will survive to maintain the foodwebs which support ecosystems.

Another ecological concern is the viability of small populations with limited genetic diversity. For closed systems with analogues to natural ecosystems, long-term evolution indicates that adaptational capacity might be constricted by insufficient genetic diversity. This may also be an issue with crop plants long-term. Solutions include seed banks for plants, frozen sperm for animals, tissue-culture methods of propagating and selecting for wider genetic variability in the initial populations enclosed in ground-based laboratories or space systems.

2.7. Humans

Humans present a set of special challenges and opportunities for closed ecological systems. The Russians, who pioneered in humans in closed ecological systems noted that the situation was unique in that people were both a part of the overall enclosed system but also had the ability to directly monitor and manage the system. On the eve of the first human closure experiment in the Biosphere 2 Test Module, when John Allen entered for a three-day experiment, we received a message from Dr. Yevgeny Shepelev, the first human enclosed in a closed ecological system at the Institute of Biomedical Problems (IBMP) in Moscow for 24 h with vats of green algae recycling his water and respiration. It said simply: “Have courage! Remember: man is the most unstable element in the ecosystem” (Allen, 1991).

2.7.1. The “human factor”: psychological dynamics in closed systems

There is a rich literature on the psychology and group dynamics of small, isolated human groups. These include studies on exploration teams, Antarctic bases, submarines and other vessels and spacecraft crews. There have been instances of crew injury or death caused by tensions/jealousies etc. which can arise during such experiences. As well, project goals and results can be adversely affected by sub-

Table 3
Water fluxes and residence times in Biosphere 2 and the Laboratory Biosphere compared to Earth's biosphere (Nelson et al., 2009; Dempster, 1992, 1993; Dempster et al., 2004; Tubiello et al., 1999).

Reservoir	Earth residence time	Biosphere 2 estimated residence time	Acceleration of cycle compared to Earth	Laboratory Biosphere estimated residence time
Atmosphere	9 days	~4 h	50–200 times	5–20 min (cycling time at least 12x faster than Biosphere 2)
Ocean/ Marsh	3000–3200 years	~1200 days (3.2 years)	1000 times	N/A
Soil water	30–60 days	~60 days	similar	~10 days (6 times faster than Biosphere 2)

conscious sabotage. The phenomenon is so well known that it is sometimes called “explorer’s cholera”.

Being informationally-open can help counteract some of the feeling of isolation. Providing access to interactions with family, friends, colleagues through electronic or other telecommunication devices can be important for morale. The maintenance of more normal socializing functions for the human crew through such interchanges can reduce “cabin fever” social stress.

Tensions can also result from a perceived “us vs. them” dichotomy between spacecraft or expedition crew and ground-based or outside mission control personnel (Allen, 2002). There is the well-known example of a Russian space station crew turning off radio communications with ground control. During a simulated Mars mission conducted at IBMP in Moscow, tensions in the crew led to physical fights and sexual harassment between a Russian man and Canadian woman. This may have prompted the selection of an all male crew in the 520 day experiment of 2011–2012. There were personality clashes and a split amongst the Biosphere 2 crew, the depth of feeling of which was intensified by the external conflict over future control of the facility which was occurring during the two year closure experiment.

Tensions, frictions and cliques are inevitable in all human groups. The goal of project planners for closed system facilities with a resident crew is to set up procedures for dealing with such problems and designing facilities and choosing crew with the goal of enhancing crew morale. For example, cosmonauts reported that working with green plants added great personal pleasure to their working days and was very relaxing. A diversity of personality types, shared language, prior training together, clearly defined roles and task responsibilities are all factors that enhance group performance. Providing for personal space, where the crew member can enjoy privacy, is also important. Good food, special treats and times of celebration were noted by the Biosphere 2 crews as greatly helping with morale (Alling and Nelson, 1993).

2.7.2. Medical needs and research opportunities

If people are part of closed ecological or mini-biospheric systems, the full range of medical issues must be considered. For ground-based systems, ultimately, it is better to have a crew member exit or a doctor enter thru an airlock, than to risk more severe health consequences. In not all cases can a qualified doctor be a part of closure teams. In those cases, training in basic medical/dental emergency procedures may be a reasonable strategy. Use of telemedicine, which has been developing quite rapidly, can also be a resource. For example, though one of the eight-member biospherian crew during Biosphere 2’s first closure experiment was a medical doctor, his knowledge was supplemented by a team of ten specialist doctors from the University of Arizona Medical School who lent their expertise from outside the facility. Modern technologies make

this far easier than it was in the early 1990s (Walford et al., 1996).

Closed systems may offer unique opportunities for research because of tightly monitored and controlled environments and the chance to decouple vectors. A good example is the decline in atmospheric oxygen which occurred in Biosphere 2. Normally such an oxygen decline is correlated with a change in atmospheric pressure (e.g. at elevations above sea level). But in Biosphere 2, atmospheric pressure remained constant while oxygen slowly declined due to CO₂ absorption in the concrete. This precipitated a host of unanticipated research on the crew to measure physiological responses (Paglia and Walford, 2005). In addition, closure allows unprecedented opportunities for tightly controlled human diet and nutrition experiments, such as inadvertently occurred when the Biosphere 2 crew followed, perforce, a reduced calorie/nutrient dense diet (Walford et al., 1992).

As Walford et al. note in their paper on “Biospheric Medicine” (Walford et al., 1996), closed ecological systems and mini-biospheric habitation open up novel challenges compared to highly-ordered, quasi-military living conditions of current space missions. In closed ecological facilities there is heightened intensity of interaction with a more complex and unpredictable environment with subtle cultural considerations in addition to regular medical needs.

2.8. Other considerations and strategies for long-term operation

Survival, well-being and full functioning of the space crew is the over-riding priority of any closed ecological system used for space life support. So, though approaching material-closure is vital in reducing the need for consumables long-term; safety and cost argue for provision of back-up supplies and interventions when necessary and possible. Of course, in space orbit or on lunar or exoplanetary surfaces, the transition to bioregenerative systems and significant closure will be gradual. Even if achievable, contingency plans must be made for obtaining life support elements from the space environment or by resupply from Earth, and for keeping such stocks of food, water, oxygen to deal with whatever contingency occurs.

The science and engineering of closed ecological systems is still too new to determine how fully recycling systems can be implemented, and at what scale, mass and volume. It may turn out that the cost and engineering/energy requirements for even approaching closure prove prohibitive or ecologically unrealistic. The buildup of “dead-lock”, refractory substances may prove to be a limiting factor to complete material recycling and self-sufficiency. In any case, the ability to utilize in situ space resources is a valuable pursuit for extending both the lifetime of initial habitations and for building and stocking extensions. Humans will not become residents outside our biosphere until we can do so using lunar, martian and other available

resources. Initial studies of Martian regolith, and the evident abundance of water (frozen), oxygen, either concentrated from the 0.13% present in Mars' thin atmosphere or accessed through electrolysis of water or freed from oxidized rocks, are promising for eventually "living off the land" rather than being dependent on initial supplies and Earth resupply.

Long-term ecological needs include creating sinks and buffers for key biogeochemical elements. Linking closed ecological system modules offers one way of increasing reservoir sizes plus the advantage of separating individual modules which suffer either natural or ecological disasters or ill-health until the problems can be solved. Sophisticated bioremediation techniques to meet contamination/pollution issues need to be available. "Modular biospheres" offer an excellent contained laboratory for developing and testing ways of restoring ecological health (Nelson et al., 2005).

3. Conclusion: closed ecological systems as unique ecological experimental tools

The advantage of a materially closed ecological system as opposed to ecological microcosms and mesocosms is that state variables can be specified, and total cycles can be followed including atmospheric interactions (Morowitz et al., 2005). When laboratory-sized, generally 1–10 liter, materially closed "ecospheres" were first developed in the 1960s, no one predicted that they would prove to be viable long-term and apparently indefinitely if stocked with sufficient microbial/algal diversity and an appropriate energy source (Folsome and Hansen, 1986).

But ecospheres, limited to fairly simple microbial terrestrial and aquatic ecosystems, can rarely sustain even small multi-celled organisms. The expanded scale of closed ecological and mini-biospheric systems offers larger testbeds and experimental facilities for ecosystem studies. Closed ecological systems permit experimentation with analogues to natural biomes and ecosystems which might not be permitted outside a research facility. Such facilities could greatly accelerate the development of ecology as an experimental as well as observational science. Though relatively large in comparison with most phytotrons and ecological microcosms, which are now standard research and educational tools, closed ecological systems can be economically reconfigured as research needs and agenda warrant.

There has been a wealth of data already obtained from such work even in these early days of biospheric research (e.g. coral reef and ecosystem responses to elevated CO₂, phonological changes, litterfall and decomposition rates, nutrient cycling, insights into global warming etc.) (Osmond, 2005; Langdon et al., 2000; Allen et al., 2003). A special interest of the Japanese CEEF (Closed Ecological Experimental Facility) has been tracking the dynamics and fate of radioisotopes through the closed system as well as functioning and integration of subsystems and modules and study of basic processes like photosynthesis and tran-

spiration offering insights into global warming feedback loops (Nitta, 2001). Though radioisotopes are not needed for such studies; the facility was funded for risk assessment of radioactive nuclei which may leak from the nuclear waste processing facility nearby and has included research on these issues.

Biosphere 2 demonstrated that small "biospheric systems" can have surprises: e.g. atmospheric oxygen declining without a change in atmospheric pressure; self-organization in the biomes such as the trend in the original desert fog/maritime desert to a more chaparral state; the health of the mangrove and coral species in radically different environmental conditions than their original habitat (Nelson and Dempster, 1996; Allen and Nelson, 1999; Walford et al., 1996). They offer a sufficiently small laboratory that sinks, sources and causative agents can be identified and studied in great detail, e.g. finding the "missing oxygen" (Severinghaus et al., 1994). Operationally, they can be easily altered for better functioning in their ground-truth checking of hypotheses and computer simulations.

In the future, applying new methods of investigation can help deepen our understanding of fundamental properties of ecological systems. These include bio-molecular techniques and improved sensing equipment which can illuminate adaptations of organisms and ecosystems to environmental changes, response to competition. How microbes, plants and animals change as their communities change or evolve in the accelerated cycling of small closed system facilities can be studied at the observational level and through sophisticated modern techniques. Closed system facilities allow a meshing of fairly complete "metabolic" information about environmental conditions with detailed genetic, organismic and ecosystem changes.

The oxygen decline at a constant atmospheric pressure in Biosphere 2 also demonstrates that some variables usually conjoined in natural Earth conditions can be separated for study in closed ecological systems. For instance, Biosphere 2 afforded the opportunity to study the response of a rainforest or coral reef in seasonal light conditions and at elevations or latitudes not encountered in their usual geographical locations; and the response of coral reef marine ecosystems to lowered ocean pH levels and to an elevated CO₂ atmosphere.

Closed systems are ideal places for studying bioremediation and environmental technics in fundamental biogeochemical cycles and to mitigate negative human impacts. To investigate air and water purification, a closed ecological system experiment could start with polluted water or specific air pollutants, and methods of cleanup by and/or impact on plant and soil communities studied. Dynamics and cycles in ecosystems behavior can be studied by adjusting some variables while maintaining others at desired levels. This allows innovative experiments with atmospheric and water cycles and composition, response and self-organizational responses from deliberate alterations of trophic chains and pathways; or responses to changes in basic state variables (Nelson et al., 2008).

There is also a unique human dimension in closed ecological systems, namely, the development of sensitivity and adaptive management of the environment (Odum, 1996). We humans have a basic difficulty understanding that we are a part of the biosphere. It is not really “external” or foreign to us. The world we live in is far from the earlier biosphere before human population, technology and activity became such powerful forces. There are no longer vast areas of “wilderness” unaffected by human activities. We are the biggest problem but also the possible solution to a healthy and durable biosphere for all of Earth’s species. Perhaps there is another advantage to the accelerated cycles and development seen in closed ecological and mini-biospheric systems apart from their functioning as a “cyclotron for the life sciences” (Allen, 1991). They present unique opportunities for humans to manage and cooperate with living systems, learning in a very immediate and unmistakable way how the health of their biosphere is crucial for their own health and well-being. As Odum noted (1996), “What Biosphere 2 showed, in a short time, is the lesson our global human society is learning more slowly with Biosphere 1, that humans have to fit their behavior into a closed ecological system”.

Learning to be adaptive participants and managers of such experimental closed ecological system can be valuable training for the roles that we can or perhaps must learn to play in the global biosphere of Earth. This may be the most important ecological challenge and opportunity of closed systems.

References

- Allen, J. *Biosphere 2: The Human Experiment*. Penguin Books, NY, 1991.
- Allen, J.P. People challenges in Biospheric systems for long-term habitation in remote areas, space stations, Moon, and Mars expeditions. *Life Support Biosphere Sci.* 8, 2002.
- Allen, J., Nelson, M. Biospherics and Biosphere 2, mission one (1991–1993). *Ecological Eng.* 13, 15–29, 1999.
- Allen, J.P., Nelson, M., Alling, A.K. The legacy of Biosphere 2 for the study of biospherics and closed ecological systems. *Adv. Space Res.* 31 (7), 1629–1640, 2003.
- Alling, A., Nelson, M. *Life Under Glass: The Inside Story of Biosphere 2*. Biosphere Press, Tucson, Published in Dutch (1993) and Japanese (Kdansha Press, 1997), 1993.
- Beyers, R.J., Odum, H.T. (Eds.). *Ecological Microcosms*. Springer, New York, 1993.
- Bolin, B., Cooke, R.B. (Eds.). The major biogeochemical cycles and their interactions, in SCOPE 21. John Wiley and Sons, NY, 1983.
- Dempster, W. Biosphere 2: Overview of System Performance During the First Nine Months. In: 22nd International Conference on Environmental Systems, SAE Technical Paper Series 921129, 1992.
- Dempster W.F. Biosphere 2: system dynamics and observations during the initial two-year closure trial, in: Society of Automotive Engineers 23rd International Conference on Environmental Systems, Society of Automotive Engineers Technical Paper No. 932290, Colorado Springs, CO, 1993.
- Dempster, W.F. Methods for measurement and control of leakage in CELSS and their application and performance in the Biosphere 2 facility. *Adv. Space Res.* 14 (11), 331–335, 1994a.
- Dempster, W.F. Water systems of Biosphere 2, in: International Conference on Life Support and Biospherics at the University of Alabama. Space Biospheres Ventures, Oracle, AZ, 1994b.
- Dempster, W.F. Airtight sealing a Mars base. *Life Support Biosphere Sci.* 8, 155–160, 2002.
- Dempster, W.F. Tightly closed ecological systems reveal atmospheric subtleties – experience from Biosphere 2. *Adv. Space Res.* 42, 1951–1956, 2008.
- Dempster, W.F., Alling, A., van Thillo, M., Allen, J.P., Silverstone, S., Nelson, M. Technical review of the Laboratory Biosphere closed ecological system facility. *Adv. Space Res.* 34, 1477–1482, 2004.
- Dempster, W.F., Nelson, M., Silverstone, S., Allen, J.P. Carbon dioxide dynamics of combined crops of wheat, cowpea, pinto beans in the Laboratory Biosphere closed ecological system. *Adv. Space Res.* 43, 1229–1235, <http://dx.doi.org/10.1016/j.asr.2008.12.005>, 2009.
- Folsome, C.E., Hanson, J.A. The emergence of materially closed system ecology, in: Polunin, N. (Ed.), *Ecosystem Theory and Application*. Wiley, New York, pp. 269–288, 1986.
- Frye, R., Hodges, C., Soil Bed Work of the ERL in Support of Research and Development of Biosphere 2. Biological Life Support Technologies: Commercial Opportunities. Synergetic Press/NASA Conf. Publication #3094, 1990.
- Gitelson, I.I., Lisovsky, G.M., McElroy, R. *Man-Made Closed Ecological Systems*. CRC Press, Boca Raton, FL, 2003.
- Langdon, C., Takahashi, T., Sweeney, C., Chipman, D., Goddard, J., Marubini, F., Aceves, H., Barnett, H., Atkinson, M.J. Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. *Global Biogeochem. Cycles* 14 (2), 639–654, <http://dx.doi.org/10.1029/1999GB001195>, 2000.
- Marino, B.V.D., Mahato, T.R., Druitt, J.W., Leigh, L. The agricultural biome of Biosphere 2: structure, composition and function. *Ecological Eng.* 13 (1999), 199–234, 1999.
- Morowitz, H., Allen, J.P., Nelson, M., Alling, A. Closure as a scientific concept and its application to ecosystem ecology and the science of the Biosphere. *Adv. Space Res.* 36 (7), 1305–1311, 2005.
- Nelson, M. Nutrient recycling systems of Biosphere 2: litterfall, decomposition and wastewater recycling, results from the 1991–1993 closure experiment. *Life Support Biosphere Sci.* 4 (3/4), 145–153, 1997.
- Nelson, M. Wetland systems for bioregenerative reclamation of wastewater – from closed systems to developing countries. *Life Support Biosphere Sci.* 5 (3), 357–369, 1998.
- Nelson, M., Bohn, H. Soil-based biofiltration for air purification: potentials for environmental and space life support application. *J. Environ. Prot.*, published online, doi: <http://dx.doi.org/10.4236/jep.2011>, 2011.
- Nelson, M., Dempster, W.F. Living In Space. results from Biosphere 2’s initial closure, an early testbed for closed ecological systems on Mars, in: Stoker, C.R., Emmert, C. (Eds.), *Strategies for Mars: A Guide to Human Exploration*, 86. AAS Publication, San Diego, CA, pp. 363–390, 1996.
- Nelson, M., Leigh, L., Alling, A., MacCallum, T., Allen, J.P., Alvarez-Romo, N. Biosphere 2 test module: a ground-based sunlight-driven prototype of a closed ecological system. *Adv. Space Res.* 12 (5), 151–158, 1992.
- Nelson, M., Burgess, T., Alling, A., Alvarez-Romo, N., Dempster, W., Walford, R., Allen, J. Using a closed ecological system to study Earth’s biosphere: initial results from Biosphere 2. *BioScience* 43 (4), 225–236, 1993.
- Nelson, M., Dempster, W.F., Alvarez-Romo, N., MacCallum, T. Atmospheric dynamics and bioregenerative technologies in a soil-based ecological life support system: initial results from Biosphere 2. *Adv. Space Res.* 14 (11), 417–426, 1994.
- Nelson, M., Finn, M., Wilson, C., Zabel, B., van Thillo, M., Hawes, P., Fernandez, R. Bioregenerative recycle of wastewater in Biosphere 2 using a created wetland: two year results. *J. Ecol. Eng.* 13, 189–197, 1999.
- Nelson, M., Allen, J., Alling, A., Dempster, W.F., Silverstone, S. Earth applications of closed ecological systems: relevance to the development of sustainability in our global biosphere. *Adv. Space Res.* 31 (7), 1649–1656, 2003.

- Nelson, M., Dempster, W.F., Silverstone, S., Alling, A., Allen, J.P., van Thillo, M. Crop Yield and Light/Energy Efficiency in a Closed Ecological System: Laboratory Biosphere experiments with wheat and sweet potato. *Adv. Space Res.* 35 (9), 1539–1543, 2005.
- Nelson, M., Allen, J.P., Dempster, W.F. Modular Biospheres – A new platform for education and research. *Adv. Space Res.* 41 (5), 787–797, 2008.
- Nelson, M., Dempster, W.F., Allen, J. The water cycle in closed ecological systems: perspectives from the Biosphere 2 and laboratory biosphere systems. *Adv. Space Res.* 44 (12), 1404–1412, 2009.
- Nelson, M., Pechurkin, N.S., Allen, J.P., Somova, L.A., Gitelson, J.I. Bioengineering of Closed Ecological Systems for Ecological Research, Space Life Support and the Science of Biospherics (chapter 11, vol. 10), in: Wang, L.K., Ivanov, V., Tay, J.-H. (Eds.), *Environmental Biotechnology in the Handbook of Environmental Engineering Series*, The Humana Press, Inc., Totowa, NJ, <http://dx.doi.org/10.1007/978-1-60327-140-0>, 2010.
- Nitta, K. The CEEF closed ecosystem as a laboratory for determining the dynamics of radioactive isotopes. *Adv. Space Res.* 27 (9), 1505–1512, 2001.
- Nitta, K., Otsubo, K., Ashida, A. Integration test project of CEEF – A test bed for closed ecological life support systems. *Adv. Space Res.* 26 (2), 335–338, 2000.
- Odum, H.T. Scales of ecological engineering. *Ecol. Eng.* 6, 7–19, 1996.
- Osmond, B. Experimental ecosystem and climate change research in controlled environments: lessons from the Biosphere 2 Laboratory 1996–2003, in: Omasa, K. (Ed.), *Air Pollution and Global Change*. Springer, Tokyo, pp. 1–13, 2005.
- Paglia, D.E., Walford, R.L. Atypical hematological response to combined calorie restriction and chronic hypoxia in biosphere 2 crew: a possible link to latent features of hibernation capacity. *Habitation* 10 (2), 79–85, 2005.
- Salisbury, F.B., Gitelson, J.I., Lisovsky, G.M. Bios-3: Siberian experiments in bioregenerative life support. *Bioscience* 47 (9), 575–585, 1997.
- Schlesinger, W.H. *Biogeochemistry: An Analysis of Global Change*. Academic Press, NY, 1991.
- Severinghaus, J.P., Broecker, W.S., Dempster, W.F., MacCallum, T., Whalen, M. Oxygen loss in Biosphere 2. *Trans. Am. Geophys. Union (Eos)* 75, 33–36, 1994.
- Silverstone, S.E., Nelson, M. Food production and nutrition in Biosphere 2: results from the first mission, September 1991 to September 1993. *Adv. Space Res.* 18 (4/5), 49–61, 1996.
- Terskov, I.A., Gitelson, J.I., Kovrov, B.G., et al. Closed System: Man–Higher Plants (Four Month Experiment). Translation of Nauka Press, Siberian Branch, Novocibirsk, Publication. NASA-TM 76452, Washington, DC, 1979.
- Tubiello, F.N., Druitt, J.W., Marino, B.D.V. Dynamics of the global water cycle of dynamics of the global water cycle of Biosphere 2. *J. Ecological Eng.* 13, 287–300, 1999.
- Vernadsky, V.I. Geochemistry and the Biosphere, in: Salisbury, F. (Ed.), *Synergetic Press*, Santa Fe, NM, 1997.
- Walford, R.L., Harris, S.B., Gunion, M.W. The calorically restricted low-fat nutrient-dense diet in Biosphere 2 significantly lowers blood glucose, total leukocyte count, cholesterol, and blood pressure in humans, in: *Proceedings National Academy of Science*, vol. 89(23), pp. 11533–11537, 1992.
- Walford, R.L., Bechtel, R., MacCallum, T., Paglia, D.E., Weber, L.J. “Biospheric medicine” as viewed from the two-year first closure of Biosphere 2. *Aviat. Space Environ. Med.* 67, 609–617, 1996.
- Wolverton, B.C. *How to Grow Fresh Air*. Penguin Books, NY, 1997.