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THE BRINE SHRIMP *ARTEMIA FRANCISCANA* IN CLOSED MICROALGAL-BASED MICROCOSMS (BIOSPHERES)

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ABSTRACT

The purpose of this study was to examine the effects of three experimental variables on the stability and fecundity of a brine-shrimp community in a closed, microalgal-based microcosm. Xenic microalgal cultures and brine shrimp (*Artemia franciscana* Kellogg) in 300-cc acrylic spheres (referred to as biospheres or microcosms) were subjected to three experimental variables: (1) the culture volume (150 ml or 250 ml), (2) presence or absence of daily culture mixing (sphere rotation), and (3) a microcosm atmosphere that was either closed or open to the surrounding atmosphere. Each sphere started with 200 brine shrimp cysts and the microalga *Nannochloris* at a density of $5–7 \times 10^7$ cells/ml in a culture medium of 35 ppt total salts. The biospheres were monitored twice a week with closeup color photography, noting culture color and transparency, oxygen bubble accumulation, settling of planktonic algae, and the density, size, and maturity of the brine shrimp over time. The brine shrimp copulated by 2½ weeks; a second generation of nauplii appeared in ca 30 days, temporarily increasing the density. These observations constitute the first report of brine shrimp sexual reproduction (ovoviviparity) in closed microcosms. The density of the brine shrimp appeared to stabilize during days 50–82, at an approximated 0.08 individuals/ml. Because all biospheres were yellow in the final weeks, i.e. showed no signs of *Nannochloris*, the brine shrimp were likely sustained by bacteria generated from the brine-shrimp feces. Blue-green algal filaments, a culture contaminant, at the air-water interface, out of the reach of brine shrimp, appeared to have provided oxygen in the absence of *Nannochloris*.

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Aquatic microcosms have been used in studying phenomena when containment is of high priority, such as in studies of genetically engineered microbes (Awong et al. 1990, Heuer et al. 1995, Leser 1995); chemical effects, particularly of hazardous materials (Johnson and Romanenko 1989, Swartzman et al. 1989); or, more basically, subsets of our biosphere to better understand ecosystems (e.g., island biogeography, Dickerson and Robinson 1985); effect of complexity on stability (Wilson and Botkin 1990). Aquatic microcosms, although in one sense contained, may still be open or closed to the surrounding atmosphere and provide information on stability and reliability of such systems that, for example, might one day provide food or waste processing for extraterrestrial outposts manned by astronauts. Studies of space biology at orbital stations are becoming routine (Eckart 1994, Nechitaoilo and Mashinsky 1993), and the brine shrimp, the focus our present study, has been used as a model system to assess microgravity effects on developing organisms (Spooner et al. 1992). Structural constraints under which brine shrimp nauplii either hatch and become free-swimming or fail to do so have been examined (Rosowski et al. 1997) and thus we are now in a better position to evaluate success or failure of hatching of nauplii in closed systems.

In a previous study (Rosowski 1989) it was shown that a microalgal-based brine shrimp microcosm of 500-ml culture volume, open to the room atmosphere and at unit gravity, could support rapid growth of hundreds of individuals of *Artemia franciscana* from encysted embryos to bisexual adults. Slower but successful growth for 1 or 2 brine shrimp was also demonstrated in 1.6-cc closed microcosms, chambers not much bigger in diameter than the length of the preadults and adults they ultimately produced (Rosowski and Efting 1992). This realization that brine shrimp could not only tolerate but thrive in small quarters led to the first embryonic development and hatching of brine shrimp from cysts in the microgravity of low earth orbit, in similarly small chambers (Rosowski et al. 1995). Experiments in the 500-ml open cultures also showed that sexual reproduction (ovoviviparity) could occur in less than two weeks. However, in the tiny 1.6-cc sealed microcosms at unit gravity, adulthood was not usually reached, or adults mostly died soon after reaching maturity. Start-

ing with no more than four cysts, when these 1.6-cc microcosms supported growth of two nauplii, they became either both males, both females, or remained immature. Surprisingly, in no case in 50 experiments did a male and female mature in the same chamber, for reasons unknown. The question thus remained as to whether sexual reproduction could occur in sealed microcosms and, more importantly, whether a biological equilibrium could be established so that a closed microcommunity could become self sustaining over time, as occurs in laboratory cultures open to the atmosphere and in natural habitats.

The earth's biosphere has maintained itself for 3.7 billion years, but success with man-made, closed-loop biospheres having light as an energy source is measured in mere months, a few years, or in a very few instances one or two decades [as in Clair Folsome's studies cited by Shaffer (1993) or in the commercial "Ecospheres" based on Folsome's prototypes]. This may be largely because closed-system aquatic biospheres have not been, in the past, the subject of many published experiments (Eckart 1994, *p. 142*), although experiments with microbial-based closed ecological systems continue to be under study (reviewed by Tamponnet and Savage 1994). The community including humans in Biosphere II (Allen 1991, Shank 1991, Stover 1990, Vergano 1996) obviously was more complex with respect to species diversity and their interactions than the algal-based brine shrimp communities we are studying. However, that does not mean that our small volume, low-density, low-diversity community cultures are "simple systems," rather we think of them as small, low cost, easily-assembled systems. From their low diversity one might predict them to be more stable than similar-volume communities of larger diversity, based on the predictions of the theoretical, sealed-microcosm models of Wilson and Botkin (1990). However, experimentally, we know little of what is essential to sustain communities of any size, for a decade to a century or more, particularly the construct that may be necessary to attain the stability required for the establishment of human settlements elsewhere in our solar system.

By most accounts (Eckart 1994, Holtzapple et al. 1989, Nechitaoilo and Mashinsky 1993, Westgate et al. 1992), microalgae may play a major role in life support systems in manned space outposts because of their "extreme compactness, ease of handling and high production efficiency" (Holtzapple et al. 1989), adaptability to bioreactor constraints (Mori et al. 1987), and utility for creating "food, potable water and oxygen, and as sinks for carbon dioxide and metabolic wastes" (MacElroy and Bredt 1984). The recent challenge to the old idea that "all energy for living communities comes ultimately from the sun," based on the discovery of a deep, subterranean bacterial biomass currently estimated to equal or exceed that of the earth's land surface (and created not from sunlight but from energy derived from chemosynthetic primary production from geothermically reduced sulfur compounds [see Gould (1996) for review], appears of little consequence to the ethos of supporting space-station life. It remains likely that the now-updated idea "all energy for living *terrestrial* communities comes ultimately from the sun" will similarly be applied to human-based communities which may one day develop beyond earth but within our solar system.

The primary purpose of the present study was to determine if we could exceed the 56 days of growth of brine shrimp to adults and sustain them and their life support system as demonstrated earlier with one or two brine shrimp in the 1.6-cc, closed, Materials Dispersion Apparatus similar to that used in Consort Sounding Rocket missions (Rosowski and Efting 1992). Furthermore, with a much larger microcosm (300 cc), and up to 100 times more cysts (200) as starting material, we likely would be providing sufficient space and brine shrimp density required for the development of a sexually active community if the biosphere chemistry was and remained so conducive. We also examined three factors that we anticipated might influence the stability and longevity of this largely endogenously regulated microcommunity. These factors were: (1) culture volume, (2) resuspension of settled matter (particularly microalgae needed for oxygen, food production and dissolved waste recycling), and (3) gas exchange (we thought gas exchange between the outside atmosphere and our biospheres might provide for a more stable community, with greater final brine shrimp density than would occur, for example, from a community with gas exchange confined to within the biosphere).

MATERIALS AND METHODS

The community culture

Xenic algal cultures were maintained in 1.5-L glass milk bottles aerated continuously at 22° C and illuminated continuously under four to six 20-watt cool-white fluorescent light bulbs in an incubator. One-liter cultures were maintained every three weeks by combining equal amounts of the old culture and newly prepared saltwater to which was added 8 cc of particulate Milorganite™ fertilizer/1.5-L milk bottle. The algal culture was one we have maintained with this procedure since 1979. It has consisted of *Nannochloris* sp. (formerly referred to as *Chlorella* sp.) plus a nonheterocystic, unbranched, filamentous blue-green alga contaminant (Rosowski 1989). Rather than trying to remove the BGA contaminant, we kept it when we found that it congregates in culture vessels at the air-water interface (meniscus) where it would contribute

oxygen and remove carbon dioxide while maintaining itself generally out of reach as food for the brine shrimp. The saltwater was made to a specific gravity of 1.025 using a dry mix of 1 part Instant Ocean™ to 3 parts NaCl by volume. Brine shrimp cysts were obtained from Sanders Brine Shrimp Company, L.C., Ogden, Utah, U.S.A. and stored at 5° C until needed.

Sphere preparation

The 8.7-cm outside diameter 300-cc acrylic spheres came as two hemispheres. For each sphere a hole was drilled into one hemisphere. The spheres assigned to be open to the surrounding atmosphere had a 9-mm hole drilled in the top hemisphere, while the spheres ultimately to be closed and sealed had a 3-mm hole drilled in the bottom hemisphere. Hemispheres were sealed together with di-ethylene chloride, forming a sphere in the following manner. The rim of one half of the hemisphere was dipped for 10 sec into a shallow dish of di-ethylene chloride. Excess di-ethylene chloride was wiped off, and the rim of one hemisphere was placed within the flange of the other hemisphere. The hemispheres were then immediately twisted to spread the solvent and make an air-tight seal. The success of the seal was determined by placing the assembled sphere into water below the seal and applying a vacuum through the hole in the sphere to see if water was drawn inside along the seam of the fused hemispheres. Those spheres that leaked were sealed with further applications of di-ethylene chloride or discarded if they could not be sealed with this solvent.

Biosphere preparation

The microcosms were prepared by adding brine shrimp cysts and the microalgal culture to the spheres as follows. According to the experimental design, either 150 ml or 250 ml of the algal culture was added with a 60-cc syringe (no needle). 200 cysts were then counted under a dissection microscope and placed in the culture of each sphere. The spheres assigned to be closed were then sealed by hot-gluing a round, 12 mm diameter, glass coverslip over the 3-mm hole in one hemisphere. Finally, a support base consisting of a 5-cm plastic Petri-dish bottom was hot-glued to the bottom of each biosphere to keep the spheres in a fixed position as would be required by the photography method of data collection.

Experimental design

After assembly, the biospheres were placed in a 28° C, continuously lighted incubator for 24 hr to allow the cysts to hatch. After 24 hr they were then placed in an incubator at 22° C under continuous light for the remainder of the experiment. Three variables were examined over time: (1) culture volume (150 ml or 250 ml), (2) effect of the absence or presence of sphere (and thus culture) rotation (inverted and up-righted daily to resuspend the sediments), and (3) the effect of the presence or absence of gas exchange with the air outside the biosphere. These three factors were arranged to make the eight treatments as shown in Fig. 1. Each treatment was replicated once (N = 16 biospheres).

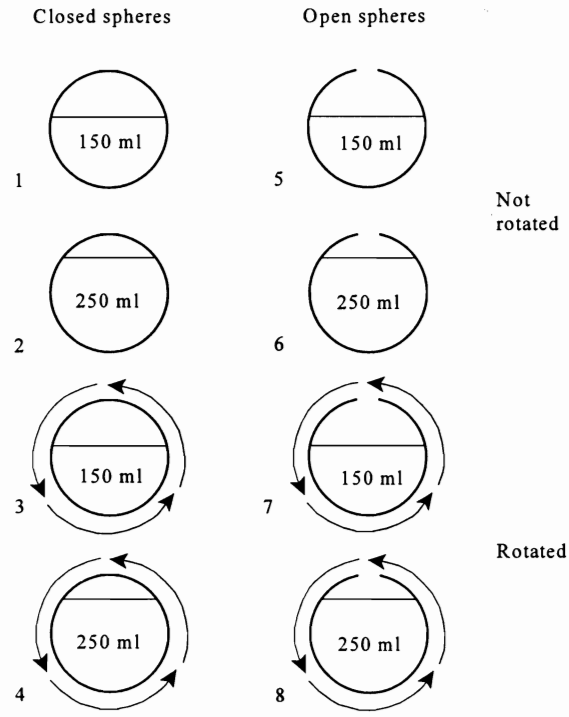


Figure 1. Biosphere treatments for the 2 × 2 × 2 factorial design replicated once (N = 16). This design produced eight unique treatments by the biosphere combinations shown above.

Biosphere monitoring

The experiment was monitored externally by direct observation through the acrylic spheres and by using closeup color photography to provide a visual record. No samples were taken from the biospheres during the course of the experiments. Photographs were taken by placing a Canon 35 mm SLR camera, a biosphere, and a 100 watt incandescent light bulb in a straight line using a series of clamps and rods to maintain specific distances between these three objects. The front of the camera was 15 cm from the "front side" of the biosphere, and the "back side" of the biosphere was 7 cm away from the light bulb (Fig. 2). The film was Kodak J Gold, 35 mm color print film, ASA 200.

Characteristics observed in the culture medium were color, particulate settling, bubbles, and culture medium transparency. Color was measured on a scale

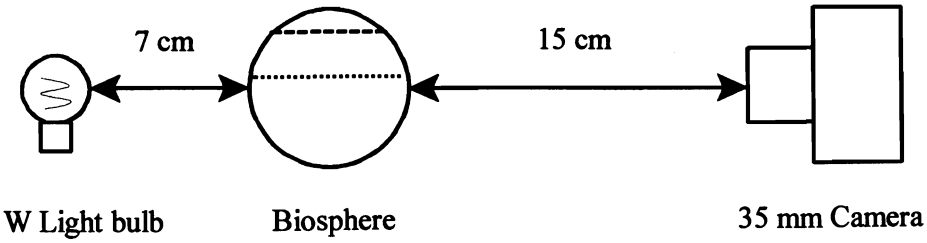


Figure 2. Diagram showing placement of equipment and biosphere during photographing. The dotted lines in the biosphere refer to the two volumes of culture medium, 150 ml (bottom dotted line) and 250 ml (top dotted line).

of 1–5, with 1 = green; 2 = yellow-green; 3 = yellow; 4 = yellow-brown; and 5 = clear. Color was recorded with photographs on an every-other-day basis for two weeks, then twice a week until the termination of the experiment. Transparency was similarly determined with a 1–5 scale, with 1 = opaque, 2 = densely cloudy, 3 = moderately cloudy, 4 = slightly cloudy, 5 = transparent. Settling was scored as having occurred or not having occurred. The eventual occurrence of a ring of blue-green algae around the biosphere culture-medium surface at the meniscus was also noted.

For brine shrimp, the average length and total number were estimated using 4 × 5 inch photographic prints. Average length was estimated by measuring each shrimp in focus in the print. These numbers were then averaged and multiplied by a conversion factor to compensate for the difference in size as photographed in the spheres in comparison to their actual size. The total number of brine shrimp was estimated by counting the shrimp that could be seen in the photograph, including those slightly out of focus. At the beginning of the experiment the culture medium was so green

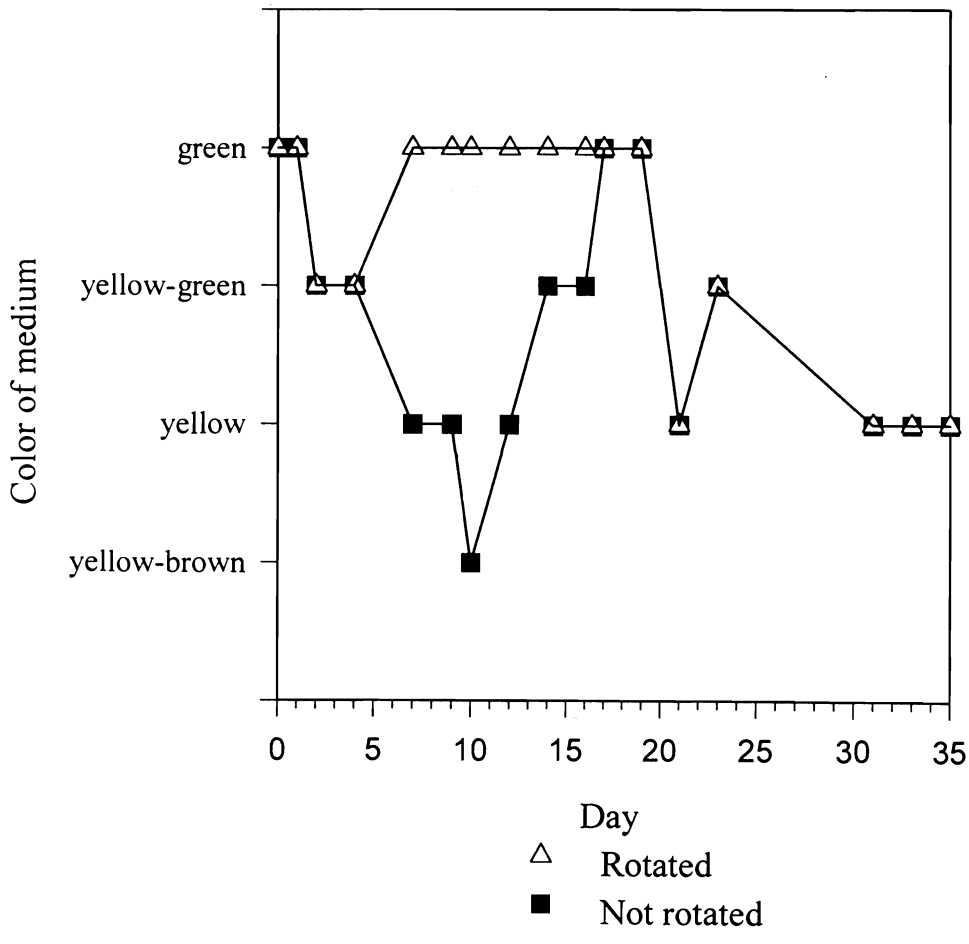


Figure 3. Changes in medium color in rotated and non-rotated biospheres for the first 35 days. After 35 days the medium was yellow and remained so until day 82.

that nauplii, and even preadults, could not be seen clearly enough to be counted, although they could be seen copulating.

Statistical treatment of the data

The experiment was a $2 \times 2 \times 2$ factorial design over time. The computer program was SAS using the general linear model procedure, which detected differences between treatments and included all combinations of treatments. Interactions between factors were examined before looking at the significance of the solitary factors. Significance was determined at the level of 0.3.

RESULTS

Culture medium

The color, transparency and settling of particulates within the culture medium of each of 16 microalgal-based brine shrimp biospheres was monitored over time to determine response to each of three experimental variables: (1) absence or presence of daily biosphere rotation bringing about (in the latter) immediate culture sediment resuspension, (2) open as opposed to isolation of the surrounding atmosphere from the biosphere atmosphere, and (3) two different culture volumes within the biospheres (Fig. 1). In the general linear model, culture color was found to have a significant two-way interaction between sphere roatation and time (p = 0.00001, Fig. 3). Culture medium color was not effected by atmospheric isolation or medium volume. Culture medium transparency varied over time (p = 0.0001) and in relation to sphere rotation (p = 0.0001). No effect was detected on transparency in relation to the factors of medium volume and isolation of the surrounding atmosphere from the biosphere atmosphere. Settling of particulates in the biospheres (brine-shrimp feces and algae) occurred indiscriminately over time in all spheres. Even though settling reduced the algal density and thus the green color, sphere rotation significantly impacted the magnitude of the decline in green color. For example, biospheres that were rotated remained more opaque over time (cf. Figs. 9–12 with 13–16), with a Least Square Means (L S Means) of 2.33, as opposed to the non-rotated spheres that had a L S Means of 2.56, on a 1 to 5 scale (p = 0.0001).

Particulate settling and consumption of the tiny microalga *Nannochloris* sp. by the brine shrimp are hypothesized as the two most important reasons for an increase in transparency of the culture medium of the biospheres over time. This loss of green color of the medium was always followed over time by a noticeable yellowing by day 21 (Fig. 3), and the yellow remained until termination of the experiment at 82 days.

Settling of particulates (algae and fecal pellets) occurred in all biospheres over time but, based on the photographs, settling was not similar for all treatments. Although fecal pellets settled at the bottom, we also noted that fecal pellets often remained attached and then became continuous, extremely long tubes by the time the cultures were 45 days old or more (Figs. 14, 16).

As previously mentioned, the transparency of the biosphere culture medium increased over time. However, sphere rotation resulted in an elongation of the period of opacity. In all spheres over time, the culture medium became increasingly transparent to the point that no green color was evident in the 21-day or older spheres except at the air-water interface, where filamentous blue-green algae grew attached to the sphere wall. Regardless of whether or not daily rotation was applied to a sphere, all spheres had sediment at the bottom within the first few days (cf. Figs. 6, 7, 10, 11). Although sphere rotation resulted in a resuspension of these sediments, the heavier particles experienced a relatively quick resettling after rotation, that is, within an hour. The culture medium color of all biospheres showed a similar trend over time. Initially the medium of biospheres was vivid grass green (Figs. 5, 9), with a density of the unicellular algae of 5–7 $\times$ 10^7 cells/ml. Then the medium yellowed, followed by a return of green (i.e. bloomed, cf. Figs. 7, 8) and finally, for most of the experiment, the medium remained yellow (Figs. 3, 14–16). While all biospheres experienced this pattern, the magnitude of the color changes and the time peri-

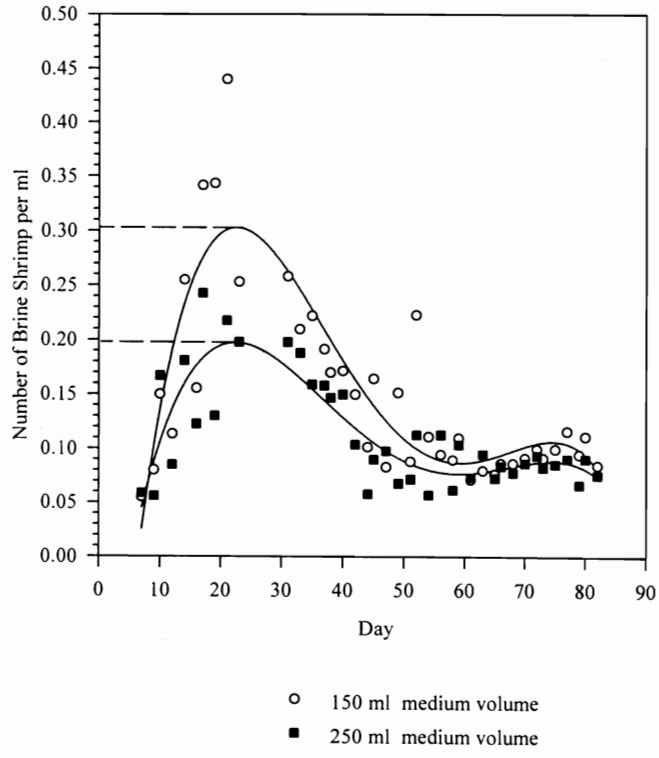
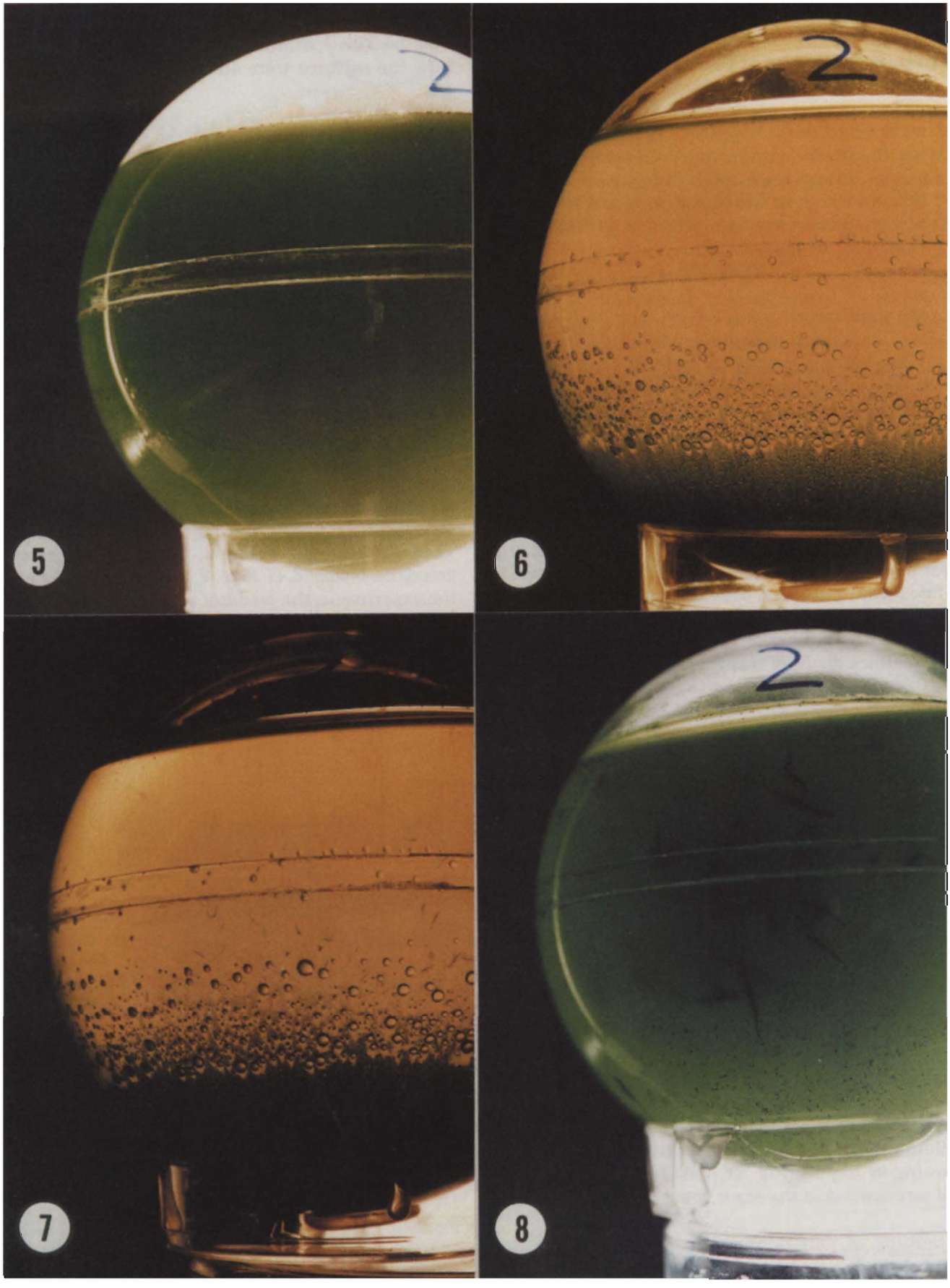


Figure 4. The density of brine shrimp over time in 150-ml and 250-ml cultures in 300-cc biospheres. The dashed line points to the likely peak density in the two culture volumes.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ods over which they occurred differed according to the rotational treatment of the spheres. The medium of the rotated spheres changed to yellow-green after only day 4, at which time the algae bloomed and returned the medium to the original green color by day 9. In the non-rotated spheres, the medium took 10 days to change to yellow-brown (Fig. 7), followed by re-greening of the medium by day 17 (Figs. 3, 8). Nonetheless, by day 21, all cultures in the spheres, rotated or non-rotated, had turned yellow, coinciding approximately with the arrival of the second generation of brine shrimp by live birth (ovoviviparity). The suspended (planktonic), green, microalgal population was largely depleted by day 21 (much more transparent and no longer green), but there were living filaments of blue-green algae that formed a blue-green ring at the water's surface, attached to the wall of the sphere. (Knowledge of the type of algae in the ring is based on previous examination of this material from spheres that were sampled). The color of the medium of the spheres suggested a loss of the microalgal population in non-rotated spheres that was greater than that in the rotated spheres (Fig. 3). Interestingly, by day 17 or 19, both the rotated and the non-rotated spheres had a green color similar to that of their first day of growth (cf. Figs. 5 and 9, with 9 and 12). However, as mentioned, by day 21 the green was gone and the medium had changed to yellow. The graph in Fig. 3 shows data only through day 35. After this day both the rotated and non-rotated cultures had the yellow color that would remain until the end of the experiment at day 82 (Fig. 3). Even though the cultures were yellow and probably had a very low density of living *Nannochloris*, the blue-green algal filaments remained robust at the air-water interface. The meniscus of the cultures had a distinct blue-green ring from day 31 on, and was significant in oxygen production as evident by the ring of bubbles near it (Figs. 14, 15). Bubbles (presumably oxygen) also adhered to the side of the sphere well below the medium surface and they were associated with the planktonic microalgae. These bubbles were conspicuously absent in areas were the planktonic microalgae had settled out (Fig. 13).

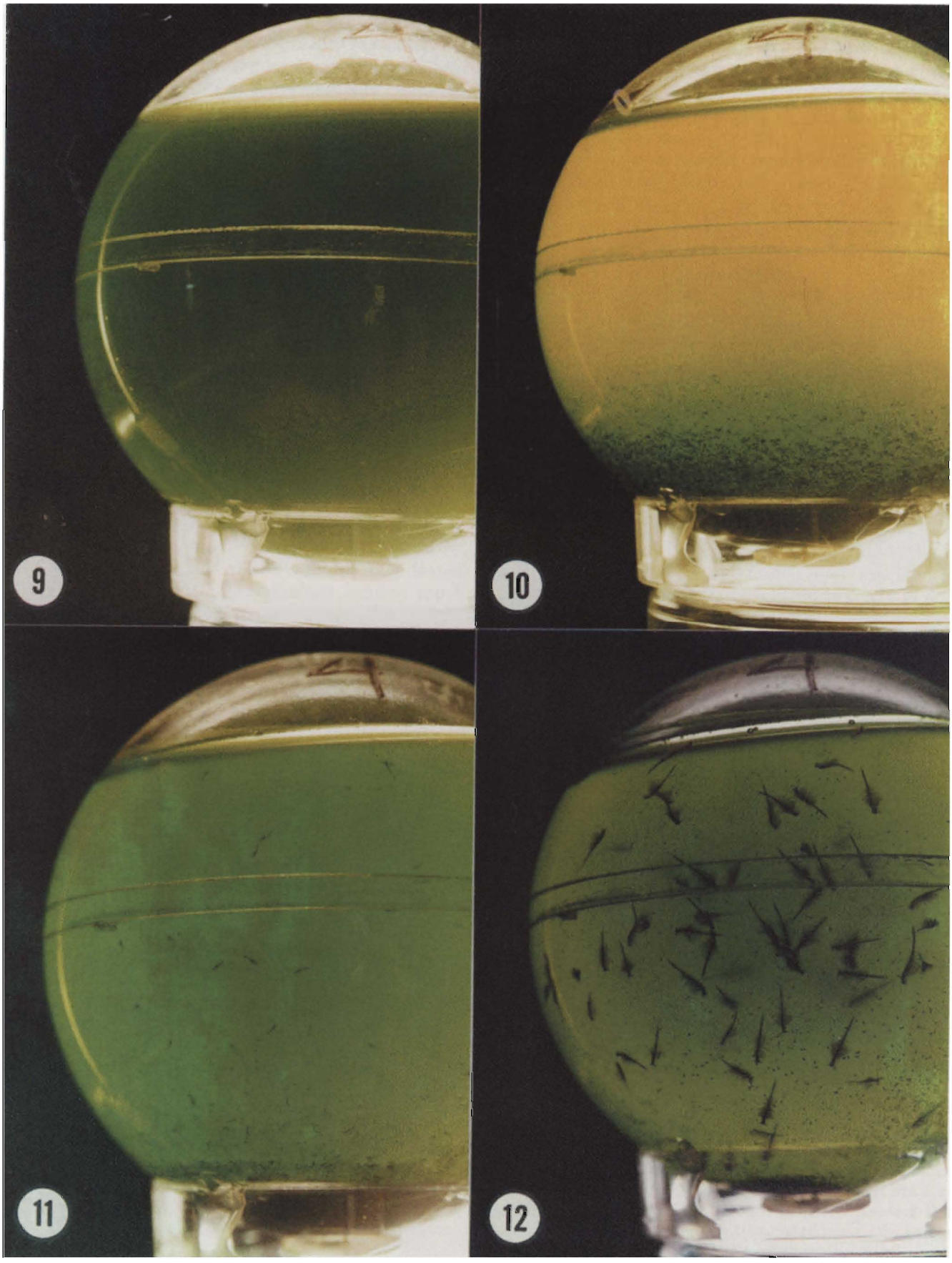
Brine shrimp

For the brine shrimp, there were two general themes over time: (1) a decrease in density (Fig. 4), and (2) an increase in average length (Fig. 17). The density of brine shrimp was moderately high at the beginning because 200 cysts were placed in each sphere. A large percentage of these cysts hatched and the nauplii grew into sexually mature adults within the first three weeks. However, the dark color of the culture medium made them difficult to observe during that period. Nonetheless, we observed copulating adults in 2½ weeks in some spheres. The combination of a high density of brine shrimp and the effects of an increase in consumption of microalgae by the growing metanauplii and preadults resulted in a planktonic algal crash by day 21. Some sexually mature females continued producing live nauplii, while others died, presumably for lack of food, which in turn resulted in a net decrease in brine shrimp density. The decrease was from 0.25 brine shrimp/ml of biosphere culture to 0.08 brine shrimp/ml from about day 20 to day 50. After this time the density (0.08 brine shrimp/ml) appeared to remain steady until termination of the experiment at day 82. During this latter time, the average length of the brine shrimp (males and females) was about 7.5 mm.

Independent of time, brine-shrimp length and density were also affected by culture volume. In the spheres with a culture medium volume of 250 ml, the mean length was 7.6 mm, and in the cultures of 150-ml medium volume, the mean length was 7.1 mm (Fig. 19). Conversely, the brine-shrimp density was higher in the 150-ml culture-volume spheres, with an average density for preadults of 0.146/ml, whereas the mean density of preadults in the 250-ml culture-volume spheres was 0.114/ml (Fig. 18).

Under the constraints of our closed biospheres (permanently united plastic hemispheres), no mass measurements could been taken, but length measurements were estimated from color photographs. The estimated length was then used to estimate the mass of the brine shrimp on any day in any biosphere. By manipulating the data, biolength density (an estimate of biomass density) was calculated for each day and then statistically analyzed, as follows. Biolength density equals the number of brine shrimp per ml divided by the average length. The biolength density for the 150-ml cultures was 1.02 mm/ml, compared to a biolength density of

Plate 1. Figures 5–8. Non-rotated biosphere with 250 ml of culture medium; all photographs in this plate are of the same sphere. **Fig. 5**. Day 0. 200 cysts added to the biosphere and barely visible in the meniscus. Oxygen bubbles (presumptive) generated by photosynthesis appear trapped in the lower third of the biosphere on the sphere surface. **Fig. 6**. Day 4. Oxygen, from previous production when the culture was green, is trapped as bubbles (evidence of super saturation). Planktonic algae have settled to the bottom one-third of the biosphere. No brine shrimp are evident but are present. **Fig. 7**. Day 9. The microalgae have settled in the lower third of the culture medium. Most of the same bubbles on the side of the sphere evident at Day 4 are still evident (cf. Figs. 6, 7) although some appear slightly smaller. Preadult *Artemia* are visible. **Fig. 8**. Day 19. Adult *Artemia* including copulating pairs (not clear in the photograph because of the high algal density). The culture has re-bloomed without sphere rotation, that is, the algae have become resuspended, likely through the swimming of the brine shrimp.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0.87 mm/ml for the 250-ml cultures (Fig. 20); ANOVA showed this difference was not significant ($p = 0.2149$).

DISCUSSION

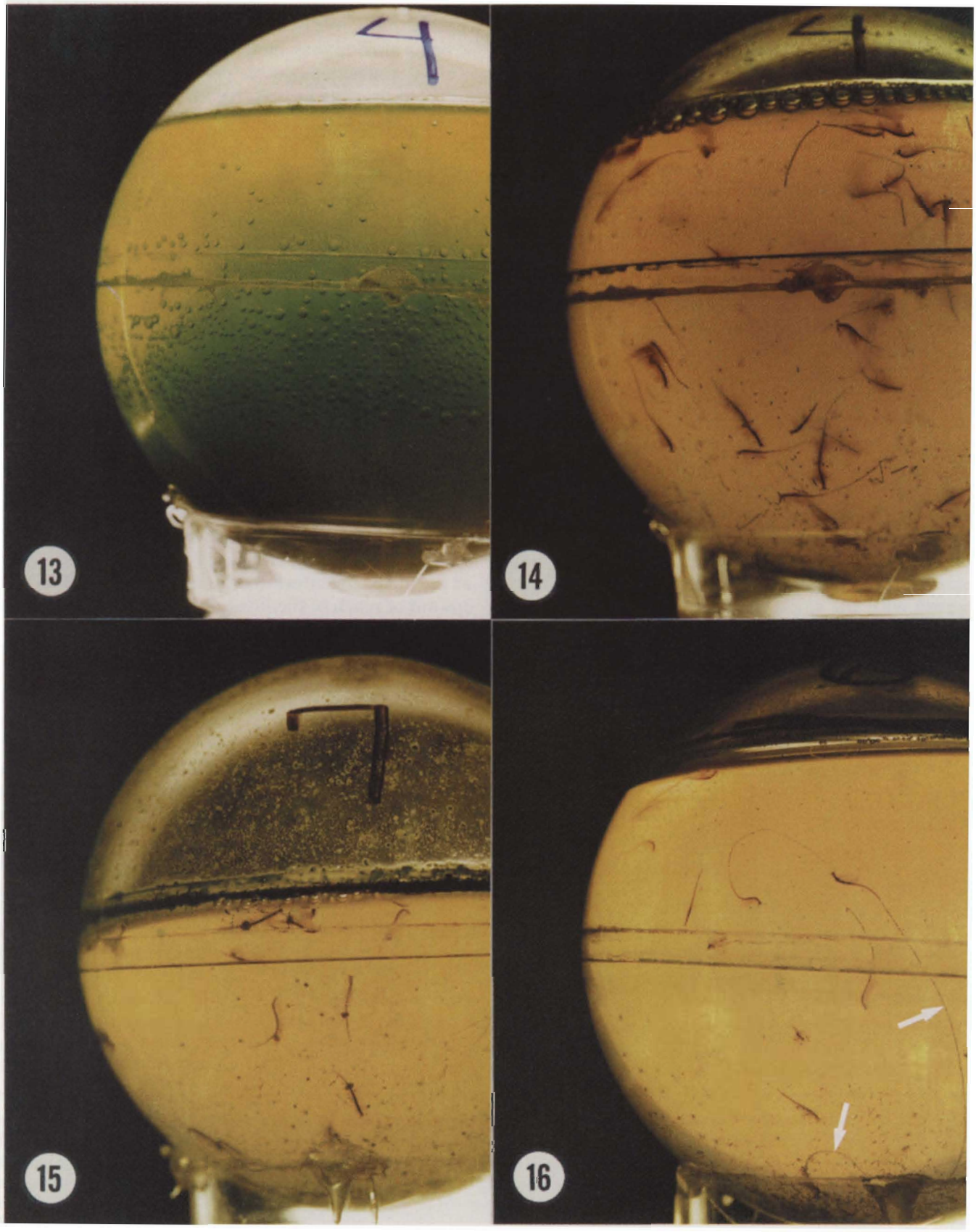
This study demonstrated that sealed, microalgal-based, 300-cc microcosms with bisexual brine shrimp hatched from cysts, were sustainable for up to 82 days during which time live birth of nauplii occurred as a result of sexual mating (presence of riders). This appears to be the first published account of sexual reproduction of brine shrimp in a closed microcosm, although earlier success in this regard was obtained in 1990, in 15-ml vials of *Nannochloris*, in which nauplii were produced by ovoviviparity from adult females just 18 days after the introduction of dry cysts into the vials (Efting and Rosowski, unpublished data).

The biospheres lost their green color after only 21 days, by removal from suspension of the planktonic *Nannochloris* by the suspension-feeding brine shrimp. The reduction of the green color coincided with the plateau of brine shrimp length (Fig. 17). These coinciding events had a deleterious effect on the stability of the microcosm. Initially 200 cysts were placed in the sphere, and although not all hatched, the hatch percentage appeared similar to what we had previously observed with the same batch of cysts (unpublished). The initial supply of algae was sufficient to support the growth of these nauplii to preadults. As they all grew to mature adults, however, the algal supply dwindled due to brine shrimp demand, leaving a population of *Nannochloris* too small to sustain itself. The emergence of live offspring by the 30th day further added to the destabilization of the culture system, and demonstrates the dynamic interactions of the microcosm community.

One surprising aspect of the present study was the re-blooming of the *Nannochloris* in the non-rotated biospheres (Fig. 8). It is likely that this re-blooming was aided by the swimming activity of the preadult and adult brine shrimp, which would have resuspended living cells thus increasing their exposure to light. We have noted for example (unpublished, since 1985, and in recent biospheres similar to those studied here), that brine shrimp stir up bottom sediments and carry particles, often green aggregates. Stirring of pond water by a paddle wheel has been shown to be effective in increasing dissolved oxygen in lower portions of a pond (Busch 1980). We believe that the swimming activities of brine shrimp may effectively accomplish on a small scale a similarly desirable distribution of oxygen (and algae) within the biospheres. Benthic cropping of the settled algae and bacteria by the brine shrimp as they ply the sediments is also likely (Savage and Knott 1998).

It is noteworthy that for approximately the last 75% of the growth period in the biospheres, i.e. the period following the reduction of the *Nannochloris* to a low level (culture medium no longer green), the dissolved oxygen presumably was largely maintained at a sufficient concentration for brine-shrimp growth by the conspicuous culture-medium surface ring of attached blue-green algae (Figs. 14, 15). We know from past experience (unpublished) that the filamentous blue-green alga contaminant occurs in the water column as well [although it is primarily periphytic in dense cultures of *Nannochloris* (Rosowski 1989)], and thus it is likely eaten along with the *Nannochloris*. The only place a dense, healthy population of this non-heterocystic filamentous blue-green alga was able to develop and survive the on-going cropping by the brine shrimp was at the air-water interface, the meniscus. Here it develops out of reach of the brine shrimp, although presumably some filaments would continuously extend into the water where eventually they would be dislodged from the ring and eaten. A previous study showed that raptorial fairy shrimp can feed on a green filamentous alga, "pulling at the algal mass with their phyllopods," and thus so-called filter feeders (suspension feeders) may exhibit more diversity in feeding behavior than previously thought (Belk and Ballantyne 1996; see also Savage and Knott 1998). In our study, filamentous blue-green algae were never observed as macrocolonies below the water's surface when adult brine shrimp were present. We hypothesize, however, that most of the food for the adult brine shrimp (after the loss of green color—*Nannochloris*—from the medium) came directly from bacteria that were recycling the culture wastes. Bacteria have developed in other brine shrimp culture systems at a density of up to 10^9 colony-forming units/ml without deleterious effects on growth (Rosowski et al. 1992) and, in fact, certain bacteria can serve as the sole food for brine shrimp (Intriago and Jones 1993)

Plate 2. Figures 9–12. Rotated biosphere with 250 ml of culture medium; all photographs in this plate are of the same sphere. **Fig. 9**. Day 0. 200 cysts and the microalgae were added and, after closure, the sphere was immediately rotated, which is why cysts are not seen at the meniscus as in Fig. 5. No presumptive oxygen bubbles are evident on the inner sphere surface. **Fig. 10**. Day 4. Microalgae have settled to the bottom third of the biosphere. Numerous attached oxygen bubbles (presumptive) are evident in the microalgal region but none above this region as for the biosphere in Figs. 6, 7. **Fig. 11**. Day 7. Pre-adult brine shrimp and their fecal pellets are evident at the bottom of the sphere. No oxygen bubbles (presumptive) are trapped on the sphere surface as in Figs. 5–7. **Fig. 12**. Day 17. Adult brine shrimp including copulating pairs. Fecal pellets are scattered throughout the biosphere but particularly towards the bottom. No oxygen bubbles (presumptive) are trapped on the sphere surface, which is similar to the situation in Fig. 8 in the non-rotated sphere.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and fairy shrimp (Dierckens et al. 1997).

In a study of 250-ml and 40-L microalgal-based communities assembled in the laboratory with invertebrate consumers, Drake (1991) showed that the order in which the species of the algal community are assembled determines the community structure. That is, one must know the historical sequences, i.e. assembly mechanics, to appreciate that even with the same set of species to create a community, one can produce "alternative community end points" depending on when the species are introduced. In our study, the species and assembly mechanics were similar in all 16 biospheres, and although there were short-term differences in community dynamics brought about by the experimental variables (culture volume, daily biosphere rotation, and a closed or open atmosphere above the biosphere culture surface), the final communities were similar. This was unexpected, and in particular we were surprised that the perturbation by daily rotation of certain biospheres did not have a dramatic effect on resilience, that is, "the rate at which a system returns to a stable equilibrium after a perturbation" (Bischi 1992). Bischi (1992) has suggested that, based on a mathematical model, nutrient recycling from organic sediments should show "very low resilience" after perturbations in "quasi-closed" ecosystems, because nutrient recycling is not instantaneous as has often been assumed. On the other hand, given the relatively short period of our experiments (82 days), perhaps "rotation" type perturbations remain insignificant because nutrient recycling is also insignificant as a source of needed nutrients in the short term of 82 days, and thus our perturbed and unperturbed biospheres produced similar end results. That is, nutrients were not likely limiting even without recycling and thus the rotation perturbation had no noticeable effect. The test, therefore, of resilience of our system, and indeed of its regenerative and self-sustaining capabilities, will require a much longer experimental period for evaluation.

Because of the apparent depletion of the *Nannochloris* in the rotated and non-rotated biospheres, until a re-bloom after 21 days, we are doubtful that we have created a self-sustaining, closed community, that would last for years or decades. Still, it may be possible for

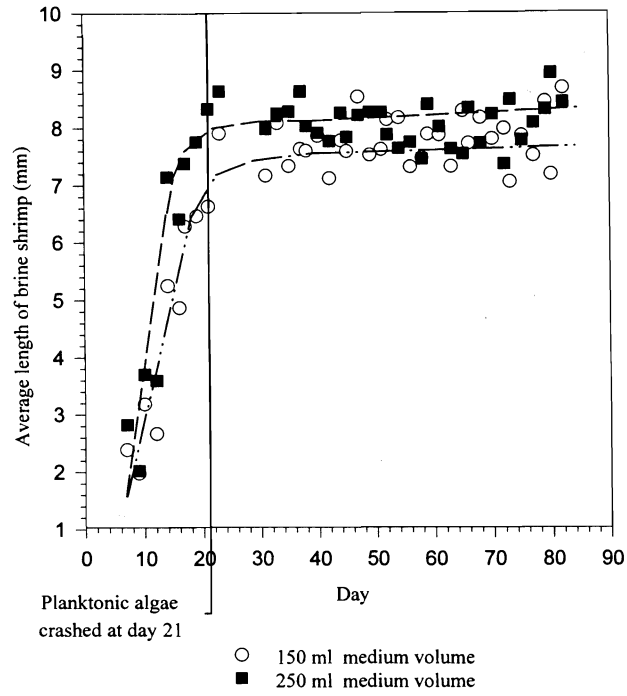

Figure 17. Average length of brine shrimp over time in 150-ml and 250-ml cultures within biospheres.

"yellow-water" communities to subsist for some time with bacteria serving as the primary food for the brine shrimp providing that the blue-green algal ring at the culture's surface continues to generate sufficient oxygen to sustain the entire community.

Finally, it should be noted that a recent reassessment of the historical context and development of the idea of "the balance of nature," has lead to "a paradigm shift in ecology" (Wu and Loucks 1995), which has significant implications for microcosm studies and efforts to develop closed, sustainable, life-support systems. To summarize salient points: "nature is not in constant balance, and patchiness is ubiquitous." Thus harmony exists in "patterns of fluctuation," and ecological persistence becomes evidence of "order within disorder." Most importantly (*p. 460*), "observed differences in process rates suggest that small-scale events induce dynamic responses, usually at a high frequency,

Plate 3. Figures 13–16. Non-rotated biospheres showing particular aspects of microalgal settling, sticking, and oxygen generation, plus short and long *Artemia* fecal pellets. **Fig. 13**. Day 1. 250 ml of culture medium; rotated biosphere. The *Nannochloris* has settled after only 24 hrs. Bubbles of oxygen (presumptive) are held to the sphere surface, mostly where the green planktonic algal cells are most dense. Cysts collect along the meniscus. **Fig. 14**. Day 47. Same rotated biosphere as in Fig. 13; 250 ml of culture medium. Some copulating brine shrimp. Some individuals have short fecal pellets; smaller fecal pellets are unattached. No planktonic algae visible; filamentous blue-green algae have produced the large oxygen bubbles (presumptive) trapped in the meniscus. **Fig. 15**. Day 56. Rotated biosphere with 150 ml of medium. Copulating brine shrimp. Oxygen bubbles (presumptive) are on the sphere surface (and away from the brine shrimp). In rotated biospheres, the top of the sphere daily is washed over with culture medium during rotation, which explains the attached algae far above the culture surface. **Fig. 16**. Day 45. Rotated biosphere; 250 ml of medium. Incredibly long, attached fecal pellets occurred in some cultures, as shown at the arrows.

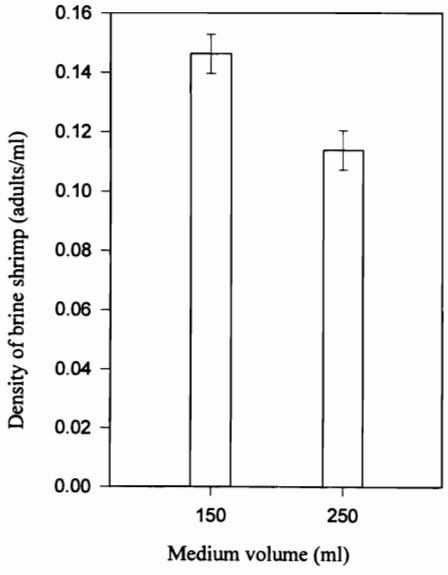

Figure 18. Density of brine shrimp over 82 days in two different volumes of culture medium.

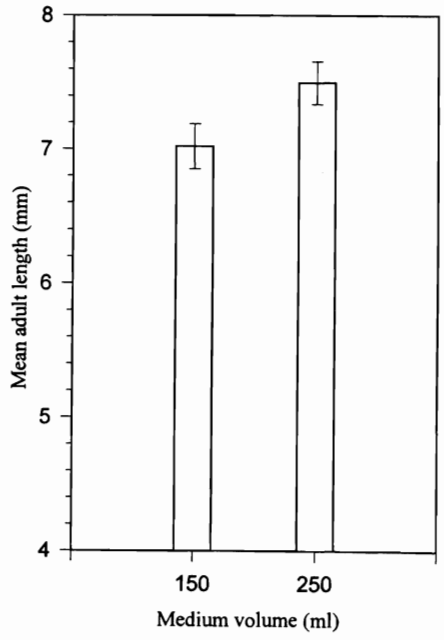

Figure 19. Brine shrimp length after 82 days in two different volumes of culture medium.

while at large scale these dynamics are at a low frequency. As a result, small ecological systems are generally subject to higher rates of extinction from either biotic feedbacks or stochastic instabilities than are large systems." With this new paradigm in mind, we see the challenge for our continuing studies to be the development of a closed aquatic community of sufficient size and species complexity to provide acceptable stability, perhaps a decade, in the wake of anticipated and unanticipated community perturbations.

DEDICATION

This paper is dedicated to Senator John Glenn, visionary and risk taker on earth (as a politician) and in space (as an astronaut), on the occasion of his recent successful flight on STS-95 in low earth orbit and return to unit gravity, October 29–November 7, at the age of 77.

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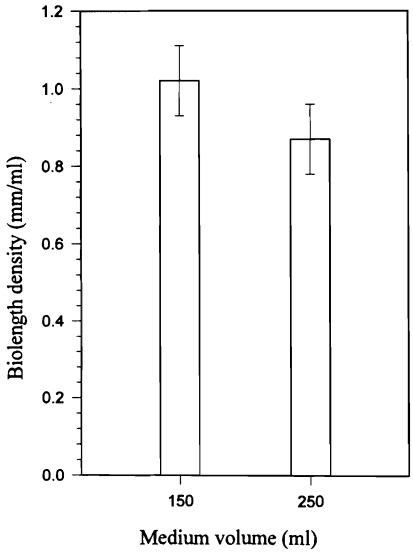

Figure 20. Biolength density of adult brine shrimp over 82 days in two different volumes of culture medium.

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