THE PHYSIOLOGY OF CARBON MONOXIDE PRODUCTION BY DEEP-SEA COELENTERATES: CAUSES AND CONSEQUENCES*

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Introduction

Coelenterates of the order, Siphonophora, are known to occur in considerable numbers in productive near-shore areas of much of the world's oceans. Particularly off southern California, these delicate, gelatinous carnivores (FIGURES 1A & B) are to be found throughout much of the water column, but are concentrated especially at intermediate depths of 300–400 meters.

At these depths the complex, acoustically reflecting population of organisms, collectively labeled the Deep Scattering Layer (DSL), is most highly developed. This biological phenomenon has been determined responsible for false bottom recordings on ships' echo sounders for many years.[1] Present theory holds that a major portion of the sonic reflectivity from this assemblage of animals is due to the presence of gas-filled enclosures such as the swim bladders of fishes.[2]

Among the Siphonophora, two suborders, the Cystonectae and Physonectae, possess gas-filled floats, or pneumatophores (FIGURES 1A & B & 2) and both groups are probably important sound scatterers at certain times. The physonect siphonophores, however, are present in greater numbers at DSL depths and are active and vigorous swimmers,[3] whereas the cystonects lack organs of locomotion and seem to occur largely at shallower depths.

A near-universal behavior for portions of the DSL is a diurnal vertical migration, often of several hundred meters, from depth to surface in late afternoon and evening, and return to depth just prior to dawn.[1,4] The total transit seldom requires more than one to two hours. Because of their well-developed swimming abilities, some members of the Physonectae undertake this migration each day, thus exposing themselves to a complex of physiological stresses rarely encountered in the terrestrial environment. During the evening ascent these organisms may traverse a vertical distance equivalent to a decline in hydrostatic pressure of 30–40 atm while traversing a temperature increase of some 10° C. Conversely, during the early morning descent the increase in pressure and decline in temperature will be of equal magnitude. During these migrations increased activity places demands upon the energy metabolism, and during descent and at depth further energy demands must be met in order to secrete fresh pneumatophore gas and reinflate the float.

The gas utilized by these organisms has been identified as carbon monoxide[5] and is apparently secreted by way of a system utilizing the terminal carbon of L-serine mediated by a tetrahydrofolate.[6,7] With the pneumatophore fully inflated and acting as a buoyancy trim tank for sensitive adjustments to depth, the physonectid siphonophore, with tentacles extended, drifts horizontally through the water awaiting the capture of small crustaceans and other organisms that form its prey.

Because of their gas-filled floats and the additional feature of expulsion through

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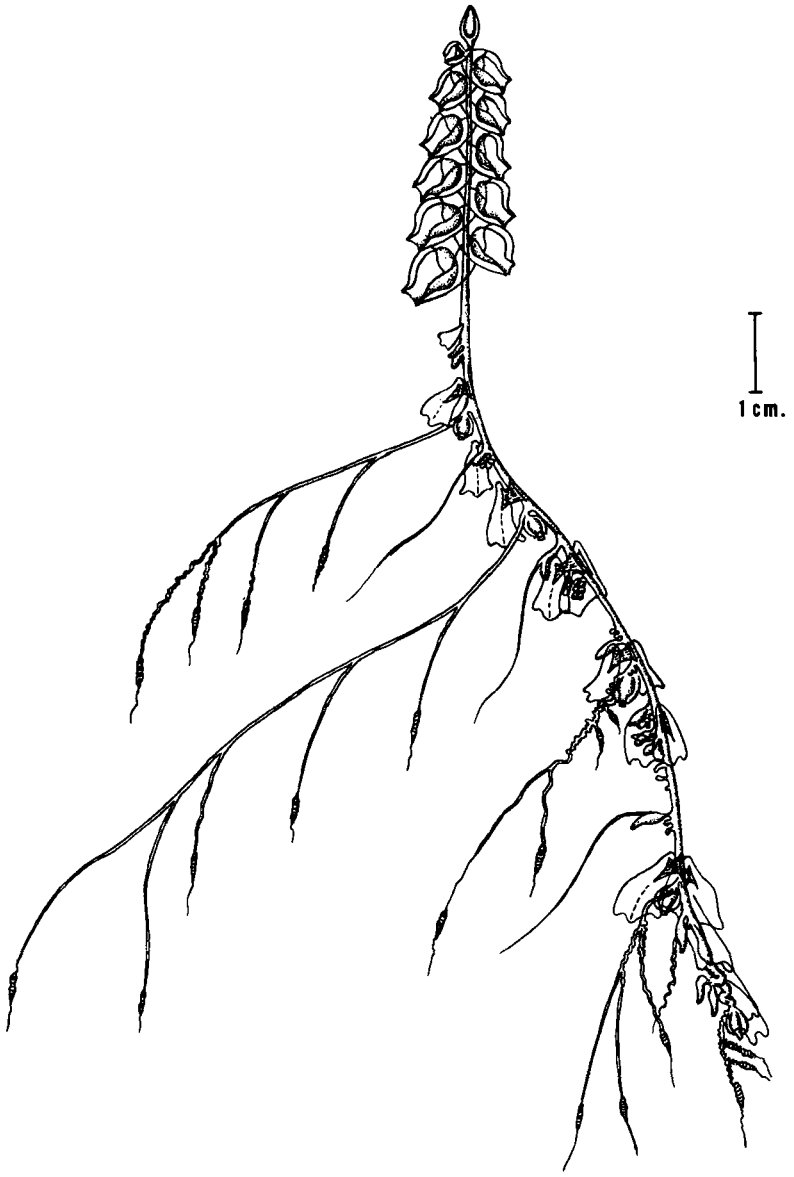

FIGURE 1A. Semidiagrammatic representation of a physonect siphonophore, *Nanomia bijuga*, showing tentacles extended in the fishing position; swimming bells (nectophores) are clustered beneath the single, apical, gas-filled float (pneumatophore). (Drawing by G. Prible)

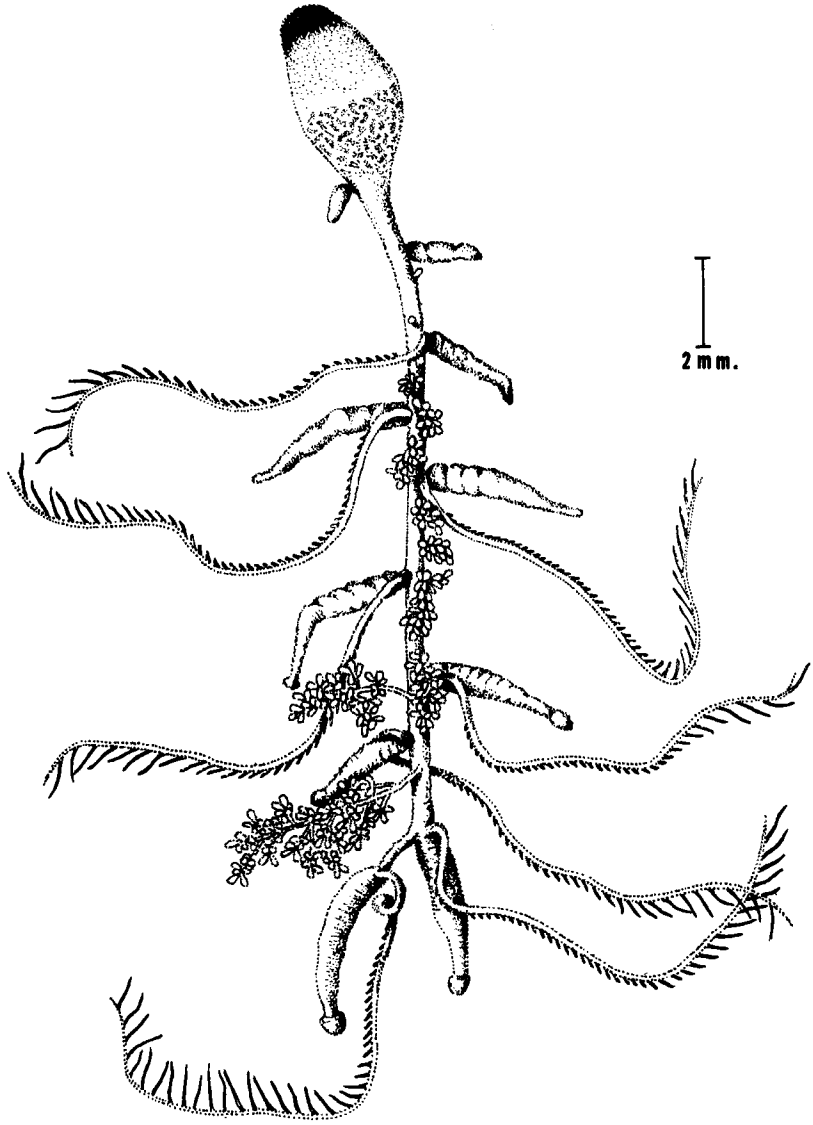

FIGURE 1B. A cystonect siphonophore, *Rhizophysa* sp. Drawing based on an intact, preserved specimen. Note lack of swimming organs beneath the comparatively large pneumatophore. (Drawing by G. Prible)

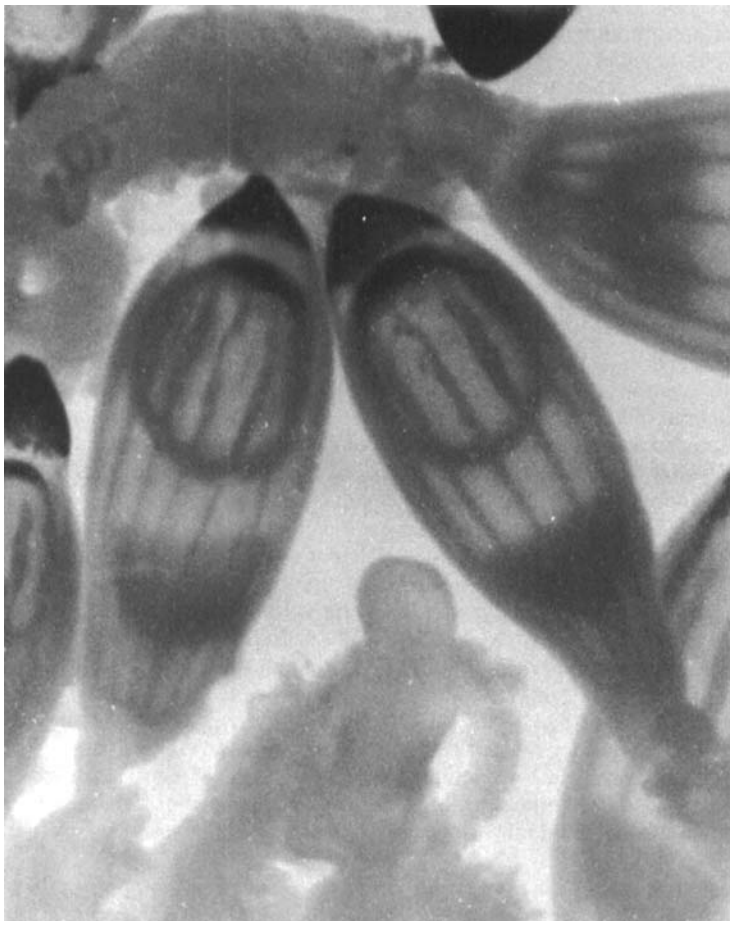

FIGURE 2. Pneumatophore specimens of *Nanomia bijuga* broken off the remainder of the organism during net collection. The cup-shaped gas glands are clearly visible through the walls of these preserved individuals. Approximate float diameter, 1 mm. (Photo by J. Sneed)

an apical pore in the float of bubbles resulting from expanding gas during upward migration,[8] the physonect siphonophores constitute important biological targets for acoustic echo sounders. They further contribute to an as yet undefined system of dissolved CO in the sea, with possible implications for atmospheric pollution,[9,10] the importance of which has still to be assessed.

The several aspects of siphonophore occurrence, migration, metabolism, energetics, and gas production presented and discussed below are intended as a further step toward understanding the functioning of these fascinating and little-known creatures. Here, the physiological requirement for neutral buoyancy obtained through gas production itself becomes a cause producing a variety of effects or consequences ranging from unique chemical substrate-governed behavior to production of the deepest naturally occurring free bubbles in the sea. Accordingly, assessment has been made of siphonophore float dimensions and

volumes, float elasticity, rate of oxygen consumption, and CO production of the pneumatophore alone, and oxygen consumption of intact siphonophores. Several related parameters having to do with the supply of energy and gas and the means by which they are utilized in coping with the physical environment have been examined.

The calculations and conclusions presented in this paper are based for the most part on information presented in two unpublished research reports[11,12]† with additional analyses and interpretations and a modest increment of newly acquired data.

Methods and Materials

The physonect siphonophores used in this study were all *Nanomia bijuga* (FIGURE 1A), collected over the San Diego Trough off southern California on on several occasions using a modified Tucker net.[13,14] Cystonect siphonophores were collected in the same area using a meter net and were diagnosed as a species of the genus *Rhizophysa* (FIGURE 1B). Although the siphonophores generally fragmented upon collection, many of the floats, when refrigerated at 5° C, remained viable for at least a week, as judged by CO secreting ability, muscle contraction, and gastrovascular fluid movements.

Dimensions of pneumatophores were taken with an ocular micrometer in a dissecting scope at 12–15X. Volume calculations were made assuming the float to be a regular prolate spheriod. Direct volume measurements of float gases were made in microgas analyzers possessing volume-calibrated capillaries or displacement spindles,[15,16] or were calculated on the basis of bubble dimensions.

Oxygen consumption of individual siphonophore floats was measured in closed tuberculin syringes (larger syringes for intact specimens) supplied with a drop of mercury for stirring. Water samples from the syringes were analyzed by the method of Scholander and colleagues.[16] Alternatively, oxygen uptake of single floats was measured in micrometer respirometers.[11,12,17] Carbon monoxide production was also determined in the syringe experiments. For detailed descriptions of the methods and modifications, as well as error analysis, see my earlier reports.[11,12]

Direct observation and photography of physonect siphonophores *in situ* was accomplished from the Westinghouse submersible research vehicle, Deepstar 4000, on several dives in the same area as the net collections.

Results and Discussion

Size of Pneumatophores

The range in measured gas volumes from a representative collection of pneumatophores (TABLE 1) was found to be approximately 0.25 to 2.5 mm.^{3} Previously[5] this gas was shown to be mainly CO generally in excess of 85 to 90% (see reference 12 for analytical procedures and confirmatory analyses). The volume of the gas phase calculated as a regular prolate spheroid is generally slightly higher than the actual measured volume of the extruded gas (both measurements made at the same temperature), although the difference is seldom greater than a few percent. This fact suggests that the gas within the float remains at or very near ambient pressure, although the float appears to be moderately elastic.

† Available to interested investigators upon request to the author.

TABLE 1

CORRECTED VOLUMES OF PNEUMATOPHORE GASES*

Pneumatophores	External Dimensions of Pneumatophores		Gas Phase†		Volume†	Volume of Extruded Gas‡
Group I	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Calculated as prolate spheroid (mm^3)	(mm^3)
1	2.50	0.83	1.70/1.64	0.80/0.74	0.57/0.47	0.52/0.41
2	3.00	1.50	2.19/2.13	1.13/1.07	1.46/1.28	1.74/1.62
3	3.80	1.13	2.59/2.53	1.03/0.97	1.44/1.24	1.23/1.11
4	2.86	1.00	2.33/2.27	0.93/0.87	1.05/0.90	0.41/0.29
5	2.46	0.86	1.81/1.75	1.22/1.16	0.48/0.39	0.66/0.54
6	3.00	1.23	2.43/2.37	1.09/1.03	1.50/1.31	1.04/0.92
7	2.90	1.20	2.16/2.10	0.97/0.90	1.06/0.88	1.70/1.59
8	2.00	0.90	1.46/1.40	0.83/0.77	0.53/0.43	0.38/0.26
9	2.63	0.90	2.16/2.10	0.83/0.77	0.77/0.65	0.49/0.37
Group II						
1	4.40	1.67	3.16/3.10	1.46/1.40	3.51/3.18	2.52/2.40
2	3.16	1.43	2.36/2.30	1.49/1.43	1.13/0.96	0.96/0.85
3	4.00	2.06	2.56/2.50	1.56/1.50	3.25/2.93	2.77/2.65
4	3.16	1.16	2.50/2.44	1.33/1.27	1.00/0.82	1.46/1.34
5	2.70	1.03	2.33/2.27	0.86/0.80	0.90/0.76	0.83/0.72
6	3.27	1.97	2.10/2.04	1.20/1.14	1.58/1.38	1.37/1.26
7	4.40	1.70	2.96/2.90	1.26/1.20	2.45/2.19	1.68/1.56
8	2.60	1.03	1.63/1.57	0.76/0.70	0.49/0.40	0.44/0.32
9	2.73	1.50	2.00/1.94	1.26/1.20	1.66/1.47	1.60/1.48
10	2.77	1.50	1.93/1.87	1.03/0.97	1.07/0.92	0.75/0.64

* These pneumatophores of *Nanomia bijuga* were collected on July 1 and 2, 1965. All measurements were made within 90 minutes of net on board. Group I was obtained from depths of 50 to 100 meters at 2300, 1 July; Group II, from 290 to 355 meters at 1030, 2 July.

† Each measurement is represented by two numbers indicating the maximum/minimum reading allowing for an ocular micrometer reading error of ± 0.03 mm.

‡ Volumes are recorded as plus or minus capillary reading error.

Oxygen Consumed and Carbon Monoxide Produced

Oxygen consumption of individual pneumatophores at temperatures similar to those at depths of capture was determined to lie in the range 0.024 to 0.129 mm^3/mg-hr. These values are in good agreement with values from intact siphonophores (TABLE 2), substantiating the validity of experimental results obtained from live floats alone. However, upon addition of the amino acid, L-serine, as a CO production substrate,[6] the O_2 consumption rose dramatically, although not unexpectedly, as increased quantities of CO were produced. Conversely, in a volumetric respirometer, the oxygen consumption increased, on the average, when both CO and CO_2 were absorbed from the gas phase as compared to when CO_2 alone was absorbed.[12] This observation tends to confirm findings from syringe experiments indicating that CO production proceeds at some level at all times probably in order to compensate for diffusive loss of this gas across the float walls and to keep the float fully inflated. Thus, if one uses representative values from the data presented, a pneumatophore possessing, for example, a gas volume of 1 mm^3 and a surface area of 10 mm^2 might be expected to lose about 4 mm^3 per hour at a depth of 300 meters in the sea,[11] if the rate of diffusion in this case is directly proportional to the partial pressure of the gas.

Note, however, that some of the pneumatophores, while on the bench top at about 20° C prior to transfer to the subambient water baths, produced CO at

TABLE 2

O_2 CONSUMPTION AND CO PRODUCTION OF SIPHONOPHORE PNEUMATOPHORES*

Pneumatophore Specimens	Wet Weight	Experiment Time	O_2 Cons.†	CO Production	Remarks
	(mg)	(hrs:min)	(mm^3/mg-hr)	(mm^3/mg-hr)	
B	3.7	11:37	0.129	0.039	In 1 cc syringe filled with sea water. No substrate added. 1 bubble produced.
F	8.8	12:49	0.024	0.027	As above, no substrate added. 8 bubbles produced.
O	1.6	23:06	0.105	0.052	As above, with added L-serine as CO production substrate. No bubbles.
P	3.1	12:23	0.089	0.040	As above, with added substrate. No bubbles.
R	1.3	13:40	0.097	0.016	As above, with added substrate. No bubbles.
Rhizophysa 1	29.3	10:00	0.037		Intact siphonophore, 5 cc syringe.
Rhizophysa 2	17.2	10:00	0.057		Intact siphonophore, 5 cc syringe.

* All at 7–8° C. All specimens living and in good condition at end of run.
† Corrected for controls.

a rate resulting in bubble expulsion (TABLE 3). When these bubbles were collected and measured for volume, the resulting rates of gas production were markedly higher than those determined from experiments in which no bubbles were expelled. When these rates of CO production were equated to the weight of the gas gland tissue from which the gas originates (see FIGURE 2), values in one case as high as 277 mm^3CO/mg tissue/hour at 20° C were attained when substrate had been added, and 115 mm^3/mg-hr in another experiment with no added substrate (TABLE 3). A series of gas glands, carefully dissected from within individual pneumatophores, weighed, on the average, 8 to 10% of the weight of the intact float.

At a normal at-depth temperature for these organisms of 7°C these rates became approximately 110 and 47, respectively, as calculated with the van't Hoff equation, assuming a Q_{10} of 2.[12] For an average intact pneumatophore of 3.5 mg possessing a gas gland weighing approximately 0.35 mg, the above rates of gas production at 7° C equal 38 and 16 mm^3CO/pneumatophore/hour, respectively.

Work of Inflation at Depth

The physical work of secreting gas against hydrostatic pressure in the sea will require some expenditure of energy. A useful formulation applied in this context to fishes possessing swim bladders[18] is written

$$W = 1.99\ nT\ 2.3 \log p_1/p_2$$

where n is the molar quantity of the gas in question, T, the absolute temperature, and p_1/p_2, the ratio of the initial and final pressures. Suitable choice of units gives W in gram-calories. As an example, the work required to compress (or conversely, to secrete against a pressure head) 30 mm^3 of CO as a hypothetical

TABLE 3

BUBBLE PRODUCTION BY *Nanomia bijuga* PNEUMATOPHORES

Pneumatophore Specimen	Total Wet Weight	Time Required to Produce Bubbles	Est. Gas Gland Weight	Temp.	No. of Bubbles Produced	Volume of Bubbles	CO in Bubbles	Maximum Est. Rate of CO Production
	(mg)	(min)	(mg)	(°C)		(mm^3)	(%)	(mm^3/mg gas gland/hr)
B	3.7	10–30 sec	0.15	21.0	1	0.68		Not estimated
F	8.8	10 for Nos. 1–3	0.30	22.5	8	1) 0.13		25.5 at 20° C
						2) 0.13		
						3) 1.40		
		5 for Nos. 4–8				4) 0.17	76.3	115 at 20° C
						5) 0.37		
						6) 0.78		
						7) 0.11		
						8) 2.51		
N*	2.3	10	0.04	21.8	3	1) 0.76	87.4	155 at 20° C
						2) 0.17		277 at 20° C
						3) 0.37		

* L-serine substrate added, 0.05 M in sea water.

siphonophore with a 1 mm^3 float swims downward from 100 to 400 meters (10–40 atm guage pressure) at a temperature of 7° C equals 3×10^{-3} gm-cal.

Taking a conservative estimate of 15 mg wet weight for a small intact *Nanomia bijuga*, and employing an average no-substrate oxygen consumption at 7° C (TABLE 2) of 0.05 mm^3/mg-hr (*Rhizophysa* only), gives a total of 0.75 mm^3 of oxygen consumed. This quantity is equivalent to about 3.8×10^{-3} gm-cal, assuming the caloric equivalent of 1cc of O_2 is 5 gm-cal.

Possible Energy Requirements for CO Secretion

Since all experiments indicated some level of CO production, a nonsecretory rate of oxygen consumption can probably best be approached using the values from the *Rhizophysa* experiments (TABLE 2). By employing a value of 0.05 mm^3/mg-hr (a rounded up average of the two experiments), it then becomes possible to perform calculations on the possible energy requirements for CO production in some experiments where oxygen consumption was measurably elevated during gas production. Such an approach is only valid, however, if the secretory process is assumed to be endergonic.

An example is Float O, TABLE 2. Here, 0.168 mm^3 of oxygen was consumed per hour. The total oxygen consumed for nonsecretory or maintenance respiration equaled 0.080 mm^3 at 7° C. The difference (0.088 mm^3) constitutes the oxygen combined with carbon to produce CO as well as an amount possibly consumed to provide the energy required in the secretory process.

In this experiment 0.083 mm^3 CO was produced per hour at 7° C. This quantity equals 0.104 μg of CO of which about 0.060 μg is oxygen. The oxygen remaining after substraction of the maintenance rate equals 0.126 μg; substracting that oxygen combined with carbon to produce CO gives 0.066 μg of O_2 which is equivalent to 2.3×10^{-4} gm-cal/hr. This calculation suggests that the actual energy cost of producing carbon monoxide in this case was approximately 3×10^{-3} gm-cal/mm^3 CO. These figures do not, however, take into account the rapid rates of CO production associated with bubble expulsion or the unknown rates of O_2 consumption during these periods.

Carbon Requirements for Metabolism and Gas Production

In the above observations, the metabolic demand for carbon to meet the oxygen consumption of 0.180 μg/hr equals 0.067 μg/hr. Added to the requirement for CO production (0.044 μg) the total hourly carbon requirement of the pneumatophore alone equals 0.111 μg. At an ocean depth of 400 meters, however, the siphonophore must secrete 40 times the float volume of gas in order to avoid pneumatophore collapse. For a 1 mm^3 float this quantity equals 40 mm^3 of CO or about 21 μg of carbon simply for gas production. Respiratory requirements and diffusive loss of CO will necessarily raise this figure much higher.

A prime source of food for these siphonophores is small copepod crustaceans. One such copepod, *Calanus helgolandicus*, has a dry weight of organic substance in the vicinty of 12 μg per copepod.[19] If 20% of this weight is protein, of which perhaps 60% is carbon, then approximately 1.4 μg of proteinaceous carbon is available per copepod. Since serine is a nonessential amino acid and can be synthesized in the body tissues from a number of precursors, a ready supply of nutrients may be all that is required to assure the siphonophore a sufficiency of this substrate. Indeed, the gastrovascular fluids of *Nanomia bijuga* have been

shown to contain serine in quantities as high as 5.3% of free amino acids and 4.5% of protein amino acids.[20] Although siphonophores are basically carnivores, a secondary source of nutrients and carbon might be the considerable particulate organic detritus appearing as "snow" largely throughout the water column.

Without knowing more of the prey-capturing efficiency of these siphonophores, it is difficult to say whether availability of carbon might be a limiting feature in determining whether a hypothetical siphonophore would perform an extensive or partial vertical migration on any given evening. Clearly, the increased energetic requirements of aerobic metabolism will impose considerable demands during vigorous upward or downward swimming, particularly when the organism is traversing several hundred meters in the short space of an hour or two. In addition, if the pneumatophore is to be rapidly refilled following or during downward migration, in order to regain neutral buoyancy quickly, an additional heavy demand will be placed upon available nutrient and substrate stores.

Energy Capabilities at Depth

The energy metabolism of these siphonophores and particularly of the severed floats, with the exception of cases of rapid bubble production, seems to suggest a marginal ability to meet energy requirements when extrapolated to conditions at depth. For example, in the aforementioned case of Float O, if the float were to be reinflated at a depth of 300 meters within an hour, the energetic require-

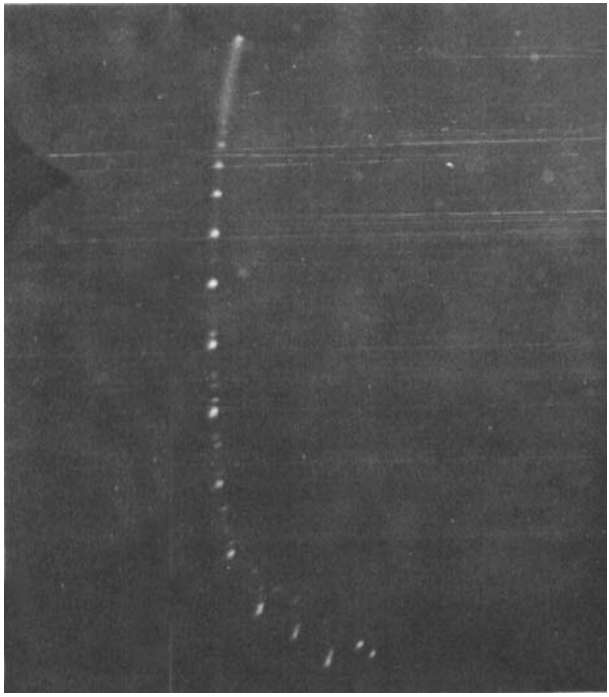

FIGURE 3. A living specimen of *Nanomia bijuga* photographed at DSL depths from Deepstar 4000. Note that organism is in extended feeding position and that light reflects from the apical float as well as pigmented areas along its length. Estimated length 60 cm.

ments that would have to be met would include the small maintenance respiratory level of about 4×10^{-3} gm-cal for a 15 mg intact individual, the energy required to perform the physical work of countering the hydrostatic pressure (3×10^{-3} gm-cal), the very large energetic demand for secreting 30 times the float volume, and replacement of the fourfold loss of float volume through diffusion, a total of about 1.1×10^{-1} gm-cal.

However, a few pneumatophores demonstrated spectacular gas-producing capabilities during bubble production (TABLE 3), and these probably can be regarded as indicative of what the float and its contained gas gland can accomplish at depth. That is, the float can reinflate in a very short time, probably less than an hour. Validity for this belief lies in the observation of these creatures at their daytime depths, shortly following the morning downward migration of the Deep Scattering Layer. In no case was the pneumatophore ever observed to be other than inflated and buoyant, and thereby, an effective light reflector in photographs (FIGURE 3).

Nevertheless, considerable time may be required to replenish energy stores.

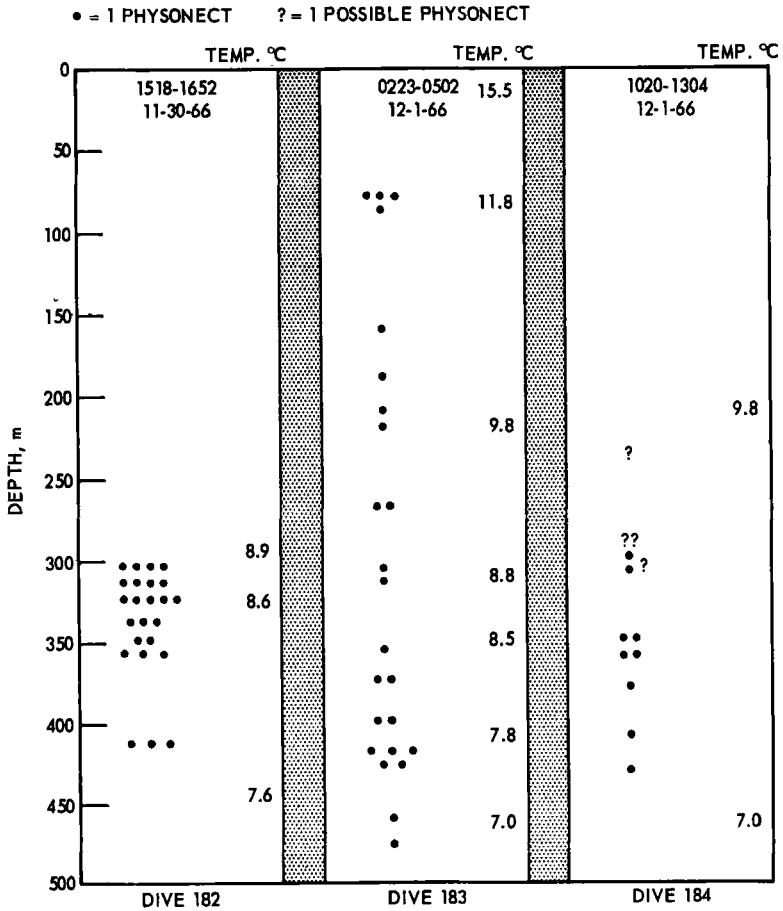

FIGURE 4. Numbers of physonect siphonophores, mainly *Nanomia bijuga*, observed from Deepstar 4000.

That is, the animal may not be able on any given day to capture sufficient prey to cover energy expenditures for extensive vertical migration. Support for this suggestion lies in an observation made on a number of dives in Deepstar 4000. Seldom were more than 25 to 50% of the physonect siphonophore population observed to migrate vertically for any significant distance (FIGURE 4).[12,21] Thus, while few siphonophores were ever seen at depths above 300 meters during the day, on night dives they were distributed virtually throughout the water column from below 400 meters to within 50 meters of the surface. The consistency of this observation on other dives strongly suggests that energy stores required for gas production and vigorous migration, or available carbon for CO secretion, or both, may be limiting factors in the organism's ability to undertake partial or extensive vertical migration.

Additional Factors

Great variability must be expected in physiological responses to varying experimental factors in invertebrates such as siphonophores, and additional difficulties might be anticipated due to the fragmented condition in which they are nearly always captured in midwater trawls. Thus, the calculations for the energy required in the actual production of CO given here in the case of Float O can only be regarded as tentative and, to some degree, speculative. However, observations indicate good survival of some specimens for periods of at least a week when held at low temperatures. Factors such as muscle contraction, pumping of gastrovascular fluid, oxygen consumption and gas production including bubble expulsion are all comparable to similar parameters evaluated in freshly captured material.

Further, many important factors relating to production of carbon monoxide in these animals have yet to be fully assessed. The expulsion of bubbles (FIGURE 5) is unquestionably undertaken during the evening ascent of those siphonophores migrating with the DSL simply to avoid bursting the pneumatophore.[21] The released bubbles, as well as the inflated pneumatophores, will act as targets across a broad spectrum of sound frequencies mostly above 10 kHz.[8,11,12] As yet, however, it is not known at what depths the first bubbles of CO will appear; this point will be governed to some degree by the starting depth of the individual organism. The majority of data presently at hand suggest that most floats of *Nanomia bijuga* will not tolerate much more than a twofold increase in gas volume without initiating bubble expulsion. Thus, for physonect siphonophores migrating upward from 400 meters, the first bubbles would be anticipated in the vicinity of 200 meters where the hydrostatic pressure is reduced to half. If this is the case, these doubtless represent the deepest regularly occurring free bubbles in the sea.

Virtually all of the CO from bubbles released at depth would be expected to pass into solution and probably most even from bubbles released near the surface. The siphonophore floats themselves transmit CO to the surrounding water by diffusion, but the greatest quantity of siphonophore-produced CO dissolved in sea water would most likely be in the upper 100 meters where rapidly decreasing pressure induces an increasingly rapid expulsion of bubbles.

These minute bubbles from a population of siphonophores, usually no more concentrated than one to ten individuals per 1000 m^3 of water, nonetheless contribute only micro-liter quantities of dissolved CO to the relatively gigantic bulk of sea water in which they occur. Thus, although dissolved CO in surface sea water sufficiently high in concentration to indicate a diffusion of this gas from the sea into the atmosphere has been recently reported,[10,22] it is difficult to ascribe siphonophores as the source of such high surface values.

Residence times for CO dissolved in sea water at various depths is not known,

but presumably, is quite short. Carbon monoxide-fixing bacteria have been identified from the marine environment[23] and the abundant particulate material occurring in suspension at most depths undoubtedly provides suitable substrate for their growth.

Lastly, the intriguing problem of the nonlethality of siphonophore CO to the cytochrome oxidase of its own float tissues has yet to be attacked. Many possible approaches come to mind, but the difficulties in obtaining even limited amounts of material in a fragmented state have thus far retarded progress on this aspect of siphonophore biology. Nonetheless, it seems truly amazing that delicate gelatinous organisms such as physonect siphonophores, observed with inflated pneumatophores at depths to at least 450 meters,[8,12,21] can survive a partial pressure of CO equivalent to 45 atm when, for the tissues of most organisms, a pCO of scarcely 1 atm would prove lethal.

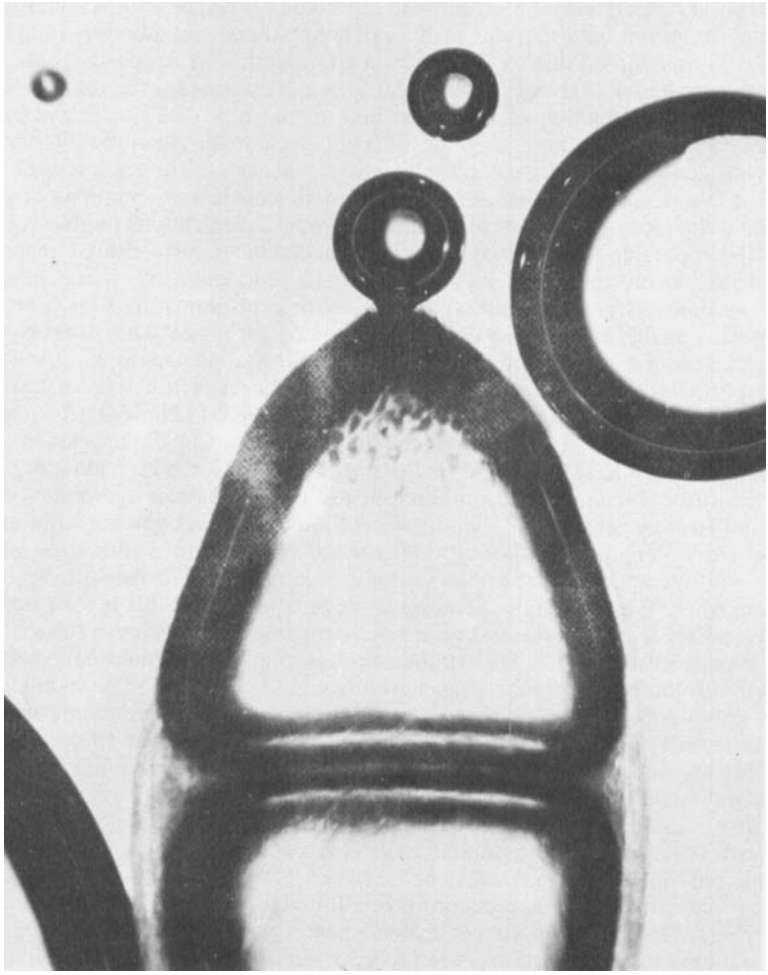

FIGURE 5. A pneumatophore of *Nanomia bijuga* in the act of expelling a bubble of CO gas. Approximate diameter of pneumatophore 1 mm. (Photo by B. Burns)

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