

**PHYSIOLOGICAL DYNAMICS OF SIPHONOPHORES
FROM DEEP SCATTERING LAYERS**

Size of gas-filled floats and rate of gas production

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THE PROBLEM

Determine the magnitude of the contribution to sonic scattering by siphonophores. Specifically, study and analyze the size of gas bubbles contained in siphonophores and the amount of gas produced by them. Determine by experimental methods rates of gas production under various conditions and time required to fill siphonophore floats.

RESULTS

1. Gases contained within fresh siphonophore floats are generally very close to ambient pressure, suggesting the probable release of numerous bubbles during vertical ascent. Volumes of contained gases in a series of fresh floats ranged from 0.5 mm^3 to 2.5 mm^3 .
2. Oxygen consumption may be elevated during carbon monoxide production.
3. Diffusion constants for siphonophore floats calculated from rates of diffusive loss of CO are close to those which have been determined for chitin.
4. Calculated energy requirements for the physical work of countering hydrostatic pressures of 30 to 40 atmospheres indicate that float refilling times are probably no more than a few hours at most.

RECOMMENDATIONS

1. Perform additional experimental determinations on oxygen consumption of siphonophore floats, especially during gas production and with added chemical precursors of the manufactured gas.
2. Determine the effects of elevated pressures, with tests conducted in small plastic and metal pressure chambers now under design.
3. Employ pressure chambers in an assessment of bubble production by siphonophores and size of released bubbles, with stepwise reduction in pressure through a range of 40 to 0 atmospheres (simulated vertical migration).

ADMINISTRATIVE INFORMATION

Work was performed under SR 011 01 01, Task 0401 (NEL Z12051). The report covers work from November 1964 to August 1965 and was approved for publication 20 April 1966.

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INTRODUCTION

The study reported here was undertaken as part of a broad, continuing investigation of the marine organisms which act as sonic scatterers and thus affect the operation of sonar systems. This effort was substantially aided in the 1950's, when the advent of the bathyscaphe TRIESTE permitted direct observation of organisms residing in zones of strong sonic reflection (deep scattering layers, or DSL). Thus, E. G. Barham was able for the first time, in 1962, to make direct visual identification of many of the most prominent organisms and to obtain some estimates of their population densities in these areas.^{1,2}

Such observations made from the TRIESTE and more recently from the Cousteau Souscoupe Sous Marine ("diving saucer") have indicated that siphonophores are among the two or three most prominent types of organisms found in association with the DSL in the San Diego Trough area and also in the region of Cabo San Lucas, Baja California -- two areas where the DSL is highly developed.³

Of the siphonophore species observed directly by Barham and also captured in net hauls, by far the most abundant in the vicinity of San Diego has been *Nanomia bi juga* (Delle Chiaje) 1841, an ubiquitous form belonging to the sub-order Physonectae found in tropical and temperate seas in many parts of the world (fig. 1). Members of this taxonomic group are characterized by the presence of a gas-filled float or pneumatophore, which possibly contributes as much or more to acoustic reflection and reverberation as the gas-filled swim bladders of fishes.¹ These organisms obviously offer a valuable potential for increasing knowledge of the dynamics of the deep scattering layer (DSL) and for making predictions concerning its acoustic behavior in areas where siphonophores are a major constituent.

¹See list of references, p.33.

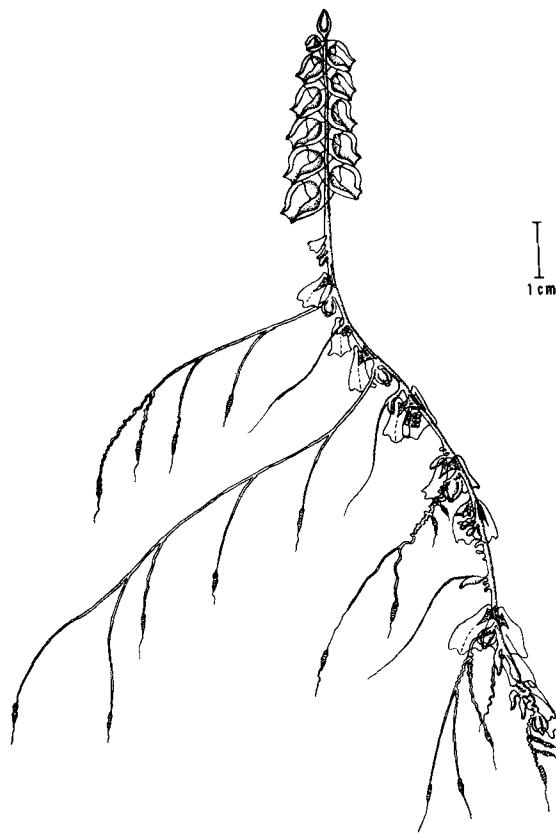


Figure 1. A single colony of *Nanomia bijuga*, showing tentacles extended in the fishing position; swimming bells (nectophores) are situated in a cluster beneath the gas-filled pneumatophore.

Since most organisms associated with the DSL must be capable of daily vertical migrations over distances of several hundred meters, any animal possessing an enclosed gas phase must be able to cope with the problems created by this expanding gas during upward swimming. *N. bijuga* possesses a pore at the tip of the pneumatophore which is said to be closed by a sphincter-like muscle.⁴ Thus it is easily capable of voiding gas during a vertical ascent.

Therefore, although the manner by which nanomians cope with expanding gas during ascent seems apparent, it is not yet known how quickly they may regain neutral buoyancy during descent or while at deep daytime levels, or how they handle the hydrostatic pressures of 30 to 40 atmospheres impinging upon the pneumatophore. These

features involve inherent rates of gas production and the energy cost of resisting high hydrostatic pressures.

It was recognized that any predictions based on the acoustic behavior of siphonophores would require investigation into their metabolism, the rates of gas secretion and diffusive loss across the pneumatophore walls, and the volumes of gas involved. Results of such studies would permit estimates of the energy equivalent to the physical work required to reinflate the float until it is again an effective sonic reflector. It further becomes possible, from volume studies, to more clearly determine the ability of the float to withstand pressures from within due to expanding gases during upward migration, and then to estimate the number of gas bubbles, constituting secondary acoustic targets, which will be voided by the rising siphonophores. The volumes of these extruded bubbles are an indication of their possible resonance at sonar frequencies.

Subsequent sections will describe a program of investigation into the physiological dynamics of siphonophores and the resulting data which contribute to understanding them as sonic scatterers.

EXPERIMENTAL METHODS AND RESULTS

The data to be presented here were obtained on cruises aboard USS MARYSVILLE (EPCER 857) in November 1964, and July and August 1965, and one cruise aboard the Scripps Institution of Oceanography R/V T-441 in May 1965, in the region of the San Diego Trough to the west and south of San Diego.

On the latter trip, a siphonophore not previously encountered by us was taken from depths ranging from approximately 40 to 80 meters in an area over the San Diego Trough about 15 to 20 miles west of San Diego. This species, which also possesses a gas-filled float, has been tentatively identified as *Rhizophysa* sp. (figs. 2-4). Data obtained from this organism provide an interesting comparison with *N. bijuga*, and show similar physiological responses. The specimens were captured whole and relatively undamaged, as compared to *N. bijuga* which invariably fragments when taken in nets.

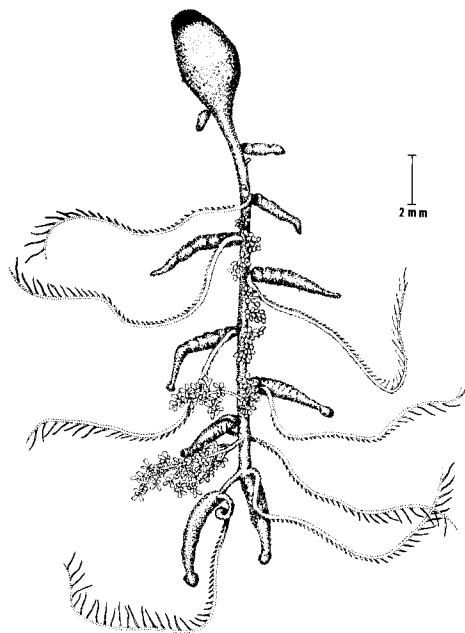


Figure 2. An intact colony of *Rhizophysa* sp. The specimen was captured by meter net and relaxed in magnesium sulfate prior to formalin fixation.

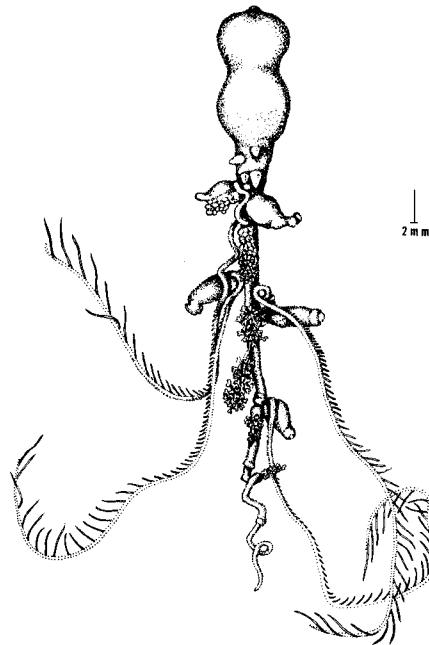


Figure 3. Intact colony of *Rhizophysa* sp., showing indentation in pneumatophore walls thought to be associated with bubble expulsion.

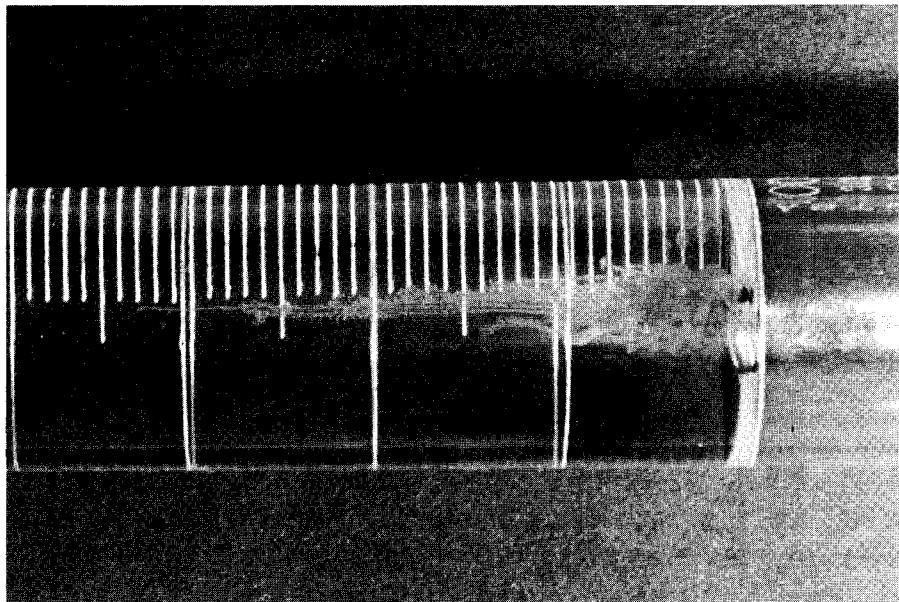


Figure 4. Two living specimens of *Rhizophysa* sp. with stolon and tentacles extended. Volume of pneumatophores was 30 to 40 cubic millimeters. Distance between lines on cylinder equals 1.9 mm.

Respiratory Metabolism of Siphonophores

In dealing with gas and bubble production by deep-sea siphonophores, rate of oxygen consumption becomes important for several reasons. First, it provides an index by which an estimate of presumably available aerobic energy can be made. This in turn can be compared to calculated physical (but not chemical) energy requirements for maintaining an inflated gas-filled float against existing hydrostatic pressures. Second, rate of oxygen consumption appears to be correlated with the act of secretion of carbon monoxide gas (see discussion below), and thus may be employed as a convenient analytical tool for the study of the chemical reactions involved (for example, the effects of various substrates or inhibitors). Third, rates of oxygen consumption are an index of metabolic level, and are therefore of fundamental value in comparative physiological studies of deep-sea organisms and their environmental relationships (e.g., effects of pressure, and ambient oxygen).

Measurements of Oxygen Consumption by Syringe

The figures for oxygen consumption of siphonophores presented in table 1 were, with the exception of Experiments 8, 11, and 12,* obtained by a simple syringe method. The organisms were placed in 1, 2, or 5 cc syringes filled with seawater without an entrapped gas phase, together with a drop of mercury for stirring. They were placed in water baths at the stated temperature (controlled to $\pm 0.5^{\circ}\text{C}$) for various periods of time. Samples of the same seawater were analyzed for dissolved gases⁵ at the start of experimental runs. Control syringes, containing seawater without organisms, were run alongside those containing siphonophores. At the end of the experiment, samples of water from each syringe were analyzed for dissolved gases. Suitable corrections were made for the seawater blanks, and final values were subtracted from starting values to give total oxygen removed from the seawater during the interval in question.

This procedure assumes a constant rate of oxygen utilization by the siphonophores. In view of the stable temperature, the large volume of water compared to the volume of metabolizing tissue, and, therefore, the large supply of dissolved oxygen, this assumption seems warranted.

In the case of the fastest metabolizing 10-hour specimen (Experiment 5), the amount of dissolved oxygen remaining at the end of the experiment would have sufficed at the measured level of utilization for more than an additional six hours. The oxygen level, therefore, probably did not become limiting.

Effects upon respiration of the carbon dioxide evolved are believed negligible because of the large volume of water relative to the siphonophore and also the buffering capacity of the seawater. In a second method described below, where carbon dioxide was absorbed by strong alkali, the measured rates of oxygen consumption were comparable to the syringe values.

* Throughout the report, experiment numbers correspond to those listed in table 1.

TABLE 1. OXYGEN CONSUMPTION OF SIPHONOPHORES

Exper. No.	Date	Species	Number of Individuals	Weight (mg)	Oxygen Cons. (mm ³ /mg/hr)	Time (hr:min)	Temp. (°C)	Method	Remarks
1	11-5-64	<i>Nanomia*</i> <i>bijuga</i>	3	7.3**	0.090*** (0.037)	3:15	20	Syringe (2cc)	Meter net, haul #2, 1410, 450 meters depth
2	11-6-64	<i>N. bijuga</i>	4	7.3**	0.078 (0.032)	1:48	20	Syringe (2cc)	Meter net, haul #7, 0800, 430 m
3	11-6-64	<i>N. bijuga</i>	3	7.3**	0.075 (0.031)	2:00	20	Syringe (1cc)	Meter net, haul #7
4	5-26-65	<i>Rhizophysa*</i> sp.	1	29.3	0.053 (0.037)	10:00	12	Syringe (5cc)	Meter net, 0300 to 0500, 5-25, 60 to 120 m
5	5-26-65	<i>Rhizophysa</i> sp.	1	17.2	0.081 (0.057)	10:00	12	Syringe (5cc)	Meter net, 0300 to 0500, 5-25
6	7-2-65	<i>N. bijuga</i>	1	1.2	0.178	3:52	7	Syringe (1cc)	Tucker net, ⁶ haul #5, 0927, 290-355 m
7	7-2-65	<i>N. bijuga</i>	1	5.4	0.095	3:40	7	Syringe (2cc)	Tucker net, haul #6, 1246, 240-330 m
8	7-2-65	<i>N. bijuga</i>	1	2.5	0.042	1:37	7	Micro- respirom.	Tucker net, haul #7, 2043, 55-110 m
9	7-3-65	<i>N. bijuga</i>	1	1.5	0.079	11:30	7	Syringe (1cc)	Tucker net, haul #8, 2144, 165-280 m
10	7-3-65	<i>N. bijuga</i>	1	0.6	0.066	11:30	7	Syringe (2cc)	Tucker net, haul #8
11	8-10-65	<i>N. bijuga</i>	1	5.0	0.091	1:44	7	Micro- respirom.	Tucker net, haul #4, 2100, 80-150 m
12	8-11-65	<i>N. bijuga</i>	1	6.0	0.088	4:40	7	Micro- respirom.	Tucker net, haul #7, 1408, 280-410 m
				0.069	Avg. at 7°C				

*In all cases only the pneumatophores of *N. bijuga* were run. The individuals of *Rhizophysa* sp. were intact colonies.

**The weights in experiments 1, 2, and 3 were obtained from an average of ten preserved specimens of similar dimensions.

***Figures in parentheses represent the observed values readjusted to 7°C by van't Hoff equation assuming a Q₁₀ of 2.

The average for the column utilizes these values.

Seawater samples in all cases were analyzed for dissolved oxygen, nitrogen, and carbon monoxide by the gasometric method of Scholander *et al.*⁵ This method generally requires a 1-cc sample from which the dissolved gases may be determined with an accuracy of 2 percent of the theoretical value (as checked against air-equilibrated water). With proper technique the precision of gas extraction from duplicate samples is better than ± 2 percent (average of four test extractions = 1.7 percent) or $\pm 0.3 \text{ mm}^3$ of the total extracted gas equivalent to $\pm 0.4 \text{ ppm}$ in the case of oxygen. The precision of duplicate analyses for dissolved oxygen, carbon monoxide, or nitrogen averages $\pm 0.3 \text{ mm}^3$ (determined from four sets of test analyses) which at its worst is an error of only about 3 percent (see Appendix A for further discussion of analytical techniques and sources of error).

All pneumatophores and intact siphonophore specimens were weighed on an analytical balance to $\pm 0.1 \text{ mg}$. Weights reported are "blotted wet weights," that is, the specimen was either gently rid of adhering surface liquid and then weighed in a previously weighed water drop; or weighed with adhering moisture, gently blotted, and the blotting (filter) paper with absorbed moisture weighed separately. Subtraction of the latter value provides the weight of the "blotted" specimen.

Measurements of Oxygen Consumption by Micrometer Respirometer

This method, devised originally by Scholander,⁷ provides a volumetric means of following the oxygen consumption of very small organisms, tissues, or cell suspensions (fig. 5). The spindle of a standard metric micrometer is replaced with a 1/16-inch drill rod which travels into a mercury reservoir. Movement of this "micro spindle" into or out of the reservoir controls the position of an indicator drop in a capillary opposite the reservoir. As an organism in the reaction vessel consumes oxygen from the air, the indicator drop travels toward the "T." Setting the drop back to the reference line by adjustment of the micrometer gives an indication of oxygen consumed in the time interval in question. The bulb, closed by a polyethylene-tipped,

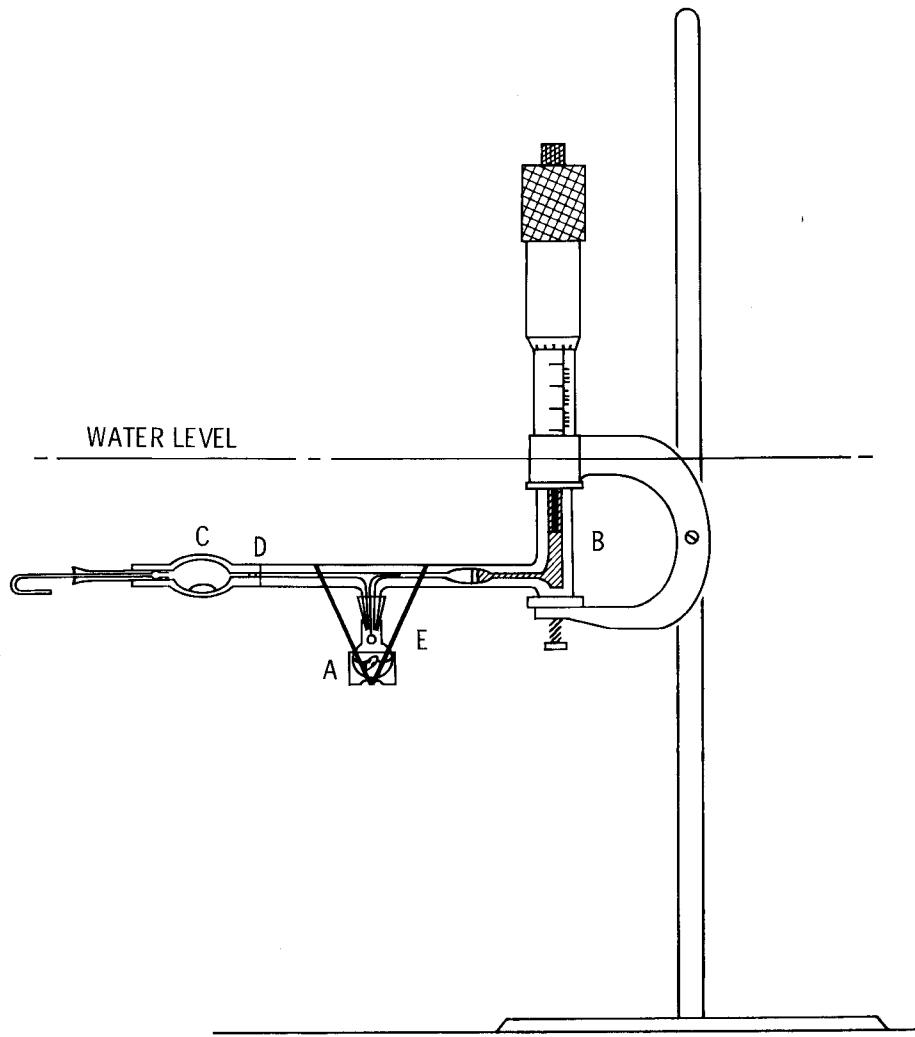


Figure 5. Micrometer respirometer. A, reaction vessel (held in place by rubber bands) containing siphonophore float in sea water; B, mercury reservoir; C, compensating chamber with a drop of water to maintain vapor pressure; D, indicator drop; E, drop of alkali in wire loop.

paper-clip plug, serves as a thermobarometer balancing the reaction vessel while a drop of alkali absorbs the CO_2 evolved. By using a 2x hand lens to read the micrometer, volume changes of as little as 0.004 to 0.007 mm^3 may be detected. A second respirometer containing seawater, but no organism, is run as a control. All equipment is mounted in a constant-temperature bath controlled to at least $\pm 0.1^\circ\text{C}$. Figure 6, obtained from Experiment 8, indicates the linearity of rates of oxygen consumption determined by this method. The rates shown in table 1 were corrected for seawater controls (see Appendix A).

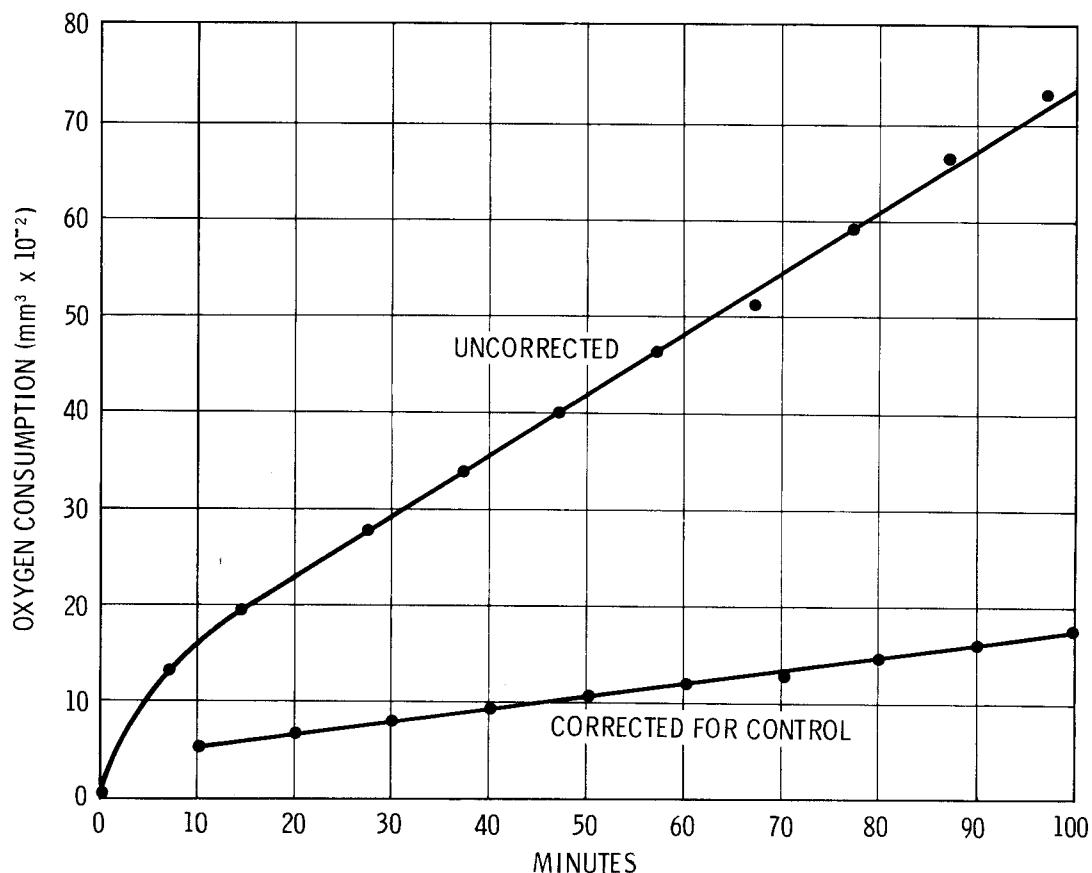


Figure 6. Rate of oxygen uptake by a siphonophore (Experiment 8). Values are corrected for seawater controls.

The only figure for intact siphonophore metabolism to be found in the literature is given by Vernon⁸ for *Forskalia contorta*, also a physonect form. His value, from a single specimen, was equivalent to 0.012 $\mu\text{l}/\text{mg}/\text{hr}$, at 16°C. This is only about one-fifth of the average of the figures reported here even without temperature compensation.

Larimer and Ashby⁹ measured oxygen consumption of the minced float tissues (presumably not including gas gland) of the Portuguese man-of-war, *Physalia*. Their experiments, performed at 25°C and with sodium succinate added as a metabolic substrate, gave a range of 0.075 to 0.105 μl per mg/hr. Although the experimental conditions are markedly different, these values closely approximate those presented in table 1.

Comparison of *Nanomia bijuga* With Other Siphonophores

The siphonophore listed for Experiments 4 and 5 is included because it provides an important basis for comparison to the comparatively common *N. bijuga*. This species, tentatively identified as *Rhizophysa* sp. (figs. 2-4) is a member of the pneumatophore-bearing Cystonectae, another sub-order of the Siphonophora. These also bear gas-filled floats* with apical pores but lack nectophores (swimming bells) or bracts (leaf-like appendages which aid in flotation). However, the specimens obtained were captured intact, and the similarity of results for oxygen consumption of this species and of *N. bijuga* indicate the validity of results obtained by performing tests upon the automatized pneumatophores of the latter.

Volume of Pneumatophore Gases

In terms of effects on acoustic energy at specific wavelengths (frequency dependence), the volume of gas

* Analysis of the gases within the float of one of these individuals after holding it for 30 hours at 4°C gave 27.3% carbon monoxide, virtually all of the remainder analyzing as inert gas presumed to be largely nitrogen.

contained within the pneumatophore is of paramount importance in estimating the total amount of gas which must be secreted to maintain float volume at various depths. Determination of volume of enclosed gases compared to volume estimates made from dimensional measurements may, in addition, allow some estimate of total number of bubbles expelled during an upward migration.

It can be seen from figure 7 that gases contained in nanomian floats more closely approximate a prolate spheroidal shape than do the floats themselves. Calculations of gas volume based upon linear dimensional measurements might therefore be expected to indicate more accurately the true volume of the gas bubble than similar calculations based upon float dimensions (see Appendix C).

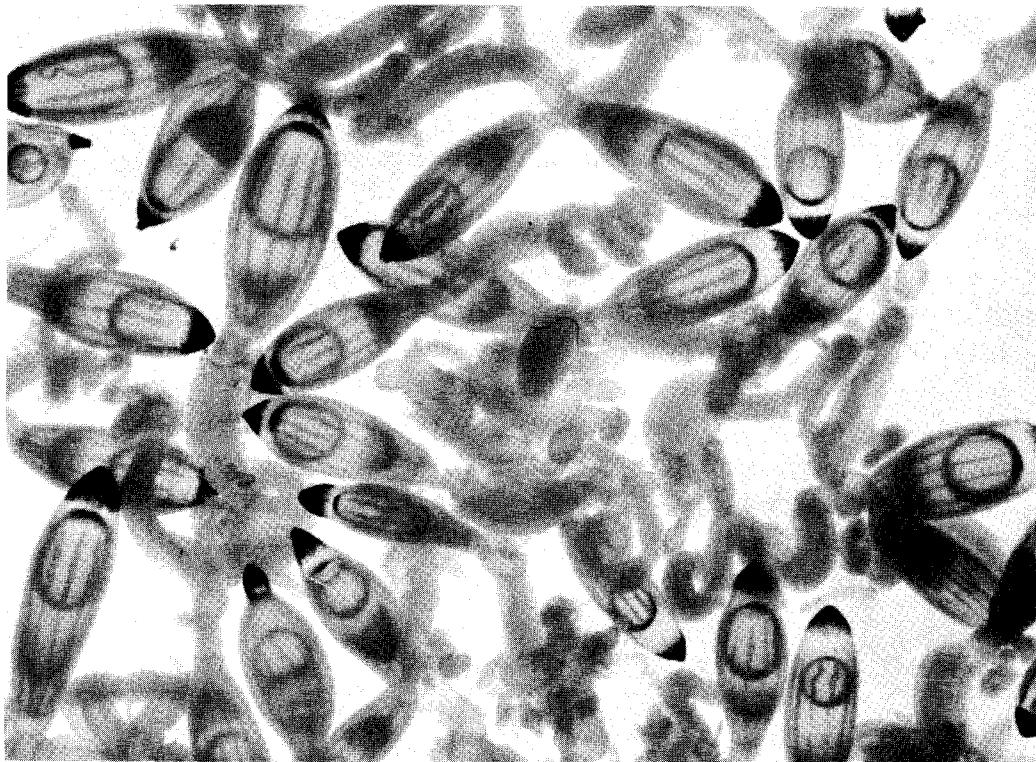


Figure 7. Specimens of *Nanomia bijuga*, photographed 30 days following formalin fixation. The stolons are contracted and most members of the colony have been shed. In general the enclosed gas bubbles more closely approximate a prolate spheroidal shape than do the external dimensions of the floats themselves. (Magnification, approximately 12x.)

Dimensions of pneumatophore gases are given in table 2.* After measurement with an ocular micrometer at 27x (maximum reading error = ± 0.03 mm, as determined from replicate measurements on a series of 10 floats), all gases contained in a single float were extruded by pressure into the capillary of a gas analyzer⁵ previously calibrated by weighed mercury delivered from a known length of the capillary, and checked for cylindricity. The analyzer was charged with a saturated solution of acidified sodium citrate, a solution having very low gas solubility. The possibility of gas volume change, resulting from a change of surface tension between the gases in contact with the moist walls of the float and the acid citrate, was checked by injecting known volumes of gas, either dry or in contact with seawater, into the analyzer capillary. No measurable volume change was detected. In general, calculation of the volume of the gas phase appears to be in excess of that obtained by direct measurement by a factor of only 0.2 to 0.3 mm³ (table 2, columns 6, 8).

Temperature differences between the float, when measured beneath the dissecting scope and when placed in the analyzer, were generally within one degree centigrade and never greater than 2.6°C. The maximum volume change that could result from a temperature difference as great as the latter is only 0.8 to 0.9 percent.

The analyzer capillary can be read with a hand lens to a volume of plus or minus 0.57 mm³. This amounts to an average error slightly less than 7 percent for Group I, table 2, and an average of slightly more than 4 percent for Group II.

The corrected ratio of calculated volume of gas to actual volume measured by extrusion was determined by calculating a pair of ratios, thus:

calculated volume range	high	high	extruded volume range
	\times	\times	
	low	low	

to give the maximum range in ratios:

<u>calc. low</u> <u>ext. high</u>	<u>calc. high</u> <u>ext. low</u>
--------------------------------------	--------------------------------------

*See also Appendix B, External Measurements of *Nanomia*.

TABLE 2. CORRECTED VOLUMES OF PNEUMATOPHORE GASES*

Pneumatophores	Gas Phase**		Volume		Volume of Extruded Gas		Corrected Ratio
	Length (mm)	Width (mm)	(Calculated as Prolate Spheroid)		Uncorrected (mm ³)	Corrected for ± Max. Error (mm ³)	Calc. Vol. Extruded Vol. (High/Low)
			Uncorrected (mm ³)	Corrected (mm ³)			
1	1.67	0.77	0.52	0.57/0.47	0.47	0.52/0.41	1.39/0.88
2	2.16	1.10	1.36	1.46/1.28	1.68	1.74/1.62	0.90/0.74
3	2.56	1.00	1.34	1.44/1.24	1.17	1.23/1.11	1.29/1.01
4	2.30	0.90	0.97	1.05/0.90	0.35	0.41/0.29	3.57/2.20
5 Two Bubbles	1.42/0.36	0.73/0.46	0.43	0.48/0.39	0.61	0.66/0.55	0.87/0.58
6	2.40	1.06	1.41	1.50/1.31	0.98	1.04/0.92	1.62/1.26
7	2.13	0.93	0.96	1.06/0.88	1.65	1.70/1.59	0.67/0.49
8	1.43	0.81	0.48	0.53/0.43	0.32	0.38/0.26	1.99/1.15
9	2.13	0.80	0.71	0.77/0.65	0.43	0.49/0.37	2.07/1.33
Average			0.91		0.85		1.59/1.07
Group II							
1	3.13	1.43	3.34	3.51/3.18	2.46	2.52/2.40	1.46/1.27
2	2.33	1.46	1.09	1.13/0.96	0.91	0.96/0.85	1.33/0.99
3	2.53	1.53	3.08	3.25/2.93	2.71	2.77/2.65	1.22/1.06
4	2.47	1.30	0.90	1.00/0.82	1.40	1.46/1.34	0.74/0.56
5	2.30	0.83	0.82	0.90/0.76	0.77	0.83/0.72	1.25/0.92
6	2.07	1.17	1.47	1.58/1.38	1.31	1.37/1.26	1.26/1.01
7	2.93	1.23	2.31	2.45/2.19	1.62	1.68/1.56	1.57/1.30
8	1.60	0.73	0.44	0.49/0.40	0.38	0.44/0.32	1.51/0.92
9	1.97	1.23	1.55	1.66/1.47	1.54	1.60/1.48	1.12/1.92
10	1.90	1.00	0.99	1.07/0.92	0.69	0.75/0.64	1.67/1.22
Average			1.60		1.38		1.31/1.02

*These pneumatophores of *Nanomia bijuga* were collected 1 and 2 July 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.

**All measurements of length and width should be read ± 0.03-mm maximum reading error. Measurements were all made at approximately 20°C. Volumes have not been corrected for STPD.

The volume range calculated as a prolate spheroid was determined by adding and then subtracting the ocular micrometer error from the values obtained.

The low ratio averages for Groups I and II (table 2, column 10), 1.07 and 1.02, respectively, suggest that the internal pressures of float gases are virtually at ambient, and that any variations which appear are due to the errors described, and possibly to the degree of deviation of the shape of the gas phase from the ideal prolate spheroid.

The fact that the floats in Group I are more homogeneous in respect to size than those of Group II (table 2, column 6) is possibly an indication that our net when collecting Group II was sampling from two more or less distinct age groups or sublayers of the DSL. This is possibly due to vertical cycling behavior of the net as shown in characteristic depth records (fig. 8). The cycling was due to the inability of the towing vessel to maintain a constant slow speed (i.e., less than about 3 to 4 knots), thus necessitating continuous engaging and disengaging of the ship's clutch.

Barham³ has observed from the bathyscaphe as many as three distinct siphonophore layers associated with sonic scattering, each made up of siphonophores of different size and presumably, therefore, of different age.

It is of interest to compare volume observations made upon specimens of *Rhizophysa* sp. This siphonophore possesses a pneumatophore some 30 to 40 times the volume of *Nanomia*, but lacks nectophores (swimming bells). Propulsion presumably is supplied by controlling gas volume by means of an apical pore, and by contraction of the comparatively short stolon. This species is probably capable of expelling gas by constricting the float as shown in figure 3, but does not appear to possess longitudinal septa in the float walls as do nanomians. It is not known at the present time how many of these interesting siphonophores are present in the San Diego Trough area, and although the float is certainly an excellent acoustic target, thus far this species has seldom been captured in net hauls made through zones of sonic scattering in this area.*

* Abundant individuals of *Rhizophysa filiformis* have been sighted during bathyscaphe dives in the Mediterranean, however. See reference 10.

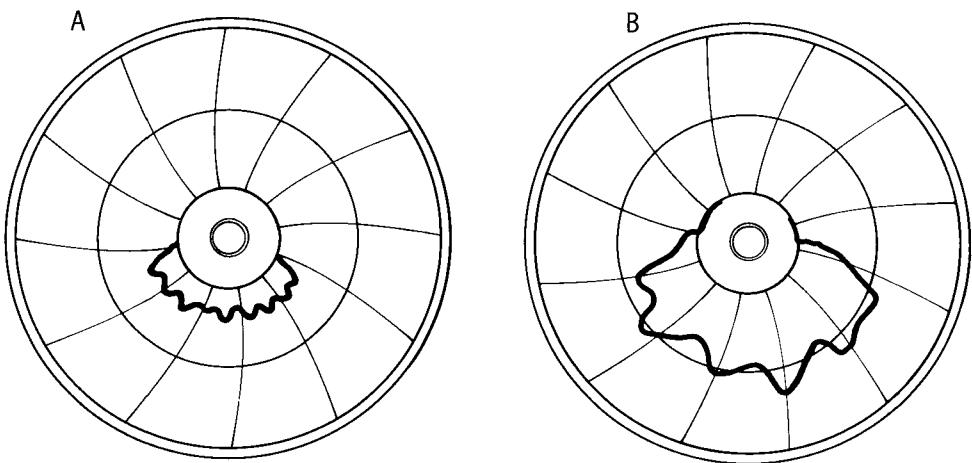


Figure 8. Depth record taken with a Benthos depth-time recorder showing vertical cycling of towed Tucker net. A, cycling amplitude is reduced at shallower depths; B, cycling amplitude increases at greater depths. Distance between two concentric lines = 50 m; time for one entire cycle of chart, about 4 hours.

Rate of Gas Production

Direct evidence for rate of carbon monoxide production in other than surface-dwelling siphonophores is largely lacking (see, however, Appendix C). Nevertheless, a few observations have been made which provide limited indirect evidence.

Jacobs,¹¹ in 1937, observed living intact specimens of *N. bijuga*, captured at Naples, performing a vertical cycling movement when placed in glass cylinders. This was accomplished by active expulsion of a bubble of gas from the pneumatophore by the siphonophore, whereupon it would sink to the bottom of the vessel. Within 45 to 90 minutes the colony had refloated itself; then it would again expel a bubble, sink, and perform the entire cycle again. This behavior was observed by Jacobs to continue for a matter of hours.

Pickwell and others¹² observed a similar phenomenon with the automatized floats of *N. bijuga* captured off San Diego. Some pneumatophores which had been degassed by gentle suction and set aside in a syringe of seawater containing no gas phase were observed to refloat themselves,

usually within an hour. Apparently some of the floats, though severed from the remainder of the colony, still possessed enough substrate to permit secretion of enough additional gas for refloating.

In neither of the above cases was all gas removed from the pneumatophores. Jacobs cites no data but presents a figure which shows about half the gas being expelled from the siphonophore float. My vacuum extraction method generally left a residual bubble of gas within the pneumatophores which was insufficient to provide flotation.

A single direct observation on rate of gas production was performed using the pneumatophore in Experiment 6. After measuring the float and enclosed gas, with an ocular micrometer at 27x, a small bubble of gas was gently extruded by mild pressure. The float was then placed in a 1-cc syringe with no gas phase and immersed in a water bath at 7°C. The extruded bubble was measured for volume and the contained gases analyzed by an ultra-micro technique¹³ (Appendix A, fig. A2).

At the end of the four-hour test period the syringe was removed from the bath. The dissolved gases in the 1-cc of water were extracted and analyzed, and the remainder of gas within the float was extruded, measured and analyzed. The results and a protocol for the rate calculations appear in Appendix C.

Secretion of additional carbon monoxide is immediately evident from the 20 percent increase in that gas within the float. By allowing for the total CO present at the start, and adding the amounts of additional CO encountered within the float and dissolved in the water in the syringe, a total production figure for additional CO of 0.31 mm³ was found. Thus, the pneumatophore produced approximately 0.08 mm³ of carbon monoxide per hour if production rate was linear for this period. This equals a total volume somewhat in excess of the starting volume and indicates that a float this size may easily fill itself in a matter of a few hours. However, this still appears slow compared to the observations noted above, even though the total gas phase was not removed from the pneumatophore under observation. In addition, it is not certain whether all of the gas was produced continuously or in a burst during some period of the experiment.

An even more important consideration is that while a pneumatophore at one atmosphere need produce only enough gas to fill its observed volume, at daytime scattering-layer depths it will be required to produce 30 to 40 times its apparent volume in order to counter the collapsing forces of hydrostatic pressure. Under such circumstances, the observed rate of CO production is entirely too slow. The probable reason for this is the nonavailability of sufficient substrate for gas secretion, unless rate of secretion varies in response to hydrostatic pressure. But even as early as 1861, Keferstein and Ehlers¹⁴ observed that filling of the float was accomplished rapidly in only a few minutes. This observation has been confirmed from time to time by later workers.¹⁵

It is also possible that the automatized pneumatophores are in a more or less declining condition from the moment they are brought on board thus possibly accounting for the apparent lack of correlation between respiration and temperature. This moribund condition could account as well for some of the variability in rate of oxygen consumption (table 1), even though care was taken to use only those individuals appearing in best condition. In this context, however, it is important to note that the float under discussion, regardless of condition, did certainly produce gas, and at the same time consumed oxygen at about twice the rate obtained for any other float observed (table 1). This single observation suggests that production of CO may in fact be an aerobic and energy-requiring process, and therefore very likely does involve an enzymatic pathway, as Wittenberg^{16,17} has indicated is the probable situation in *Physalia*. Without further confirmatory data, however, such a conclusion can be regarded as only tentative.

Diffusion of Gases Across the Float Walls

Diffusive loss of float gases can have important consequences upon the buoyancy of the siphonophore colony, and upon the rates of gas secretion necessary to maintain buoyancy and counter ambient pressures.

Absolute rates of diffusion for carbon monoxide across the walls of the pneumatophore from inside to outside are listed in table 3. In determining total surface area the floats were treated as regular prolate spheroids. Dimensional measurements are subject to the errors listed above, but have comparatively less significance here. The total volume of carbon monoxide dissolved in the syringe water was corrected by means of blanks run on fresh seawater.

It can be seen that rates of loss of CO across the float walls are roughly equal to the rate of CO production determined above. This further suggests that the float in Experiment 6 (tables 1 and 3) was at best producing gas only at a maintenance level.

It is of interest to consider the nature of the diffusion barrier presented by the delicate-appearing pneumatophore walls. The pneumatophore of the pelagic siphonophore, *Physalia*, has been shown to possess a double layer of chitin.¹⁵ The pneumatophore of *N. bijuga* also has a double wall with intervening septa and gastrovascular fluids.¹⁸

TABLE 3. DIFFUSION OF CARBON MONOXIDE ACROSS PNEUMATOPHORE WALLS.*

Experiment Number**	Surface Area of Float (mm ²)	Total Dissolved CO in Syringe (mm ³)	Length of Run (hr:min)	Absolute Diffusion Rate (mm ³ /mm ² /hr)	Krogh Diffusion Constant (Start/End)	Remarks
6	4.79	0.23	3:52	0.012 ≈ 0.01	0.027 0.013	CO produced
7	11.66	0.29	3:40	0.007 ≈ 0.01	0.013 0.019	No CO
9	13.42	1.43	11:30	0.009 ≈ 0.01		
10	10.14	0.91	11:30	0.008 ≈ 0.01		

*All at 7.0°C.

**Experiment numbers coincide with those of table 1.

† Values for dissolved CO were corrected for reagent blanks.

Possession of a chitinous lining on either or both of the walls would be of obvious value and would explain the low rates of diffusive loss for CO.

Krogh¹⁹ defined a diffusion constant, K , for gases across animal tissues as the number of cubic centimeters of gas reduced to standard conditions, dv , which traverses a thickness of 1 micron, dx , and an area of 1 square centimeter, A , in one minute, dt , when the pressure difference, dp , (i.e., the partial pressure gradient of the gas in question) is one atmosphere. The equation may be written

$$K = \frac{dv}{dt dp/dx A}$$

The units are cm^2 per minute as in the case of diffusivity determinations.*

Krogh gives a K for oxygen across chitin as 0.013, a figure lower by at least an order of magnitude than his diffusion constants for oxygen across muscle, connective tissue, gelatin, or water. He found carbon monoxide in general to have a diffusion constant through animal tissues of 25 percent less than that of oxygen.

Diffusion constants for the floats, in two experiments from which there was sufficient data, are included in table 3 (see Appendix D for calculations). Since the partial pressure difference for carbon monoxide across the float walls was not constant, K 's have been calculated for partial pressures at the start and finish of the respective experiments. The ranges given, 0.027 to 0.013, and 0.013 to 0.019, agree well with Krogh's oxygen value for chitin. This suggests that a chitinous lining provides the diffusion barrier in the pneumatophores of *N. bijuga*. It still remains to be seen what a partial pressure differential of several atmospheres will do to the diffusion constants. At present, however, it seems unlikely that they will be much elevated at higher pressures.

* Millington²⁰ has performed a valuable service by clarifying certain conflicting points arising from confusion in the use of the standard Fick equation and the Krogh diffusion constant.

The Work of Countering Hydrostatic Pressure

Given realistic figures for siphonophore float volumes and colony weights, and measured values for oxygen consumption, it is now possible to arrive at an estimate of the physical work required to prevent collapse of the pneumatophore, or conversely, the work required to compress the necessary volume of gas to the volume of the float at depth.²¹

Kanwisher and Ebeling²² have utilized an equation which is said to give the work necessary for a vertically migrating fish to maintain its swim bladder at a constant volume and therefore keep the fish at neutral buoyancy:

$$W = 2.3 v p_2 \log \frac{p_2}{p_1}$$

where v is the volume of the swim bladder of a fish migrating downward from pressure p_1 to p_2 . W is in ergs when v is in cc and p in dynes/cm².

If the assumption of neutral buoyancy maintained by continuous gas secretion is taken for a downward migrating siphonophore possessing a pneumatophore volume of 1 mm³, and the total distance traversed is from 100 to 400 meters, W equals approximately 0.001 gm-cal. It is readily apparent from figure 9 that the total work of countering the hydrostatic pressure increases with increasing depth for each unit increment in gas content of the pneumatophore.

If the siphonophore, on the other hand, performs the vertical descent without maintaining buoyancy, at its daytime depth it must secrete up to a maximum of 30 mm³ of gas under the above conditions. Production of this quantity of gas against the high pressure will cost more energy than would continuously maintained neutral buoyancy during descent; however, the energy cost of swimming downward can be expected to be greater in the latter case.

The work required to compress a given amount of carbon monoxide across a known pressure range, and at a stated temperature, is given by

$$W = 1.99 nT 2.3 \log \frac{p_1}{p_2}$$

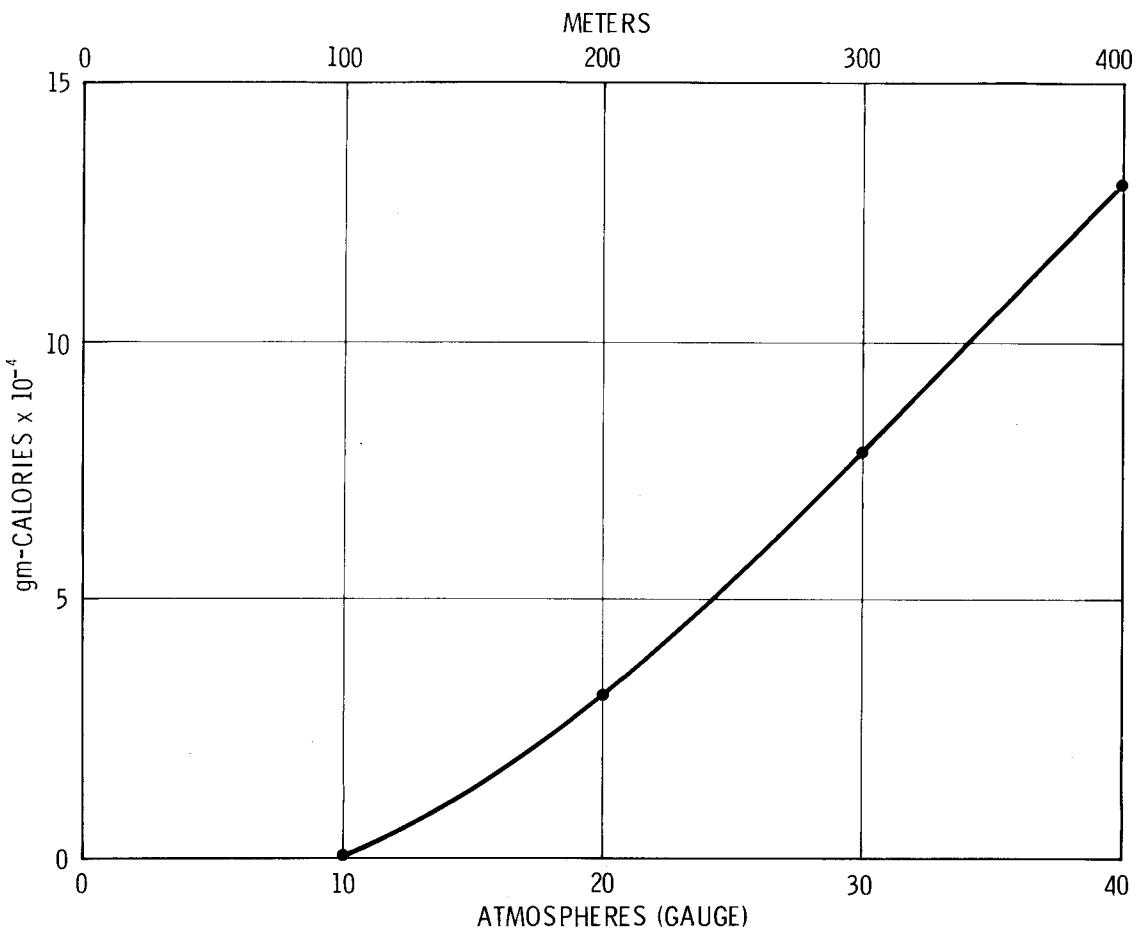


Figure 9. Energy equivalent of physical work required to keep a siphonophore float of 1 mm^3 volume inflated while migrating from 100 meters to various lower depths.

in which n is the molar quantity of the gas in question and T , the temperature absolute.²¹ W here will be read directly in gram-calories. The work required to compress 30 mm^3 of CO from 10 to 40 atmospheres at 8°C for a siphonophore possessing a 1 mm^3 float equals 0.003 gm-cal .

Average oxygen consumption from table 1 is $0.069 \text{ mm}^3/\text{mg/hr}$. For a nanomian colony weighing 15 mg this equals 1.04 mm^3 of oxygen utilized per hour, equivalent to about 0.005 gm-cal , * slightly less than twice that required

* Caloric equivalent of 1 cc of oxygen is taken as 4.8 gm-cal .

for the float to initially resist the hydrostatic pressure. The various metabolic functions of the colony, as well as vigorous swimming, will necessarily place an additional drain upon the available energy. Furthermore, as yet we know nothing of the energy requirement of the enzymatic machinery responsible for gas production. And, in addition, the diffusive loss of carbon monoxide, which may be as high as 0.1 mm³ per hour (table 3), will demand that gas production be sustained at a maintenance level at least this high, as indeed it was in Experiment 6. It thus appears likely, on the basis of these observations, that a siphonophore colony may require a period of several hours in which to complete the process of filling its pneumatophore. If, however, instead of an average oxygen consumption, we employ the elevated rate shown to be associated with gas secretion (Experiment 6), the work required to counter hydrostatic pressure in one hour becomes less than 25 percent of the energy available from respiration (oxygen consumed).

DISCUSSION AND CONCLUSIONS

It is apparent from the foregoing that our knowledge regarding the gas dynamics of *N. bijuga* is still fragmentary and in some cases quite inadequate. Nevertheless, we are still able to make some predictions concerning its role as a sound scatterer.

On the basis of volume measurements, it now seems most likely that nanomians are comparatively sensitive to internal gas pressures rising above ambient. There is little evidence at present to suggest that the pneumatophores are very elastic, and in any case it is unlikely that they could tolerate an expansion equivalent to more than 10 to 20 percent of the float volume. The structure of the float itself indicates that even much smaller additional quantities of gas are probably intolerable and must be voided. We are faced, therefore, with a population of organisms each of which must void gas during its diurnal vertical ascent. That this is in fact the case has been suggested previously.^{1,12} It is not yet certain how many gas bubbles are likely to be expelled by a rising nanomian, but an estimate of one per atmosphere of reduced ambient pressure does not seem unwarranted. Furthermore, some of these transient

secondary acoustic targets are certain to pass through a size permitting a brief period of resonance in response to the sonar frequency in use. The additional sonic targets then, multiplying the regional populations of scatterers at any given moment and with some of them in resonance, must contribute to the increase in scattering intensity which is so routinely seen on precision depth recorders during the crepuscular rise of the DSL.

Conversely, when the scattering layer migrates downward in the early morning hours it is often seen to diminish in intensity, and occasionally to fade out entirely for some period of time. A major cause of this phenomenon, of course, must be inverse-square loss resulting from increased range between signal source and target. However, it is not impossible, in the light of estimates of refilling time for the float based upon physical work equations, that the siphonophores undergo a period when, in fact, their pneumatophores are not fully inflated and therefore respond poorly as sound reflectors. The same postulate could also be put forth in the case of fishes associated with the DSL which possess swim bladders.

Maximum possible rates of gas secretion still remain a mystery, but on the basis of diffusion studies it is reasonable to assume that some production of carbon monoxide must go on at all times simply to counter diffusive loss. If this is true, then the lack of evidence for CO production by most of the floats presented in tables 1 and 3 suggests the unavailability of a suitable substrate for gas secretion. This means that the figures presented for rate of oxygen consumption, although evidencing considerable spread, are representative (under the circumstances of probable continuous decline in float condition) of a "basal" or nonsecretory respiratory rate. In the sea the siphonophores may be nonsecretory (although possibly still resisting diffusive loss) only during the vertical ascent and while in the ascended layer. It may well be that during the remainder of their existence they possess a respiratory rate more closely approximating or very likely surpassing that of the pneumatophore in Experiment 6.

The miscellaneous observations of rapid filling times for siphonophore floats, made by various workers during the last 100 years, have necessarily all been made

at atmospheric pressure. The effects of 30 to 40 atmospheres of pressure upon the rate of carbon monoxide production by a pneumatophore possessing sufficient substrate remains a matter of conjecture. Future studies of these important and interesting organisms might well include this aspect of their physiological capabilities.

SUMMARY

The major findings to date relevant to the effects of siphonophores on sound propagation in the sea may be summarized as follows:

1. Volume of gases contained in siphonophore floats can most accurately be calculated from dimensional measurements treating the bubble as a prolate spheroid.
2. Sizes of floats vary, probably depending upon age of the colony; their gas volumes, determined by extrusion, range from approximately 0.5 to approximately 2.5 mm³ as measured at atmospheric pressure.
3. Adjustment of gas pressures within the siphonophore float closely follows the ambient pressure, suggesting that release of numerous bubbles takes place during a vertical migration. It is very likely that some of these released bubbles will pass through a size where they will briefly resonate if insonified by a particular sound frequency.
4. Measured rate of gas production by a siphonophore float was too slow to account for observations made by others of rapid filling of the float. This is probably a function of float condition and available substrate for gas production, but may be affected by other variables as well.
5. Measured rate of gas production in one instance was rapid enough to balance total diffusive loss of CO by the float, suggesting that normal floats probably sustain gas secretion at some minimum maintenance level.

6. Measured rates of diffusive loss of carbon monoxide from siphonophore floats, when used to calculate diffusion constants for the float walls, give values very close to those determined for chitin, indicating that it is most likely this material which provides the diffusion barrier in the float wall.

7. Calculations of the energy required to perform the physical work of countering hydrostatic pressures at the daytime depths of the DSL indicate that the siphonophore may very well require more than the observed one hour needed for refilling the float at a pressure of one atmosphere. This is not surprising since the volumes of gas which must be produced are 30 to 40 times as great.

8. It should be noted that work estimates have been based upon what may actually be "basal" or nonsecretory levels of oxygen consumption. Observations from a single experiment indicate the possibility that energy production from aerobic respiration may rise to at least double the "basal" level while CO is being produced. The time required for refilling the float at high pressures may thus be somewhat less than calculations indicate.

9. Comparisons of data obtained from intact siphonophores and from automatized floats indicate the validity of results obtained from tests performed upon the latter.

RECOMMENDATIONS

1. Conduct further studies of oxygen consumption of siphonophore floats, especially during secretion of carbon monoxide. Such data should provide a better idea of the energy likely to be available for countering the collapsing effects of high pressure, and of probable refilling times.
2. Provide possible chemical substrate materials to floats in an effort to determine maximum rates of carbon monoxide secretion.
3. Conduct procedures 1 and 2, above, in small pressure chambers to directly determine the effects of elevated pressures on respiratory rate and gas production rate.
4. Utilize pressure chambers in a study of bubble release by siphonophore floats; attempt to learn size and frequency of bubble release as the pressure is reduced by steps.

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APPENDIX A: NOTES ON ANALYTICAL METHODS

Micrometer Respirometer

The pneumatophore within the reaction vessel of the micrometer respirometer (fig. 5 in main text) floats in the seawater in such a way that occasionally a portion of its surface is above water. Since the total surface of the pneumatophore diffuses gas, some carbon monoxide will escape through the float membranes and adhering liquid and go directly to the gas phase within the chamber, while some, generally the greater part, will diffuse first into the seawater.

Assuming the surface area of a hypothetical float to equal 10 mm^2 the approximate rate of diffusion of CO across the float walls equals $0.01 \text{ mm}^3/\text{mm}^2/\text{hr}$ (table 3, main text) which then equals a total of 0.10 mm^3 per hour for the entire float.

From table 1(in the main text), an "average" oxygen uptake (exclusive of Experiments 1 through 3, and 6) is equal to $0.74 \text{ mm}^3/\text{mg}/\text{hr}$ and an "average" float weight (Experiments 8, 11, and 12, only) equals 4.5 mg. The hypothetical float then consumes $0.333 \text{ mm}^3 \text{ O}_2$ per hour.

If all diffused CO is quickly lost to the gas phase of the reaction vessel, thus countering movement of the indicator drop, true O_2 uptake in one hour would equal $0.433 \text{ mm}^3 = 0.096 \text{ mm}^3/\text{mg}/\text{hr}$.

Thus it cannot be stated with certainty that Experiments 8, 11, and 12 would not have shown somewhat higher rates of oxygen consumption if diffused CO had been absorbed by suitable reagents as was CO_2 , nor can it be shown conclusively that no CO was produced during these experiments. From the above example, however, it can be seen that even allowing for the maximum measured rate of diffusion for CO, the resulting rate of oxygen consumption falls very close to the range of values obtained by the syringe method. Since even the comparatively slow rate of carbon monoxide production demonstrated for the float in Experiment 6 resulted in a respiratory rate accelerated to double the next highest rate, it seems unlikely that any significant gas secretion occurred during Experiments 8, 11, and 12.

Scholander Gamma-Burette

This instrument¹³ (fig. A1), so named because the smallest division on the micrometer approximates a volume of one γ -liter (= 0.001 mg of water) or slightly less, analyzes minute quantities of gas by differential chemical absorption to an accuracy of ± 2 to 3×10^{-4} mm³.

Samples of the gases contained within the pneumato-phores of *N. bijuga* were invariably analyzed by use of this instrument. The precision of duplicate or triplicate analyses performed with care will generally lie within a range of 0.3 to 0.7 percent by volume for a given gas.

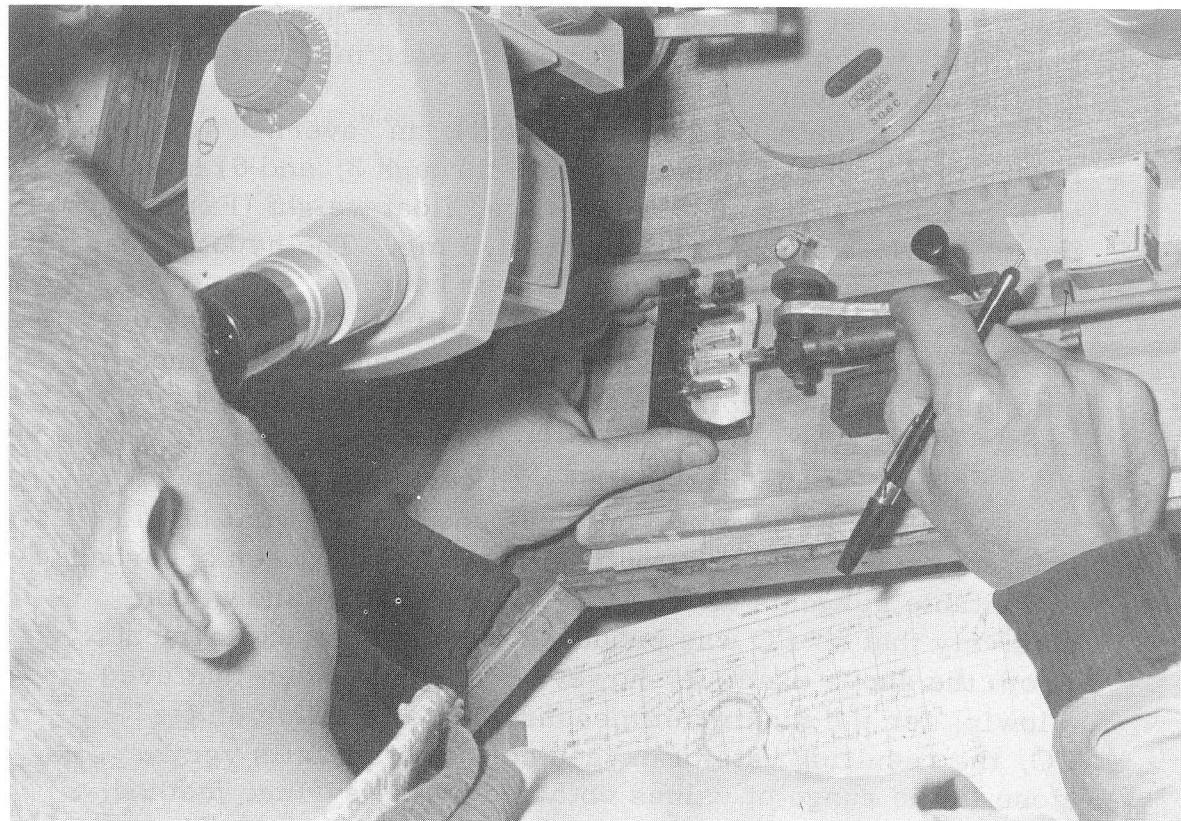


Figure A1. Scholander gamma-burette micro gas analyzer; the gas bubble is partially extruded from the glass burette tip into each successive liquid absorbent. The micrometer may be read to ± 2 to 3×10^{-4} mm³ by use of a hand lens.

Scholander Dissolved Gas Analyzer

The analyzer for dissolved gases employed in this study (fig. A2) was originally devised for the purpose of performing analyses for oxygen and nitrogen in samples of 1 cc of water.⁵ In expert hands the accuracy of results obtained by this method are comparable to results from the unmodified micro Winkler titration and have the advantage of immunity from the possible effects of interfering substances. To the best of the author's knowledge this is the first reported instance of the use of this versatile instrument for the determination of dissolved carbon monoxide. Precision of duplicate analyses is nearly always within 0.2 to 0.3 percent by volume, the accuracy generally of the same order.

The precision bore capillary graduated in 100 divisions, employed in the analyzer for this study, was found upon calibration to have a volume equal to 28.5 mm³. The meniscus within the capillary could be read by use of a hand lens to ± 0.2 to 0.3 division making the reading error at best equal to ± 0.057 mm³.

Because accuracy for analytical results obtained by this method can be claimed to be no greater than about ± 0.06 mm³, the data given in Appendix C are expressed to two significant decimal figures only. The data arising from analyses performed with the gamma-burette, however, are actually accurate to ± 0.001 mm³ or better.

All analyses discussed in this report, with the exception of the confirmatory analysis upon *Rhizophysa* sp. and some of the blanks and calibrations, were performed aboard ship under varying, though usually mild, sea states.

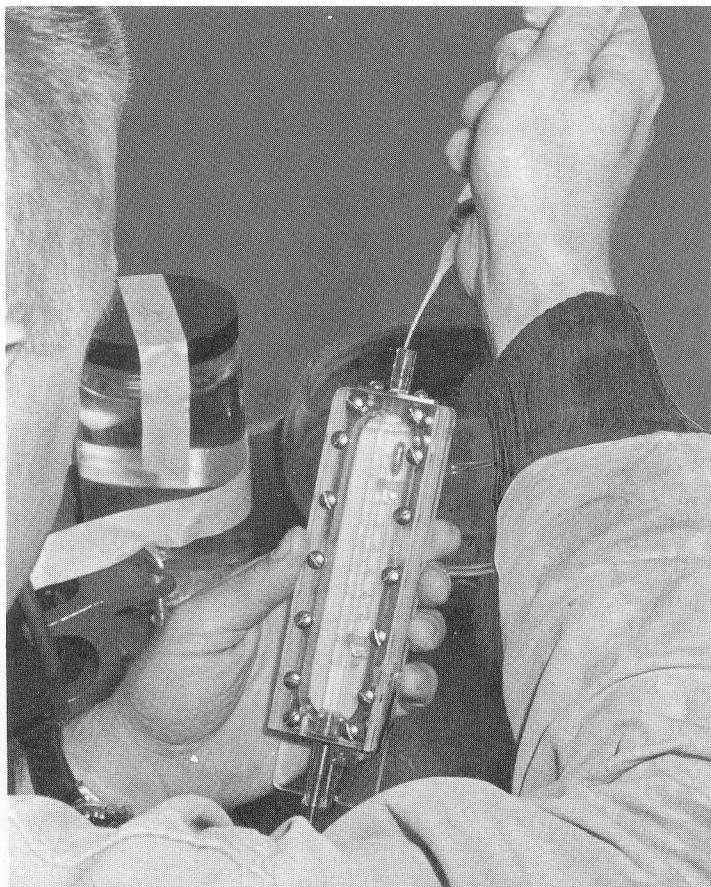


Figure A2. Scholander analyzer for dissolved gases. The capillary shown inside the lucite water jacket is calibrated in 100 divisions equivalent to a total volume in this case of slightly less than 30 cubic millimeters. The threaded plunger partly shown at bottom permits precise control of the gas bubble and liquid absorbents within the capillary.

APPENDIX B: EXTERNAL MEASUREMENTS OF NANOMIAN PNEUMATOPHORES

It is of interest to know the volume of the pneumatophores of *Nanomia*. Several series of these floats were measured by ocular micrometer at 27x; a representative tabulation is presented in table B-1. Actual volumes of the pneumatophores were obtained by measuring meniscus displacement of a drop of water in a glass capillary of diameter just sufficient to accommodate the float being measured. The difference in length of the drop of water before and after introduction of a float gave the true float volume. Volumes calculated as spheres (diameter obtained by averaging length and width) were found to be high over displacement volume by a factor of 1.5 to 3, while volume calculated as prolate spheroids are more uniformly low by a factor of 0.5 to 0.8 (table B-2).

The possibility that the severed floats were imbibing or extruding water through their basal areas where adhering fragments of stolon had been removed was considered. This was checked by constructing a small solid glass model which was measured in the usual way, and whose volume was determined by the capillary displacement method. The ratio of volume calculated as a prolate spheroid to that actually measured by the capillary method was 0.7. This is very near to the average for this ratio given in table B-2. Therefore, the capillary displacement method for determining the volume of intact floats was deemed reliable.

TABLE B-1. EXTERNAL DIMENSIONS OF PNEUMATOPHORES*

Pneumatophore	Length (mm)	Width (mm)	Volume as Prolate Spheroid (mm ³)
Group I**			
1	2.50	0.83	0.90
2	3.00	1.50	3.52
3	3.80	1.13	4.45
4	2.86	1.00	1.50
5	2.46	0.86	1.10
6	3.00	1.23	2.35
7	2.90	1.20	2.18
8	2.00	0.90	0.84
9	2.63	0.90	1.11 Avg. 1.99
Group II			
1	4.40	1.67	6.38
2	3.16	1.43	3.36
3	4.00	2.06	8.90
4	3.16	1.16	2.22
5	2.70	1.03	1.49
6	3.27	1.97	6.63
7	4.40	1.70	6.63
8	2.60	1.03	1.44
9	2.73	1.50	3.20
10	2.77	1.50	3.25 Avg. 4.35

*These are uncorrected measurements. A discussion of suitable corrections will be found in the main text, p. 10, dealing with measurement of the float gases.

**Groups I and II and the pneumatophore numbers coincide with those given in table 2 in the main text.

Errors (Float Volume)

The error in reading the ocular micrometer at 27x was no greater than ± 0.03 mm. This will give rise to maximum error in volume estimates of about 8.5 percent for the smallest volume in Group I and a minimum error of about 2.7 for the largest volume in Group II (see table B-1), if both length and width are read erroneously high. The maximum range of errors in volume estimate then becomes about 2 to 9 percent.

The position of the meniscus in the volume-displacement measurements could be read with a hand lens to ± 0.2 mm, an error of approximately 1.5 percent for an average water-drop length of 15 mm in a capillary of 1.85 mm ID.

If, for example, in the case of pneumatophore number 8, table B-2, the estimated volume was a maximum of 9 percent greater than that shown and the displaced volume a maximum of 2 percent less than that shown, the resultant increase in the ratio of calculated to displaced volume in this case changes from 0.064 to 0.072, a maximum discrepancy of only 12 percent.

TABLE B-2. EVALUATION OF PNEUMATOPHORE VOLUME MEASUREMENTS*

Pneumatophore**	Length (mm)	Width (mm)	Volume Estimated as a Prolate Spheroid (mm ³)	Volume Determined by Capillary Displacement (mm ³)	Ratio (Est. Vol.: Dis. Vol.)
1	4.3	1.2	3.2	3.8	0.8
2	4.3	1.5	4.8	8.0	0.6
3	3.6	1.2	2.6	4.9	0.5
4	3.9	1.2	2.8	4.4	0.6
5	4.2	1.8	6.8	10.5	0.6
6	4.0	1.4	4.1	7.9	0.5
7	4.0	1.6	5.5	7.3	0.7
8	4.8	1.5	5.6	8.7	0.6
9	4.3	1.6	5.9	7.1	0.8
10	4.2	1.3	3.9	6.3	0.6
Glass Model	9.3	0.8	3.1	4.6	0.7

*Dimensions were measured with an ocular micrometer at 9x, and an accuracy of ± 0.1 mm.

**These were preserved pneumatophores, collected July 1963. All pieces of attached stolon were removed.

APPENDIX C:

RATE OF CARBON MONOXIDE PRODUCTION

Protocol Statement for Calculations

(Example from Experiment 6, table 1)

Original volume of pneumatophore gases calculated from dimensional measurements (all volume measurements and analyses performed at about 20°C):	0.29 mm^3
Bubble extruded at start:	0.05 mm^3
Analysis of bubble gases: ¹³ CO 15.6%, 0.008 mm^3 (O ₂ 2.3%, 0.001 mm^3 ; N ₂ 82.1%, 0.044 mm^3)*	$\approx 0.01 \text{ mm}^3$
Original volume of carbon monoxide: $0.01 + 0.156 \times (0.29 - 0.05) =$	0.05 mm^3
After 3.87 hours, CO dissolved in syringe water (volume 1 cc) corrected for reagent blanks: ⁵	0.23 mm^3
Total gas phase extruded from pneumatophore at end of experiment:	0.36 mm^3
Analysis of gases: CO 35.0%, 0.126 mm^3 (O ₂ 1.8%, 0.007 mm^3 ; N ₂ 63.2%, 0.227 mm^3)*	$\approx 0.13 \text{ mm}^3$
Total volume of additional CO produced: $0.23 + 0.13 - 0.05$	0.31 mm^3

* Nitrogen was obtained by difference.

Rate of carbon monoxide production	0.08 mm ³ /hr
0.31 ÷ 3.87 =	
(mm ³) (hours)	Approximately = 0.07 mm ³ /hr STPD
Weight of pneumatophore:	1.2 mg
Final rate of CO production (see below):	
0.08 ÷ 1.2 =	0.07 mm ³ /mg/hr
(mm ³) (mg)	Approximately = 0.06 mm ³ /mg/hr STPD

Note Regarding Rate of CO Production

Experiments designed to determine the rate of CO production by *Physalia*, the Portuguese man-of-war, have been conducted by Wittenberg¹⁶ and by Larimer and Ashby.⁹ The former obtained values of 5 to 20 $\mu\text{l}/\text{hr}/\text{gas gland}$ for carbon monoxide produced, while the latter determined a range of 7.5 to 120 $\mu\text{l}/\text{hr}/\text{animal}$. While these values appear superficially high compared to the observations reported here, a direct comparison is not possible since neither dimensional measurements suitable for comparison, nor weights, were given by those authors.

However, in more recent work Hahn and Copeland,²³ while testing the effects of aminopterin, a folic acid inhibitor, upon the carbon monoxide secreting capabilities of the excised gas gland of *Physalia*, found their control specimens, when incubated at 22°C with L-serine added as a substrate, produced CO at rates ranging from 0.280 $\mu\text{l}/\text{mg}/\text{hr}$ to 1.075 $\mu\text{l}/\text{mg}/\text{hr}$. My single figure for rate of CO production incorporates the weight of the entire nanomian float and is, therefore, not readily comparable to the above figures. However, a series of dissections performed previously for other purposes disclosed the weight of the goblet-shaped nanomian gas gland to be in the range of 3 to 17 percent of the total float weight after removal of all attached stolon; that is, about 0.1 to 0.6 mg in floats weighing 3.0 to 4.3 mg. When the value reported here is regarded as representing

the gas production of a tissue approximately 10 percent of the total float weight (for example), then it equals $0.7 \mu\text{l/mg}$ gas gland tissue/hr. The average obtained by Hahn and Copeland was 0.6. Furthermore, the determination of CO production reported here was performed at 7°C , whereas the above authors made their determinations at 22°C . If the Q_{10} of the enzymatic reactions involved in gas secretion is above 1, as is almost certainly the case, then the single observed value in this report will become relatively greater. For example, at a Q_{10} of 2 for the process of secretion, my figure of $0.7 \mu\text{l/mg}$ gas gland/hr when adjusted to 22°C becomes approximately $2.5 \mu\text{l/mg}$ gas gland/hr.

APPENDIX D:

CALCULATIONS FOR DETERMINING THE KROGH DIFFUSION CONSTANT FOR THE PNEUMATOPHORE WALLS OF NANOMIA

Below is the protocol followed in calculating K for the float in Experiment 6:

Float dimensions: 1.7 mm \times 1.0 mm

Surface area, A , of a regular prolate spheroid:

$$A = 2\pi b^2 + 2\pi \frac{ab}{e} \sin^{-1} e$$

where a and b are the major and minor semi-axes, respectively, and e is the eccentricity.

$$A = 6.28(.5)^2 + 6.28 \frac{(.85)(.5)}{0.863} 1.04 = 4.79 \text{ mm}^2 \\ = 4.79 \times 10^{-3} \text{ cm}^2$$

$$K = \frac{dv}{dt} \cdot \frac{dx}{A} \cdot \frac{760}{dp}$$

From table 3: $dv = 2.3 \times 10^{-4} \text{ cm}^3$

$$dt = 2.3 \times 10^2 \text{ min}$$

Measured thickness of float wall (float width minus gas phase width, $\div 2$),

$$dx = 0.2 \text{ mm} = 2 \times 10^2 \mu$$

Percent carbon monoxide in float gases (partial pressure gradient across float walls):

$$\text{at start} = 15.6 = 119 \text{ mm Hg p CO}$$

$$\begin{aligned} \text{at end} &= 35.0 = 266 \text{ mm Hg p CO less } 13 \text{ mm Hg p CO} \\ &\quad \text{dissolved in syringe} \\ &= 253 \text{ mm Hg p CO} \end{aligned}$$

$$\begin{aligned} \text{That is, } dp &= 119 \text{ at start} \\ &= 253 \text{ at end.} \end{aligned}$$

Thus,

$$\begin{aligned} K_{119} &= \frac{2.3 \times 10^{-4}}{2.3 \times 10^{-2}} \cdot \frac{2 \times 10^3}{4.79 \times 10^{-2}} \cdot \frac{760}{119} \\ &= 1 \times 10^{-6} \cdot 4.17 \times 10^3 \cdot 6.39 \\ &= 2.67 \times 10^{-2} = 0.027 \end{aligned}$$

and

$$\begin{aligned} K_{253} &= 1 \times 10^{-6} \cdot 4.17 \times 10^3 \cdot 3.0 \\ &= 1.25 \times 10^{-2} = 0.013 \end{aligned}$$

These figures are uncorrected for standard conditions since barometric pressure at time of experiment aboard ship was not taken. However, a barometric fluctuation amounting to even as much as 20 mm would have no more effect on the final value of K than:

$$K_{119} \pm 0.007,$$

$$K_{253} \pm 0.004.$$

Experiments were conducted at constant temperature, 7.0°C. It is assumed that diffusion across pneumatophore walls can be in either direction and will move according to the partial pressure gradient.*

*Larimer and Ashby⁹ have reported the results of their efforts to determine the diffusion rate of carbon monoxide across the pneumatophore walls of the Portuguese man-of-war, *Physalia*. Their absolute rates, unfortunately, are not presented in a manner permitting direct comparison with the values given here for *Nanomia*. However, the diffusion rate which they found for CO relative to that of oxygen was 17 to 25 percent lower than the latter, agreeing with the observations of Krogh¹⁹ for chitin.