as a new experimental organism with many desirable characteristics. Its cells are small $({\sim}2 \mu)$, it may be grown as easily and reliably as Chlorella, it contains phycocyanin as an auxiliary photosynthetic pigment, it can be studied over a wide range of temperatures up to 41° C, it shows no inhibitory effects of the Warburg buffers, and it has an unusually low rate of dark respiration.

SUMMARY

Photosynthesis and respiration of three blue-green algae, Anabaena variabilis, Anacystis nidulans, and $Nostoc$ muscorum G, have been studied by conventional Warburg manometry.

Rates of photosynthesis at light saturation were measured in the Warburg series of carbonate-bicarbonate buffers and in bicarbonate-carbon dioxide buffers in the pH range ⁶ to 10.5. Evidence was sought, but not found, that rate of photosynthesis is any simple function of bicarbonate concentration. In terms of their light intensity curves the three algae show no feature markedly different from those of Chlorella. Limited response of respiration to added organic substrates is considered related to the obligate photoautotrophic character of the three algae.

Attention is directed to Anacystis nidulans which has several features making it particularly useful as an experimental organism for studies on photosynthesis.

LITERATURE CITED

- 1. EMERSON, R. and LEWIS, C. M. The photosynthetic efficiency of phycocyanin in Chroococcus, and the problem of carotenoid participation in photosynthesis. Jour. Gen. Physiol. 25: 579-595. 1942.
- 2. KRATZ, W. A. and MYERS, J. Nutrition and growth of several blue-green algae. Amer. Jour. Bot. 42: 282-287. 1954.
- 3. LEWIN, J. C. Obligate autotrophy in Chlamydomonas Moewusii. Science 112: 652-653. 1950.
- 4. LEWIN, J. C. Heterotrophy in diatoms. Jour. Gen. Microbiol. 9: 305-313. 1953.
- 5. MYERS, J. Influence of light intensity on photosynthetic characteristics of Chlorella. Jour. Gen. Physiol. 29: 429-440. 1946.
- 6. MYERS, J. and CLARK, L. B. An apparatus for the continuous culture of Chlorella. Physiol. 28: 103-112. 1944.
- 7. RABINOWITCH, E. I. Photosynthesis, Vol. I. Pp. 1-599. Interscience Publishers, Inc., New York. 1945.
- 8. RABINOWITCH, E. I. Photosynthesis, Vol. II. Part Interscience Publishers, Inc., New York. 1951.
- 9. SOROKIN, C. and MYERS, J. A high temperature strain of Chlorella. Science 117: 330-331. 1953.
- 10. STEEMAN-NIELSEN, E. Experimental carbon dioxide curves in photosynthesis. Physiol. Plantarum 5: 145-159. 1952.
- 11. WEBSTER, G. C. and FRENKEL, A. W. Some respiratory characteristics of the blue-green alga, Anabaena. Plant Physiol. 28: 63-69. 1953.

PERSISTENT RHYTHMS OF O2-CONSUMPTION IN POTATOES, CARROTS AND THE SEAWEED, FUCUS^{1,2}

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It has been widely known for a long time that numerous plants and animals normally display overt daily rhythms of various processes and, if they live in the littoral regions of the seas, have also primary lunar cycles (tidal) of various physiological processes. In general, it has been customary to think of these as direct responses to rhythmic external factors. Similarly, the numerous instances of semi-lunar and lunar breeding cycles, common for marine animals and plants, have generally been considered to be simple responses to illumination or tides, or some combination of these.

It is gradually becoming increasingly apparent, however, that daily rhythmicity in animals possesses

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an important persistent aspect. The rhythmic changes persist, more or less temperature-independent as to frequency, under constant conditions of light, temperature, and humidity. It is also becoming evident that there are similarly temperature-independent persistent rhythms of primary lunar frequencies.

Beliefs- as to relationships between lunar phases and human and other organismic behavior are as old as the beginnings of the human cultural heritage, but these have seldom found solid scientific support. Even when finally supported and used to predict accurately such phenomena as occurrence of plankton pulses of the Illinois River (18), the swarming of the grunion (24), or the palolo worm (17) the lack of an adequate explanation has usually left the matter in the hands of the naturalist rather than the experimentalist.

Biologists who have had occasion to concern themselves with the measurement of $O₂$ -consumption in either plants or animals have almost invariably been plagued by what appeared to be a wide range of random variability in the rates of $O₂$ -utilization even under conditions constant with respect to all factors known to influence the organisms. Gompel (16) found variations in $O₂$ -consumption correlated with tidal times in a number of marine animals in the laboratory away from tidal changes. Brown et al (6) made analyses of the variations in rates observed in the fiddler crab, Uca pugnax, under constant conditions and found them capable of being resolved into a complex of rhythms. One of these correlated with the primary solar cycle of 24 hours. Another correlated with the primary lunar cycle of 12.4 hours. A third cycle of about 15 days appeared to result from the periodic reinforcement of the primary solar by the primary lunar one. A fourth cycle of about ^a month was observed but it was not possible with the data of the limited period of the investigation to determine decisively whether this correlated closely with the synodic month of 29.5 days or possessed another cycle length. Preliminary work with two species of snails suggested strongly that the same types of persistent rhythms of $O₂$ -consumption were to be found in them (21).

Although the amplitudes of persistent cveles seem clearly to be a function of temperature as are the rates of biological processes in general, the frequencies of the persistent cycles appear in every case so far carefully investigated to be independent of temperature. This independence extended over as wide a range as from 6 to 26° C for the primary solar cycle and at least from 14 to 30° C for the primary lunar one in the fiddler crab (9, 10). Temperature-independent endogenous rhythms with closely similar properties have also been described for Drosophila (20) and for Avena coleoptile (1). Although the temperature-independence of the frequency of the rhythms was shown using criteria other than respiration, it seems highly likely that the frequency of the rhythms of $O₂$ -consumption are similarly temperature-independent. This seems likely inasmuch as the rhythm frequencies correlated closely with the cosmic ones at any arbitrarily selected constant temperature used within an experiment, which was performed under conditions constant with respect to all factors now known to influence organisms.

The experiments to be reported constituted an attempt to learn whether plants possess persistent rhythms of respiratory rate comparable to those found in animals as described earlier. A brief summary of some of the results have been published (7, 8).

Among the more recent reviews of the subject of organismic rhythmicity are those of Caspers (13) and Korringa (19). The relative roles of persistently rhythmic mechanisms, on one hand, and direct induction by variations in light, temperature, O_2 -tension, water and similar obvious factors, on the other, in determining the normal periodic behavior of organisms constitute an almost wholly unexplored field.

MATERIALS AND METHODS

Potatoes (Solanum tuberosum) and carrots (Daucus carota) were procured from a local grocery store in Evanston, Illinois. With a large cork-borer, short cylinders about two centimeters in diameter, each bearing an eye and weighing about ⁵ gm were cut from the potatoes. The carrots were cut transversely into sections similarly weighing about 5 gm. The pieces of the vegetables were placed in automatic continuous recording respirometers (3) and their rates of $O₂$ -consumption measured continuously for the 29-day period, May 12 through June 9, 1954.

An experiment with the brown alga, Fucus, was performed in Woods Hole, Massachusetts. Fresh Fucus was collected every ³ to ⁵ days. Five gm of terminal portions of thalli were placed in each of several respirometers. $O₂$ -consumption was determined continuously for the 35-day period, July 27- August 30, inclusive, 1954.

The respirometer was of such a character that the organisms were maintained in essentially constant $O₂$ -tension during the course of the experiments since it operates on the principle of continuous O_2 -replacement. The organisms were subjected continuously to the natural variations in barometric pressure.

The respirometer consisted essentially of a 50 ml Soxhlet distilling flask as the plant-containing chamber. The chamber also contained a vessel of saturated KOH as a $CO₂$ -absorbent. The flask was closed by a rubber stopper which was perforated by a 27-gauge hypodermic needle; attached to the top of the stopper was a collapsible, O_2 -impermeable, Saran sack which was filled with O_2 (about 50 ml). The plant was maintained in air with its base immersed in a small amount of water. The flask-sack ensemble, constituting a "diver," was weighted with lead just sufficient to sink it in water and then suspended by fine wire into a constant-temperature bath. The wire activated a delicate, ink-recording, spring scale which recorded the changing, weight of the submerged diver continuously upon a strip of paper drawn over a roller by a telechron clock motor. For each milliliter of $O₂$ -consumed by the organism, the diver obviously increased in weight one gram. The walls of the bath were opaque and painted black on the inside. The apparatus was operated in a light-proof enclosure in which six 7.5-watt frosted incandescent lamps provided a constant illumination of about ¹ fc at the level of the water-surface of the baths.

The pieces of potato and carrot were prepared and placed in respirometers at about 4 P.M. (all times for potato and carrot are Central Daylight, and for Fucus, Eastern Daylight) on May 10, 1954. One respirometer, possessing nine divers, was operated in conjunction with a bath maintained at 19.8° C. A piece of potato was paced in each of eight of the divers, the ninth being left as a blank control. Another respirometer, similarly possessing nine divers, had its constant temperature bath operating at 19.6° C and a carrot section was placed in each of eight of these divers with the last one left blank as a control, as for the potatoes.

The plants were permitted to remain in the respirometers but the records for the first 31 hours were not used. Thereafter, beginning at ¹¹ P.M., May 11, 1954, an essentially complete, 29-day record of $O₂$ -consumption for 8 carrots and 8 potatoes was obtained.

The potatoes were all replaced by freshly cut pieces at 4 P.M. on May 14, and half of them, or four, were replaced by new ones at ¹¹ A.M. on May 30. The O_2 -reservoirs were replenished and the CO_2 absorbent was replaced at about 3- to 4-day intervals. Freshly cut pieces of carrot were substituted for the old at ⁵ P.M. on May 17; and thereafter only two carrots were replaced because of accidental contamination while changing $CO₂$ -absorbent. As for the potatoes, the O_2 reservoirs were refilled and the CO_2 absorbent replaced at 3- to 4-day intervals.

Fucus was placed in each of 8 divers on the afternoon of July 26, and hourly records were taken from midnight of that day through midnight of August 30. The bath temperature was 19.6° C. Illumination at the bath surface was kept constant, about ¹ fc. The O_2 and CO_2 -absorbent reservoirs were refilled at each time the plants were replaced (3- to 4-day intervals).

It had been the original intention to utilize the control blank divers in order to compensate for the influences of the changes in barometric pressure upon the plant-containing respirometers but this turned out not to be feasible. When the recording system was adjusted in its sensitivity so that the full scale would permit 3 to 4 days of uninterrupted continuous recording of the $O₂$ -consumption of the plants, the random rises and falls in barometric pressure, though qualitatively followed quite faithfully, were quantitively of smaller extent than were to be expected upon the basis of the gaseous volume and the known changes in pressure simultaneously recorded upon a precision barograph.3 In fact, a careful correlation of 29 days of blank diver data with barometric pressure showed the divers to exhibit only about 27 $\%$ of the expected amplitude. This was finally traced to a very small freedom of movement in certain pivotal bearings of the spring scales. Since the blank divers were almost completely free from gaseous escape, the normal pressure changes produced only minute right and left excursions of the ink-writing arm about a mean. A large fraction of these excursions which were of the order of tenths of millimeters was absorbed in the pivotal bearings of the pen rather than being translated to the pen point.

This blank-diver error was confirmed also by attaching blank divers to greatly sensitized spring scales; the pivotal freedom became, thereby, smaller relative to the total barometric-pressure-change induced excursion of the pen. The blank divers under these circumstances became greatly improved barographs.

³ Data generously provided by the U. S. Weather Bureau.

The plant-containing divers, on the other hand, appeared to behave as nearly perfect barographs during the experiments. In these instances the pen lever arm was ordinarily in constant slow movement in one direction as a result of the $O₂$ -consumption. Here the variations in barometric pressure expressed themselves as variations in apparent rates of $O₂$ -consumption, and the pivot of the pen arm was under continuous, essentially uniform, unidirectional tension. In figure ¹ are to be found sliding averages (three consecutive hours) for 3 three-day periods of data for potatoes and carrots uncorrected for barometric pressure. Plotted directly beneath each, on the same scale, is the variation calculated for the divers on the basis of the known barometric pressure changes. It

FIG. 1. A comparison for general form and amplitude, of the hourly values for three 3-day periods of A) uncorrected data for carrots, B) uncorrected data for potatoes, and C) the calculated expected change in blank divers due to the concurrent barometric pressure changes. All are plotted on the same scale.

FIG. 2. A. Plotted on the same coordinates are the 29-day mean daily cycles of 1) uncorrected carrot data, 2) uncorrected potato data, and 3) the calculated variation for the respirometers due to the actual barometric pressure changes. B. The same for 29 days of data 1) for Fucus and 2) calculated barometric pressure influence.

will be noted that the general form and amplitude of the major variations are as would be expected on the basis of the divers behaving as nearly perfect barographs.

In figure ² A are illustrated the mean daily variations of uncorrected respirometer data for the 29-day period for the carrots and the potatoes, and the calculated daily rhythm to be expected solely on the basis of the hourly barometric pressure changes during the same 29-day period. Figure ² B illustrates the same for Fucus. All are plotted as sliding averages using three consecutive hourly values. The great similarity of not only general form and phase relationships, but also of amplitude, for the plant-containing respirometers and the calculated barometric-pressure-induced changes again gives adequate support for the assumption that the respirometers are behaving as practically perfect barographs.

In the preceding computations of barometric pressure effect, an average diver volume of 100 ml was always used and the corrections were calculated on the base value of barometric pressure, 30.00 in. Hg. Less than a 2% error was introduced in using this arbitrarily selected value. Assays of the divers indicated a mean diver volume of about 100 ml for the 3- to 4-day respirometer runs. During this period there was a gradual volume reduction from about 110 ml to about 90 ml. Therefore, about a 10% error was introduced through the use of this mean volume. The general drop in volume of the respirometers as the oxygen was utilized over 3 or 4 days would not contribute any rhythmic component to the frequencies being investigated. The general validity of this method is borne out by the gross good fit of the observed respirometer fluctuations and the calculated fluctuations based upon the concurrent pressure changes (fig. ¹ and 2).

However, since there is a clear mean daily rhythm of barometric pressure with a range of 0.03 to 0.07 in. Hg, it would be expected that there would be a mean daily rhythm imposed upon the volume of the respirometers and hence be reflected in the data corrected for the pressure on the basis used here. At those times of day when the barometric pressure is above the average the corrections used would be too large, and for those times the pressure was below the average, the corrections would be too small. The cyclic influence of the barometric pressure would be expected to be slightly amplified, as a result of the correlated slight variation of the diver depth in the water bath and also through the operation of Henry's Law operating with respect to the water in the bottom of the respirometer chamber. A slight error is also introduced in correcting the volume as for a dry gas.

All the data used in the analyses to follow were corrected for the concurrent hourly actual changes in barometric pressure on the basis of a diver mean volume of 100 ml and a uniform pressure of 30.00 in. Hg.

The fact remains, that when one considers the form of the daily variation expected on the basis simply of the mean daily variations in barometric pressure effecting the respirometers as described, one finds in no case the form of the mean daily cycle of $O₂$ -consumption to correspond. The rhythms appear to possess distinct species-specific components.

In the analysis of the hourly correlation between the barometric pressure rate and direction of change and the concurrent rate of $O₂$ -consumption, the values used in the calculation of each point included indiscriminately the use of both overcorrected and undercorrected data resulting from any imposed barometric-pressure daily cycles. Hence the barometric pressure daily cycles could not have been responsible for the good correlations found.

It should also be pointed out that in setting up afresh the respirometers at three- to four-day intervals, the respirometers were closed a number of times in each 15-day or 30-day series, and in a random manner with respect to the actual pressures at the times of closure.

A diurnally rhythmic component due to the uncorrected mean daily barometric pressure cycles would be the result of overcorrection when in the cycle the pressure was above the daily mean and undercorrection when it was below the daily mean. Since there are either undercorrections or overcorrections to both rising and falling pressure changes during any period of pressure divergence from the daily mean, there would be expected to be either two or four maxima in any imposed spurious daily effect, depending upon whether the particular mean daily pressure cycle exhibited one or two diphasic variations about the mean.

RESULTS

The rates of $O₂$ -consumption for the potatoes during each hour of the 29-day period of observation (May 12-June 5) are found in table I. The rates are the averages for the eight pieces run concurrently. It is quite evident that the pattern of change in rate of respiration differs from day to day with maxima and minima occurring at different times. There is no overt daily rhythm. From lowest to highest, values found during the lunar period of observation involve increases of several hundred percent.

The hourly rates of O_2 -consumption for the carrots for the 29-day period are found in table II. Here it is evident that injury to the carrot in preparation of slices evidently resulted in greatly accelerated initial rates of O_2 -consumption which were 2 to 3 days in subsiding. The general pattern of daily changes also changes markedly from day to day in a manner comparable to that for the potatoes. From lowest to highest values over the 29-day period involved similarly an increase of several hundred percent.

A casual study of the data for the carrots and potatoes revealed that despite the fact that the mean rate of O_2 -consumption of the carrot (67.33 \pm 0.86 m /kg \times hr) was substantially higher than that for the potato $(39.78 \pm 0.64 \text{ ml/kg} \times \text{hr})$ there was a correlation in the forms of the minor variations in rate during the whole 29-day period. A determination of the

TABLE I

coefficient of correlation for the hourly data of the two yielded a value of 0.37 ± 0.033 . Since the coefficient is more than 11 times its standard error the reality of a correlation cannot be questioned. It should be emphasized here, that the carrots and potatoes were being run in different constant temperature baths, at slightly different temperatures, and at different locations.

The hourly rates of $O₂$ -consumption for the alga, Fucus, for the 35-day period, July 27-Aug. 30, are found in table III. As with the other species there appear to be relatively large, apparently random,

FIG. 3. A.The average 29-day diurnal cycles of 1) potato, 2) carrot, and 3) Fucus. B. Average 15-day daily cycles for the 1) potato, 2) carrot, and 3) Fucus, over a period extending from first to third quarter of the moon. C. Same as B but for a period extending from third to first quarter of the moon.

TABIE III

 $0₂$ -consumption in the <u>Fucus</u> (ml./Kg.x hr.)

		Α. м.											۲. м.												
JULY			2	3			6		8	9	10	11	12		2	3	4	5	6	7	8	9	10	11	12
	27	$\overline{\mathbf{35}}$	$\overline{55}$	38	45	39	$\overline{12}$	35	41	52	60	37	31	50	$\overline{25}$	37	53	28	38	32	$\overline{19}$	50	38	32	40
	28	44	40	32	37	37	20	52	24	48	45	38	37	43	41	35	15	27	38	39	53	29	13	40	53
	29	54	43	29	38	45	45	48	37	31	57	13	24	39	54	38	51	42	50	47	42	50	53	45	53
	30	37	67	63	49	52	57	43	50	--			53	57	45	43	48	38	77	41	46	57	52	55	56
	31	48	60	67	65	63	56	72	55	62	50	70	73	62	64	64	57	82	57	52	67	59	55	55	75
AUGUST	ı	72	37	72	70	55	69	50	53	65	52	55	79	$\overline{42}$	45	57	35	72	41	46	70	51	42	65	53
	2	58	43	57	55	50	56	58	48	39	43	53	5	53	42	48	57	49	47	38	48	48	37	49	45
	3	45	60	50	35	36	47	36	60	53	--	37	48	40	52	44	39	45	38	43	35	36	38	28	41
		30	35	31	42	39	35	25	37	30	--	50	50	50	46	59	41	50	35	45	45	30	55	43	39
	5	55	43	44	55	38	40	58	39	62	45	47	65	55	69	45	71	5	75	58	35	53	37	68	34
	6	52	42	33	45	43	53	36	40	44	41	33	56	26	58	--	43	43	48	32	56	45	50	40	52
	7	43	50	57	48	48	41	40	42	52	48	57	60	61	47	35	35	52	40	55	43	44	67	41	57
	8	50	48	48	58	41	$\overline{41}$	32	43	53	λ	48	55	41	43	48	45	53	59	38	45	51	50	60	49
	9	45	58	53	57	53	48	43	50	--	--	53	41	41	89	62	13	45	47	48	48	35	27	26	--
	10		--	50	23	33	45	27	42	28	30	43	35	25	32	39	30	28	33	30	70	20	44	25	50
	11	29	32	44	34	33	26	35	48	32	43	35	45	35	--	34	25	31	55	44	43	53	70	64	70
	12	70	72	80	76	57	60	61	63	62	89	105	96	95	90	84	61	84	83	81	69	79	79	81	77
	13	92	98	90	73	79	67	80	83	--	--	62	60	50	48	49	43	40	46	35	45	30	51	45	48
	14	38	40	48	36	41	40	33	52	35	43	38	37	37	32	42	34	30	35	44	40	35	55	38	40
	15	33	46	35	40	44	37	38	42	37	37	44	45	39	45	38	45	30	27	25	51	17	50	44	32
	16	29	48	41	15	57	32	44	39	42	30	14	32	31	69	39	30	54	29	29	35	35	46	49	28
	17	6	25	22	35	13	18	27	27	--		60	70	60	67	52	57	55	63	42	52	47	57	51	50
	18	48	40	60	47	51	44	56	53	43	52	53	54	53	38	55	55	55	55	50	55	35	56	$\mathbf{47}$	46
	19	48	45	43	49	62	49	43	53	58	48	48	52	63	57	50	60	70	50	60	40	--	57	55	63
	20	63	52	77	65	42	70	61	57	60	--	--	75	62	45	57	62	48	62	48	42	60	50	43	51
	21	35	44	40	55	45	32	51	29	53	37	53	62	45	38	40	42	38	42	43	70	27	37	38	41
	22	41	33	28	77	57	12	35	28	42	45	42	35	44	59	35	50	\mathbf{A}	38	28	45	33	48	48	28
	23	33	45	40	38	50	27	48	31	29	--		60	76	69	59	64	60	57	50	49	57	53	48	68
	24	56	43	49	62	52	53	42	58	40	60	43	62	53	55	48	57	56	42	45	47	46	57	58	49
	25	45	64	48	43	49	41	52	57	53	48	53	57	42	61	37	43	51	60	42	53	56	38	52	49
	26	63	57	55	61	60	45	39	55	50	61	50	40	67	45	76	55	41	76	59	35	46	28	48	46
	27	37	41	48	50	32	56	19	41	46	51	32	43	39	29	24	38	30	49	50	48	27	35	48	40
	28	41	32	48	43	35	44	36	56	32	36	60	33	46	40	42	36	45	28	31	49	25	28	38	38
	29	25	37	43	40	53	44	42	41	51	41	34	35	51	43	35	60	53	40	60	41	46	37	46	51
	30	44	54	61	53	79	56	53	34	59	57	29	41	44	--	52	41	44	59	40	31	47	43	55	38

fluctuations in rate through days and also from one day to the next.

The mean rate of O_2 -consumption was found to be 48.29 ± 0.59 ml/kg x hr. A study of possible correlation of the hourly changes with those for the potato revealed there to be no significant one (0.0527 ± 0.039) despite the fact that the hourly correlations were made in such a manner as to bring as closely into alignment as possible any existing solar and lunar cycles for the two series of data, by synchronizing not only times of day but phases of the moon.

The random character of the day-by-day variations in O_2 -consumption was clearly evident when one attempted to correlate the hourly rates of $O₂$ -consumption of the carrot and the potato, but now after dislocating the two series of data by one day (i.e., the hourly data of day n for the carrot was correlated with the hourly data of day $n+1$ for the potato). Despite the similarity of the phases of the solar cycle, and the close similarity (51 minutes off) of any lunar cycle a determination of a coefficient of correlation over the 29-day period yielded a value of -0.016 ± 0.04 , obviously indicating no measurable correlation.

ANALYSIS OF THE RESULTS

To determine whether rhythms of any frequencies of known external physical cycles were present in the potatoes, carrots, and Fucus, the data were first analyzed in such a manner that any existing primary solar cycle of $O₂$ -consumption would be essentially stripped of any complicating primary lunar one. This was done through the use of the whole 29 or 30 days of data. In such a synodic monthly period every phase of a primary lunar cycle would have just scanned once the hours of a primary solar cycle. Hence, for example, the ¹ A.M. hour over such a period would have within it every phase of the 24.8 hour cycle, as would have every other hour of the day. In figure ³ A are plotted the average rates of $O₂$ -consumption for every hour of the day for the monthly period, for potatoes, carrots, and Fucus. There is a distinct daily rhythm of $O₂$ -consumption in the potato with ^a maximum about ⁵ A.M., ^a general tendency to remain high through the day with secondary maxima in the late morning, and middle afternoon, and a tendency for a drop after 3 P.M. with ^a small maximum in the late evening.

A comparable analysis of the data obtained for the carrot similarly reveals an unequivocal daily rhythm. In this case, the maximum for the day similarly occurs about 5 A.M., and there is ^a minimum just after noon. There is a steady but irregular increase in rate from the noon low to the next morning high.

Fucus similarly exhibits a persistent daily rhythm with conspicuous maxima at ³ A.M. and ¹ P.M., but

 $\ddot{}$

with lesser ones at 6 and 11 P.M. Broad minima occur about 7 A.M. and 8 P.M.

In figure ³ B are found the daily rhythms of the three plants calculated on the basis of a 15-day period, from first quarter to third quarter (centering on full moon) of the moon. Here, each half of the solar day is scanned by a different portion of a lunarday cycle-a zenith or a nadir tide. In figure $3C$ are comparable daily rhythms for the three plants for the 15-day period from third quarter to first quarter of the moon (centering on new moon).

In all three species it is evident that the form of the daily cycle differs between the two semi-lunar periods. The predominant difference for all three is that the mean daily rate of $O₂$ -consumption is lower in the fortnight centered on new moon than in that centered on full moon. Also, the afternoon or early evening values, or both, tend to be relatively higher, compared to other times of day, in the 15-day period straddling new moon, than for that straddling full moon.

In order to attempt to understand further the significances of these variations, or at least to learn their correlation with external cosmic events, figure 4 was prepared. In this figure are found for the three plants the relationship between phase of moon and 1) average daily rate of $O₂$ -consumption (individual daily means) 2) average rate of $O₂$ -consumption for two 3-hour periods, $5-6-7$ A.M. and $5-6-7$ P.M. (sliding averages of three davs). All the plants exhibited considerable fluctuations in rate over the month of observation. The potato in both the daily averages and the averages for the two selected periods of the day tended to show general maxima which were, except for the 6 AM. period, at or near the times of full and new moon. The time of new moon was correlated with a relatively low rate at 6 A.M. This difference was obviously the basis of the change in form of the average daily variation over full moon, on the one hand, and new moon on the other. From lowest to highest values for the month, even for these sliding averages which obscure extreme variations, was an increase of more than 100 $\%$.

The values for the carrot correlated to some extent with those of the potato. Full moon, however, was correlated with a relatively low value. The high values just following this time are probably, in part, artifacts of an injury-induced increase in $O₂$ -consumption. Although such injury probably resulted in higher values than otherwise for the values for May $17-May$ 21, inclusive, the data suggest that a lesser maximum would probably have occurred at about the same time had not the carrots just then been replaced with freshly-cut pieces. The carrot showed another distinct difference from the potato in having a maximum near the time of third quarter of the moon, a time when respiration in the potato was near minimal for the 29-day period. But, as for the potato, the carrot showed a 6 A-M. minimum about the time of new moon when on the same day a relatively high rate was found for 6 P.M. This latter would

FIG. 4. A. The variation in mean daily rates of O_z -consumption for 1) carrot and 2) potato. The mean rates for the 5-6-7 A.M. period and the mean rates for the 5-6-7 P.M. period, for the carrots and potatoes and the variations in the 5-6-7 A.M. and 5-6-7 P.M. values of barometric pressure are also shown. B. The comparable relationships for Fucus. Except for the mean daily rates, all are 3-day sliding averages.

clearly account for the differences in the general forms of the two fortnightly average daily variations for the carrot.

Just as the carrot differed from the potato in the rate for the two periods of the day (6 A.M. and 6 P.M.I) so did it also differ in the variation in mean dailv rates. Here the carrot tended toward high rates not only about the time of full and new moon, but also about the time of third quarter of the moon.

Compared with the potato and carrot, the form of the daily variation in respiration of Fucus differed less between the two fortnights. Both the variations in the daily mean values and the mean values for the two periods of the day showed a periodic fluctuation with maxima occurring at 6- or 7-day intervals and with amplitude of variation from low to high values, of the order of 40 %.

Plotted also in figure 4 are sliding averages of the values for barometric pressure for two 3-hour periods, 5-6-7 A.M. and 5-6-7 P.M. For the potato there is suggested a rough inverse correlation with barometric

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pressure. The carrot tends to show maxima correlated with both relatively high and relatively low values of mean barometric pressure. For Fucus there seems to be, as for the potato, an approximate inverse correlation between mean barometric pressure and mean rate of $O₂$ -consumption.

To determine next the character of any existing primary lunar rhythm, it was necessary to render random the persisting daily cycle. To do this, the 24 hourly values for the second day of the 29-day series for each plant were placed directly under those of the first day. The data for the third day were placed below those of the second, but now every value was shifted one hour to the left so that, for example, the 2 P.M. value for the third day lay beneath the ¹ P.M. value for the second. The ¹ A.M. value was then displaced to the opposite end of the line under the 12 P.M. value for the second day. The data for the fourth, fifth, and sixth days were similarly each shifted an hour to the left relative to the lines directly above them, and the early morning hours which no longer lay beneath the values for the first day were for each day removed as a block to the hight hand end of the same line to complete a 24-hour row. The remaining days of data of the 29-day series were used in the same manner to move successive days of data backwards over earlier days at the rate of five hours in each six days or, in other words, at an average rate of 50 minutes a day. It was now clearly evident that a primary solar cycle had just scanned once the 24 vertical columns of data, and hence was neutralized. On the other hand, any cycles of primary lunar frequency were now closely synchronized in the successive days of data. In figure ⁵ A are shown the primary lunar variations in $O₂$ -consumption for the potato and carrots and for Fucus, studied 3 months later. The values in figure 5 are sliding averages of 3 hours. The approximate times of lunar zenith and nadir relative to the variations found are indicated in the figure legend.

It will be noted that for all three species the lowest rate of respiration for the lunar day preceded lunar zenith by ¹ to 3 hours and all showed a lesser minimum ¹ to 3 hours before lunar nadir. It is evident that for respiration in these organisms lunar zenith and nadir are not correlated with quantitatively similar variations. The pattern of the lunarday (24.8-hour) cycle of $O₂$ -consumption for the carrot resembles in a general manner that of the potato and Fucus except for a greater tendency to exhibit what might be referred to as quarter lunarday cycles (i.e., with minima between the primary ones correlated with zenith and nadir).

In view of the possibility that the rhythms of respiration which were here observed under "constant" laboratory conditions might be directly induced by rhythmic variations of the same frequency of some external factor or factors other than light, temperature or humidity, an analysis was made of the relationship between the hourly rates and direction of barometric pressure change and the rate of respiration. The coefficients of correlation for the hourly values for these two phenomena for 29 days of data were determined for the three plants. All three plants showed significant negative coefficients. The value for the potato was -0.585 ± 0.035 ; for the carrot it was -0.176 ± 0.037 ; and for Fucus it was -0.306 ± 0.034 . In other words, in a general way the more rapid the rate of barometric pressure rise, the lower the rate of respiration and the more rapid the rate of fall, the higher the rate of respiration. Just as there were species differences in the forms of the daily cycles, so were there substantial differences in these correlations.

The relationship between rate and direction of barometric pressure change and the concurrent rate of respiration are shown in figure 6 A. The potato shows a reduction in $O₂$ -consumption in proportion to the rate of pressure rise and an increase in proportion to the rate of pressure fall. An increase or decrease of about 10 $\%$ for each 0.01 in. Hg/hr change is seen.

The same general form of relationship is found for Fucus, except that there is only about a 5 to 6% change in O_2 -consumption for each 0.01 in. Hg/hr change in barometric pressure.

FIG. 5. A. The mean 29-day primary lunar cycle in respiration of 1) potato, 2) carrot, and 3) the 30-day primary lunar for Fucus. B. The concurrent mean lunar-day barometric pressure cycle for the 1) 29-day period of the potato and carrot study and 2) the 30-day period of the Fucus study. Lunar zenith lies above the E of zenith and lunar nadir above the ^I of nadir.

FIG. 6. A. The relationship observed between the rate of rise or fall of barometric pressure in 0.01 in. Hg/hr and the mean rate of respiration for 1) potato, 2) carrot and 3) Fucus. The pressure rate changes, 0.03, 0.04, and 0.05, have been combined because of the few data. The size of the circle denotes the standard error of the means. B. The mean barometric pressure cycles for the same periods for which the mean daily rhythms were determined for the carrot and potato. 1) The fortnight centered on full moon, 2) the fortnight centered on new moon, and 3) the 29-day period. C. The comparable mean daily cycles for the period study of Fucus.

The carrot which, it will be recalled, exhibited the lowest general correlation with barometric-pressure change, shows a qualitative difference from the other two plants. The carrot displayed a reduction in respiration rate in response to both rise and fall in barometric pressure. To rise in barometric pressure there was a reduction in $O₂$ -consumption of about 7-8 % for each 0.01 in. Hg/hr. On the other hand, there was a drop of about 3 $\%$ for each 0.01 in. Hg/hr fall in pressure. It is interesting at this point to recall that the carrot tended to show maxima in the various earlier plots of variations in $O₂$ -consumption with time, not only nearly synchronously with potato maxima but also often at the times of potato minima.

Very similar daily and primary lunar rhythms and correlations with barometric pressure change have been found for a vertebrate, Triturus, and for two species of fiddler crabs among the invertebrate animals (unpublished work). Furthermore, the fiddler crabs which were studied for two months continuously exhibited not only the same kind of general form shift in the daily cycle in going from the first fortnight straddling full moon to a second fortnight straddling new moon, but repeated the difference the second month.

That the daily and lunar rhythms with their monthly variation is not an artifact due to improperly corrected respirometer data is found by work on the oyster (4) , whose activity was automatically recorded for 45 continuous days under constant conditions by simply measuring the mechanical opening and closing of the shells through threads attached to them and to ink writing levers recording on a continuous strip of paper. The daily, lunar, and monthly cycles were of the same character as for the plants.

It resembled, in considerable detail, including the hourly correlation with barometric pressure change, the relationships found for Fucus.

It is, therefore, evident that at least one component in the persistent daily rhythmicity in $O₂$ -consumption is a direct induction by some external factor. This fact was supported by correlating the hourly rates of $O₂$ -consumption for a month with the hourly changes in $O₂$ -consumption displaced by exactly 24 hours. When this was done for Fucus, the value of the coefficient of correlation was only $+0.115$ \pm 0.038. A comparable study for the potato yielded a value of 0.217 ± 0.038 . These values are of the general order of size as found for correlating pressure changes, day n, with pressure changes, day $n + 1$, (0.176 ± 0.036) on day n + 2, (0.136 ± 0.037) . It will be recalled that carrot and potato respiration failed entirely to show any significant correlation when the data for day n for one was correlated with day $n + 1$ for the other (-0.016 ± 0.04) . Similar lack of hourly correlation was found between the values of Fucus and the potato studied at different times, even when the data were synchronized with respect to time of day and phase of moon (0.053 ± 0.039) . These last lacks even of suggestions of a correlation again points to the species differences in the cycles.

The 15-day and 29-day rhythms of barometric pressure for the periods of study of the carrot and potato, on the one hand, and for the period for Fucus, on the other, are illustrated in figures ⁶ B and ⁶ C, respectively. It is obvious that the forms of the cycles are slightly different for the two locations and times of year. It is apparent here, as is well known, that there is a daily cycle of signs and magnitude of rates of change of barometric pressure which are statistically almost as precise as are the daily variations in illumination. It is also known, however, that there are larger random, hour by hour, and day to day, variations than are true for visible light under natural conditions.

It will be recalled from figure 4 that the day to day variations in mean $O₂$ -consumption appear to be, in general, inversely correlated with the slow gradual rises and falls in mean daily barometric pressure in a comparable manner to that of the hour-by-hour However, on an hour-by-hour basis throughout the experimental periods, a correlation with the actual barometric pressure was either small or could not be seen. For the potato a correlation of -0.024 ± 0.038 was obtained; for the carrot, $+0.162 \pm 0.038$; and for Fucus $+0.005 \pm 0.039$.

It has been known (13a) that there are barometric pressure rhythms of primary lunar frequency. These are of much lower amplitude than the primary solar; on the average of the order of 0.004 in. Hg, and only capable of rough approximation as to form and frequency even with 29 or 30 days of hourly barometric-pressure data. In figure ⁵ B is plotted the primary lunar variation in barometric pressure, for the period May ¹² through June ⁹ at Evanston, Illinois, and also the comparable variation for the period July 22 through August 20 at Woods Hole, Massachusetts. The primary lunar variation in respiration of Fucus at Woods Hole might be in some measure accounted for in terms of direct induction by some external factor correlated with barometric pressure since maximum rates of barometric rise (cf. respiration reduction) occur shortly before lunar zenith and lunar nadir and maximum rates of fall (cf. respiration increase) tend to occur 5 to 6 hours after lunar zenith and nadir respectively. Any primary lunar cycle of pressure was much lower in amplitude for the month, May ¹² to June 9, in Evanston, Illinois. Furthermore, the general form of the mean lunar-day variation which is present has phase relationships which differ greatly from those obtained at Woods Hole at the later dates. Hence, this cannot account for the carrots and potatoes both having mean primary lunar cycles, essentially in the same phase relationships to the actual lunar cycles as with Fucus, and, in the case of the potato, having even greater amplitude.

Qualitatively, the form of the 29-day daily variation in respiratory rate of Fucus is explained fairly well in terms of the hourly correlation of rate with concurrent barometric pressure change. The early morning barometric pressure drop is correlated with an early morning maximum, the middle A.M. rise in barometric pressure is associated with a minimum in respiratory rate, and the early afternoon rapid fall, with a peak in respiration. The early evening rise in barometric pressure is correlated with a second minimum in respiration. It is very important to recall from the earlier discussion that this is not the mean cycle form expected from any insufficiency of correction of the respirometer data for barometric

pressure changes, although some of the minor fluctuations in the general form of the cycle may be so explained.

In the carrot it is apparent by inspection of the plots of the daily rhythms of barometric pressure and respiration, that, as expected from the relationship of respiratory rate to rate of pressure change, the highest rates of respiration occur during the hours of relatively little pressure change, particularly during the early morning and early evening hours.

For the potato, it would appear that the morning rise in pressure is correlated with a reduction in respiration and that the steep afternoon drop is correlated with one of the daily peak rates.

Also, the general form differences between the first and second fortnights in all three plants clearly reflect the differences in the forms of the pressure daily rhythms for the same periods, when interpreted in terms of the discovered relationships between rates of pressure change and respiration.

A full explanation of the form of the respiration cycles would appear to require the interpolation of a more purely biological component, perhaps, e.g., including periods of compensatory accelerated respiration following periods of directly induced reduction in rate. Also, whatever external factor operative, the organism may be capable of exhibiting accommodation, adaptation, or other relatively rapid regulatory reaction.

In view of the fact that a purely endogenous 24 hour cycle can occur in an animal, even a lower, coldblooded one (11), it seems quite possible that the plant may similarly not be entirely passive in its response to the external cyclic factors. Some of the divergence from the form of response to be predicted from the pressure changes may be due to such endogenous cyclic contribution. The phenomenon of photoperiodicity as, it is known, of wide importance through both the plant and animal kingdoms, implies the possession of a relatively accurate and temperature-independent physiological time-sense, since in this phenomenon, there is, within rather broad limits, a relative independence of the illumination intensity and often even of simultaneous temperature.

Also, the highly precise solar and lunar rhythmicity that synchronizes gamete liberation in certain of the marine algae (e.g., Dictyota) even when directly shielded from normal full daylight or moonlight would appear to require time-measuring mechanisms of considerable accuracy to serve as insurance mechanisms for unusual meteorological conditions. There is some evidence that diatoms do possess such lunar biological clocks (14). These clocks could normally maintain the frequency of the cosmic cycles by such a mechanism as is described in this report.

This research gives little suggestion as to what external rhythmic forces are involved. Virtually every known physical factor of the type that could be operative here exhibits some degree of correlation with barometric pressure.

It is quite reasonable to postulate that the fre-

quency of these cycles of $O₂$ -consumption of daily and lunar frequency in the potato, carrot and Fucus are independent of random temperature variations during the month. This has been shown to be true for rhythms of similar forms and/or frequencies, those of activity in the oyster (4), of color change in the fiddler crab (10), and for rhythms of emergence in Drosophila (12, 20). Despite the fact that the carrots and potatoes had been kept at room temperature and also in a refrigerator at different times over a period of days, all seemed when placed in constant conditions of light, temperature, and humidity at the arbitrarily selected temperatures of 19.6 and 19.8° C to possess cycles of the same frequencies and a rhythm having not only the same frequency as the primary lunar cycles but even the same phase relations. It must be presumed that the frequencies would have been the same whether the temperatures during the experiments had been held, for example at 15 or at 25° C. The form and amplitude, however, may well be influenced by temperature.

The absolute rates of $O₂$ -consumption, on the other hand, undoubtedly are a function of the temperature with a $\mathbf{Q_{10}}$ in the expected range for biological processes. It seems likely that the differences in form of the daily cycles of the several plants can be interpreted in some measure in terms of their specific responses to some fluctuating physical factor or factors which, in turn, are correlated with barometric pressure. It is possible that pressure changes themselves may serve as stimuli.

It is tempting to speculate that the organisms are responding primarily to some factor other than pressure. Suggesting this is the similarity of the lunar rhythms of the three plants despite considerable differences in the form, amplitude, and phase relationships of the simultaneous mean lunar day cycles. The individual daily patterns of cosmic-ray bombardment of the earth (22) show the same more or less irregular gradual changes of form from day to day, and these appear to be capable similarly of resolution into cycles of primary solar length (2) and in addition into lower amplitude cycles which could well be of primary lunar frequency (23) . The cosmic-ray variation at ground level is in some degree influenced by the barometric pressure variations and hence have forms of daily patterns which are in part correlated with the barometric pressure. Suggestive of a possible role of cosmic-ray showers, whose intensity would be correlated with barometric pressure, and yet independently possesses a clear daily rhythm (15) is the demonstration of a measurable physiological response of an organism to experimental variation in intensity of cosmic-ray induced showers (5).

SUMMARY

1. The O_2 -consumption of sections of carrot, pieces of potatoes containing eyes, and tips of the thallus of Fucus was measured continuously over 29 day or lunar month under constant conditions. Slow variations ranging over as much as 3- to 5-fold differences were observed during this period, with the pattern of variation changing from day to day.

2. The mean daily rate of respiration of the potato showed a 15-day cycle with maxima near the times of full moon and new moon. The carrot exhibited a minimum about the time of full moon and maxima at about third quarter and new moon. Fucus displayed cyclic variations in rate of respiration with maxima at 6- to 7-day intervals.

3. Both the carrot and the potato, while exhibiting maxima in mean daily rates of $O₂$ -consumption at the time of new moon, showed minimum rates about this time of the month for the 5-6-7 P.M. period.

4. Through the use of 29 complete days of data it was possible to obtain the form of the mean daily cycle of $O₂$ -consumption, since in 29 days all phases of a primary lunar cycle are present at each hour of the day.

5. The average daily pattern for the 29-day period for the potato was found to show a maximum at 4 to 5 A.M. and lesser maxima at 10 A.M., 3 P.M. and 10 P.M., highest rates were observed in the morning hours and the rate gradually decreased through the day to the lowest ones in the evening.

6. The average daily pattern for the 29-day period for the carrot was found to show a conspicuous maximum at 4 to 5 A.M. and a minimum just after noon.

7. Fucus exhibited maxima at 3 to 4 A.M. and 1, 6, and 11 P.M. with principal minima at 7 A.M. and 8 P.M.

8. In all three species the mean daily rate and form of the daily variation for a fifteen-day period straddling full moon differed from that for the fifteenday period straddling new moon, and in the same qualitative manner.

9. An analysis of the data performed in such ^a manner as to determine the character of any existing persistent primary lunar cycle of $O₂$ -consumption revealed that all three plants possessed such cycles, and the cycles were similar in form and phase relationships. All showed a primary minimum one to three hours before lunar zenith, and a lesser minimum ¹ to ³ hours before lunar nadir.

10. A significant correlation was found between the hourly rates of respiration and the concurrent rate of change in barometric pressure for all three plants.

11. The rate of $O₂$ -consumption of the potato was decreased about 10 $\%$ for each 0.01 in. Hg/hr rise in barometric pressure, and increased by about the same amount for each 0.01 in. Hg/hr fall.

12. Fucus respiration was decreased or increased about 5 to 6 $\%$ for each rise or fall, respectively, in barometric pressure of 0.01 in. Hg/hr.

13. The respiration of the carrot was decreased by both rises and falls in barometric pressure, decreasing about 7 to 8 % for each 0.01 in. Hg/hr rise and decreasing about 3% for each 0.01 in. Hg/hr fall.

14. The forms of the daily and lunar rhythms of respiration are discussed in terms of these correlations and the measured daily and lunar-day barometric pressure cycles.

15. There is also some suggestion of a correlation between the mean daily rate of respiration and the gradual rising or falling of barometric pressure over hours or days.

16. There is a brief discussion of what might be the nature of the operating external factors, of the possibility of an endogenous rhythmic component in the plants, and of possible functional significance of these rhythms to plants.

LITERATURE CITED

- 1. BALL, N. G. and DYKE, I. J. An endogenous 24-hour rhythm in the growth rate of the Avena coleoptile. Jour. Exptl. Bot. 5: 421-433. 1954.
- 2. BARNOTHY, J. and FORRO, M. Diurnal and sidereal effects and the meteorologic influences on shower and vertical intensity of cosmic rays. Physical Rev. 55: 868-870. 1939.
- 3. BROWN, F. A., JR. Simple, automatic, continuousrecording respirometer. Rev. Sci. Instr. 25: 415- 417. 1954.
- 4. BROWN, F. A., JR. Persistent activity rhythms in the oyster. Amer. Jour. Physiol. 178: 510-514. 1954.
- 5. BROWN, F. A., JR., BENNETT, M. F., and RALPH, C. L. An apparent influence of alternation in cosmic-ray-induced showers on a living system. Anat. Record 120: 796. 1954.
- 6. BROWN, F. A., JR., BENNETT, M. F., and WEBB, H. M. Persistent daily and tidal rhythms of 02-consumption in fiddler crabs. Jour. Cellular Comp. Physiol. 44: 477-505. 1954.
- 7. BROWN, F. A., JR., FREELAND, R. O., and BENNETT, M. F. Persistent rhythms of O2-consumption in carrots and potatoes. Biol. Bull. 107: 305. 1954.
- 8. BROWN, F. A., JR., SANDEEN, M. I., and WEBB, H. M. Solar and lunar rhythms of $O₂$ -consumption in the seaweed, Fucus. Biol. Bull. 107: 306. 1954.
- 9. BROWN, F. A., JR., WEBB, H. M., BENNETT, M. F., and SANDEEN, M. I. Temperature-independence of the frequency of the endogenous tidal rhythm of Uca. Physiol. Zool. 27: 345-349. 1954.
- 10. BROWN, F. A., JR., and WEBB, H. M. Temperature relations of an endogenous daily rhythmicity of the fiddler crab, Uca. Physiol. Zool. 21: 371-381. 1948.
- 11. BROWN, F. A., JR., WEBB, H. M., and BENNETT,

M. F. Proof for an endogenous component in persistent solar and lunar rhythmicity in organisms. Proc. Nat. Acad. Sci., U.S. 41: 93-100. 1955.

- 12. BRETT, J. W. Persistent diurnal rhythmicity in Drosophila emergence. Doctoral dissertation, Northwestern Univ., Evanston, Illinois. 1953.
- 13. CASPERS, H. Rhythmische Erscheinungen in der Fortpflanzung von Clunio marinus (Dipt. Chiron.) und das Problem der lunaren Periodizitat bei Organismen. Arch. Hydrobiol. Suppl. 18: 415- 594. 1951.
- 13a. CHAPMAN, S. Tides in the atmosphere. Sci. Amer. 190: 36. 1954.
14. FAURÉ-FREMIET, E.
- The tidal rhythm of the diatom, Hantzschia amphioxys. Biol. Bull. 100:
- 173-177. 1951. 15. FORRO, M. Diurnal variation of cosmic ray showers. Nature 139: 633. 1937.
- 16. GOMPEL, M. Recherches sur la consommation d'oxygene de quelques animaux aquatiques littoraux. Compt. rend. acad. sci., France 205: 816-818. 1937.
- 17. HUNTSMAN, A. G. Odontosyllis at Bermuda and lunar periodicity. Jour. Fisheries Research Board, Canada 7: 363-369. 1948.
- 18. KOFOID, C. A. Plankton of the Illinois River. Illinois State Nat. Hist. Lab. Bull. No. 8: 2-361. 1908.
- 19. KORRINGA, P. Relations between the moon and periodicity in the breeding of marine animals. Ecol. Monographs 17: 347-381. 1947.
- 20. PITTENDRIGH, C. S. On temperature independence in the clock system controlling emergence time in Drosophila. Proc. Nat. Acad. Sci., U.S. 40: 1018- 1029. 1954.
- 21. SANDEEN, M. I., STEPHENS, G. C., and BROWN, F. A., JR. Persistent daily and tidal rhythms of oxygen consumption in two species of marine snails. Physiol. Zool. 27: 350-356. 1954.
- 22. SEKIDO, Y. and YOSHIDA, S. On the diurnal variation of cosmic rays. Part I. The disturbance in cosmic-ray diurnal variation. Rept. Ionosphere Research (Japan) 4: 37-42. 1950.
- 23. SEKIDO, Y. and YOSHIDA, S. On the diurnal variation of cosmic rays. Part III. Semi-diurnal variation and magnetic disturbance. Rept. Ionosphere Research (Japan) 5: 43-46. 1951.
- 24. WALKER, B. W. A guide to the grunion. California Fish and Game 38: 409-420. 1952.