LUNAR ORIENTATION OF ORCHESTOIDEA CORNICULATA STOUT (AMPHIPODA)

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The celestial navigation accomplished by Talitridae (Amphipoda) was first reported by Pardi and Papi (1953) and Papi and Pardi (1953). These two papers contain numerous observations of the solar navigation of *Talitrus saltator* and, in the second paper, certain night-time observations suggested the existence of lunar navigation in this species, *i.e.*, appropriately time-shifted angles of orientation with the moon. The existence of solar navigation has been confirmed with several other species of Talitridae: *Talorchestia megalophthalma* and *Talorchestia longicornis* (Menaker, 1958); *Talorchestia deshayesei* (Pardi and Grassi, 1955); *Orchestoidea corniculata* and *Orchestoidea benedicti* (unpublished qualitative observations of the author). The existence of lunar navigation, however, had not been completely documented until the recent paper by Papi and Pardi (1959) which presents results supporting the contention that, under the experimental conditions described, *Talitrus saltator* oriented to moonlight with an angle that varied with lunar position. This resulted in a relatively constant and ecologically "correct" compass orientation. On the basis of this evidence, the authors conclude (p. 596) "dass zwei verschiedene physiologische Rhythmen die Sonnen- und die Mondorientierung von *Talitrus* bedingen." (Two different physiological rhythms are responsible for the solar and lunar orientation of *Talitrus*.)

Because of the significance of such a conclusion to the general theory of endogenous rhythms, the present study was undertaken to determine whether *Orchestoidea corniculata* Stout, a related amphipod which is capable of solar navigation, is similarly able to navigate by moonlight.

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Methods and Materials

The experimental animals were adult and sub-adult specimens of *Orchestoidea corniculata* Stout, collected usually on the morning before the experiment. In a few cases, as indicated in Table I, the animals were collected one or two weeks before the experiment and kept at constant temperature until the day of the experiment. Following collection, all animals were kept in light-tight, one-liter bottles containing moist sand. The animals can survive two weeks in such containers with no evidence of oxygen shortage. No control of the temperature was attempted on the day of the experiment, but the range was not greater than 16° to 28° C. Collections were made on the beach in front of the Scripps Institution of Oceanography, La Jolla, California. The shoreline there is oriented north 15° east; the "correct" azimuth orientation to return the animals to the water is thus 285° from north.

In order to avoid fog, city sky-glow, surf sounds, etc., the experiments were conducted in a level, open field about 40 km. northeast (inland) of San Diego.

TABLE I

Orientation of Orchestoidea corniculata with moon: animals kept in constant darkness

Date	Time	Lunar stage: days	Lunar azimuth	Orientation azimuth	Vector length	N	Angle with moon
1960							
*9 July	2241	16	137°	258°	0.74	26	121°
9 July	2306	16	141°	266°	0.77	32	125°
**9 July	2330	16	148°	277°	0.40	28	129°
***10 July	0055	16	171°	260°	0.62	29	89°
*10 July	0133	16	183°	280°	0.64	30	97°
10 July	0153	16	189°	307°	0.62	25	118°
*10 July	0330	16	216°	001°	0.64	26	145°
10 July	0355	16	222°	349°	0.65	29	127°
16 July	0144	22	093°	212°	0.59	30	119°
30 July	2003	8	228°	352°	0.53	31	124°
30 July	2055	8	239°	348°	0.56	25	109°
30 July	2105	8	241°	359°	0.62	23	118°
30 July	2147	8	248°	010°	0.58	26	122°
30 July	2158	8	250°	041°	0.65	13	151°
6 August	2201	15	144°	314°	0.72	24	170°
7 August	0005	15	181°	299°	0.85	25	118°
7 August	0017	15	185°	293°	0.61	26	108°
7 August	0240	15	224°	329°	0.65	25	105°
7 August	0252	15	226°	338°	0.79	29	112°

* Animals kept for one week at 20° C.
** Animals kept for one week at 10° C.
*** Animals kept for two weeks at 10° C.

The observation chamber was an opaque, white, circular plastic tray 25 cm. in diameter, 4 cm. deep, over which an unmarked sheet of glass was placed. No attempt was made to control the humidity within the chamber; Papi and Pardi (1959, p. 585) state that this is usually unimportant.[1]

The camera was mounted 1.4 meters above the chamber. Photographic lighting involved a single electronic photo-flash bulb ("strobe light") fixed 1.2 meters above the observation chamber, and at a constant position relative to it. The results suggest that the photographic equipment did not affect orientation. As later discussed, the flashes necessary for picture-taking did have an effect.

[1] See, however, Papi and Pardi (1953, p. 501, 502).

A compass and a clock, out of view of the animals, were included in each photograph.

Results are summarized by the method described by Pardi and Papi (1953, p. 463): a vector sum of the positions of the individual animals is calculated (using 16 equal sectors), and this is divided by the number of animals. The orientation of the resultant vector gives the average orientation direction and its length gives an indication of the degree of scatter about the average. Such a vector can range in length from zero, indicating an even distribution of directions around the center, to unity, indicating all animals within the same sector. Random distributions will, of course, result in vectors larger than zero.

In order to determine the vector length which would result from random, independent orientation of the animals, 20 "experiments" were performed by assigning positions to 30 hypothetical "animals" by use of a table of random

TABLE II

Orientation of Orchestoidea corniculata *with moon: animals not kept in constant darkness*

Date	Time	Lunar azimuth	Orientation azimuth	Vector length	N	Angle with moon
1960						
6 August	2015	122°	288°	0.59	25	166°
6 August	2025	124°	291°	0.87	21	167°
6 August	2225	151°	339°	0.77	25	188°
6 August	2235	153°	299°	0.72	25	146°
*6 August	2245	156°	319°	0.87	25	163°
*6 August	2255	159°	325°	0.86	30	166°
7 August	0025	187°	321°	0.68	22	134°
7 August	0045	193°	286°	0.47	31	93°
*7 August	0100	198°	325°	0.77	25	127°
*7 August	0110	201°	321°	0.75	26	120°
7 August	0305	229°	280°	0.44	27	51°
7 August	0315	231°	298°	0.64	28	67°
*7 August	0325	233°	341°	0.64	26	108°
*7 August	0335	235°	320°	0.52	25	85°

* "Redarkened" animals; all others "Natural Light."

numbers. Eighty per cent of the resultant vectors were less than 0.30 in length and none was greater than 0.37. This suggests that a vector of length 0.35 or less based on the positions of 20 to 35 animals, the number usually used in the actual experiments, is of doubtful meaning. As there is clearly a lack of independence in the behavior of the individual animals, which predominantly cluster around the edge of the container, and often crowd and crawl over one another, a vector of greater length, perhaps 0.50 or more, is required to indicate definite orientation. No observations were used in the tests of the lunar navigation hypothesis (Tables I and II) if the calculated vector was less than 0.40 in length. The interaction invalidates conventional statistical techniques for more rigorously evaluating variability on the basis of an individual experimental result.

All angles are measured clockwise; azimuths are measured clockwise from north.

Results

Moonlight as the orienting stimulus

In order to determine whether the observed orientations were due to moonlight, a group of amphipods, which had shown a given orientation relative to the moon, was shaded and an image of the moon was presented at about 180° from the actual position of the moon by means of a mirror. The results, shown in Figure 1, clearly demonstrate that the apparent lunar position is the dominant orienting stimulus for *Orchestoidea corniculata* under these experimental conditions.

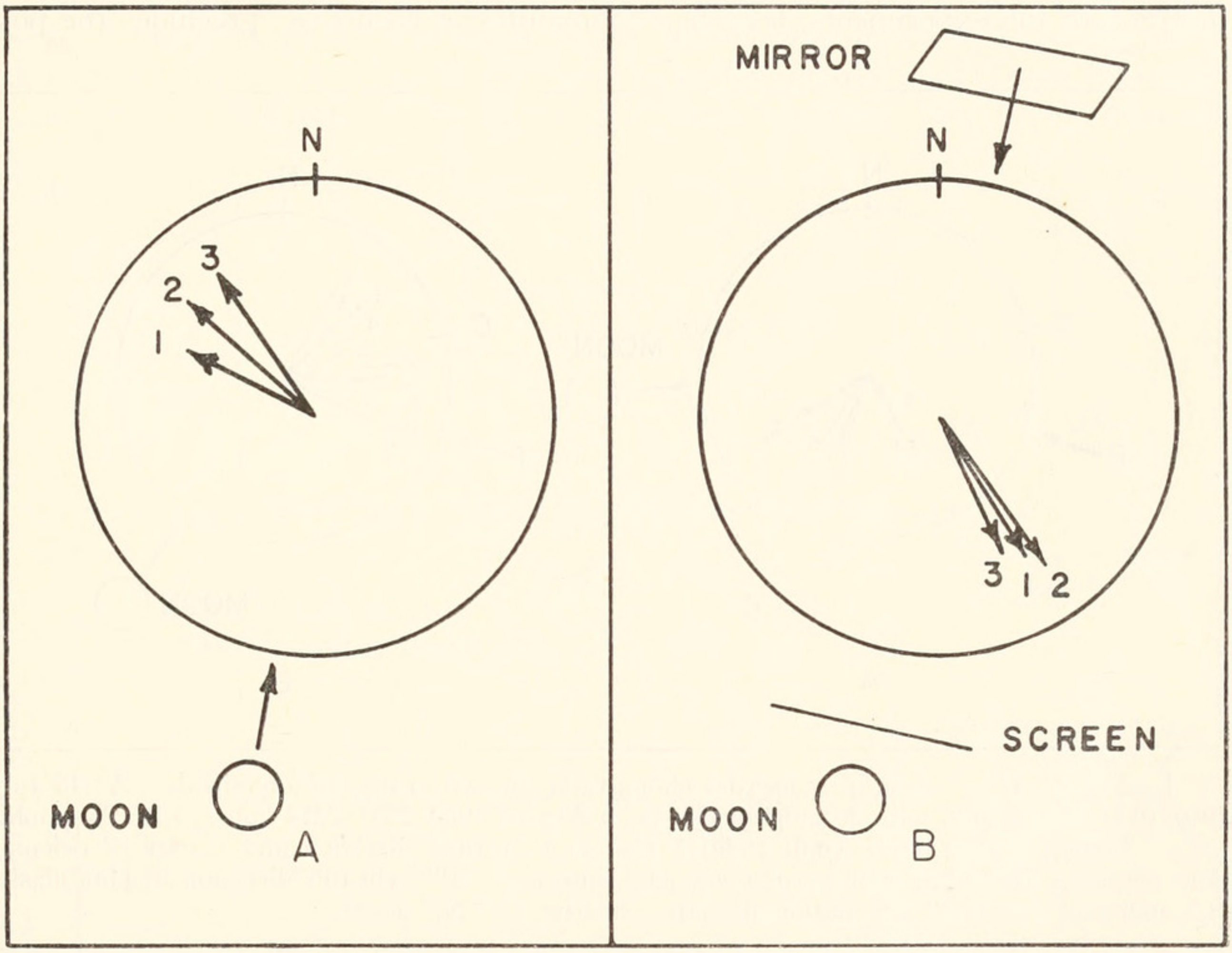

FIGURE 1. Orientation of a group of *Orchestoidea corniculata* to apparent lunar position. A: Initial orientation with the moon; B: orientation with moon reflected at about 180° from true position. Arrows within circle (which has unit radius) indicate average direction and scatter of orientation. Numbers adjacent to arrows indicate sequence in which pictures were taken. Observations made 10 July 1960, 0153–0209 hours, with 25 amphipods.

Serial observations with electronic flash

In initial experiments, a series of three photographs of each group of animals was taken. It was noted that groups of amphipods frequently showed a consistent shift in average direction of orientation, presumably as a reaction to the electronic flash. These results, while too few to be statistically significant, indicated a trend for the animals to orient with successively larger angles with the moon. (See, for example, Fig. 1A.) There was significant evidence of an increase in scatter from Photograph 1 to Photograph 3. For example, in 19 sets of 3 photographs, Photograph 1 showed least scatter in 14 cases and Photograph 3 showed most scatter in 12 cases.

Two series of ten photographs were taken to determine whether the shifts in direction and increase in scatter are cumulative (Fig. 2). In both cases, the ultimate angle of orientation with the moon after ten photographs was much smaller than the initial angle (contrary to the initial trend, over sets of only three photographs), with a net shift of 85° to 90°. In neither case is the final orientation more "correct" than the initial (although an average of the vectors of Figure 2B would be much closer to "correct" than either initial or final results). The short duration of the experiments, less than 15 minutes in each case, precludes the pos-

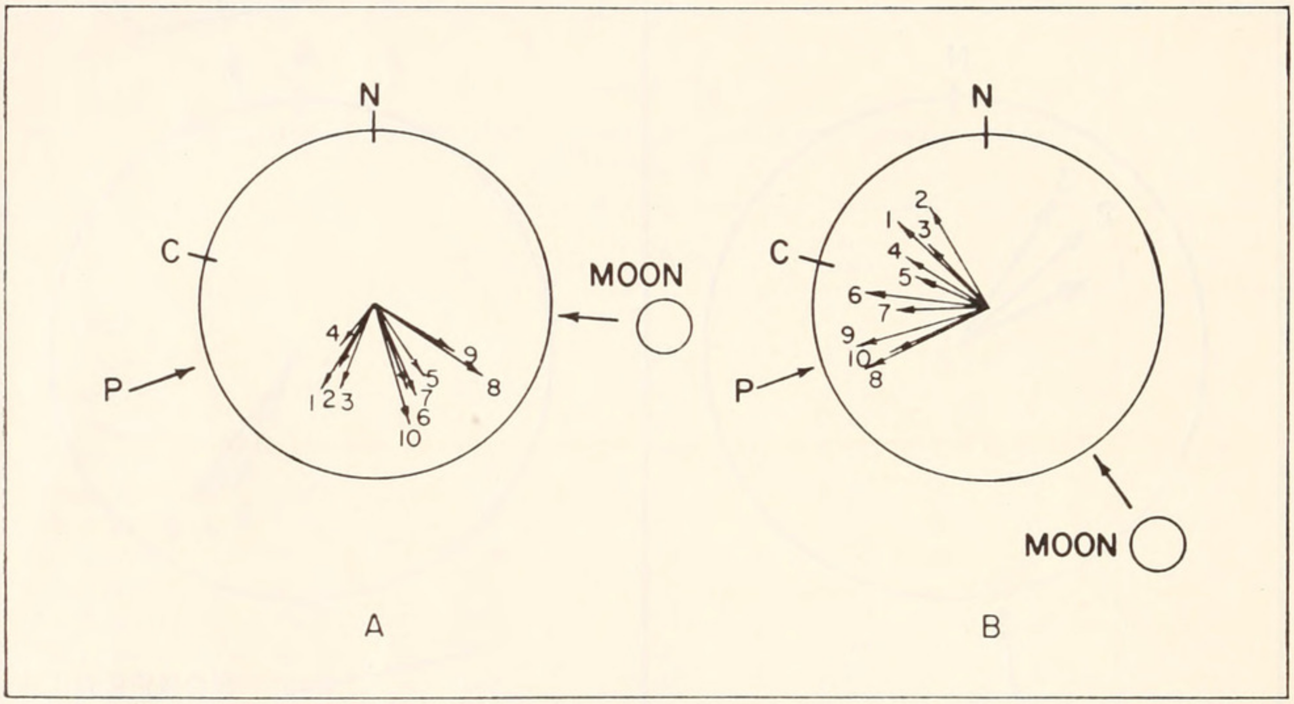

FIGURE 2. Orientation in sequential photographs of two groups of amphipods. A: 16 July 1960, 0144–0158 hours, with 30 amphipods; B: 6 August 1960, 2201–2214 hours, with 24 amphipods. Arrows within circle (unit radius) represent average direction and scatter of orientation; numbers associated with arrows indicate sequence. "P" indicates direction of photoflash; "C" indicates "correct" orientation direction relative to "N," north.

sibility that the shift is due to some normal compensation for changes in lunar position. Furthermore, the shift apparently does not take place without photography: qualitative continuous observation of a group of unphotographed amphipods, kept in the observation chamber for 15 minutes, indicated no noticeable orientation shift. (2 September 1960, 2315–2330 hours; lunar day 12; lunar azimuth 206°–210°; estimated orientation azimuth, initial and final, about 340°; angle with the moon, about 130°.)

The results in Figure 2 seem best interpreted as a resetting effect of light on a rhythmic system, a phenomenon previously demonstrated using similar millisecond electronic flashes[2] (Pittendrigh, in press). The lengths of vectors in

[2] See Pardi and Papi (1953, pp. 466–467) for an alternative explanation of a superficially similar result with solar orientation.

Figure 2 indicate that, after an initial increase in scatter over the first three to five photographs, there is a recovery so that final orientation, after ten photoflashes, is strongly directed, but in a different direction from the initial.

Because of these complications with multiple photographic observations of the moonlight orientation of *Orchestoidea corniculata,* all data subsequently cited consist only of results from first photographs of the initial, unshifted orientation, and no group of amphipods was used twice.

Orientation of amphipods kept in constant darkness

Observations of the orientation of *Orchestoidea corniculata,* kept in complete darkness until just prior to observation, are summarized in Table I and Figure 3. As Figure 3 shows, the data can be explained by the hypothesis that this amphipod, when exposed after ten or more hours of constant darkness, orients to the moon

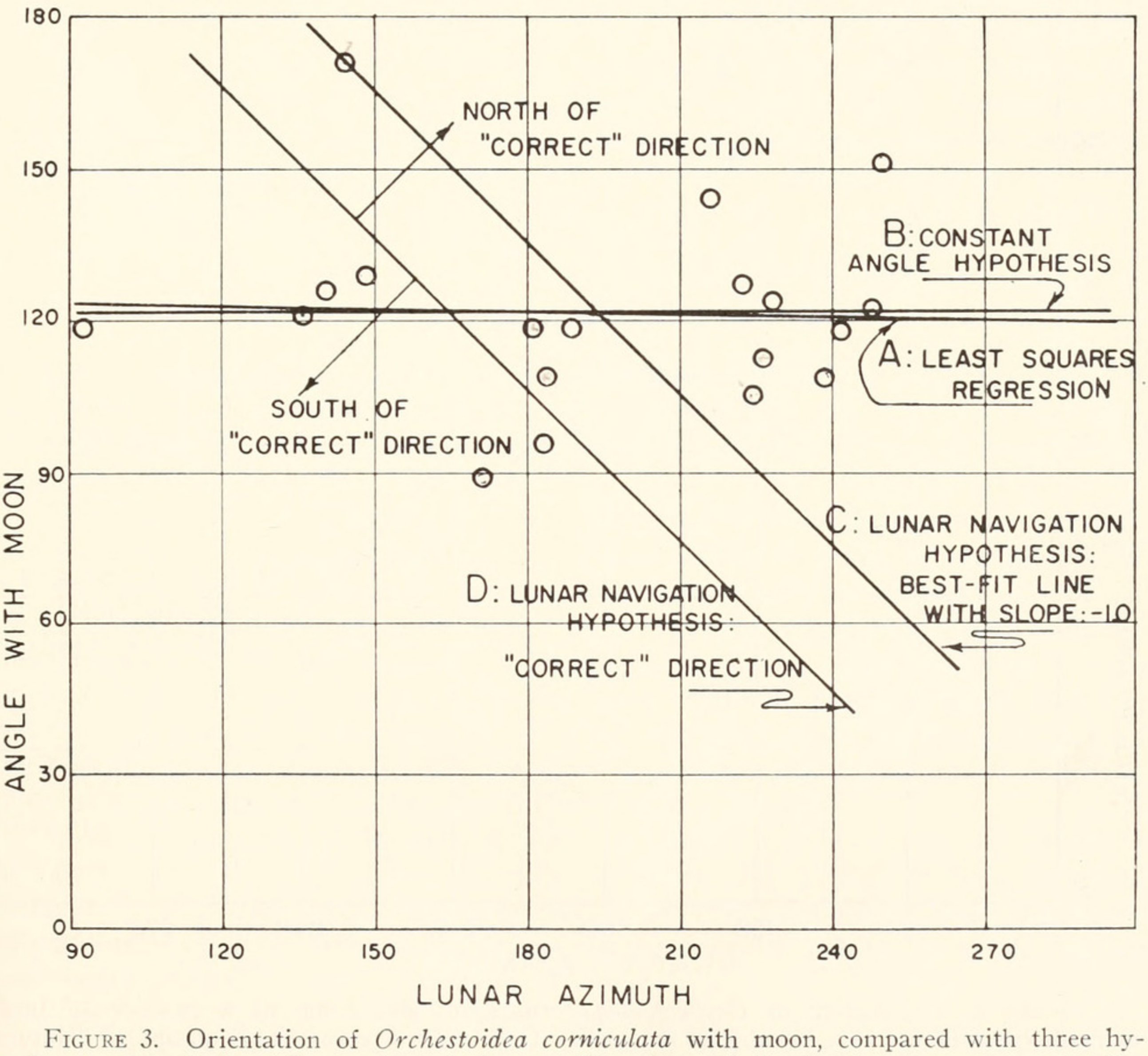

FIGURE 3. Orientation of *Orchestoidea corniculata* with moon, compared with three hypotheses: animals kept in constant darkness. The slope of the least-squares regression line (A) is −0.015. This is not significantly different from zero (line B); ($p > .50$). The probability that the slope of the regression line is as large as −0.50 is less than .001; steeper slopes (−1.0 for line C) are even less likely; and the departure from line D is even greater.

with an average angle of about 120°, regardless of lunar stage or position (Lines A and B). The hypothesis of a *continuously-operating* endogenous lunar physiological rhythm in this species, similar to that claimed for *Talitrus saltator,* appears unnecessary and is contradicted by the data (Lines C and D).

Orientation of amphipods exposed to sunset and moonrise

Additional observations were made with amphipods placed in constant darkness at the time of collection, and then re-exposed, in the sand-containing bottles, to

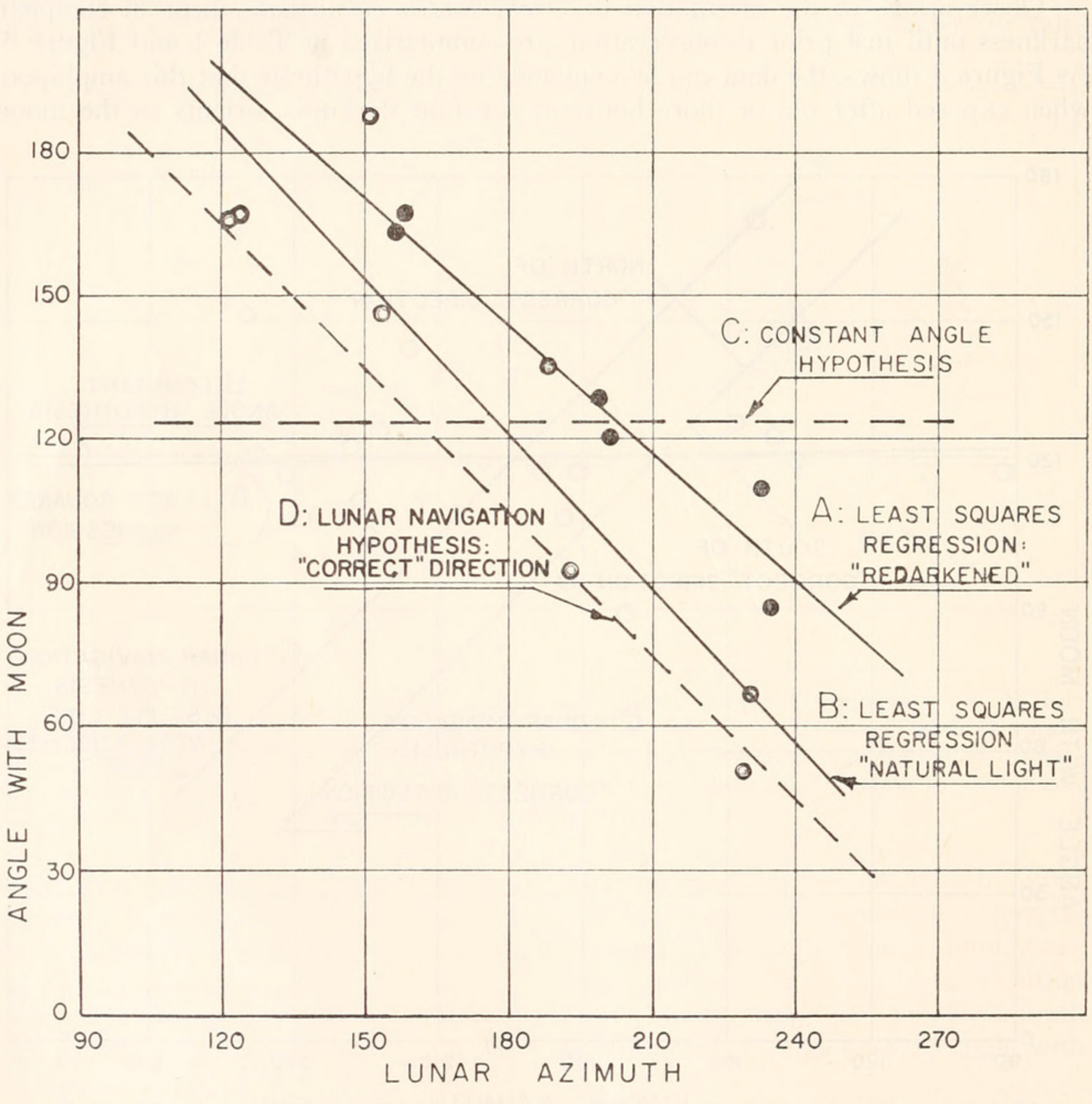

FIGURE 4. Orientation of *Orchestoidea corniculata* with moon as a function of lunar azimuth: animals not kept in constant darkness. Open circles represent orientation of "Natural Light" animals; solid circles represent orientation of "Redarkened" animals. The slope of line A is −0.888; and of line B is −1.078. The probability of the slope being −1.00 is > .20 for line A, and > .50 for line B. Points to the right of line D are north of "correct" orientation.

natural light about one hour prior to sunset. Those which were then continually exposed until observation are designated "Natural Light"; others which were replaced in constant darkness two hours after moonrise are designated "Redarkened." The observations, made August 6 and 7 (full moon), are summarized in Table II and Figure 4. The orientation resulting from this treatment of the animals does not seem adequately explained by the constant-angle hypothesis (Line C); the probability that the slope of the regression is 0 is extremely small in both cases ($p < .001$).

The directions of orientation, particularly those of the "Redarkened" animals, are generally somewhat north of the "correct" 285° azimuth, but the slopes of both regression lines are not significantly different from -1.0, the expected value if the animals compensated appropriately for shifts in lunar position.

A possible explanation is that sunset and/or moonrise (or perhaps removal from constant dark) can initiate a single cycle of appropriately time-compensated lunar orientation in these organisms. Such a system, with no information carry-over from the preceding nights, would afford the animals a crude but workable orientation mechanism (except at first quarter?). It would not require the organizational complexity and long-term precision necessary for the *continuously-operating* endogenous lunar periodicity claimed for *Talitrus saltator*. Further investigation may indicate that "night-sun" orientation (Pardi, 1953/54) or some other by-product of solar navigation is involved. No investigation of the effect of this treatment on the solar navigation of the amphipods was made.

Summary

1. The orientation of *Orchestoidea corniculata* in direct moonlight, compared with the orientation when moonlight is reflected from a mirror, demonstrates that apparent lunar position is the dominant night-time orienting stimulus for this organism.

2. An analysis of the effects of repeated photographic recording of the orientation indicates that this species changes its angle of lunar orientation and the scatter about the average as a result of repeated electronic photo-bulb flashes.

3. Using only single observations of each group of animals, in order to avoid these artifacts, it was determined that this species, kept in total darkness for ten or more hours before observation, orients at a relatively constant angle with the moon, regardless of lunar stage or position. This result is not compatible with the hypothesis that *O. corniculata* possesses a *continuously-operating* lunar physiological rhythm similar to that claimed for *Talitrus saltator*.

4. When the amphipods were exposed to sunset and moonrise on the night of observation, there was an indication of subsequently time-shifted angles of orientation with the moon. The tentative hypothesis of a single-cycle night-time orientation rhythm, re-initiated by the appropriate stimuli each night, would explain such observations.

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