CHAPTER 1

SOLAR AND LUNISOLAR RHYTHMS IN MARINE ENVIRONMENTS

SOME of the earliest correlations between rhythmic animal behaviors and rhythmic environmental factors arose in studies of marine invertebrates, especially those living in the littoral zone along the oceans' coasts, where lunar tidal rhythms interact with daily rhythms of light and darkness to create special temporal environments. To survive these changing conditions, where illumination or exposure at low tides subjects organisms to predation and desiccation, multiple adaptive rhythms were needed, owing to the temporal complexity of the overlap of daily, tidal, and lunar periodicities. These conditions created opportunities for naturalists to investigate the timing of both adaptive color changes and activity patterns—feeding, burrowing, spawning, egg laying—in connection with the cosmic factors that were thought to be the exogenous causes of biological rhythms. Such complex adaptations also bore on the question of inheritance of instinctual behaviors.

LUNISOLAR (TIDAL) RHYTHMS

Tidal rhythms are complicated, owing to the interaction of the oceans and other terrestrial bodies of water with the gravitational forces of the sun, moon, and earth and to the effects of local geographical features. Typically, a given location will exhibit two high tides and two low tides per 24.8-hour "day," but many places experience only one high tide and low tide. The extremes of the tidal movements depend on the location—some coasts have little daily variation in water height and others have significant tidal movement—and on the time during the lunar month. When a tidal movement coincides with a full moon or a new moon, the sun, earth, and moon are more or less lined up, and the lunar and solar gravitational forces reinforce each other and accentuate the difference between high and low tide. This is called a "spring tide," owing to the springing forth of the tide. When the tide occurs near the half-full moon, when the moon is at right angles to the earth–sun line, the tidal differences are less, and these are called "neap tides." In terms of rhythm, the fundamental period of the tidal rhythm is therefore 12.4 hours (half of the 24.8-hour "daily" component), modulated by a synodic lunar period, which varies about an average of approximately 29.5 days. Other factors complicating tidal height are the angle of the moon's orbit with respect to the angle of the earth's revolution around the sun, the tilt of the earth's axis, and the rhythm of the movement of the moon closer to the earth and farther away, but these have less pronounced effects on the tidal height than do the daily and monthly variations. In all, the complexities of these factors defied attempts to predict tides accurately until modern times, and local charts are needed to accommodate local geographical effects.

Shortly after the turn of the century, British marine biologists Frederick W. Gamble and Frederick W. Keeble began to collaborate on a study of the rhythmic behavior of two littoral zone inhabitants—*Hippolyte*, a kind of prawn, and an oblate marine flatworm called *Convoluta roscoffensis*.[1] *Roscoffensis* takes its name from Roscoff, a town on the north coast of Brittany, somewhat west of the marine laboratory at Tregastel, where Keeble worked seasonally during the decade before he published his monograph *Plant-Animals: A Study in Symbiosis* (1910). This flatworm is particularly interesting because it bears within it invasive algal cells and incorporates the algae's ability to use light for photosynthesis, bringing it to the surface during the day. The resultant daily migration of *Roscoffensis* to gain exposure to sunlight combined with the worm's behavior as resident of a tidal zone reflects both solar and lunar rhythms characteristic of many residents of the littoral environments. Discovering that *Roscoffensis* only lays eggs during neap tides in its normal environment, Keeble removed them to the laboratory and discovered that this rhythmic reproductive behavior persisted in the absence of the physical changes in water depths that were associated with the tides.[2]

During this time, French marine biologist Georges Bohn was also studying *Roscoffensis*, first in Normandy and then at Saint-Jacut-de-la-Mer, east of Tregastel on the coast of Brittany, and he also recognized that *Roscoffensis* migrates vertically in the sandy beaches, coming up for sunlight and going down for shelter.[3] He published his findings "On the oscillatory movements of *Convoluta roscoffensis*" in a French journal in 1903, where he described the plant-animal's behavior in the wild. He also noted that it maintained its "spontaneous oscillations" in phase with the tides for up to fourteen consecutive tides after it was removed from the beach and placed in an aquarium. This was true even when lighting conditions were reversed, demonstrating that this rhythm did not depend on the solar day.[4] Bohn concluded that the oscillatory movements were really twofold, one corresponding to the 12.4-hour rhythm of the tide, which served to protect the animal from the pounding surf, and a second corresponding to the daily rhythm of illumination, which presented both opportunity for photosynthesis and risk of desiccation. He commented that the annelid *Hediste diversicolor* and other coastal animals exhibit similar rhythms, but he did not elaborate.[5]

Keeble acknowledged Bohn's work when he published his monograph seven years later, but he rejected Bohn's Neo-Lamarckian hypothesis that the spontaneous rhythm of oscillation arose as a kind of memory imposed through the repeated experience of the shock waves of the surf. He thought that Bohn's hypothesis could explain why *Roscoffensis* migrated downward, but not the timing of its return to the surface, which logically could not be an acquired characteristic response to the environmental stimulus.[6] Keeble apparently did not wholly reject the idea that spontaneous rhythm might be the result of a remodeling of the animal's internal organization in response to the environment, inasmuch as he cited Richard Semon's *mneme* theory of memory, which Semon had applied to the analogous daily rhythms of plant leaf movements.[7] But, Keeble was inclined to see this spontaneous memory as a property of the organism, in this case specifically arising as an adaptation of the protoplasm, rather than as a learned response.[8]

Like Bohn, Keeble had studied the behavior of *Roscoffensis* in the laboratory and recorded that "as on the shore in the *roscoffensis* zone, so in the laboratory the upward and downward movements of *Convoluta* march with the movements of the tide. . . . In the absence of all apparent external stimulus, *C. roscoffensis*, obedient to its custom, yet keeps time with the tide." It would do this synchronously for about eight consecutive tides, but then the rhythm would begin to shift from a twice-daily migration to a once-daily movement, which he interpreted as the animal's attempt to conform to a solar rhythm.[9] He theorized that the daily light cycle affected the natural irritability of the living matter, causing it to respond differently to gravitational stimulus: "In its simplest form, the hypothesis involves the assumption that prolonged light-exposure and prolonged dark-exposure modify the tone or state of nervous irritability of the animals, and that these changed conditions manifest themselves by a changed mode of response to gravitational stimulus."[10] Thus, despite his and Bohn's attempts to connect the worms' activity behavior to a specific littoral environment, a fundamentally ecological project, questions of inherited behaviors or acquired physiological transformations were salient.

One year after Keeble published his monograph, the American zoologist Samuel Jackson Holmes incorporated his and Bohn's findings in *The Evolution of Animal Intelligence* (1911), viewing them in light of Ivan Pavlov's behaviorist physiology and placing the phenomenon of spontaneous rhythm more clearly in the context of Neo-Lamarckian inheritance of acquired characteristics: "We have in these periodic variations of behavioral habits of action in relation to different influences of the environment which have been acquired by the experience of the organism. . . . It does not seem improbable that all of them may be dependent upon some general modifications of the organism as a whole rather than upon merely the mechanism of response to stimuli."[11] He was articulating a point of contention that was current at the time. Jacques Loeb and other strict reductionist mechanists viewed individuals as physico-chemical structures that respond to environmental stimuli in determined ways. Others, like his contemporary Heinrich Menke, believed that experiences transform organisms in ways that mean new behaviors—such as specific periodic behaviors that reflect rhythmic environmental factors—become heritable characteristics.

Research on intertidal rhythms was facilitated by the establishment of marine biology stations and laboratories, which gained momentum in the last quarter of the nineteenth century. Keeble, whose academic home was at University College Reading, engaged in physiological research at the marine biology stations at Roscoff in Brittany and at Dohrn's zoological station in Naples and collaborated with Gamble, who focused mainly on descriptive natural history and morphology at Manchester University and at the newly established marine biological station at Port Erin, on the Isle of Man.[12] Besides studying the coastal marine life in the intertidal zone, scientists at these early marine biology laboratories were harvesting, identifying, and cataloguing offshore species by dredging and dragging nets, returning some specimens to onshore tanks for observation and experimentation under controllable laboratory conditions.

The account of Benjamin Moore, who worked at the Port Erin station during the spring, summer, and early autumn of 1908, writing up his results for publication in early November, reveals how an awareness of endogenous rhythmic behaviors could emerge unforeseen from systematic zoological investigation. The title of his paper, "Observations on Certain Marine Organisms of (a) Variations in Reaction to Light, and (b) a Diurnal Periodicity of Phosphorescence," aptly conveys both the serendipity of his stumbling upon diurnal periodicity during the course of his study of the physiological responses of photoluminescent animals to externally imposed light—their tropisms or movements toward or away from sources of light, for example—and also his recognition that this rhythmicity warranted careful study.[13]

Phototropism and phototaxis had been subject to extensive research by this time, much of it in service to materialist-mechanist explanations for basic behaviors by Jacques Loeb and like-minded behaviorists, bringing into consideration questions about how organisms react physiologically to light and how these reactions fit into phylogeny. Moore's research was organized around the hypothesis that, if the development of vision in higher animals was a product of evolution by variation and natural selection, then one might expect to find vestigial sensitivities to light in primitive cells, functions that were superceded by specialized organs.

Referring to Thomas Finsen's research on therapeutic uses of light in Denmark and his recent findings that cells react to different wavelengths of light, which supported this hypothesis, Moore reasoned that organisms that themselves produce light internally might also exhibit interesting reactions to external light, and that knowledge of this process could benefit medicine: "Recent discoveries have proven the value of light treatment as a practical adjunct of medicine, and the study of light effects upon the simpler organisms must sooner or later yield a key, both for the rational understanding of such effects, and their extension to further utility."[14] Against this background, and with this hypothesis in mind, Moore examined samples he collected by tow net, bringing them back to the laboratory to see if they would produce photoluminescence under controlled conditions. But, before he wrote up his findings for publication, he discovered a rhythmic pattern to this behavior and began a literature search for precedent observations. A sense of his process of discovery remains in diary records he included in his paper.[15]

Discovering a persistent, endogenous diurnal rhythm of luminescence of marine copepods kept in continual darkness, Moore began a search for precedents, finding a description of its occurrence in *Pyrophora noctiluca* by M. Aubert and Horace Raphäel Dubois, but he was particularly drawn to Jean Massart's 1893 study of the dinoflagellate *Noctiluca scintillans*, which Moore translated on account of the similarity to his own observations: "Fact still more curious, whether the organisms are submitted to the alternations of day and night, or whether they are maintained in constant illumination or constant obscurity, they still remain much more excitable during the night than during the day. . . . everything looks as if the *Noctilucæ* preserved the recollection of the regular succession of days and nights."[16] Moore noted that Massart had compared these diurnal alterations in photoluminescence to the diurnal leaf movements of *Oxalis* and some of the *Papilionaceæ* (legumes), but that whereas these persisted for only a few days in the plants, in *Noctiluca* they lasted until the death of the plankton.[17] Moore understood that his findings, which sustained those of Massart fifteen years earlier, were relevant to contemporary discussions about acquired characteristics and the nature of memory, citing Francis Darwin's 1908 presidential address to the British Association in Dublin, which prompted him to comment that "whether this diurnal periodicity has the same physical basis in a rudimentary fashion as memory in higher animals, is still an open question, for it is open to believe that the alternating play of light and darkness upon those cells which produce the phosphorescence may have induced in them a periodicity of activity and rest which still persists after the alternating stimulus is withdrawn."[18]

Moore's conclusions witness his shift from Loeb's approach to the physiology of phototaxis as a mechanistic organismic reaction to external stimulus toward grasping the importance of endogenous causes of rhythm. He had shown that the movements of the phosphorescent copepods he experimented with were unaffected by light coming from without the organism, which ruled out simple phototaxis. Additionally, he recognized that, although the rhythmically alternating "periods of activity and rest in regard to phosphorescence follow respectively the hours of daylight and darkness," this "alternating diurnal periodicity" is not immediately dependent on the external factors but "can persist for a long period (twelve days) in absence of the accustomed recurring stimulus of the light and darkness of day and night."[19] Moreover, by physically agitating the samples to provoke luminescence he had produced another aspect of this phenomenon, which he left unexplained: He observed that just prior to the onset of evening luminescence and just after its cessation in the morning, there were periods of about a half hour during which stirring the quiescent animals could provoke luminescence, but that during the intervening "daytime" period they had "become completely refractory," unresponsive to the stimulus.[20] This phenomenon, the rhythmic variation in organisms' susceptibility to imposed stimulus, became especially important when rhythmic biological behaviors were modeled as oscillators. This, along with the complexity of the timings of bioluminescence in marine organisms in general, would be subjected to careful chronobiological study in the second half of the twentieth century, when scientists turned to the marine flagellant *Gonyaulax* as a model organism.

RHYTHMS OF SWARMING AND SPAWNING

About the same time that Benjamin Moore was netting phosphorescent copepods and speculating on how they "remembered" the environmental rhythms of light and dark when placed in continual darkness, John W. Scott speculated that the periodicity of egg laying that is characteristic of the segmented marine worm *Amphitrite ornata*—always within a day or two of the spring tides of the summer months—was somehow "induced by the conditions that depend upon the tide."[21] Scott dismissed the possibility that the moon acted directly on the organism and also doubted that the tide itself was immediately causal and suggested that higher beach temperatures and the availability of food during spring tides played a role in the timing. Keeble and Gamble had also noted a fortnightly rhythm in the egg-laying habit of *Convoluta roscoffensis* in addition to its daily tidal migrations in the sand, and this coincided with the neap tides. This behavior, too, persisted in the laboratory, so it clearly was not dependent on actual water pressure changes. *Convoluta paradoxa*, a related species that was not bound to one small beach area, but mobile, also laid and hatched eggs on a lunar-tidal cycle.[22] Such lunar rhythms had been noted before but were not subjected to sustained study until the twentieth century, when Europeans learned about the Palolo worm, an annelid, from the inhabitants of the Samoan and Fiji Islands—who took advantage of their rather precise lunar rhythm to harvest them for food in the late autumn.[23]

Among the earliest scientists to study the Pacific Palolo (*Eunice viridis*) were two Germans, Benedict Friedländer and Augustin Krämer, who conducted research in Samoa in the late 1890s.[24] Friedländer thought that the moon acted directly on the worm in some way, perhaps via changes in atmospheric electrical charge, as Arrhenius had postulated for lunar rhythms in humans. Looking back on the discussion almost half a century later, Pieter Korringa judged that Krämer was closer to the truth—namely, that one should look for a biological (ecological) explanation for the timing of the Palolo's swarming based on how the annelid functioned in its environment, in this case being adapted to activity during the night, when it would be protected from predation in shallow reef waters. Korringa thought that the conditions of low tide coincident with dark night coordinated the spawning during the last quarter of the lunar cycle.[25]

Almost simultaneously with Friedländer and Krämer, Alfred Mayer made a close study of the Atlantic Palolo (*Eunice fucata*), which likewise swarmed in a three-day period during the last quarter, but in the Dry Tortugas off Florida this occurred in late June and July, when the tidal and temperature conditions were similar to those in Samoa and Fiji in October and November. Mayer explained the peculiar breeding behavior as an adaptation that maximized fertilization by aggregating the worms' reproductive parts in the same place at the same time and thus shortening the mean distance between ova and sperm, an adaptation that he interpreted as favored by natural selection. He noted that Friedländer rejected this interpretation, cautiously conceding that "the worm no doubt responds to some physical stimulus which is dependent upon the condition or position of the moon, but the exact nature of this stimulus remains to be discovered."[26] Other related *Polychæte* worms had similar repro-

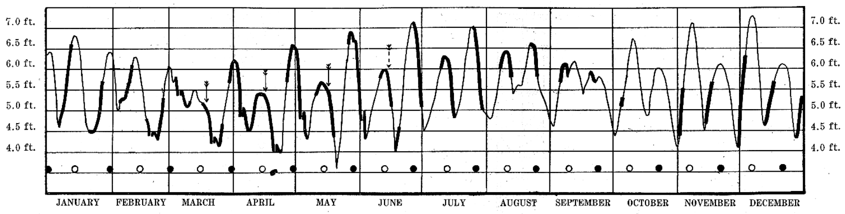

Fig. 6. Highest levels reached by tides each day at San Diego. Those occurring at night shown by heavy lines, those during the day by light lines. The phases of the moon indicated. The observed spawning times of *Leuresthes tenuis* indicated by arrows. Year 1919.

FIG. 1.1. Will and Julia Thompson found that the spring spawning of the California grunion is timed to a night following close upon the high tide occurring with the full moon, when the female and male fish deposit and fertilize the eggs in the sand near the shore. The eggs hatch and the hatchlings remain buried in the sand, out of sight of predatory birds, until the next high tide washes them free. Since the next high tide, coinciding with the new moon, is higher than the one occurring at full moon, it washes out the hatchlings more efficiently; if the eggs were deposited at the new moon, then the next tide would be lower and not reach them to wash them out. In their figure 6, new moons for the year 1919 are indicated with a solid black circle and full moons with an open circle. The thicker lines in the graph of the tide fluctuation represent nighttime, and the grunion spawn is indicated by arrows, March through June. *Source:* Will Francis Thompson and Julia Bell Thompson, *The Spawning of the Grunion (*Leuresthes tenuis*)* (Sacramento: California State Printing Office, 1919), 14, fig. 6.

ductive rhythms, which attracted the attention of scientists to this phenomenon in the first decades of the century.[27]

Equally remarkable was a similar spawning behavior in a kind of smelt called the grunion. Will Francis Thompson and Julia Bell Thompson (1919) reported on the reproduction of this fish, which swarmed inshore on California beaches at the spring tides on the second, third, and fourth nights after the full moon during the months of March through June (indicated by the arrows in figure 1.1). The males fertilized the eggs, which the females deposited on the beach at high tide, where they matured for two weeks. Then, when the next spring tide exposed them, they hatched, and the fry swam away. If not, they would abide another fortnight until the following spring tide.[28] Arthur S. Pearse, in his 1926 *Animal Ecology,* interpreted this behavior as a Darwinian adaptation to a specific ecological niche:

> The grunion offers a remarkable instance of adaptation to lunar rhythms as represented by tidal fluctuations. If spawning occurred just before the highest tides, when the high beach was being eroded, instead of just after, when the beach was being built up, the eggs would be washed out of the sand before they had developed for a fortnight. If spawning occurred at the very highest tides (dark of the moon) the eggs might not be exposed for a month, or even two months. If grunions laid their eggs during the day, they would be ex-

> posed to the attacks of gulls and other predaceous animals.[29]

Thus, spawning at these particular conjunctions of the sun and moon provided an important measure of protection.

RHYTHMS OF COLOR CHANGE IN CRUSTACEANS

Much more ubiquitous than daily phototropic and phototactic behaviors among invertebrates are the rhythmic adaptive color changes in the shells and chiton of some arthropods, and even the skin of some lower-order vertebrates—reptiles, amphibians, and fishes. These alterations of color density are produced by cells called melanophores, if they mainly serve to lighten and darken the animal, or more generally chromatophores. As we have seen, the adaptive color changes of crustaceans had come to the attention of Frederick Keeble and Frederick Gamble and other marine zoologists, some of whom also recognized that these changes were sometimes rhythmic, but the morphology and histology of chromatophores and the manner of their action has not yet been worked out.

The action of these cells conveys fitness on individual organisms by blending them with the background and making them less visible to predators. Inasmuch as the colors and brightness of the backgrounds and the general visibility of the individual are affected by ambient light, which fluctuates on a daily basis, the rhythm of these changes represents an adaptive anticipation of changing illumination. These adaptive color changes were of great interest to ecologists and animal behaviorists and also became the focus of much research in physiology, as the nature of chromatophores and the systemic mechanisms that control them came under scrutiny in the early twentieth century. These apparently rhythmic color changes were to play an important role in making a connection between biological rhythms and endocrine systems both in these simple creatures and in mammals.

Modern scientific natural history of color change began perhaps with Antonio Vallisnieri's record of his observations of amphibians in 1715, but in the early nineteenth century investigators began to examine organisms and their parts in terms of physiology, to determine the relationships between structures and functions.[30] In 1819 Giosuè Sangiovanni observed what he believed to be the organs responsible for color change, which he termed *cromoforo*, chromatophores. He thought that these operated like muscles by expanding (diastole) and contracting (systole), and that these movements must be controlled by nerves. Identification of chromatophores in chameleons by Henri Milne-Edwards (1834), frogs by Ferdinand Moritz Ascherson (1840), crustaceans by Henrik Nikolai Krøyer (1842), and fishes by Karl

Theodor Ernst Siebold and Reinhold Wilhelm Buchholz (1863) established this phenomenon in the main groups of invertebrates and lower-order vertebrates, which would be subject to much closer study in the twentieth century.[31] The foundation of marine biology laboratories in the nineteenth century facilitated study of fishes and crustaceans, and the relationships between the latter and their local habitats came under particular scrutiny. Morphologists and histologists were curious about the structure and action of the chromatophores themselves; how they were controlled and coordinated to serve the animal as an effective adaptation presented a fertile field for neurologists and endocrinologists in the 1920s and 1930s and bears directly on the history of chronobiology.

Observation of the periodicity of color changes first arose in the course of investigation of the adaptation of organisms to their environments and did not come under specific systematic study as biological rhythms until the work of Frederick Gamble and Frederick Keeble. Their investigation of color changes in the small chameleon prawn *Hippolyte varians* at the turn of the century is an early example of what became an extensive investigation of pigment changes in crustaceans and amphibians, which laid the foundations for study of biological rhythms in endocrine production more generally.

The Danish zoologist Henrik Krøyer had remarked on the color changes of *Hippolyte* already in 1842, but it was the French zoologist Charles Henri Georges Pouchet who pioneered close experimental observation of this feature of crustaceans during the early 1870s. His 1876 monograph *Des changements de coloration sous l'influence des nerfs* (Changes in coloration under the influence of the nerves) identified the role of pigmentation spots (chromatophores) in the animals' color changes, connected these with background colors, and determined that the crustacean's eyes were necessary to mediate this process.[32] Keeble and Gamble followed up on Pouchet's study of the littoral crustaceans *Palæon*, *Crangon*, and *Hippolyte* in the late 1890s, focusing on *Hippolyte*.[33]

Keeble and Gamble submitted their early findings to the Royal Society of London in October 1899. They related the basic phenomena of color change from lighter daytime hues to "a wonderfully beautiful transparent blue or greenish-blue colour" soon after nightfall, which under natural conditions persists until dawn. They noted that other crustaceans likewise took on a coloration that was specific to the nighttime and vanished by day.[34] Their findings differed from those of Pouchet chiefly in the connections they made between these color changes and both the changes in lighting conditions and a temporality that evidently was not wholly dependent on the daily alternations of light and dark—namely, a native rhythm of some sort. They agreed with Pouchet that color change occurred in the context of neurological control of physiological functions and that this control facilitated a kind of camouflage that helped the animal blend in with its environment, but their work also went beyond Pouchet's in recognizing that a prawn in nighttime color will in time assume its daytime color even if it is kept in the dark, and that if maintained under artificial illumination, it will again transition to nighttime coloration.

In part Keeble and Gamble's interest in the periodicity of color changes they had discovered in *Hippolyte*, which they believed to be a general phenomenon of animals with variable chromatophores, was based on their suspicion that it constituted a source of experimental error that needed to be understood and taken into account.[35] In other words, their initial motivation for the study of rhythmicity as a temporal phenomenon, beyond concern for its physiological basis and behavioristic interpretations, was methodological. But they plainly understood the periodicity of color change in their subjects to be an autonomous and innate rhythm, not necessarily immediately dependent on lighting conditions, although this affected it. They demonstrated this experimentally, and although they did not grasp the concept of free-running periodicity, their experiments produced results that would later lead to this important concept—namely, they observed that although the cyclical changes of color phase persist in continuing darkness, the darkness "has some effect in retarding the normal times at which these phases recur, and in weakening them." They concluded that in *Hippolyte varians* "the complete colour-cycle, from diurnal phase to nocturnal and back again, is completed in about twenty-four hours."[36]

It was almost sixty years later that Franz Halberg would reify the importance of this phenomenon of an "about twenty-four hours" rhythm as decisive evidence of the endogenous nature of this rhythm by renaming it circadian rhythm. But for Keeble and Gamble, who were working within the specific context of animal physiology, rhythm was an experimental problem to be overcome, not something to be subjected to study on its own merits. Under the subheading "Periodicity: A Possible Source of Error in Interpreting Records" they wrote: "We do not propose, however, to enter into a thorough discussion of the phenomenon of periodicity; but to consider more particularly the disturbing effects of periodicity on the results of any given stimulus," reflecting the predominance of stimulus-response in studies of behavior at the beginning of the twentieth century.[37]

Taking a cue from the findings of Keeble and Gamble regarding color changes, Heinrich Menke undertook a chronobiological investigation of chromatophores that is strikingly sophisticated for the first decades of the century, in part because he was able to conceptualize his research problem within the context of the intellectual exchanges between his contemporaries Semon and

Pfeffer and apply their experimental approaches. Writing in 1911, Menke did not have the benefit of Pfeffer's later, better developed arguments for autonomous biological rhythms or the fertile dialectic between these arguments and Rose Stoppel's claims for non-photic exogenous sources of rhythm, but he did cite Pfeffer's papers of 1907 and 1908 as part of what he referred to as a literature on periodic motions in plants that was "swollen to a considerable extent" (*zu einem beträchtlichen Umfange angeschwollen*). He pointed to the widespread rhythmicity in the animal kingdom, too, but noted that it was little studied, beyond limited attention to color changes and vertical movements of planktonic organisms, which he regarded as two manifestations of an underlying rhythmic nature.[38]

Menke framed the causes of these rhythmic behaviors in terms similar to Pfeffer and Semon, but he used the term "stimulus movement" (*Reizbewegung*) in place of their *aitionome* (exogenously caused): "A movement taking place on an external stimulus impulse, such as light, temperature change, change in the composition of the water, we will for brevity call a stimulus movement. Let us call one taking place under total constancy of external conditions, thus a movement according to internal stimulus, an autonomous movement. The question therefore arises whether the periodic motion of the chromatophores represents a stimulus movement or an autonomous movement, or whether it is in the final analysis a combination of both types of movement."[39] Menke went on to apply this approach to the study of rhythmic migrations of marine plankton.

VERTICAL MIGRATION RHYTHMS OF PLANKTON

Contemporary with the Thompsons' work on the lunisolar rhythm of the grunion, Calvin Esterly summarized research on daily phototropisms that he had conducted on seven species of marine plankton at the Scripps Institute for Oceanography at La Jolla, California, and the Occidental College in Los Angeles beginning in 1907. It was common knowledge that plankton tend toward deep water during the day and approach the surface during the night, and it was the nature and causes for this movement that he aimed to elucidate. His point of departure was a difference of opinion between Jacques Loeb and Heinrich Menke on the relative importance of external stimuli and internal metabolic factors. Loeb supposed that four kinds of factors might affect plankton tropisms—temperatures, carbon dioxide concentrations in the ambient water, illumination (heliotropism), and "a fourth factor, possibly, is found in periodic variations in the internal chemical processes."[40] According to Esterly, Loeb ascribed vertical migration chiefly to heliotropism, thus to a direct response to diurnal changes in light as the external stimulus. However, the fourth factor—fluctuations in internal chemical processes, which Loeb had likened to the sleep movements of plant leaves—had received support from Menke, whose study of chromatophores in the small crustacean *Idothea* showed that the daily color change produced by the expansion and contraction of the chromatophores persisted in constant conditions and could even be inverted by inverting the light/dark (L:D) phases. The inverted rhythm itself would persist in continual darkness for a week.[41]

Menke thought that the results of his study of chromatophore rhythms probably applied also to plankton migrations, since the underlying cause was an autonomous periodicity in metabolism, which he believed to be a characteristic of all living matter. Turning to consider the vertical migrations of plankton, Menke posed the same question: Is this rhythm driven by the rhythm of changes in illumination or by some internal cause? One might well suppose the former, since plankton had in instances been shown to be phototactic.[42] To answer this question Menke attempted to apply the same methods he had used for *Idothea* chromatophores to study a species of the marine crustacean *mysid* (*Hemimysis lamornæ*) in the fjord waters at the Kristineberg marine biology station on the west coast of Sweden. Kristineberg's northerly latitude provides natural, relatively constant light conditions near the solstices, conditions in which such a rhythm would appear to have no function.[43] Menke concluded that these plankton migrations must be autonomous, like the rhythm of *Idothea* chromatophore changes he had studied, but in some way connected with the twenty-four-hour rhythm of day and night. He speculated that the underlying causes of these rhythmic behaviors were rhythmic metabolic changes, perhaps connected to osmotic pressure changes in the plankton, but that whatever heliotropic or geotropic irritating factors are present act as triggers to coordinate the rhythm with the daily cycle, so that specifically dark-period chemical metabolic processes can operate during periods when the plankton are resting.[44]

Calvin Esterly attempted to sort out the differences between the explanations for daily plankton migrations given by Loeb and Menke—phototactic and geotactic responses to rhythmic environmental stimuli versus internal metabolic "physiological rhythms"—in his 1919 paper. He systematically harvested plankton from both ocean surfaces and depths and then experimentally subjected them to varying temperatures and illuminations in water columns in the laboratory, compiling an impressive amount of data on phototaxis, geotaxis, and diurnal rhythms. With the exception of two species of *Acartia* copepods, for which he had reported a possible autonomous diurnal migration rhythm in 1917,[45] his conclusions were hesitant and indeterminate: "Owing to specific differences in behavior no general explanation of diurnal migration can be given at present. It is suggested by the experiments that each kind of organism will have its own way, so to speak, of performing the vertical movement, as each has its own peculiar responses in the laboratory."[46] Moreover, he now stated his earlier results from *Acartia* cautiously, noting that the daily rhythm of plankton migration persisted in the absence of external stimuli but without offering an explanation as to its cause:

> It is not desired to discuss at this time the question of what effects, varying periodically previous to constant darkness, may have been responsible for the rhythm under practically uniform conditions, or whether the rhythm is to be accounted for at all by the action of antecedent recurring stimuli. . . . But there is a marked increase in relative numbers in the upper parts of the column, as compared with the lower portions, from 6 to 8 p.m., and not at other times of the day. This may be repeated on the second day although the animals have been in darkness all the time.[47]

The following year (1920), Esterly wrote a report on his research for the first issue of the new journal *Ecology,* which was just as inconclusive and meandering, dominated by his concern that inconsistencies in findings might be owing to discrepancies between observations made in the laboratory and behaviors in natural conditions. This prompted the editor to comment somewhat apologetically: "The problem of the influence of laboratory conditions on plants and animals is becoming more and more pressing with the increasing need for exact experimentation. Although the problem may be more urgent in the case of animals than plants, it still applies to plants. . . . It is hoped that Professor Esterly's paper will stimulate further research along these important lines."[48] Esterly's caution about offering a clear hypothesis on the causes of plankton migration suggests that he was avoiding engagement in the already controversial question of endogenous versus exogenous causes of plant and animal behaviors, which he clearly understood from his reading of Loeb and Menke. This interpretation finds support in the introduction to his 1919 paper, where he declared that his chief aim was "to learn how the direction of [plankton] movement is affected by various external conditions. The actual experimental facts were sought rather than the laws or principles underlying them. Since the experimental work was not concerned with the physiology of the movements controversial matters connected with that phase of the study of behavior are not discussed."[49]

PHYSICAL CAUSES FOR LUNAR BIOLOGICAL RHYTHMS

Debate about whether rhythmic behaviors are material and mechanical responses to external stimuli or autonomous of these stimuli and produced internally by some kind of irritability is readily understandable when it comes to daily behaviors, inasmuch as the sun exerts a powerful and obviously physical stimulation that affects the inhabitants of the biosphere. Parallel consideration of non-daily rhythms, ones that do not correspond to any similarly obvious physical stimuli presented a different situation. In particular, the modern scientific search for and interpretation of lunar biological rhythms was haunted by the specter of astrology—namely, the lingering suspicion among scientists that pronouncements about the influence of the phases of the moon on terrestrial life have an odor of pseudoscience about them. By the twentieth century, the long history of appeals to astrological interpretations had been safely exorcized as "medieval" superstition, apart from the well-documented and understood relationship between the lunar phases and variation in solilunar gravitation, which generates the tides. Reference to explanations that sounded astrological in serious scientific discussion therefore sounded atavistic and unenlightened.

The taint of pseudoscience affected the reception of Svante Arrhenius's pronouncement that there was a connection between lunar motions and human rhythms, even though he was careful to offer a rational explanation on the basis of the principles of physics. With the development of ecology and evolutionary biology as a framework for understanding the relationships between individual organisms and their environments, explanations for the lunar rhythms of littoral creatures and marine annelids could be based on physical consequences of lunar position with respect to local illumination and tides, adaptation to which granted fitness to *Convoluta* and the Palolo worm within their niches. In this context the work of Harold Munro Fox is especially interesting, inasmuch as he took the rich lore of lunar phase relationships into account as background for his investigation of the periodicities of reproduction in marine animals.

Fox undertook close study of the Red Sea urchin *Centrechinus* (*Diadema*) *setosus* at Suez during the summer months of 1920 and 1921, its breeding season, with the aim of finding out whether there was a scientific basis for the common belief among Mediterranean fishermen that the urchins, along with other echinoderms and mollusks, were "full" (enlarged) at the time of the full moon during the summer and therefore made for better eating (see figure 1.2). Such beliefs were as old as Aristotle and had been repeated by natural historians down to Francis Bacon. Fox's research revealed that, indeed, the Red Sea urchin did have a reproductive rhythm, with maximum gonad size and spawning coinciding with the full moon, but that this feature had been mistakenly generalized to urchins in the Mediterranean and European Atlantic seaboard.[50]

Fox's survey of literature on lunar rhythms turned up mostly negative results for mollusks and crustaceans, with some positive reports, such as for the Palolo annelids and the grunion (and among plants, algae).[51] He under-

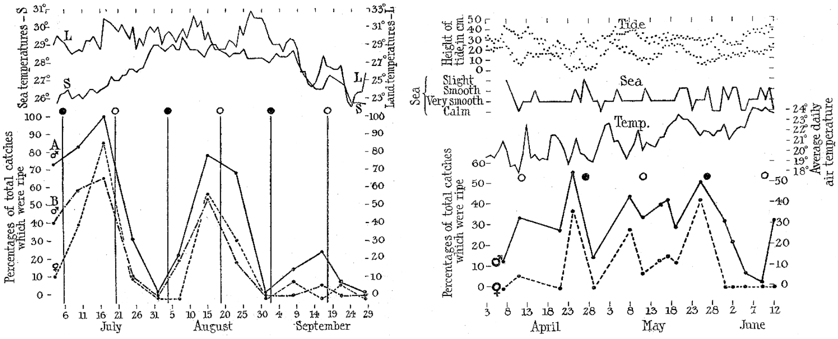

FIG. 1.2. H. Munro Fox determined that Red Sea urchins exhibit a lunar rhythm of reproduction, becoming “ripe” around the full moon. In a 1924 paper he graphed the production of the urchin’s sperm and eggs against the lunar phase and both land and sea temperatures recorded at Suez during the summer of 1921 (left). Full moons are indicated by open circles. The figure to the right adds consideration of tide levels. Inasmuch as the tide is small at Alexandria and the spawning did not obviously correlate with other environmental factors, he concluded that there must be some other lunar effect at work. *Source:* H. Munro Fox, “Lunar Periodicity in Reproduction,” *Proceedings of the Royal Society B* 95, no. 671 (1923–24): 523–50, figs. 2 and 4.

stood that, in antiquity, lunar growth cycles were conceptualized as part of a larger cosmological model that associated increase (youth, growth, humidity, and heat) with the waxing of the moon and decrease (harvest, senescence, decay, cold, and dryness) with its waning, and he tested local Egyptian beliefs about rapid vegetable growth on moonlit nights by measuring daily the length and curvature of a local squash (*Cucurbita pepo*). Finding no such correlation, he dismissed the claim for other fruits as well. Still, the phenomenon of the Red Sea urchin required explanation in terms that were acceptable to modern science. Here he tacitly criticized Arrhenius’s claim that there existed a statistical correlation between human menstruation and birthing and the lunar sidereal period (27.32 days), by pointing out that the average synodic period, which was associated with the phases and thus the physically real tides and illumination, was 29.53 days.[52]

Fox assumed that any relationship between the lunar phases and the urchin’s reproductive rhythm must be physical. He hypothesized that the tides were in some way causal, as Keeble and Gamble had argued for *Convoluta* in Brittany and Scott for *Amphitrite* at Woods Hole; but he pondered the fact that while there was a double tide cycle at Suez (two spring tides per month), the urchin cycle was single, with a reproductive maximum at the smaller full-moon spring tide. Along with his observations that the difference between high and low tides at Suez was only 58 centimeters and that urchins were mobile and thus not dependent on tidal changes in hydrostatic pressure (and thus dissolved gas concentrations), this led him to discount the direct physical influence of the tides. Moreover, his observations of sea urchin activity on dark nights and moonlit nights showed no difference, ruling out the direct effect of full-moon illumination.[53] Despite these results, Fox remained committed to an environmental rhythm of some sort as providing a physical stimulus, possibly an antecedent lunar-cycle fluctuation in the urchins' plankton food supply: "But not only are we ignorant of the nature of the periodic external factor, but the causes of spawning in echinoids, periodic or non-periodic, are unknown."[54]

Fox's initial assessment that the lunar rhythms of the Red Sea urchin were exceptional among the echinoderms and mollusks, pointing to the mussel *Mytilus* and oysters as negative examples, was corrected by subsequent research.[55] Already in 1924 James H. Orton observed a lunar periodicity in the spawning of the oyster *Ostrea edulis*.[56] Then in 1932 Helen Irene Battle found a clear correlation between lunar phase, tide height, and reproduction in *Mytilus edulis* on the Atlantic coast of Canada (see figure 1.3).[57] Pieter Korringa began to study their reproduction at the marine biology station in the oyster basin Oosterschelde in 1937, sampling daily the number of *Ostrea edulis* larvae and recording the water temperature and lunar phase throughout the duration of World War II. His data showed clear maxima in larvae swarms about ten days after both the full and new moon during the summer breeding season, with the greatest maximum falling between June 26 and July 10. From this he concluded that spawning must occur at the full and new moon, when the spring tidal difference reached 3.9 meters, 90 centimeters more than at neap tide.[58]

Placing his findings for *Ostrea* in the context of the variety of annelids, mollusks, and fish that had been found to have breeding rhythms that correlated with the daily and lunar cycles during specific seasons, Korringa explained the complexity of these rhythms as the interplay of several factors: Breeding was confined to a seasonal interval that varied from one species to the next, and within this interval the lunar rhythm of neap tides and spring tides and variations in nocturnal illumination modulated the daily tidal rhythms to coordinate reproduction at particular times of particular days.[59] He did not venture an opinion or hypothesis regarding the physiological causes of these rhythms, but his emphasis on the tides suggests that he was assuming the stimulus was environmental, related to water temperatures and pressures that were determined by the physical actions of the sun and moon.

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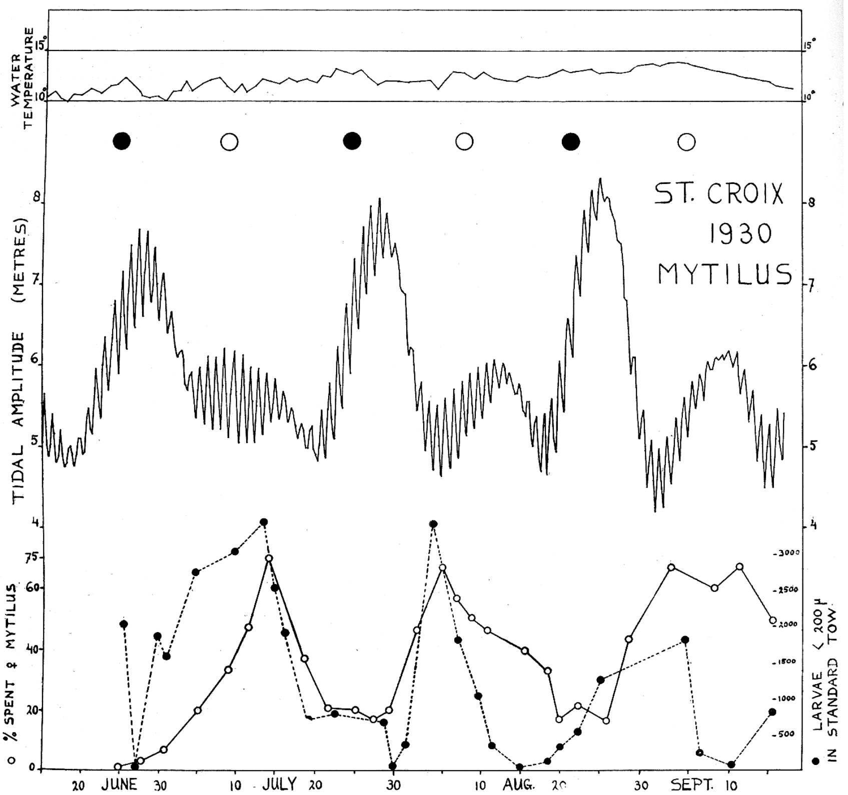

FIG. 4. Battle's data on reproduction in *Mytilus edulis* in Passamaquoddy Bay.

FIG. 1.3. Pieter Korringa graphed the data Helen Battle had collected in 1930 on the production of the mussel *Mytilus edulis* for comparison with the results he obtained from research on Dutch oysters in 1947. *Source:* Pieter Korringa, "Relations between the Moon and Periodicity in the Breeding of Marine Animals," *Ecological Monographs* 17, no. 3 (1947): 364, fig. 4.

Plankton migrations, like the reproductive behaviors of urchins, oysters, and grunion, are rhythmically complicated; the varying and sometimes contradictory reports for these rhythmic behaviors stymied biologists' efforts to attribute them to a physical cause, complicating arguments over whether these rhythms were internal or external in origin. Field observations and replication of observed natural behaviors in water columns in the laboratory subsequent to the early twentieth-century research of Keeble, Gamble, and Bohn had proved inconsistent. Esterly's results were sufficiently contradictory to cause him to doubt the direct applicability of experiments in the laboratory to behaviors in the wild. John Calhoun noted in his extensive review of animal rhythms in 1944 that "many of the Crustacea in the Gulf of Maine region near Woods Hole, Massachusetts, show little or no vertical migration," but he cited

George L. Clarke's observation of rhythm in a species of *Acartia* in Bermuda; a variability of rhythm within a single species of the copepod *Calanus finmarchicus*, which showed rhythm in one location, but not in another; and also Clarke's conclusion that the bioluminescent copepod *Metridia lucens* maintained its rhythmic migrations in a specific subsurface light zone, where the change in illumination was the only identifiable variable to serve as an environmental cue (or *Zeitgeber*, as the Germans called it) to synchronize the biological rhythm.[60] Reconsidering Esterly's 1917–1919 findings for *Acartia tonsa* in 1942, William Schallek found that even a four-hour migratory rhythm could be induced, but it persisted only a day or two when artificial stimulus was removed and therefore it could not be considered endogenous: "No evidence was found for a diurnal rhythm other than that caused by the normal alteration of day and night. Migration ceases under constant conditions."[61] He concluded that the migration must be a direct response to exogenous rhythms in the angle of incident light, with minimal persistence.

The inconsistency of all these results led Calhoun to suspect that "there must exist a minimal threshold of intensity that diel [twenty-four-hour] fluctuating influences must reach before the vertical migration rhythm of Crustacea assumes an endogenous character," but he also noted that recent findings on hormonal control of crustacean chromatophore and activity rhythms indicated that there might be a seasonal difference that had not been taken into account by earlier researchers.[62] The case for an endogenous rhythmicity that could be accounted for by natural selection remained elusive for the complex lunisolar tidal periodicities and served for a long time to justify the search for an exogenous Faktor X that was the causal stimulus for these well-synchronized behaviors. Closure on this problem would have to await a more detailed understanding of the heritable physiological mechanisms behind timings and how these interact with environmental factors to enhance fitness.