BIOLOGICAL CLOCKS OF THE TIDAL ZONE

Endogenous clocks set to the rhythm of the solar day are known throughout the biological world. Many organisms that live along the shore also have a clock set to the rhythm of the lunar day

by John D. Palmer

In the sands between the tide marks on the north shore of Cape Cod lives a microscopic golden brown alga, the diatom *Hantzschia virgata*. The protoplasm of this single-celled plant is encased in an elongated glassy cell wall perforated in places by pores and slits. Through some of the end pores is exuded a mucuslike substance that serves to slowly jet-propel the diatom through its subterranean habitat. During each daytime low tide the tiny motile organism glides up through the interstices between the grains of sand to the surface. There it remains throughout the ebb tide, its photosynthetic machinery bathed in sunlight. In midsummer the diatoms are so abundant that in spite of their microscopic size they form a prominent golden brown carpet over the beach. Moments before they are inundated by the returning tide they move down into the comparative safety of the sand.

A fascinating aspect of this vertical-migration behavior becomes more apparent when sand bearing the diatoms is transferred from the north shore of Cape Cod to the Marine Biological Laboratory at Woods Hole on the south shore. The samples are placed in an incubator where the temperature is held constant and the light is left on continuously. In this new environment, which lacks days, nights and tidal changes, the diatoms continue their periodic excursions up to the surface of the sand in virtual synchrony with the diatoms 27 miles away. Their movements in the laboratory are sufficiently punctual so that when we plan a collecting trip to Cape Cod Bay, we sometimes observe the diatoms in the incubator instead of consulting tide tables. Since the rhythm of the diatoms persists in the absence of the environmental periodicities that would be expected to govern such behavior, it seems that within the plants there is a biological clock that directs the temporal aspects of their lives.

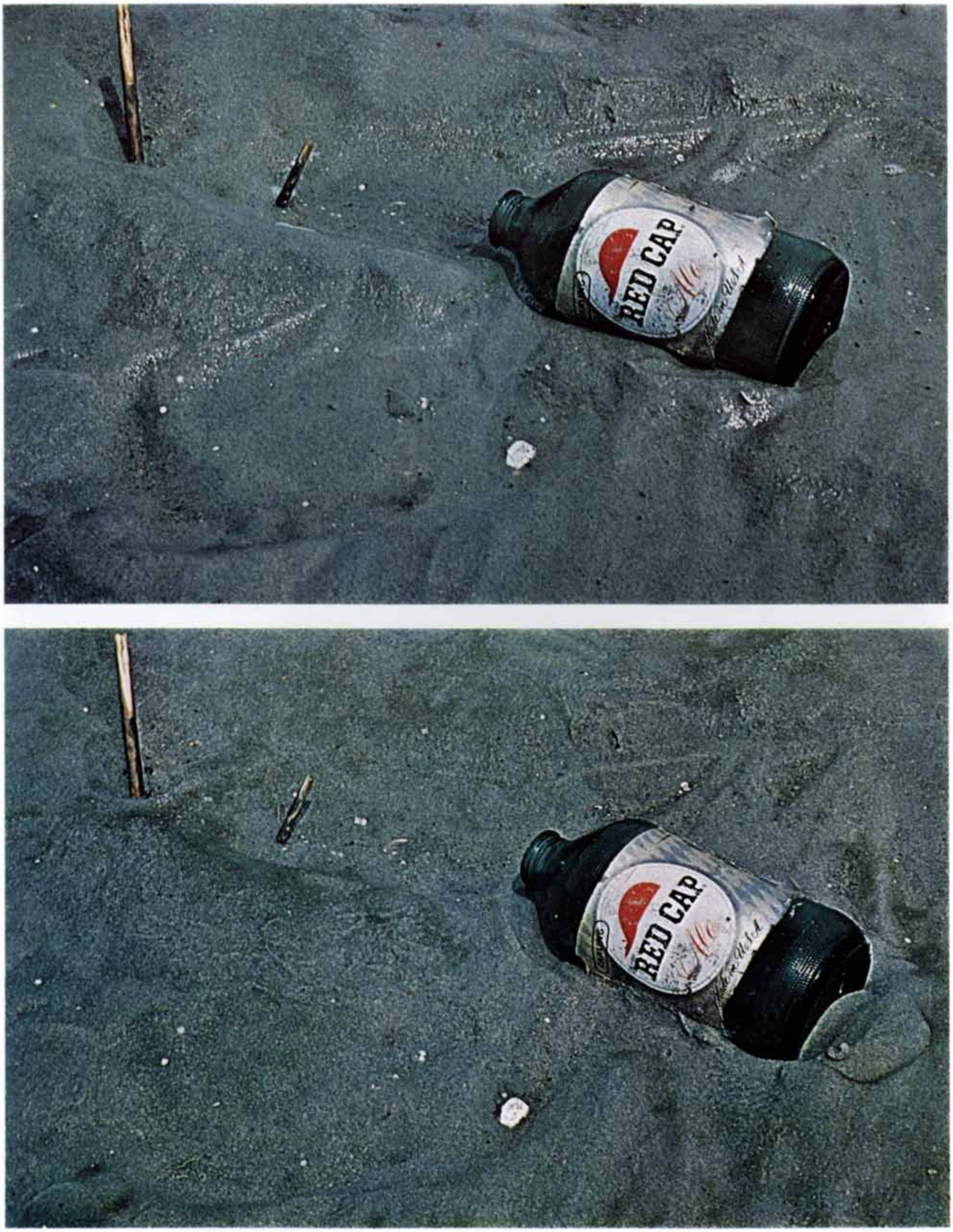
This account is not just another amusing anecdote about a rare occurrence in nature. Clock-controlled rhythms are displayed by most inhabitants of the tidal zone. The rhythms are characterized by the repetition of some behavioral or physiological event, such as a flurry of activity, synchronized with a particular phase of the tide. Since there are two tides each lunar day (a lunar day is 24.8 hours in length, the interval between successive moonrises), the rhythms are called bimodal lunar-day rhythms, in contrast to the unimodal solar-day rhythms of organisms geared to the 24-hour solar day. The biological clocks related to both the lunar-day and the solar-day rhythms are apparently important as an aid to survival in that they give advance warning of the regular changes in certain periodic aspects of the environment, such as nightfall or the return of the flood tide. Under unchanging conditions in the laboratory the clocks continue to function, and thus biological rhythms persist for a considerable length of time.

The fiddler crab, a common denizen of mud flats and sand flats on North American coastlines, emerges from its burrow at low tide. It scurries sideways around the flat eating detritus. The males feign battles with one another and try to entice females into their bachelor burrow with awkward beckoning movements of their enormous fiddle claw. With each flood tide all the crabs retreat back into their burrow, where they sit out the deluge.

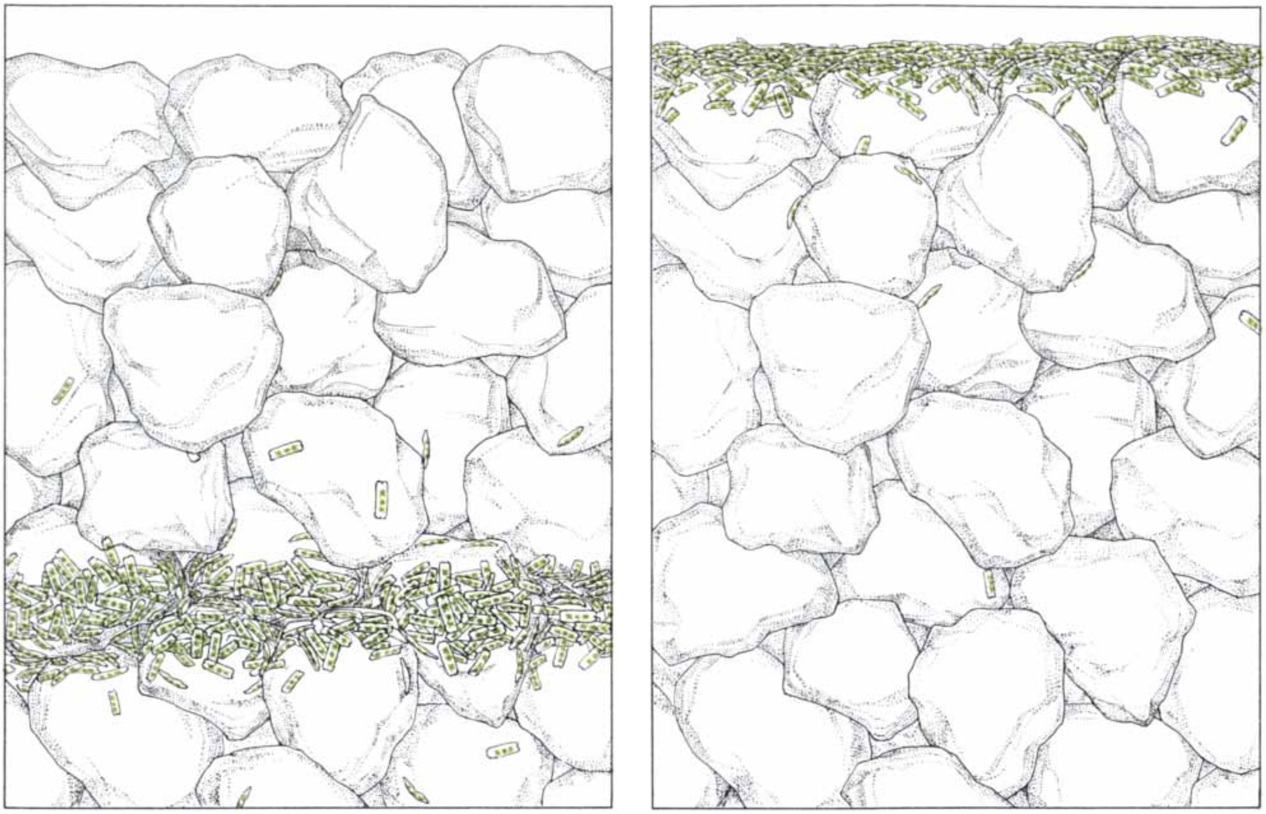
In the laboratory quantifying the locomotor behavior of fiddler crabs is quite simple. Single crabs are placed in plastic boxes (the kind in which fishing lures are bought, and therefore a common commodity in Woods Hole). The boxes are balanced on a knife-edge fulcrum, and as the incarcerated crab moves between ends of this improvised actograph the box teeters, closing a microswitch that causes a deflection of a pen on a chart recorder. The actograph is placed in the unchanging environment of an incubator, and the crab is allowed to perform spontaneously for days. In these monotonous surroundings the crab's clock continues to operate and dictates almost, but not entirely, the same ambulatory pattern found in nature. The difference is slight but significant: in the laboratory the period of the bimodal lunar-day rhythm is slightly longer or slightly shorter than the period displayed in nature. This change in periodicity when an organism is placed in constant conditions is a property of almost all clock-controlled biological rhythms. Since tidal rhythms follow the lunar day, they are called circalunadian (about a lunar day).

The rhythms in some species of fiddler crab will persist for as long as five weeks in the laboratory, but more often they are damped out rather quickly. The crabs must occasionally be exposed to periodic immersion in seawater if their tidal rhythm is to be maintained. Even in nature whenever small populations of fiddler crabs become established along the margins of pools not subject to tides, they lose their tidal rhythm and display only a solar-day rhythm. When the crabs are returned to a tidal flat, they quickly reestablish a lunar-day rhythm that will then persist for some time even after the animals are removed from the tidal location.

Living side by side on the same flats



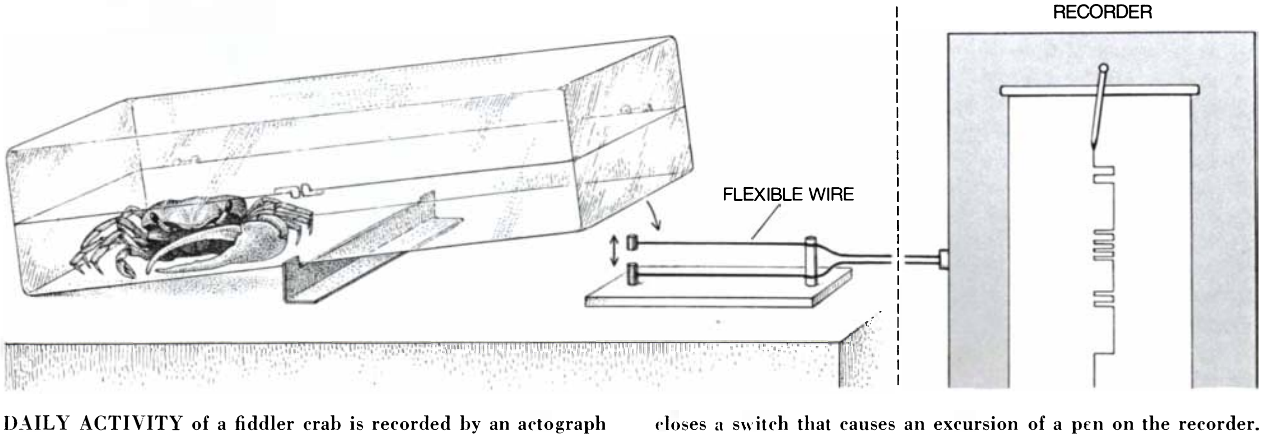
BEACH ON CAPE COD is inhabited by *Hantzschia virgata*, a species of diatom. These algae live in the damp sand between the high-tide mark and the waterline at low tide. The upper photograph shows a section of the beach that has just been exposed by an outgoing tide. The lower photograph, made a few minutes later, shows golden brown patches formed by diatoms that have migrated to the surface of the sand. When sand containing these diatoms is placed in an incubator where the light and temperature are held constant, the organisms continue to migrate to the surface synchronously with the periods of the daytime low tides at their home beach. The familiar shoreline object seen at the right in both photographs served to mark the location for purposes of comparison.



MIGRATORY BEHAVIOR of *Hantzschia virgata* is depicted in vertical section. Diatoms of this species normally reside about a millimeter below the surface of the sand (*left*). Each diatom has two *X*-shaped chloroplasts, which are the site of photosynthesis. During daytime low tides the organisms are propelled upward to the surface by mucus that is forced through pores at the end of their elongated glassy cell wall (*right*). The diatoms remain in the sunlight until moments before sand is inundated by returning tide.

with the fiddler crab are the green crab and the penultimate-hour crab. Both of these crustaceans display tidal rhythms, but they differ from the fiddler crab in that their activity is synchronized with the times of high water. The rhythms of the two species will persist in constant laboratory conditions for about a week before being damped out. In the case of the green crab it has been found that animals that have lost their rhythm in the laboratory need not be subjected to the tides to reestablish the rhythm. Instead it can be reinstated by cooling the crabs to a temperature of four degrees Celsius (39 degrees Fahrenheit) for six hours.

This technique has also been used to demonstrate that tidal rhythms are not learned or otherwise impressed on crabs by the tides themselves. Barbara Williams, working with Ernst Naylor at University College of Swansea, had the perseverance and the rare skill necessary to raise green crabs in the laboratory from eggs through several larval stages to adults. During the entire maturation process the crabs were exposed only to the alternating day-night changes in the laboratory. When the crabs were large

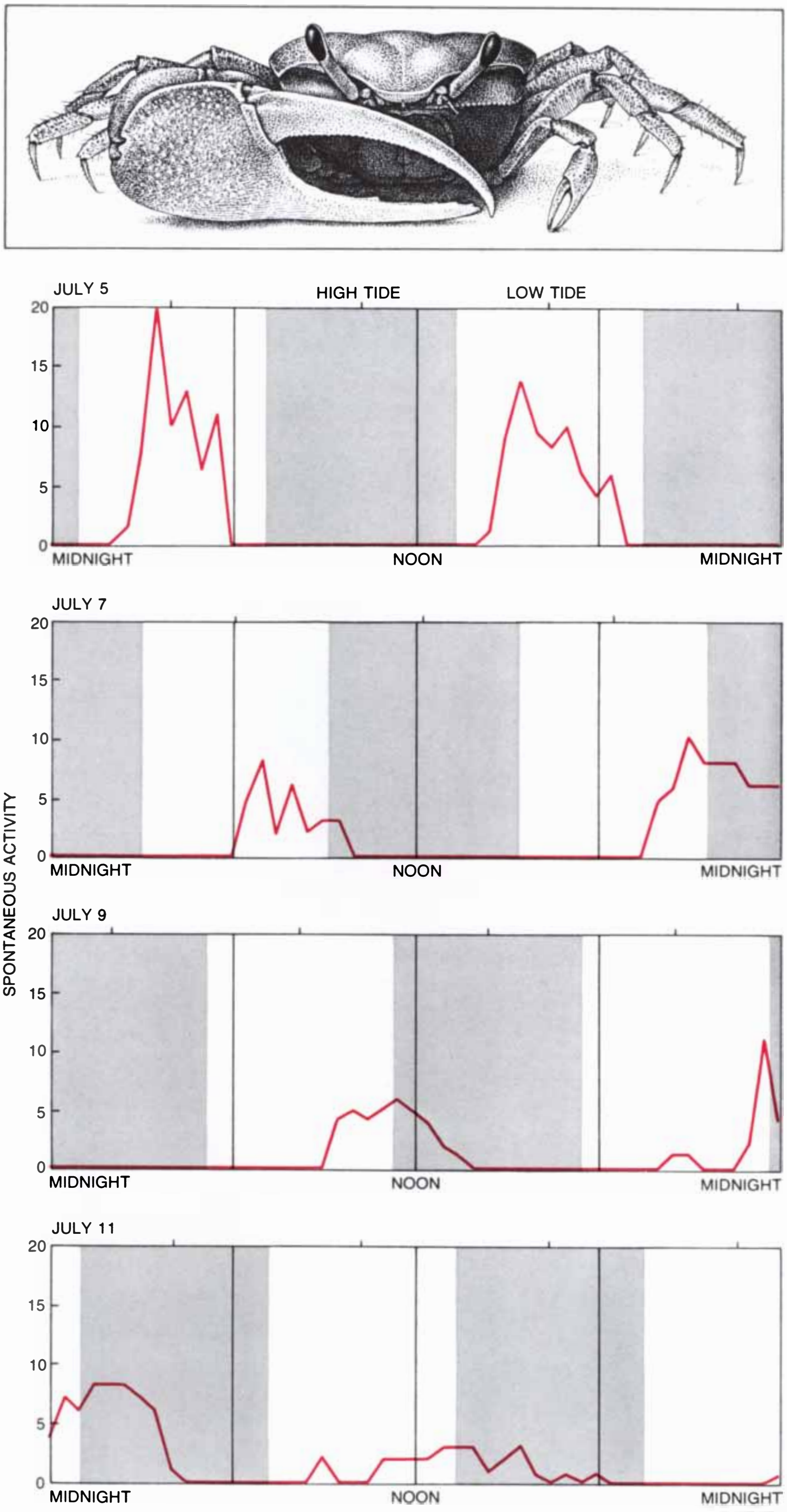


DAILY ACTIVITY of a fiddler crab is recorded by an actograph that consists of a plastic box balanced on a knife-edge fulcrum. When the crab moves to the near end of the box, the box tilts and closes a switch that causes an excursion of a pen on the recorder. The number of daily back-and-forth movements of the crab is determined by counting the number of excursions made by the pen.

enough for their activity to be studied, it was found that their locomotor activity was limited to the daylight hours. Williams then gave the crabs one 15-hour cold treatment and recorded their subsequent locomotor behavior. A distinct tidal component appeared in their activity. Since a single 15-hour cold spell could not have provided the crabs with any information about the 12.4-hour cycle of tides, it is reasonable to conclude that the clock that measures the tidal frequency is innate, and that it merely needs to be activated by some environmental stimulus for its first expression.

Under natural conditions both the penultimate-hour crab and the green crab display in their locomotor activity a clear-cut solar-day rhythm as well as a lunar-day one. In the penultimate-hour crab the solar rhythm appears as a broad peak of activity spanning the hours of darkness. In the green crab the solar rhythm is represented not as an individual peak but as a decrease in the amount of activity at the crest of the daytime tide. The combination of solar-day and lunar-day rhythms is rather common in intertidal organisms, and it raises the question of whether such organisms have a solar-day clock for one rhythmic component and a separate lunar-day clock for the other, or whether a single horologe drives both rhythms. A single-clock mechanism might be regarded as analogous to the kind of wristwatch worn by surf fishermen, in which a single movement is transmitted to present on the dial both the time of day and the time of the tide.

Processes other than locomotor activity are also controlled by the crab's biological clock. Color-change rhythms have been investigated in the fiddler crab, the green crab and the penultimate-hour crab by Frank A. Brown, Jr., Marguerite Webb and Milton Fingerman at the Marine Biological Laboratory and by B. L. Powell of Trinity College in Dublin. Within the hypodermis of these crabs are star-shaped chromatophores that contain granules of dark pigment. When the pigment granules are tightly aggregated in the center of these cells, the coloration of the crabs is light. When the granules are evenly dispersed throughout the extensions of the cells, the coloration is dark. All three species of crab blanch during the night and darken during the daylight hours, even when they are placed in constant conditions in a laboratory. The color-change pattern of the fiddler crab has

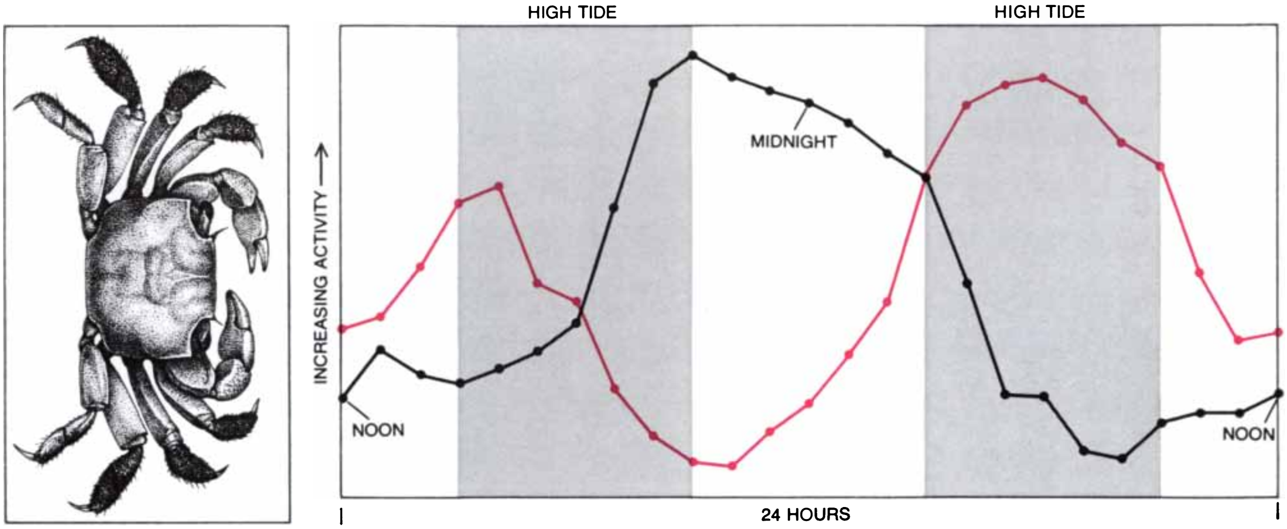


FIDDLER CRAB (*top*) is an inhabitant of tidal mud flats and sand flats. At high tide it remains quiescent in its burrow; at low tide it emerges to look for food. When a fiddler crab is taken from its natural habitat and put in an incubator where the light and temperature are constant, its periods of peak activity initially correspond to the times of low tide at its home location (*top curve*). The period of the crab's rhythm then begins to lengthen, and by the end of a week it is no longer synchronous with the times of the tide (*bottom curve*).

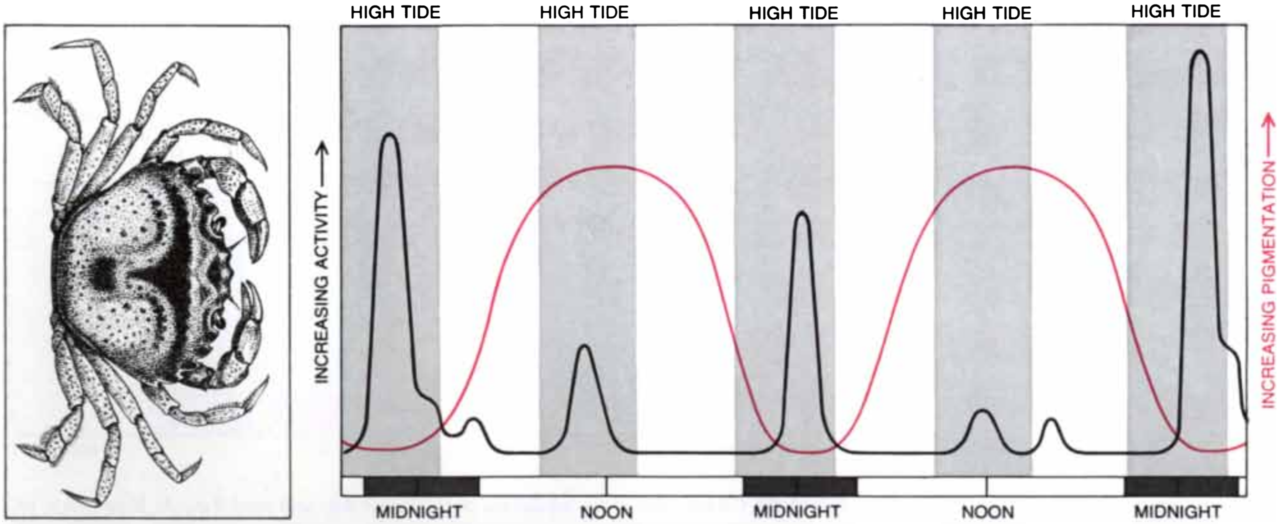
also been found to have a tidal component that gives rise to additional darkening at times of low tide.

The eyes of crabs are mounted on movable stalks. The stalks also house a neuroendocrine unit called the X-organ sinus-gland complex, which secretes a hormone that causes the pigments to disperse within the chromatophores. Powell found that the removal of the eye stalks from the green crab (and thus the X-organ sinus-gland complex as well) destroyed the color-change rhythm of the crab. Furthermore, Powell showed that the rhythm can be restored in a stalkless crab by implanting in it the stalk glands from another crab. These findings strongly suggest that in the green crab the eye stalks are the site of the clock that controls the color-change rhythm. In the penultimate-hour crab and the fiddler crab, however, removal of the eye stalks only reduces the amplitude of the color-change rhythms.

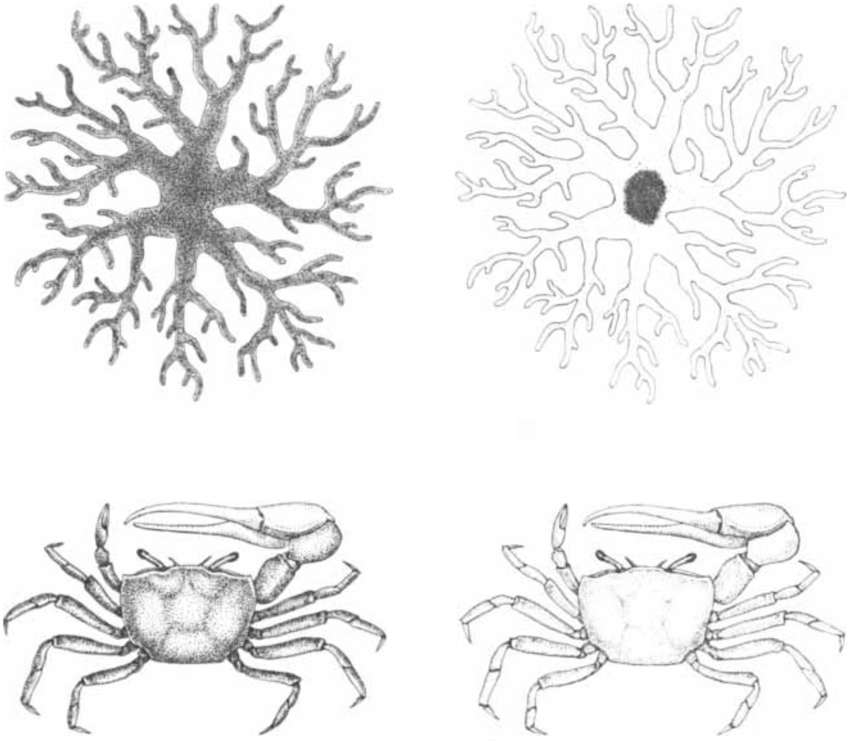
The neuroendocrine system of the eye stalks also exerts some control over the locomotor-activity rhythm of the green crab. When the eye stalks of green crabs were removed in experiments conducted by Naylor and Williams, all locomotor activity ceased, and it returned only gradually over the next six days. Since in green crabs the tidal rhythm normally vanishes after about a week in constant laboratory conditions, it was not surprising to find a lack of rhythm in the stalkless crabs when they resumed their activity. Attempts to reinstate the rhythm, however, by immersing the crabs in cold water were unsuccessful. On the other hand, crabs from which only the retinas were removed, not the stalks, returned to



PENULTIMATE-HOUR CRAB lives in close proximity to the fiddler crab on tidal flats. (The name of the crab is derived from the fact that the activity of a newly caught animal peaks one hour before midnight.) The black curve shows the mean solar-day activity of 30 penultimate-hour crabs over a period of a month, which is expressed as a broad peak of activity during the hours of darkness. The penultimate-hour crab also has a mean lunar-day activity rhythm (*colored curve*) that corresponds to the times of high tide.



GREEN CRAB, which also lives on tidal flats, has a basic pattern of locomotor activity that corresponds to the times of high tide. Its activity greatly decreases when the high tide comes during the hours of daylight. This pattern of activity continues when the crab is placed under constant conditions in the laboratory (*black curve*). In addition the green crab displays a solar-day rhythm in its body color, which blanches at night and darkens during the daylight hours. The color-change rhythm (*colored curve*), which persists when the green crab is kept under constant conditions, is thought to be controlled by a neuroendocrine system in crab's eye stalks.



CHANGE IN COLOR of the fiddler crab is the result of aggregation or dispersal of pigment granules in cells in the crab's hypodermis that are called chromatophores (*shown greatly enlarged at top*). In some chromatophores there are only dark pigment granules; in others there are white or orange granules. The fiddler crab blanches at night and darkens in daylight. Tidal component in the rhythm produces additional darkening at times of low tide.

their normal rhythm when they were immersed in cold water.

In further experiments Naylor and Williams found that subjecting the entire crab to a cold dip was not needed. If an arrhythmic crab is placed in water with a temperature of 15 degrees C. (59 degrees F.) but tethered so that its eye stalks protrude above the water, the tidal rhythm of the crab can be reinstated simply by dripping iced seawater onto its eye stalks for a brief period. Finally, Naylor and Williams made extracts from the eye stalks of green crabs in the quiescent phase of their locomotor-activity rhythm and injected the extracts into active stalkless crabs. The injection caused a significant reduction in the level of activity of the stalkless crabs, showing that there is an inhibitor substance that is periodically liberated from the stalk glands.

I have conducted similar experiments with the penultimate-hour crab. When the eye stalks were removed from these crabs, all locomotor activity stopped. I made eye-stalk extracts from rhythmic crabs during either the active phase or the quiescent phase of their locomotor activity and injected the extracts in various concentrations into crabs that had become arrhythmic because of long-term storage in constant conditions. No consistent alterations in the activity levels of the recipients were observed.

Since the neuroendocrine glands in the eye stalks of the penultimate-hour crab did not appear to be involved in the control of the crab's locomotor-activity rhythm, I carried out an experiment to determine if there was a chemical messenger coming from somewhere else in the crab's body. I joined two crabs, one strongly rhythmic and the other arrhythmic, by cutting small openings in their dorsal exoskeleton and cementing the openings together with sealing wax. In crabs most of the blood is not confined to vessels but flows freely through the spaces between organs; therefore when two crabs are joined, the blood of one mixes freely with that of the other.

I also capitalized on an anatomical peculiarity of crabs, the process called autotomy. When a crab is attacked, the attacker usually grabs one of the animal's 10 legs. The crab's defense is to cast off the leg and dash away before the predator can grab another. The leg separates from the body at a predetermined breaking point. Excessive loss of blood from the open stump is prevented by a self-sealing mechanism, and the sacrificed leg is regenerated during successive molts.

Taking advantage of this self-amputation mechanism, just before I joined two crabs I made the rhythmic crab cast off all its legs. The joined crabs therefore consisted of an ambulatory arrhythmic crab on the bottom and a rhythmic amputee upside down on the top [*see illustration on next page*]. Any rhythmic locomotor activity recorded thereafter would have been the activity of the legged member, signifying that some substance in the blood of the legless crab had induced the rhythm. In 47 fusion-pair experiments not one rhythm was found. On the other hand, a rhythm was always displayed when two rhythmic crabs were joined in control experiments, indicating that the fusion procedure was not responsible for the lack of expressed rhythmicity.

It is clear, then, that whereas the endocrine system is involved in rhythms of color change and locomotion in the green crab, it is not necessarily involved in such rhythms in other crabs. Nor is it mandatory that an endocrine or a neural mechanism form the basis of any physiological rhythm, since a single-cell level of organization such as that found in the diatom *Hantzschia* is sufficient for the expression of all the known properties of clock-controlled rhythms.

The capacity for rhythmicity is not learned or impressed on organisms by the environment; it is the expression of a genetic potential. Heredity also determines whether the crab will be active at high tide or at low tide. This is not to say, however, that the environment does not play a significant role in the overt manifestation of a rhythm. It is the schedule of the tides on a particular stretch of coastline that determines the hour-to-hour settings of the rhythm. (The relation between a biological clock and the environment is similar to that between a pendulum clock and its owner. The rate at which the pendulum clock runs is determined by the escapement mechanism and the pendulum, but the owner can set the time to any hour by moving the hands on the face of the clock.) Thus a green crab will soon be active at high tide and a fiddler crab at low tide even when they are transported to an unfamiliar beach on a different ocean.

In the sand high on the beaches of southern California lives the sand hopper *Excirolana*. At the peak of each high tide, when the waters flood the habitat of this tiny isopod, it emerges from the sand to swim and feed in the breaking waves. Two or three hours later, when the tide turns, it burrows into the sand

and awaits the return of the next flood tide. James T. Enright of the Scripps Institution of Oceanography found that when he kept sand hoppers in a jar of seawater in constant conditions, they swam actively during the times corresponding to peak tide and remained in repose at the bottom of the jar at other times.

In southern California the tidal pattern changes greatly with the phases of the moon. Over a single month the tides change from one crest per lunar day to two per lunar day. Furthermore, during the transitions from one tide per day to two tides and back to one tide the height of consecutive tidal peaks also changes. L. A. Klapow, who was then working at the University of California at San Diego, showed that the pattern of the tides at the time sand hoppers were collected was reflected in the form of the activity rhythm the animals displayed in the laboratory [*see illustration on opposite page*]. In separate experiments Klapow and Enright also demonstrated that it is the pounding waves and the swirling waters that determine the pattern of the sand hopper's rhythm.

It therefore seems that inhabitants of beaches exposed to the open sea have their activity patterns shaped by the action of the surf. Intertidal organisms that live in protected bays are not normally exposed to a pounding surf and so we must look elsewhere for the elements that help to set their rhythms. The possibilities are numerous, including periodic inundation and periodic changes in temperature, hydrostatic pressure, the chemical composition of the water or the availability of oxygen. Of all these possibilities only two have been shown to play an important role. As a clear-cut example I shall cite another study of the green crab by the prolific team of Naylor and Williams.

One of their most surprising findings was that the principal feature of the tide, the periodic inundation of the shoreline, was not itself an important agent in synchronizing the locomotor-activity rhythm to the tides. This fact was demonstrated by bringing crabs into the laboratory and subjecting them for five days to 6.2 hours of immersion in seawater followed by 6.2 hours of exposure to air. The immersion in seawater was timed to correspond to low tide at the crabs' home beach, in effect reversing the animals' tidal schedule. The temperature of both the water and the air was held constant at 19 degrees C. After this treatment the crabs were placed in actographs, and their locomotor-activity patterns were measured for the next three days at the same constant temperature. The treatment did not rephase the crabs' rhythm. The procedure was repeated, but this time the air temperature was maintained at a level 11 degrees higher than the water temperature. Five days of this treatment did rephase the crabs' rhythm, and the change persisted in constant conditions.

The final version of the same experiment omitted the seawater-immersion portions of the cycle. Crabs were exposed to air at 13 degrees C. for 6.2 hours and then to air at 24 degrees for the same length of time. Complete and persistent synchronization resulted. Recently I have educed the same behavioral changes in fiddler crabs and penultimate-hour crabs. It is therefore the drop in temperature brought by the flood tides that plays an important role in setting the phase of the crabs' rhythm.

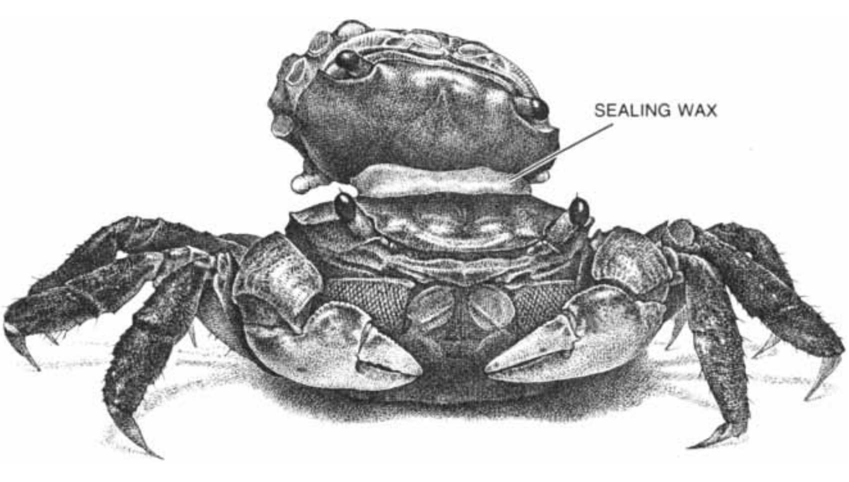
Hydrostatic pressure is the other environmental force that is known to synchronize organismic rhythms to local tides. In one experiment arrhythmic crabs were exposed for five days to a cycle of high pressure (1.6 atmospheres) for 6.2 hours followed by 6.2 hours at normal sea-level pressure. The crabs responded with an increase in activity during the high-pressure periods, and this periodicity persisted when the crabs were kept in constant conditions.

So far we have no firsthand knowledge of how the living horologe actually works. In the search for the elusive timing mechanism, however, several of its properties have been elucidated.

When rhythms in biological processes such as oxidative metabolism, photosynthesis and the like were first discovered, and it was found that these rhythms would persist without external stimuli, the controlling clock was thought to be simply some oscillatory step in the chain of chemical reactions underlying the process. As a result early attempts at locating the clock consisted in dissecting the chain of relevant reactions in the hope that the oscillatory segment could be identified. The rhythmic component was not found, and subsequent experiments showed that it probably does not exist. In fact, the clock is now known to be quite distinct from the process it makes rhythmic.

One of the many observations leading to this conclusion was conducted with the green crab. When the body temperature of the crab was lowered to 10 degrees C., all locomotor activity stopped for the duration of the chilling. When the body temperature was allowed to return to a normal level, activity resumed, and the locomotor rhythm was in exact phase with that of control crabs that had not been chilled. Clearly the crab's clock had continued to run accurately even when no rhythm was being expressed. This finding shows that the clock and the processes it causes to be rhythmic are separate and must be joined to each other in such a way that they can be uncoupled from each other and recoupled.

The disengagement of the coupling between the clock and the driven process may also be responsible for the even-



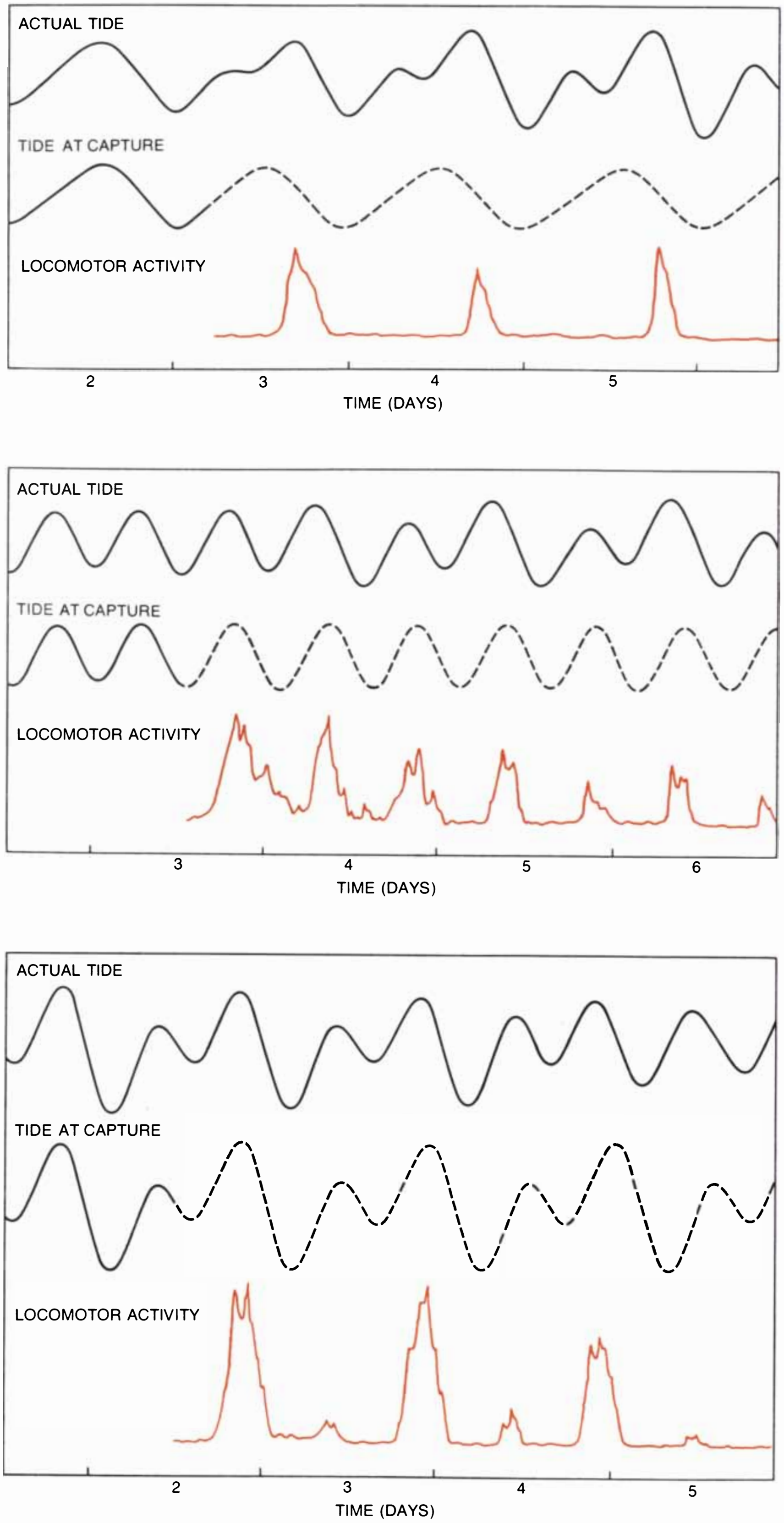
PENULTIMATE-HOUR CRABS ARE JOINED parabiotically so that their blood can continuously mix. Small openings are cut in the dorsal exoskeleton of each crab, and the openings are cemented with sealing wax. The top crab has cast off all its legs through autotomy, the process by which crabs shed a leg when it is seized by a predator. Before the union the locomotor activity of the top crab was synchronous with the tides. The activity of the bottom crab was arrhythmic. In experiments with fused crabs no rhythmic locomotor behavior was found, indicating blood does not contain a chemical messenger that induces such behavior.

tual loss of overt rhythmicity in animals that are maintained in constant conditions in the laboratory. Speaking somewhat teleologically, when intertidal animals are taken away from their tidal environment, there is no longer any pressure for them to maintain a tidal rhythm. Their life processes are emancipated from the clock and become arrhythmic.

The validity of the notion of a coupling between a clock and vital processes is enhanced by the fact that rhythms once lost by crabs, either in the laboratory or in nontidal natural habitats, can be reinstated by a single short-duration stimulus such as being chilled. Since the treatment provides no information about tidal intervals, the simplest interpretation is that the stimulus recouples the clock, which had continued to run, to the processes governing locomotor activity, causing such activity to become rhythmic again.

As we have seen, the vertical migration rhythm of the diatom *Hantzschia* demonstrates that a biological clock needs only the level of organization characteristic of a single cell to express itself. Two other unicellular organisms provide even better examples. The marine dinoflagellate *Gonyaulax* is known to simultaneously display different rhythms in four processes: photosynthesis, luminescence (it glows at night), irritability and cell division. Five different rhythms have been detected in the single-celled green alga *Acetabularia,* and all the rhythms persist even when the nucleus of the cell has been removed by microsurgery. There is evidence that in multicellular plants and animals the clock is also to be found in single cells. When organisms are subdivided and the parts are kept alive in tissue culture, the cells continue their original rhythm. Indeed, the plausible place to look for the living clock is within the single cell, where one would expect to find it in the form of some physiochemical entity. In spite of intensive investigation, however, neither the clock nor any of its components have been located. The search has nonetheless revealed two unusual aspects of the horologe: the rate at which it runs is almost completely insensitive to temperature, and the rate also is not affected by a wide variety of potentially disruptive chemical agents.

In general increasing the temperature increases the rate at which chemical reactions proceed. One would expect that the living horologe, with its chemical clockwork, would be accelerated in a similar way. To test this assumption we subjected groups of crabs to increasingly higher constant temperatures in the lab-



PATTERN OF ACTIVITY OF THE SAND HOPPER (also called the beach flea) found in the beach sands of southern California is adapted to the peculiarities of the tides of the region. The tides alternate every month between one peak per lunar day and two peaks per lunar day. When sand hoppers are kept in constant laboratory conditions, their pattern of activity (*colored curves*) tends to mimic the form of last tidal pattern to which they were exposed (*broken curves*) rather than the form of the actual tidal pattern (*black curves*).

SCIENCE/SCOPE

A new ultra-lightweight radio for tactical field operations, developed by Hughes, employs micro-miniaturized circuits including LSI (Large Scale Integration) to provide high reliability, plug-in modules for easy maintenance, and an AM mode for compatibility with current military systems. Called the HC-191 Manpack, it is a version of the AN/PRC-104 single-side-band transceiver Hughes is building for the U.S. Marine Corps. It has a frequency range of 2 to 30 MHz and 280,000 channels to make enemy jamming difficult. Another significant combat advantage is its completely silent automatic electronic tuning.

The complete Manpack radio weighs only 12½ pounds including a battery pack that gives 16 hours of service before recharging. With its built-in 8-foot whip antenna, the HC-191 has a range of up to 30 miles in the most difficult jungle or mountain terrain. For a copy of the HC-191 brochure, write: Marketing Department, Hughes Aircraft Company, Bldg. 600/C231, P.O. Box 3310, Fullerton, Calif. 92634.

Telephone users in the United Kingdom will benefit from the computer-controlled FACT-II wiring analyzer system recently delivered to Standard Telephones and Cables, Ltd., of International Telephone and Telegraph in Northern Ireland. The 68,000-lb. system can test 34 different products in any of over 5,000 electrical configurations. A special connector developed by Hughes makes it possible to simultaneously access 25,600 circuit terminations in less than 15 seconds.

FACT-II is an adaptation of the system Hughes originally developed to pinpoint and troubleshoot electrical problems in aircraft fire-control systems. Hughes has built 11 FACT systems for European users and scores more for North America, the Middle East, and Japan.

Laser rangefinders for the U.S. Army's M-1 battle tank are being developed by Hughes for prototypes by both Chrysler Corp. and General Motors Corp. Following a competitive evaluation in mid-1976, the Army is expected to select a single contractor. Hughes currently produces laser rangefinders for the Army's M60A2 tank and M551 Sheridan vehicle and is developing a full-solution laser fire control system for an improved version of the M60A1. A tank with a laser rangefinder can fire far more quickly and with a much higher first-round hit probability.

Hughes Ground Systems Group needs Senior Systems Programmer/Analysts and Communication Systems Development Engineers to join the technical staff due to growth of current research and development programs. Applicants must have a BS or MS in electrical engineering or computer science and U.S. citizenship. Qualified applicants should write or send resume to: M. F. Duggins, Hughes Aircraft Company, P.O. Box 3310, Fullerton, CA 92634. An equal opportunity M/F employer.

A solid-state watch module for ladies-size digital watches -- now in production at Hughes -- contains the equivalent of more than 1,500 transistors. It overcomes the size limitations of ladies' watches with a unique time-readout that flashes the hour for about a second, then gives the minutes. The new module supplements the men's watch modules now made for leading name-brand manufacturers by Hughes, one of the largest producers for the watch industry.

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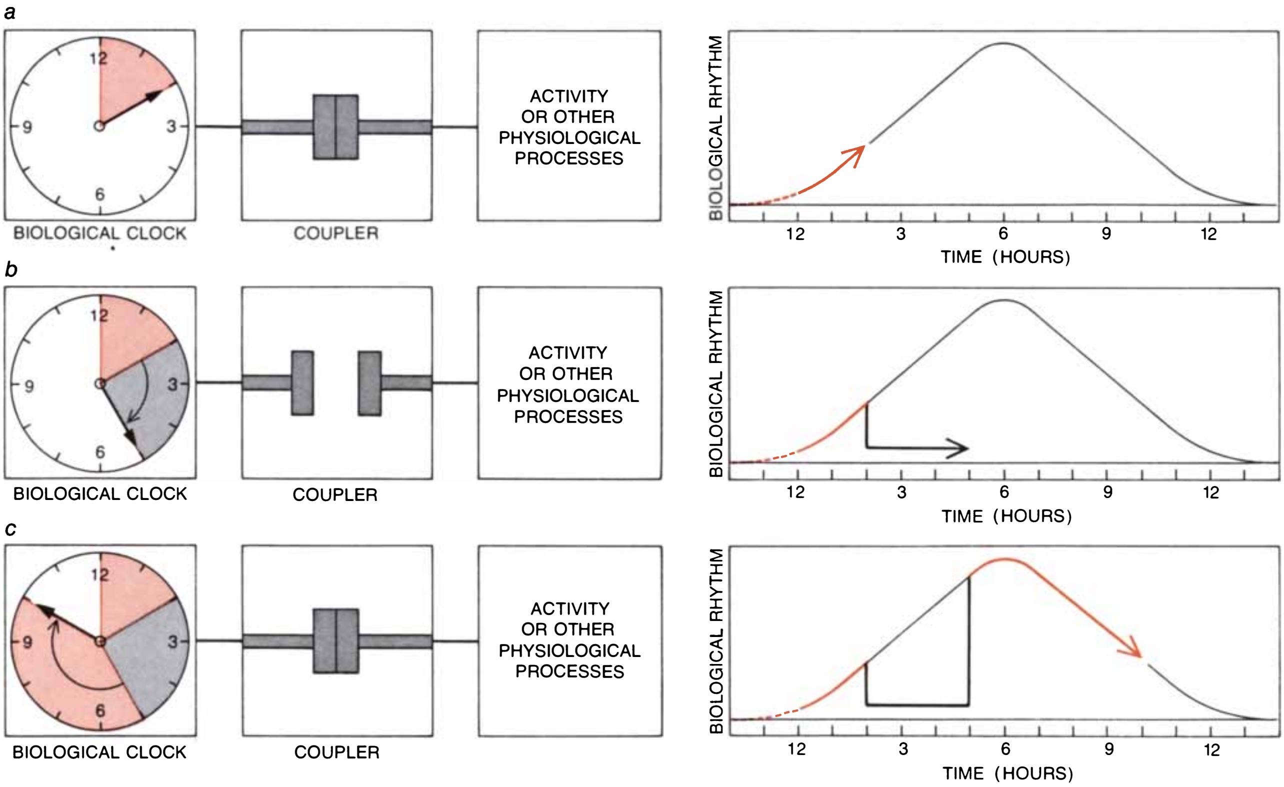
oratory and observed their rhythms over periods of several days. Since the period of an expressed biological rhythm is believed to closely mimic the driving frequency of the clock, a temperature-induced change in the rhythm is assumed to indicate a change in the frequency of the clock. The usual result obtained in such experiments is that there is no change in period at all. If any change is recorded, it is only a fraction of what one would expect from a chemical system.

Attempts to disrupt the rhythms of organisms with chemical substances such as inhibitors of protein synthesis, stimulants, metabolic inhibitors and narcotizing agents have proved to be almost equally futile. Out of hundreds of substances tested only four—deuterium oxide, ethyl alcohol, valinomycin and lithium ions—have been found to alter the period of a rhythm. In view of the variety and number of substances screened, it appears that biological clocks, unlike most other pacemaker systems in organisms, are virtually immune to chemical manipulation.

From a pragmatic point of view these insensitivities might have been predicted. Certainly one of the most important attributes of any clock—living or manmade—is accuracy, and a clock whose rate of running is altered by changes in the temperature or chemistry of its environment would not meet this requirement. In fact, if the clock responded to every change in ambient temperature, it would not be a clock at all but rather a thermometer that signaled ambient temperatures by the rate at which it ran.

The accuracy of biological clocks is even more amazing when one takes into account the fact that precision must be maintained during cell division, when presumably not only the cell but also the clock is replicated. The ease with which this replication is accomplished has been demonstrated in a study of the single-celled protozoan *Paramecium* by Audrey Barnett of the University of Maryland. In the strain of paramecium she worked with the sex of each animal changes from one mating type to another and back again each day. Barnett placed in constant darkness eight paramecia whose clocks had been set to regular day-night cycles. The cells in the population divided 2.2 times per day, and at the end of six days they had given rise to slightly more than 121,000 cells. On the seventh day the sex-reversal behavior of the entire population was examined and was found to be rhythmic. The phase of the rhythm was close to that of control cells that had remained under the regular day-night cycle. Since only the original eight cells had been subjected to a day-night cycle, it appears that each of the original cell clocks had been replicated time and time again with very little loss in accuracy. An alternative interpretation is that each cell contains many clocks, some of which are replicating themselves while others are still coupled to cell processes, causing them to be rhythmic.

We still know very little about the mechanism of living horologes in the tidal zone, and the properties of clocks that have been elucidated in some ways compound the problem. The continued search for such mechanisms is nonetheless a worthwhile endeavor because clock-controlled rhythms are found not only in intertidal organisms but also throughout the kingdom of life. One may hope that the continued effort will eventually lead to discoveries that will enable us to perceive the fundamental principle that underlies the operation of all biological clocks.



COUPLER that has not yet been identified is believed to join biological clocks and biological processes. When the coupler is engaged (*a*), the biological rhythm is expressed. The disengagement of the coupler (*b*) may be responsible for the loss of rhythmic behavior of organisms that are kept under constant laboratory conditions. The clock continues to function, however, and when recoupling occurs (*c*), the rhythm takes up not at the point where it left off but at point that corresponds to "time" determined by the clock.