A Crab in the Lab that Identified High and Low Tides in the Sea Two Miles Away

The Rediscovery of Tidal Rhythms in India

M K Chandrashekaran

The author describes his 'choice' of a Ph.D. dissertation topic in the sixties, the interactions with his advisor, the rhythms of the mole crab and the serendipity of success in research, in a candid and humorous fashion.

M K Chandrashekaran has studied biological rhythms in plants, fruitflies, crabs, mice, bats, squirrels and humans. Having established an active school of research in chronobiology at the Madurai Kamaraj University, he has just moved to the Jawaharlal Nehru Centre for Advanced Scientific Research at Bangalore and plans to focus his attention on rhythms in social insects.

I became a chronobiologist by the fortuitous re-discovery of tidal rhythms in the swimming activity and oxygen consumption of the mole crab, *Emerita asiatica* at Madras (*Figure 1*). Books stated that this crab lived in the intertidal region of the sea always under a few centimetres of water. This would hardly be logically possible for the sea rises by about 1.5 to 2m every 12.4 hours causing high tides. Similarly, the sea ebbs once every 12.4 hours causing low tides. Thus identical tides occur roughly 12 hours apart. It therefore follows that the crab must migrate up and down the beach in order to be able to live constantly under a few cm of sea water. It strains off tiny copepods and protozoans with its feather like antennae especially from the seaward washes of waves. Another prominent tidal migrant is a mussel called *Donax cuneatus* in the Madras coast.

Figure 1 Photograph showing ventral (left) and dorsal (right) view of an adult female Emerita asiatica.

The Setting

In the early sixties the Zoological Research Laboratory of the University of Madras was still housed in a lovely building (now flanking the southern side of the Centenary building) with facilities for running sea water. There was a separate aquarium building with capacious water tanks with heavy glass face containing sea water. Professor C P Gnanamuthu,

M.A., D.Sc., F.Z.S. was the Director and ran the laboratory rather sternly. The faculty members and research scholars were much in awe of him. Dr. Gnanamuthu seemed to secretly like young people but appeared too shy to give expression to this sentiment. I can vouch for the fact that he enjoyed teaching M.Sc. classes and put a great deal of effort into his lectures. He spoke and wrote chaste English and had a good sense of humour.

"It is not for nothing that the scholar invented the Ph.D. thesis as his principal contribution to literary form. The Ph.D. thesis is the perfect image of his world. It is work done for the sake of doing work - perfectly conscientious, perfectly laborious, perfectly irresponsible"

Archibald Macleish

It was very difficult in those days to become a research scholar and register for the Ph.D. degree. Only B.Sc. (Hons) and M.A. candidates with a first class made it. There was just *one* U.G.C. scholarship in a year and the students were drawn mostly from the Southern states. It was never clear to any of us what awaited us after the Ph.D. degree was awarded. The whole undertaking was one big gamble. Three copies of the Ph.D. thesis were sent to three foreign (generally English) examiners many of whom were Fellows of the Royal Society. The examiners generally, having little idea of the hardships under which the research was carried out, were invariably harsh with criticisms, particularly in matters of methodology. Roughly 50 percent of the theses were turned down of which some were recommended for resubmission after suitable modifications. Since the process often took close to a year the candidates would have left and the thesis just remained in the laboratory.

It was my Ph.D. research mandate to study the 'basal' metabolism of tropical poikilotherms. It was fairly obvious by the time I began my research in 1960 that there was no basal metabolism in poikilotherms.

Research Leading to Ph.D.

To return to the beginning. In zoology during the tenure of Prof. Gnanamuthu as the Director of the Zoological Research Laboratory, which lasted from 1946 to 1963, the research scholars did not choose the topic of their own Ph.D. research but were given topics. The M.Sc. 'by examination' candidates were especially unsuited even to suggest a topic for themselves. Similarly, it was a matter of sheer chance as to

M J Wells visited us from U.K. sometime in 1962-63 and after discussing my research plans with me, told me that he was sorry for my plight.

which faculty member one ended up with for research. It was my lot to work with Prof. Gnanamuthu who, on several counts, deserved to be better known and appreciated as a scientist.

It was my Ph.D. research mandate to study the 'basal' metabolism of tropical poikilotherms. The latter ponderous word refers to all animals save birds and mammals. It was fairly obvious by the time I began my research in 1960 that there was *no* basal metabolism in poikilotherms. Ever since the monograph on respiration by the great August Krogh, published in 1919, students of physiology knew that the basal metabolism of poikilotherms was called 'standard metabolism'. The stipulated conditions for measurement of the 'basal metabolic rate' (BMR) in humans were a light breakfast 8 hours prior to measurements of oxygen consumption of subject, a relaxed (reclining) posture and listening to light music. These were hardly conditions I could have imposed on my crabs. Prof. Gnanamuthu strongly felt (as he did on most other matters) that the extreme variabilities characterising O_2 consumption values of invertebrates and lower vertebrates were 'part and parcel' of the business of life and therefore 'the minimum energy compatible with the maintenance of life' and therefore 'basal'. I was a helpless pawn in this ideological warfare between my research supervisor and the school of the Canadian fish physiologist F E J Fry and his students, prominent among them S V Job, then reader in the laboratory and sitting just four rooms away from the Director's office. M J Wells visited us from U.K. sometime in 1962-63 and after discussing my research plans with me, told me that he was sorry for my plight.

Photograph of Dr. C P Gnanamuthu with the author made in early 1964

A Mad Crab

Among the several animals I examined (an estuarine anemone, an estuarine annelid, an intertidal bivalve, a fiddler crab, a land crab and an anomuran crab) was a mole crab *Emerita asiatica,* which was totally aquatic. I came every day

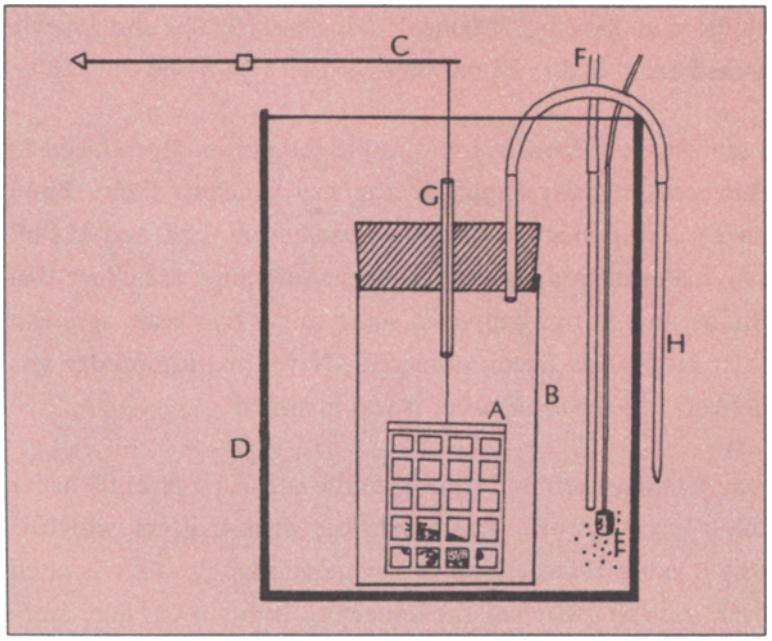

Figure 2 The simple plastic cage device used to record the locomotor activity of the crab Emerita asiatica with the continuous flow of sea water arrangement to enable oxygen consumption estimations. (A-activity cage, B-animal chamber, C-marking lever, D-constant water level trough - not drawn to size, G-inlet, H-outlet). When the crab swam up into the water space off the bottom of the activity cage, the cage rose inside the animal chamber. Such bouts of activity registered themselves through the writing stylet on the kymograph drum.

around 9 a.m. to the laboratory in the aquarium building. I had a row of continuous flow respirometers in which the respiration chamber was a wide- mouthed glass jar. One could therefore see the mole crabs. Some mornings when I came in, the crabs would swim agitatedly and on other days they sat still. The idea was to draw samples of water from the respirometers when the crabs were inactive. Since it was suspected that the movements of the investigator may cause excitement, the jars were painted over black. Then of course I could not inspect the state of activity of the crab.

I then constructed a simple but very effective actograph (*Figure 2*) with which I could continuously record the swimming activity of the crab and simultaneously measure the oxygen consumed. Dr. M J Wells was much impressed with the contraption. The recordings were traced on student kymograph drums, which, at the lowest gear, completed one revolution in 6 hours, therefore the recordings were made in several different heights, usually four. The drums had to be

adjusted at 06.00 hr, 12.00hr, 18.00hr and 00.00hr and a newly sooted drum had to be put on once every 24 hours.

I am not very clear in my mind if the events reproduced in this account truly happened the way I narrate them. But I have told this story to successive batches of M.Sc. and M.Phil candidates in this manner and have now come to believe that this is how things happened some thirty five years ago. But then, as Francis Bacon observed, "Never any knowledge was delivered in the same order it was invented".

At this stage in my research I too ought to have been in a position to shout "eureka, eureka"! But I was a victim of the condition Louis Pasteur described in the words "In the field of observation, chance only favours those minds which have been prepared".

One Monday evening I came into the aquarium at 21.00 hr for the overnight work. The day of the week matters, which is why it is mentioned. It was a new moon night and it was pitch dark, until I switched the aquarium lights on. There were twenty sea water troughs in my laboratory of about 30 cm diameter with some 4-5 cm sea water and twenty *Emerita* female crabs (ca 3-4 cm long) in each trough. These anomuram crabs have a distinctive and fascinating way of moving - burrowing backwards into the sand in the beach but always facing the sea. In laboratory troughs with water they swam backside up exposing their whitish undersides. Their carapace was slate grey. So what met my eyes were 400 crabs swimming in their troughs all around me (I tell school students 'like the host of golden daffodils dancing in the wind' when the poet saw them). This was duly noted down by me as 'lights on' effect on the crab. The activity continued and became more muted after a while. I took this to indicate some manner of 'habituation' and don't recall having given much thought to the matter for the rest of the night. But I do remember talking of the 'lights on' effect to my colleagues but none was so thrilled that he wanted to see it. Atleast not until the next Monday. A compassionate colleague, now in a university in the U.S.A., offered to stay with me for the night to see the spectacular sight. We both came to the aquarium building, again at 21.00 hr, tip-toed inside after opening the

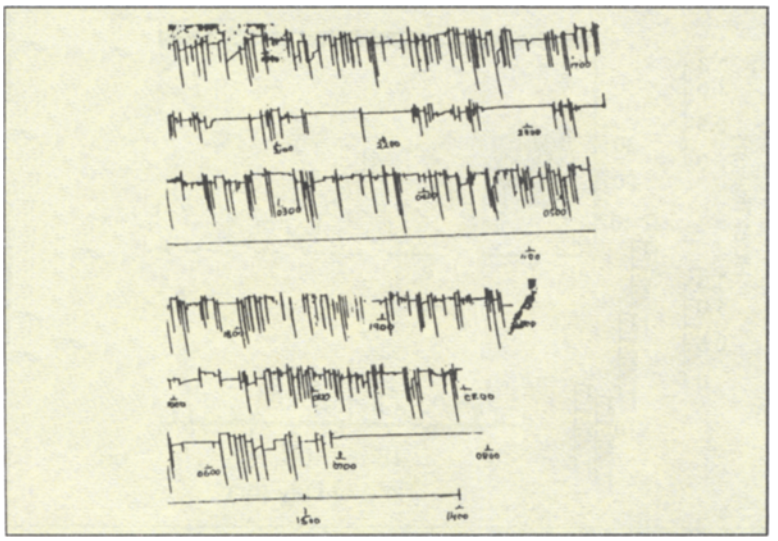

Figure 3 Typical kymograph trace indicating activity of the crab while swimming (vertical markings) and while at rest (horizontal traces).

door with bated breath, and I switched the lights 'on'. There were twenty troughs, with twenty crabs in each, and not a single crab moved! My embarrassment can be imagined. I took a pencil and touched several crabs to verify that they were alive. The crabs, thus perturbed, scampered a few cm and stayed still. A mad crab indeed.

At this stage in the story, I stop to ask my students if they can guess what was happening. At this stage in my research I too ought to have been in a position to shout "eureka, eureka"! But I was a victim of the condition Louis Pasteur described in the words "In the field of observation, chance only favours those minds which have been prepared". Not only was my mind not prepared, it was set against the rediscovery of anything as fairy tale like as *tidal rhythms*. Scientists the world over were skeptical about biological rhythms of any kind in the 1960s. Fortunately for me, I had made the crabs write their own story on kymograph drums (*Figure 3*). The activity traces were 'fixed' in liquid shellac and stored away safely. I had not plotted the data. So I did not know what the mad crab was up to.

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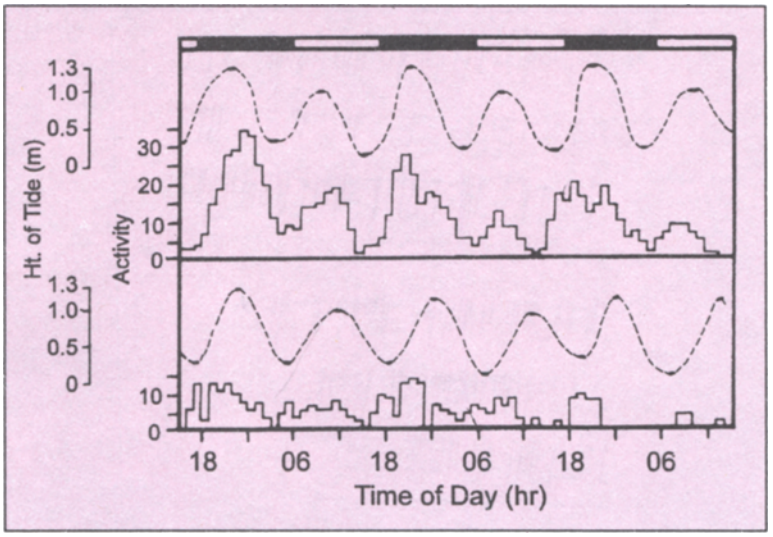


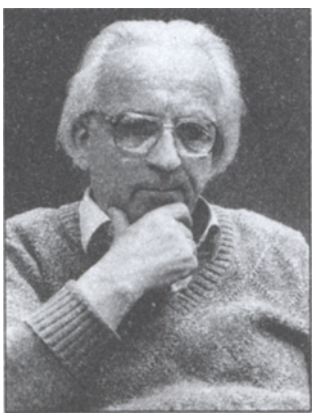
Figure 4 Tidal rhythms in the spontaneous swimming activity of Emerita asiatica *in the constant conditions of the laboratory and its waning over six days. Dark bars denote hours of darkness outside.*

Tidal Rhythms at Last!

Prof. Gnanamuthu later confided in me "between you and me, I say, I don't really believe there are rhythms".

The moment of truth dawned on me some eighteen or so months later when I decided to plot time series histograms from the swimming activity data. I was doing this late into the night and to my considerable surprise lovely and regular peaks and troughs took shape and the rhythms continued for as long as the experiments lasted! The peaks were about 12 hrs apart (*Figure 4*). During troughs the crabs had not moved for 2-3 hrs. Were these tidal rhythms?! The time was 02.00 hr. There was no one in the laboratory. Worse still we did not have a copy of the 'tide tables' published annually by the Geodetical Survey of India from Calcutta. The 'tide tables' gave in hours and minutes the times of high and low tides at various places and the heights of the tides. The first thing I did in the morning was to buy a copy of the tide tables from Lawrence and Mayo in Mount Road, rush back to the lab and draw the smooth tide curves, which filled the activity data. I breathlessly stormed into the room of Prof.Gnanamuthu and blurted out "Sir, tidal and diurnal rhythms in the activity of *Emerita!!* " or something to that effect. He also rejoiced with me and complimented me on having produced a fine piece of research.

Prof E Buenning (1906-1990).



To revert to the queer behaviour of the crabs from one Monday to another Monday. Hindsight tells me that on the first Monday it was high tide at 21.00 hr two miles away on the beach. Since the moon rises 50 min later and the lunar day is 24.8 hr, the high tide would have moved 50 min everyday over the next 7 days and been 7 x 50 = 350 min or about 6 hr later on the second Monday. This means the sea at Madras was experiencing low tide on the second Monday. The crabs on both Mondays were just reflecting or reenacting high tide (high activity) and low tide behaviour (low activity or nil activity) in the laboratory. These tidal rhythms slowly wane with time and disappear after 8-10 days of the constant conditions of the laboratory. Superimposed on this tidal rhythmicity was a nocturnal component which showed itself as exaggerated night time high tide activity. One of the tides in a semidiurnal tidal environment such as Madras must occur during hours of darkness. In the Ph.D. thesis and in my first publication (Z Vergl Physiol 1965) I did not use the word circadian which had been newly coined but used sparingly. Furthermore Prof Gnanamuthu later confided in me "between you and me, I say, I don't really believe there are rhythms". Which explains why my first paper is a single author publication. I then sent an S.O.S. to Prof. Erwin Buenning (1906-1990) with photocopies of the kymograph traces stating that no one in India wanted to believe my story, or that of the crabs. This first chronicler of chronobiology knew what I was up against and wrote back post-haste: "proceed to Tuebingen". The rest, as they say, is history.

It is true that if the tides had not moved 50 min a day, hence making my freshly captured crabs appear erratic in swimming behaviour, I might have rediscovered tidal rhythms earlier. Tidal rhythms, for the record, were first discovered in the behaviour of the flatworm *Convoluta roscoffensis* (Turbellaria) by C Bohn in 1904. The moral of the story may also be: you don't discover any kind of rhythms if you work 9 to 5.

Suggested Reading

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