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Modifications to an optocardiographic method for measurement of heart rate in a range of invertebrate species

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A non-invasive technique developed by Depledge & Andersen (1990) on a crab and a lamellibranchiate was used in this study to measure heart rate activity in a millipede, centipede, spider, two scorpion species, two crab species, three insect species and the garden snail. A novel technique to confine smaller arthropods in an aluminium foil bag provided with a 7 mm by 7 mm opening allowed heart rate measurements to be done on spiders, insects, centipedes and scorpions without direct body contact of the probe. For the crab and the garden snail a plastic device to hold the reflective optocoupler (ROC) probe was glued externally over the heart region. The amplitude of the heart rate signals in 1 mm depth water as medium was about 8% less than those in 1 mm of air. Four millimetres of water as medium reduced the amplitude signal by 90% when compared to zero thickness. With 4 mm of air as medium the amplitude signal decreased by 80%. The erroneous electronic circuit diagram published by Depledge & Andersen (1990) is corrected and redrawn.

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Extrinsic factors that affect physiological functioning in animals include temperature, salinity, oxygen partial pressure, water availability, light intensity and anthropogenic stressors (metals, petroleum hydrocarbons, halogenated hydrocarbons and radionuclides). It is known that these factors have a pronounced effect on an animal's metabolism, particularly on its respiratory physiology (Ansell 1973; Uglow 1973; Vernberg, Calabrese, Thurberg & Vernberg 1977; Bayne, Brown, Burns, Dixon, Ivanovici, Livingstone, Lowe, Moore, Stebbing & Widdows 1985). Kestler (1991) measured discontinuous CO_2 release in insects and found this parameter to be a physiological stress indicator. However, the effects of these extrinsic factors on the heart rate of stressed animals, an important parameter to measure the animal's physiological status, are less well known. Furthermore, the lack of long term heart rate data on arthropods can be ascribed to the fact that the heart rate measurements are done by impedance techniques where the two electrodes are usually inserted through perforations made in the body wall (Buchan, Peck & Tublitz 1988; Ansell 1973; Uglow 1973). Although this invasive technique does not unduly harm larger animals with sturdy carapaces, invasive impedance measurements on smaller animals such as insects and arachnids are less successful. This is particularly true when measurements are to be made for long periods lasting hours or days. For instance, puncturing of the body wall in scorpions results in rapid death owing to an excessive loss of the non-clotting haemolymph (van Aardt 1991). The Depledge monitor (Depledge & Andersen 1990) permits heart rate activity of arthropods and other animals to be measured non-invasively for long periods. It also has the added advantage that the animals can be released in their natural habitat. After a period they can be caught and their heart rates re-measured. In this way heart dysfunction caused by pollu-tants and other external harmful agents can be measured.

In this study, we modified the optical plethysmography system developed by Depledge & Andersen (1990) and used it to monitor heart rate in a range of African invertebrates. To achieve satisfactory performance we modified the circuit diagram published by Depledge & Andersen (1990).

Materials and Methods

Live, mature arthropods collected in the vicinity of Potchefstroom were taken from the laboratory aquarium or terrarium where the animals were kept for at least a few weeks. In Figure 4 the scientific and ordinary names of the arthropods tested, are displayed. The scorpion, *Parabuthus villosus*, was collected in the Namib Desert (Namibia) and kept for several years in the laboratory. Ghost crabs, *Ocypode ceratophthalmus*, were collected at Leisure Bay, Natal South Coast, in December 1994. The heart rate was measured within twelve hours after *O. ceratophthalmus* was caught. For the larger arthropods such as the two crab species and the garden snail the reflective optocoupler (ROC) with probe size 6 × 7 × 7 mm was clipped on to a square polyvinyl chloride holder (Figure 1) glued onto the shell or carapace with cyanoacrylate ('Super glue'). To immobilize the smaller arthropods and scorpions, individual animals were placed inside a bag made out of 0,02 mm thickness aluminum foil. The sides of the bag were gently pressed against the lateral sides of the animal. If a particular area on the body was identified for heart rate measurements, the aluminum foil was opened (7 × 7 mm) at this area. The probe was clamped on a micromanipulator and then carefully lowered down to about 0,5 mm above the opening of the aluminum foil. Slight body movements of the animal will be picked up by the ROC and will be registered as a false heart beat. For the animals investigated, heart rate measurements were made 15 min after the probe was positioned on the animal.

For the two beetle species, the cockroach, and the centipede the pulsating dorsal vessels were visually observed through a stereo microscope. Immediately before optocardiograms were made the pulsating rate of the dorsal vessel of the same animal was measured with a stop watch. For the three

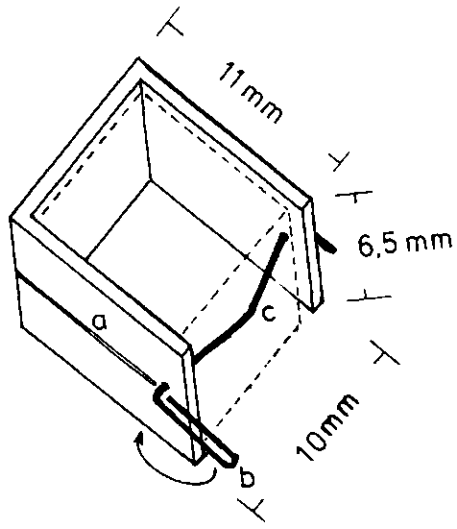

Figure 1 Polyvinyl chloride holder (mass 0,41 g) to insert and fasten the reflective optocoupler (ROC) (mass 0,54 g) on the animal. The holder is glued onto the body surface of the animal with cyanoacrylate ('Super glue'). Broken lines indicate the position of the optocoupler during the measurements. a – stop groove for the wire handle, b – wire handle, c – wire lock.

insects it could be achieved by cutting the wings at their bases. After the wings were removed the pulsating dorsal vessel, usually at the level of the first abdominal segment, could be seen. The contraction rate of the dorsal vessel was assumed to be the same as the pulsating heart (which could not be seen) of the two beetles and the cockroach. These manoeuvres were carried out only to check the heart rates recorded non-invasively by opto-plethysmography. All measurements were done between 23°C and 25°C.

In order to measure the effect of the medium thickness on the amplitude of the electrical signal collected by the reflective optocoupler from the heart, a special polyvinyl chloride holder (Figure 1) was made so that the probe face could be lifted discrete distances above the body or carapace surface. In this manner the thickness of the medium (water, air or carapace) was measured against the electrical signal or amplitude from the reflective optocoupler. The thickness of the carapace was tested for infra red light penetration by inserting an extra piece of carapace (0,79 mm thick) between the probe and the heart.

A 510N oscilloscope (Tectronix, USA) fitted with a D13 dual beam storage, SB10N time base and SA20N differential amplifier was used to record the heart pulses. In order to measure heart rates continuously for 3–4 h to see if the heart rate in the two crab species changes during a rest period, the heart rate was recorded with the optocoupler aided by a computer using the Sable Systems data acquisition hardware and software facilities (Lighton 1991). The oscilloscope images were displayed with a 1 s time base at different voltage (0,1 to 2 V) settings. Drawings were made directly by hand on graph paper from the fixed image on the oscilloscope screen. A new circuit diagram was drawn (Figure 2) to rectify the circuit diagram error published by Depledge & Andersen (1990).

Results

The resistor pair P1 and R5 in the paper by Depledge & Andersen (1990) should connect to pin 2 of the TL074 package. The function of these two resistors is to control the gain of the operational amplifier U1A as depicted in Figure 2. This explains why the circuit as presented by Depledge & Andersen (1990) could not sufficiently amplify the heart signals picked up by the phototransistor. Thus, with the original circuit set at 0,1 V, a very small signal peak of less than 1 mm in height was registered on the oscilloscope compared with more than 50 mm with the newly designed circuit.

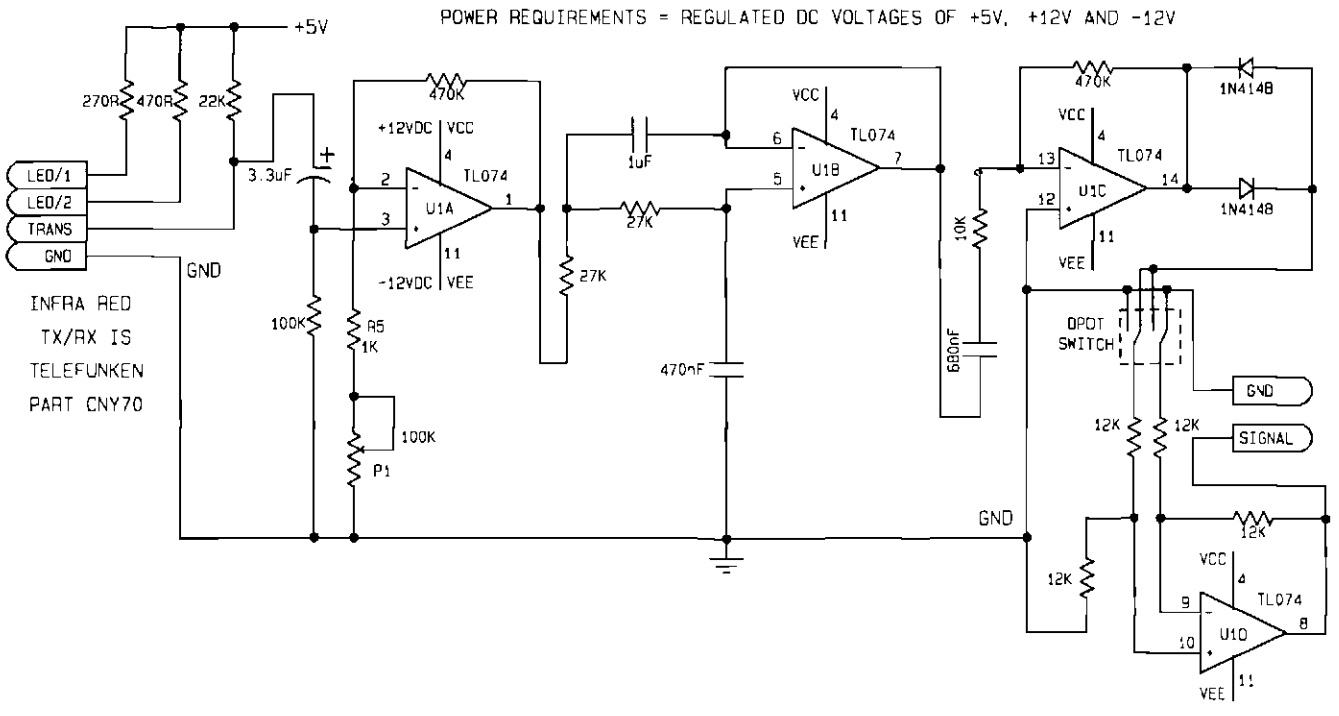

Figure 2 Electronic circuit diagram of the heart rate monitor.

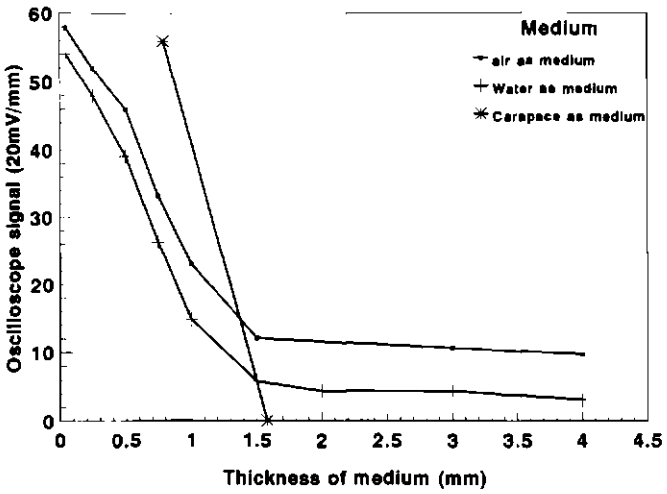

Figure 3 Oscilloscope amplitude signal (20 mV/mm) of the heart pulse made by the reflective optocoupler (ROC) for *Potamonautes warreni* in the three different media. The distance between the ROC face and carapace surface is presented in millimetres on the abscissa. The mass of the crab is 18,9 g live mass and the thickness of the carapace above the heart region is 0,79 mm.

The reflective optocoupler, type CNY70 (7 mm × 7 mm × 6 mm), is made by Telefunken (Germany). The infrared emitter (1,5 mm in diameter) and the photo transistor (1,5 mm in diameter) are embedded on the same surface plane, 2,5 mm apart.

A complete heart rate monitor unit with variable amplification can be made at a university electronic service department for the fraction of the price of commercially available units or using the pulsed-Doppler technique to measure heart rate.

Results of the oscilloscope amplitude signal of the ROC with increasing medium thickness shows (Figure 3) that if the ROC is positioned 1 mm above the carapace surface of the freshwater crab, *Potamonautes warreni*, the amplitude of the optocardiogram decreases 75% in water as medium and 62% in air as medium. At a thickness of 4 mm, signals could still be picked up both in air and in water. As expected, water reduces the amplitude of the heart rate more than air, presumably because of the higher density and lower transparency of water. The thickness of the carapace over the heart region for a 18,9 g freshwater crab is 0,79 mm. This gives an oscilloscope signal amplitude of 56 mm at a 20 mV/mm setting (Figure 3). If a piece of carapace (6 × 7 mm) with a thickness of 0,79 mm was placed directly over the measuring area between the probe and the surface of the carapace no pulses could be recorded. This observation suggests that the extra carapace piece effectively blocks infrared light from reaching the phototransistor. Alternatively, it is possible that the extra piece of carapace scatters the infrared light to such an extent that it can not be picked up by the phototransistor.

The largest heart rate amplitudes recorded were for the spider, the two crab species, and the garden snail (Figure 4). The smallest amplitudes were recorded for the two beetles, the millipede, centipede and the cockroach. In all the animals tested except the millipede the ROC was placed so that the infrared and the phototransistor 'eyes' were lined up with the elongated heart. The millipede optocardiogram could only be measured if the infrared and phototransistor 'eyes' were

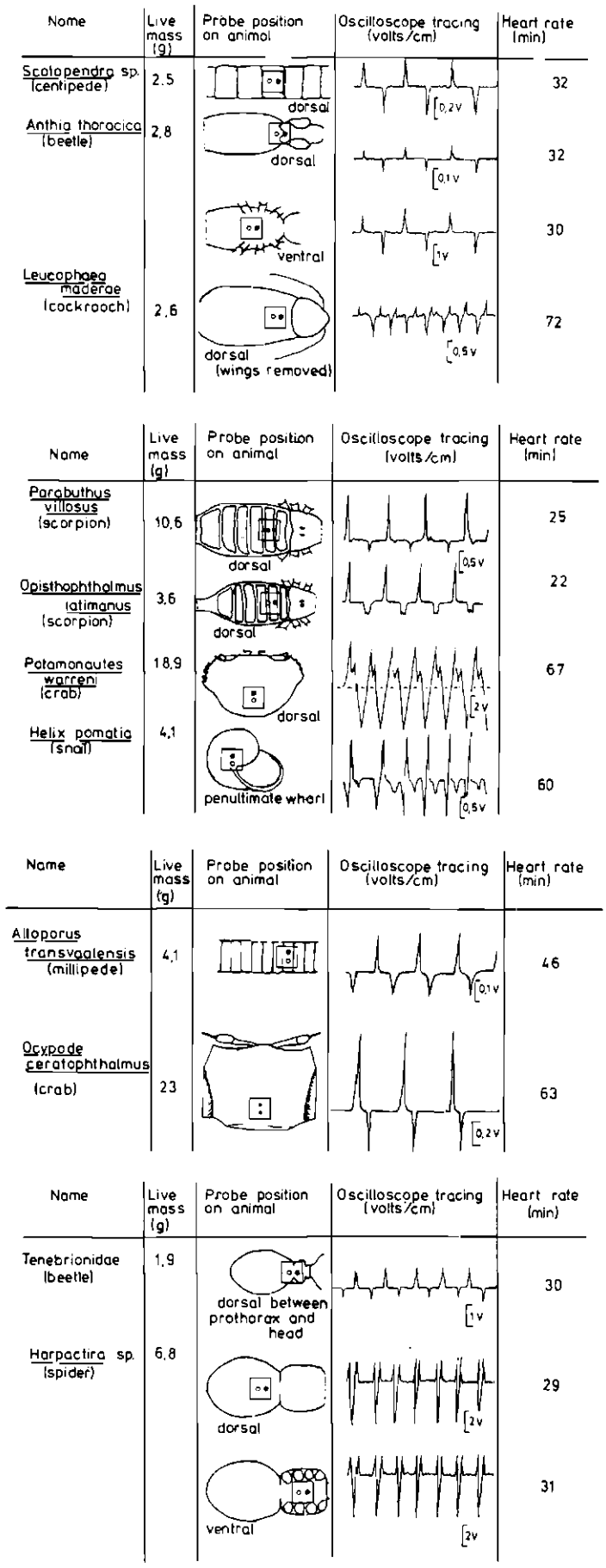

Figure 4 Oscilloscope amplitude signals and body diagrams to show the ROC position on the different animal surfaces. The black circle represents the infrared emitter on the ROC. The fixed image from the oscilloscope screen from each animal was drawn directly by hand on graph paper.

placed 90° across the dorsal heart or aorta (Figure 4).

The results show that if the visual observations of the pulsating rate of the dorsal vessel are compared with the heart rate picked up by the optocoupler no difference in frequency could be found. Furthermore, it is possible to discriminate between pulses originating from the ventilation movements of the insect's body or from the heart of the insect. The pulses originating from the heart of the three insects and the centipede are much smaller in amplitude and about five to ten times faster in frequency than the rhythmic ventilation movements of the animals' bodies.

In the centipede the dorsal aorta was visible along its entire length under the stereo microscope. In this case the visual rhythmic contraction rate of the dorsal aorta correlated exactly with the heart rate measured by the ROC apparatus. No pulses were observed on the oscilloscope when the centipede was measured by the ROC on its ventral side.

The heart rate values found for the different arthropods show considerable variation (Figure 4). The four animals with the highest heart rates, *viz.* the two crabs, the cockroach and the snail, were also active during the measurements, even after the 15 min resting period. The profile of the snail's cardiogram was completely different from cardiograms obtained from insects or scorpions.

The heart rates of animals kept in the aluminum bags rapidly decreased after a 15-min confinement. However, it was found that a sudden tactile stimulus (touching the animal or the bag) in all insects investigated, but not the snail, immediately caused an increase in their heart rates. This increase subsides, after a few minutes, back to normal levels. For *Potamonautes warreni* the heart rate decreased from an average of 67 beats per minute to 15 beats per minute after a 2 h rest period.

Discussion

The effective operative distance in water and air of the ROC is 4 mm. With this knowledge the ROC can be used to measure heart rates of animals without direct contact. This is a considerable advantage because the heart rate of a disturbed animal (handling and vibration) takes time before it returns to resting or normal values. From the results of the infrared light penetration experiment it is clear that hearts lying deeper than 4 to 5 mm below the body surface, including the 0,79 mm thick carapace, can not be reached by the infrared light of the ROC. Hetz (1994) could make heart rate measurements on insect pupae by placing the infrared emitter under the pupa and the receiver on the opposite side.

If the ROC were further miniaturized and the distance of 2,5 mm between the infrared light and the phototransistor eyes reduced to 1,5 mm it would be possible to measure the heart rates in insects as small as 5 to 10 mm in length because the heart in insects always lies just below the cuticle. A prerequisite however, would be to keep the animals motionless during the measurement. The technique of using an aluminium foil bag to immobilize the insect, in combination with the ROC probe fastened to a micro-manipulator, should be exploited further.

Long-term (hours to days) monitoring of the heart rate in arthropods and other small animals was successful using the ROC and a computer as recorder. For the data acquisition a sampling time of 0,05 s for collecting 200 signals was sufficient to discriminate between two consecutive heart rate signals. Long term heart rate measurements are essential in assessment of pollutants or physiological stress. Recent studies have shown that the heart in diapausing insects beats intermittently (Tartes & Kuusik 1994; Wasserthal 1980) and that heart rate and stroke volume in crabs can in time change in opposite directions (Airriess & McMahon 1994)

The uses of this relatively inexpensive heart monitoring system are limitless. In the area of pollutant testing, both in aquatic and terrestrial environments, the lethal and sub-lethal effects of insecticides, for example, on the heart rate of harmful and harmless insects can be measured. Our experience with the Delpedge heart rate monitor on crabs also indicates that this technique can be used to indicate the level of the crab's physiological activity. Thus heart rate data from this investigation show that it usually takes *Ocypode ceratophthalmus*, after exercise, at least 30 min before the heart rate decreases from 360 beats per minute during exercise to 60 beats per minute during rest.

Acknowledgements

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