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M. H. Depledge · A.-K. Lundebye · T. Curtis
A. Aagaard · B. B. Andersen

Automated interpulse-duration assessment (AIDA): a new technique for detecting disturbances in cardiac activity in selected macroinvertebrates

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Abstract An Automated Interpulse Duration Assessment system (AIDA) is described which permits detection of irregularities in cardiac rhythms in selected invertebrates. The sensitivity of AIDA was demonstrated by its ability to detect handling stress in mussels (*Mytilus edulis*) that was not evident when measuring heart rate alone. Changes in cardiac activity patterns of crabs (*Carcinus maenas*) held in the laboratory for up to 10 wk was also examined using the new technique. The frequency distribution of interpulse duration changed significantly as the nutritional state changed. Potential applications of the AIDA system are discussed.

Introduction

In the past, the study of physiological adjustments exhibited by organisms exposed to environmental disturbances has largely been confined to the laboratory (see reviews by Dorigan and Harrison 1987 and Holwerda and Opperhuizen 1991). More recently, technological developments have permitted investigations to be carried out in the field (see for example Aagaard et al. 1995). Some physiological parameters are more amenable to continuous monitoring in situ than are others. In particular, measurements of cardiac activity can be readily obtained (Depledge and Andersen 1990). Heart-rate data are useful in that they can provide an indication of the general well-being of most bivalve molluscs (Coleman 1974) and decapod crustaceans (Cumberlidge and Uglow 1977; Depledge 1985). Indeed, aerobic metabolic activity and nutritional state can often be inferred from heart rates in particular-sized individuals held in standard laboratory conditions.

In addition to ecophysiological investigations, there is growing interest in in situ physiological monitoring as a tool for detecting exposure to chemical contaminants and ensuing toxic effects (Depledge and Andersen 1990; Vedel et al. 1994; Depledge et al. 1995). New techniques now permit recording of physiological data over long periods from several test organisms simultaneously without imposing undue stress. For example, Depledge and Andersen described a computer-aided physiological monitoring system (CAPMON system) capable of recording cardiac activity from several aquatic invertebrates for indefinite periods. Infrared emitters/detectors monitor the heart's cycle of action, and heart rate is recorded every minute and stored on disk for later analysis. This basic system has since been improved and applied to tackle a host of physiological monitoring tasks. Oxygen consumption, locomotor activity and the heart rate can now be monitored simultaneously (Aagaard et al. 1991). Sensors have been applied to bivalves to monitor heart activity and shell opening (Depledge unpublished data), to gastropods (both limpets and whelks) to monitor heart rate and locomotor activity (Aagaard and Depledge 1995), to U-shaped glass tubes containing polychaete worms to monitor ventilatory movements (Vedel et al. 1994) and to barnacles to monitor filtering activity (Depledge unpublished data).

Although the CAPMON system is proving useful and versatile (see for example Vedel et al. 1994 and Depledge et al. 1995), it is limited to providing information concerning means of rate functions (for example, mean heart rates, mean ventilatory rates, etc.). However, this ignores the fact that irregularities not associated with rate function may occur that are of

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M. H. Depledge (✉) · A.-K. Lundebye · T. Curtis
Plymouth Environmental Research Centre and Department of Biological Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA, Devon, England

A. Aagaard · B. B. Andersen
Institute of Biology, Odense University, DK-5230 Odense M., Denmark

biological significance. Detailed examination of heart-rate traces reveals that during some periods of recording beating may be regular, while in other periods it may become irregular. Mean heart rates for each of these periods, however, may be identical. A monitoring system which addresses this problem, in addition to measuring mean rate functions, would be useful.

The present report describes a cardiac monitoring system that has been developed, which constantly monitors interpulse intervals thereby facilitating assessment of the regularity of beating and identification of factors which induce irregularities. Common mussels (*Mytilus edulis*) and shore crabs (*Carcinus maenas*) were chosen as test species to evaluate the sensitivity of the technique.

Materials and methods

Experimental animals

Six mussels (*Mytilus edulis*) and six male shore crabs (*Carcinus maenas*) were collected from the estuaries of the River Exe, Devon and the River Lyhner, Cornwall, UK, respectively, and transferred to the marine aquarium at the University of Plymouth. They were maintained in aerated, filtered seawater at 15 °C and 34‰ S in 20-litre tanks. A 12 h light:12 h dark regime was employed. The animals were acclimated to these laboratory conditions for 1 wk prior to use. Mussels were not fed during either the holding or experimental periods. Crabs were fed only during the last 6 wk of the 10 wk monitoring period.

Transducers

For the mussels, infra-red emitters/detectors (Depledge and Andersen 1990) were glued (Loctite 314) directly to the shell, on the mid-dorsal line just behind the posterior termination of the hinge. Circular collars (~ 1 cm i.d.) were affixed to the cardiac region of the dorsal carapace of the crabs. Transducers were then secured to the collars using small retaining screws. Infra-red light penetrated the mussel shell or the carapace to illuminate the heart. During the heart's cycle of action, varying amounts of light were reflected back to the detector in the transducer. The electrical signal from the transducer was connected via a fine (~ 1 mm diam) flexible cable to a purpose-built interface (see following subsection) linked to a computer. Thus, recordings of cardiac activity were obtained non-invasively, with only minimal disturbance of test animals.

Transducer interface and software program

Fig. 1 shows the circuitry incorporated in the interface, and Fig. 2 the A/D converter.

A software programme written in Turbo Pascal controls the operation of the A/D converter and detects every event (for example, each heart beat) by level detection with hysteresis. All interval (interpulse) durations are saved on disk in real-time format. The starting time for the PC internal clock is stored. When an event occurs, a software timer (loop counter) starts. Each time a new event (pulse) is detected, the count is stored in memory and the loop counter is reset. Up to ten markers can be recorded by typing a number from 0 to 9. These can be used by the researcher to denote the timing of important changes during the course of an experiment (e.g. the addition of a pollutant). Markers are recorded by setting the most significant byte (MSB) in the actual counter (long integer). When the recording session is terminated, the computer's internal-clock value is stored in memory. By dividing the total sum of numbers from the loop counter with the time difference from start to finish, the software timer is calibrated. If the quantity of data exceeds 30000 numbers within a single file, a second file is created with the same name but with an increasing extension (e.g. *.1, *.2). A software programme has been developed to analyse and compress the files into a format facilitating their import into spread sheets such as "Excel" or "Lotus".

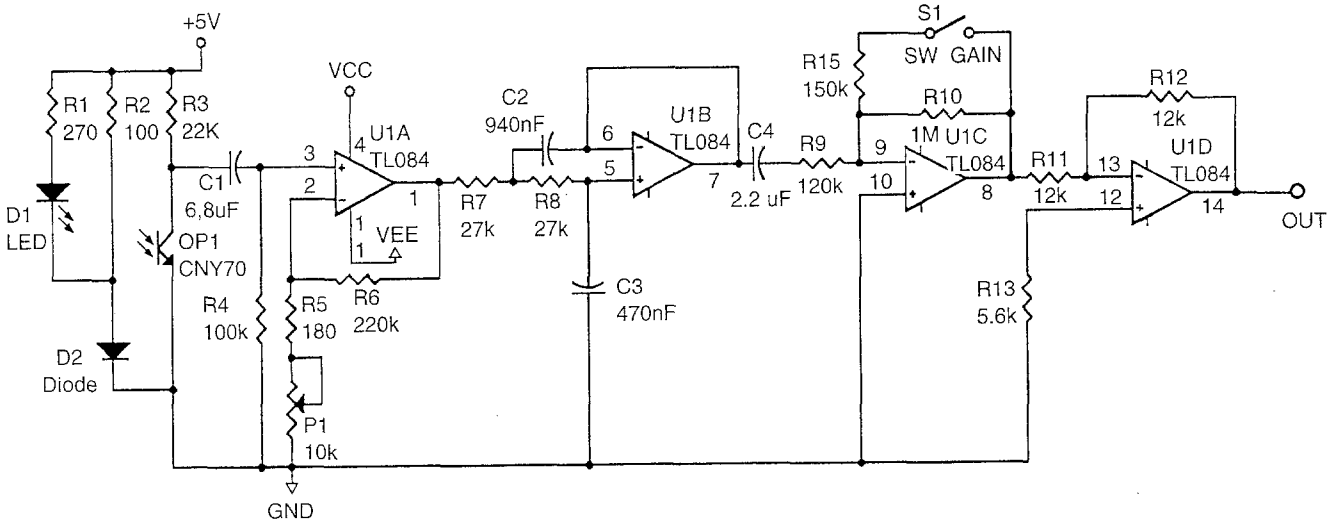

Fig. 1 Layout of circuitry incorporated in AIDA system (*R* resistor; *C* capacitor; *S* switch). Operational amplifier, U1A (part of TL084), is coupled as standard, non-inverting amplifier with adjustable gain (*P1*) from 22 to 1223 times. C1 and R4 are coupled as a high-pass filter with F_{3dB} at 0.23 Hz; U1B is coupled as a two-pole low-pass filter with F_{3dB} at 10 Hz; C4 and R9 act as a high-pass filter with F_{3dB} at 0.13 Hz; U1C is coupled as inverting amplifier with a gain of 1.1 times (S1 closed) or 8.3 times (S1 open). U1D is coupled as buffer, so that best signal to noise ratio can be selected

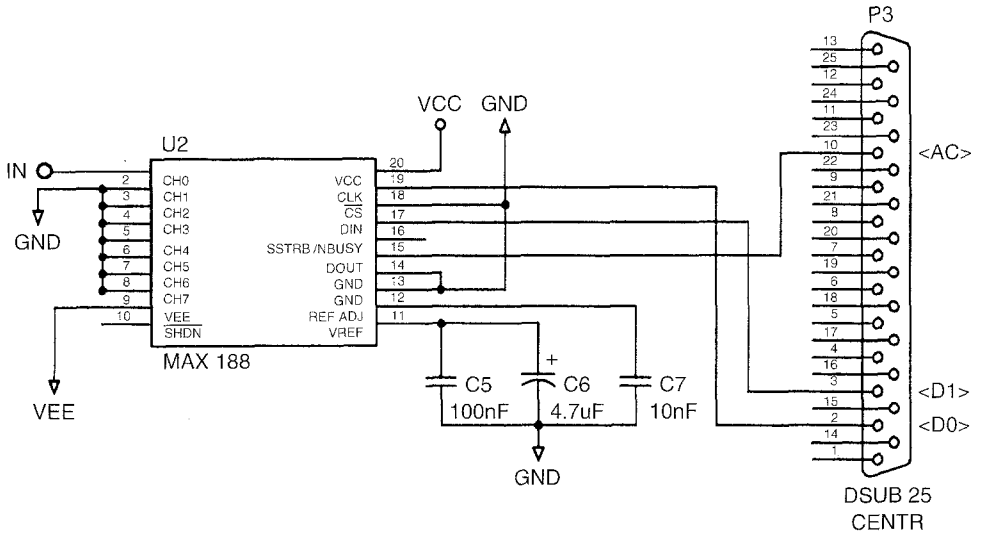

Fig. 2 Coupling of 12-bit eight-channel A/D converter to centronic (printer port). Channel 1 (*CH 1*) receives signal from filter/amplifier section. A/D converter functions as follows. Printer port Pin 2 (Data 0, *D0*) sends clock signals; Pin 3 (Data 1, *D1*) sends serial data to MAX186; Pin 10 (Acknowledge, *AC*) receives serial data from MAX186. A sequence of clock pulses (Pin 2) and data (Pin 3) program MAX186 to start a conversion, and select channel, bipolar mode, single-input mode and internal-clock mode. A sequence of clock pulses (Pin 2) and processed data (Pin 10) constitute output from A/D converter

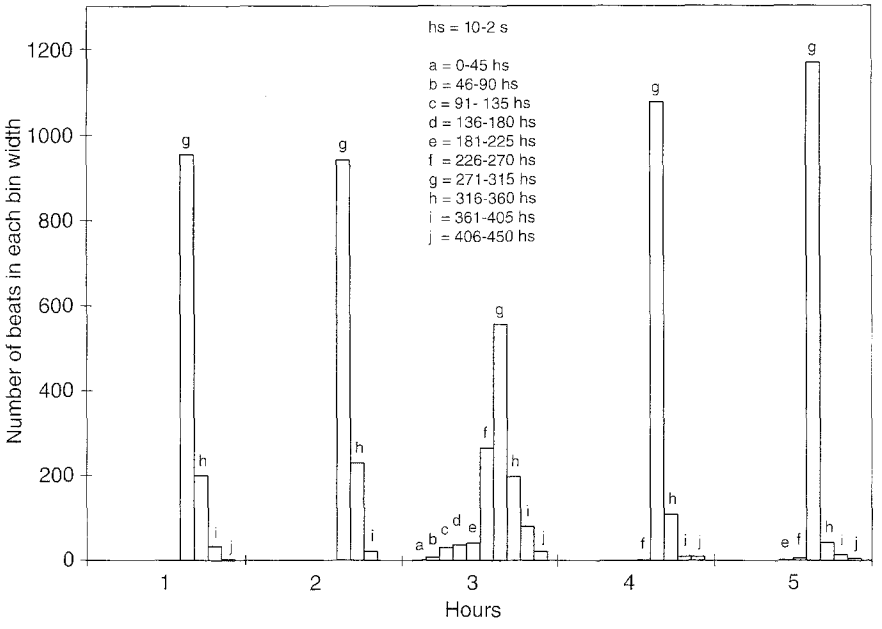

Fig. 3 *Mytilus edulis*. Effect of 30 s handling stress on regularity of heart beats (*Histograms 1 and 2* recordings during 2 h prior to handling; *Histogram 3* during hour immediately following handling; *Histograms 4 and 5* during 2 h recovery period)

Experimental protocols

Mussels. Individuals were held individually in 20-litre tanks. Recordings of cardiac activity were initiated 1 h after transducer attachment. After 12 h the mussels were handled for 30 s, simulating sensor attachment, and this was followed by a further 12 h of recording to monitor their recovery.

Crabs. Shore crabs were transferred from the holding tank to individual 2-litre aquaria following transducer attachment, and left undisturbed for 24 h. Interpulse durations were then recorded for 6 h, after which time the crabs were returned to the holding tank. This procedure was repeated after 4 wk and after 10 wk.

Results

Two versions of the software programme were developed which provided different degrees of accuracy with regard to measurement of interpulse duration. In the first version, the graphic display of the computer is used as an oscilloscope so that each heart beat can be visualised. Two adjustable horizontal lines on the screen can be positioned to ensure triggering of the counting mechanism. If the heart-beat trace passes

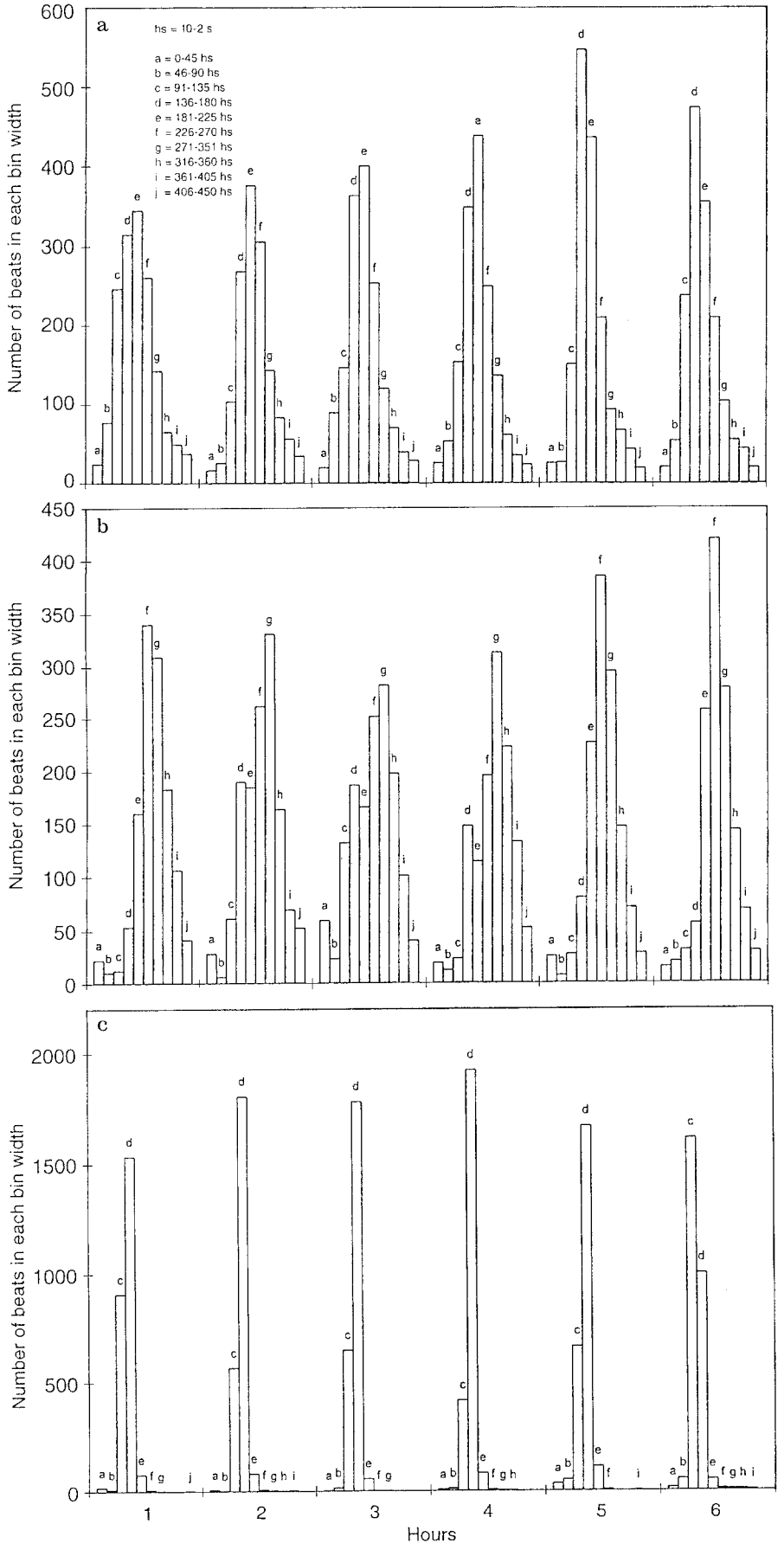

Fig. 4 *Carcinus maenas.* Frequency distribution of interpulse intervals recorded for three 6 h periods: 24 h (**a**) 4 wk (**b**) 10 wk (**c**) after transducer attachment

through both upper and lower trigger-levels, the beat will be counted. Both graphic- and audio-trigger indicators confirm that the beat has been recorded.

Fig. 3 shows a typical frequency distribution of interpulse intervals recorded for *Mytilus edulis* prior to and following a brief period of handling. Data are presented for a 5 h block of time; 2 h before handling (Histograms 1 and 2), 1 h immediately following handling (Histogram 3) and during 2 h recovery (Histograms 4 and 5). Preceding the handling period, the interpulse durations were very regular, with the majority falling between 271 and 315 10^{-2} s. Clearly, heart beats became irregular after handling stress; this is depicted by the wider distribution of interpulse intervals in Histogram 3. Histograms 4 and 5 demonstrate that when the mussel was allowed to recover undisturbed, the distribution of interpulse intervals rapidly returned to normal. The trends displayed in Histograms 1, 2, 4 and 5 are characteristic of recordings made before and after the 5 h period, therefore the remaining data are not shown. Despite the effect of handling on heart-beat regularity, there were no significant differences in mean rates between any of the 1 h recording periods.

Fig. 4 presents typical data for a single *Carcinus maenas*, and shows the distribution of interpulse intervals for six successive hourly recordings. These were initiated 24 h, 4 wk and 10 wk after transducer attachment (Fig. 4). The shapes of the frequency distributions within each 6 h recording period were relatively constant. However, it is noticeable that the distributions altered between the various monitoring times. Intervals between heart beats were irregular 24 h and 4 wk after sensor attachment. Over the 4 wk period, the heart beats became more variable, with standard deviation around mean interpulse interval increasing from 9.9 to 14.9 10^{-2} s. This was also accompanied by a decrease in heart rate, with mean interpulse duration changing from 224 to 263 10^{-2} s. After 6 wk recovery, during which the crabs were fed weekly, the heart beats became increasingly regular (standard 5.9 10^{-2} s). Furthermore, the heart rate was elevated, with mean interpulse duration falling to 142 10^{-2} s

Discussion

The concept of measuring irregularities in cardiac activity to detect chemical imbalances is relatively new. Medical scientists have found it useful in the detection of the early onset of diabetes in children (Ewing 1992), where changes in excitable cell function occur. There is also considerable interest in developing the application of chaos theory to determine when to administer drugs to control cardiac arhythmias (Garfinkel et al. 1992). To date, studies of this kind have received little attention from comparative physiologists. This is perhaps surprising in view of the extensive database which is available concerning cardiac and other rate-function abnormalities in lower animals.

All molluscan hearts studied to date are capable of beating rhythmically and spontaneously in isolation or when denervated (i.e. they are myogenic: Jones 1983). Heart rate and the regularity of beating are determined by spontaneously depolarising, diffuse pacemaker cells (Krijgsman and Divaris 1955; Hill and Welsh 1966; Irisawa 1978), although chronotrophic and inotropic control of the heart is also partially mediated by nervous innervation. The heart of *Mytilus* spp., as well as the hearts of other bivalves, is innervated by nerves from the visceral ganglion (Carlson 1905a). Electrical stimulation of the ganglion or visceral commissure results in acceleration or depression of cardiac activity (Carlson 1905b; Greenberg 1970), suggesting that the heart is innervated by at least two types of nerves, excitatory and inhibitory (Stefano 1990). The principal cardioexcitatory and cardioinhibitory transmitters in molluscan hearts are thought to be, in general, 5-hydroxytrypamine (5-HT) and acetylcholine (ACh), respectively (Stefano 1990). In addition, the neuropeptide FMRF-amide (phenylalanine-methionine-arginine-phenylalanine-NH_2) and catch-relaxing peptide may also be important in regulating cardiac activity in *Mytilus* spp (Painter and Greenberg 1982; Hirata et al. 1989).

In decapod crustaceans, stimulation to contract is provided by rhythmic impulses from neural pacemaker cells in the cardiac ganglion, located on the inner dorsal wall of the heart. Heart rate and regularity of beating are determined by two pairs of acceleratory fibres and one pair of inhibitory fibres (Cumberlidge and Uglow 1977). Nervous impulses are mediated by chemical neurotransmitters. Since the decapod heart is under neurogenic control, cardiac output depends largely on the activity of neurons within the cardiac ganglion (Maynard 1960). However, cardiac activity can also be modified by hormones released from the pericardial organ (PO), a bilaterally symmetrical group of nerves situated in the lateral pericardial sinus (Maynard and Welsh 1959; Kravitz et al. 1980; Cooke and Sullivan 1982). The POs of crabs are known to contain five cardioexcitatory compounds. These include the biogenic amines 5-HT, dopamine and octopamine (Cooke and Goldstone 1970; Evans et al. 1975; Sullivan et al. 1977), the pentapeptide proctolin (arginine-tyrosine-leusine-proline-threosine), and crustacean cardioactive peptide (custeine-asparagine-alanine-phenylalanine-threonine-glycine-cysteine-NH_2; Sullivan 1979; Stangier et al. 1986, 1987; Wilkens and Mercier 1993). Another hormone released by the pericardial glands may have an inhibitory and stabilising effect on cardiac activity (Alexandrowicz and Carlisle 1953). Since hormone-induced changes are longer-lasting than those caused by cardioregulatory nerve stimulation, it has been suggested that such hormones may mediate changes in response to environmental stressors or changes in metabolic demand (McMahon and

Wilkens 1983; Wilkens 1987; Wilkens and Mercier 1993).

The systems controlling cardiac activity in molluscs and decapod crustaceans can be influenced by a wide variety of natural and anthropogenic agents. For example, changes in the heart rhythm of bivalve molluscs that result from exposure to air have been studied in *Mytilus edulis* (Helm and Trueman 1967; Coleman and Trueman 1971). Aerial exposure of *M. edulis* results in partial or complete valve closure. This is accompanied by reduced heart beat frequency (bradycardia) and occasionally leads to a complete suppression of the heart beat (Helm and Trueman 1967). Prolonged valve closure during immersion produces the same effects on heart rate (Coleman 1973). This may occur, for instance, when bivalves are exposed to pollutants (Scott and Major 1972; Akberali et al. 1981). Bradycardia has also been identified as a response to a reduction in the oxygen tension of the water in the mantle cavity (Trueman and Lowe 1971).

In decapod crustaceans, Cumberlidge and Uglow (1977) identified three major patterns of cardiac activity associated with different degrees of sensory input: (i) Elevated beating: rapid, regular beating associated with severe disturbance (i.e following handling, etc); (ii) active beating: continuous regular beating associated with routine behaviour, activity and mild to moderate disturbance (for example, movement of another crab in close proximity); (iii) resting beating: arhythmic, slow beating in undisturbed, inactive crabs. Superimposed on these basic patterns are the effects of a number of other modulating factors. For example, feeding is associated with a rapid transition to regular beating, while exposure to severe hypoxia results in bradycardia and an increased frequency of cardiac arrests (Depledge 1985). Starvation is associated with slow, irregular beating, although disturbance will induce short periods of more rapid, regular beating (Depledge 1985). With regard to chemical contaminants, exposure to 10 mg $Cu^{2+}l^{-1}$ results in arhythmic beating and a transitory decline in heart rate, while exposure to 1 mg $Hg^{2+}l^{-1}$ results in even more pronounced arhythmia and bradycardia (Depledge 1984).

The AIDA system has proved easy to use and has enabled long-term cardiac recordings (several weeks) to be obtained from mussels (*Mytilus edulis*) and crabs (*Carcinus maenas*) in the laboratory. It was evident that the test animals were not unduly disturbed by the transducer attachment procedure. For instance, when sensor attachment was mimicked in mussels, short-lived changes in interpulse durations were detected, and the mean heart rate was unchanged. This is an important demonstration of the improved sensitivity of the new monitoring system. Interpulse duration appeared to reflect the nutritional state of the crabs, which were not fed during the 1 wk period of acclimation to laboratory conditions or for the first 4 wk of the experimental period, but were fed at weekly intervals for the last 6 wk of the study. The results indicate that starvation is associated with increasing irregularity of beating as well as a general decline in heart rate in *C. maenas*. This is consistent with the earlier findings of Depledge (1985), although the mechanisms underlying such changes have yet to be elucidated.

Since the non-invasive, infra-red transducer system is the same as that used in the CAPMON system (Depledge and Andersen 1990), it is reasonable to assume that studies with the new system will be feasible with organisms in situ where the CAPMON system has also proved effective (Aagaard et al. 1995). Despite the increased sensitivity of the AIDA system, a current limitation for in situ studies is that only one animal can be monitored at a time, compared to four with the CAPMON system. However, a 16-channel version of AIDA is under development which will run on a portable 486dx, 33Mhz, 4MB computer. In common with the CAPMON system, AIDA has excellent potential as a "biological early warning system" (BEWS). In contrast to other biomonitoring techniques, BEWS are specifically designed to provide a rapid response to the presence of toxic pollutants within a period of minutes to hours (Baldwin and Kramer 1994). To date, the development of BEWS for the detection of pollutants has centred upon the freshwater environment, particularly for industrial-effluent monitoring (Gruber and Diamond 1988). However, the versatility of the AIDA system would also permit biomonitoring in marine and estuarine conditions. In addition to cardiac monitoring, the AIDA system is suitable for a host of physiological monitoring tasks and can be applied to a wide range of aquatic and terrestrial invertebrates including bivalve molluscs, decapod crustaceans, polychaete worms and insects. The sensitivity of the technique is currently being explored with regard to the influences of locomotor activity, nutritional state, thermal and salinity stress and pollutant exposure on the regularity of heart beat in a variety of aquatic invertebrates.

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