

## Time Series Analysis and Model of Heart-Beat Rate, Oxygen Consumption, and Locomotor Activity from the Sand-crab *Portunus pelagicus* (Linnaeus)

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### ABSTRACT

Auto-correlation, cross-correlation, and Fourier transform decomposition were applied to data on parameters of perfusion, respiration, and behaviour in *P. pelagicus*. Cardiac activity, although discontinuous in buried crabs, formed a regular alternating time-series with a stable periodicity. Cross-correlation showed a close temporal linking between cardiac activity and oxygen consumption over 24 hours. Similar temporal patterns were observed in locomotor activity. Pausing (bradycardia) and pulsing (tachycardia) were investigated by "power spectrum" analysis and summarised in the form of a Fourier transform model.

**Abstracting keywords:** *Portunus pelagicus*, crab, heart-rate, oxygen consumption, behaviour, time-series, analysis, modelling.

### INTRODUCTION

*Portunus pelagicus* in common with many other large decapod crustaceans are remarkable in their ability to stop their hearts for short periods, and furthermore to maintain a regular pattern of heart stoppages over extended periods. This phenomenon, termed "pausing" (McMahon and Wilkens, 1972), consists of a period of bradycardia associated with cessation of ventilation, followed by a period of tachycardia which coincides with resumption of ventilation. As pointed out by McMahon and Wilkens (1977) from studies of *Cancer productus* and by Taylor (1984) from studies of *Atelecyclus rotundatus*, pausing and pulsing appears to be associated with aerobic metabolism. Furthermore the regular pattern was seen in undisturbed crustaceans but not in active or exercising animals (Ansell, 1973; McMahon and Wilkens, 1977; Bradford and Taylor, 1982; McMahon and Wilkens, 1982; Gribble, 1989). Oxygen consumption during pausing and pulsing reported from *Cancer pagurus* was reduced by between 30%

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(Bradford and Taylor, 1982) and 40–50% (Burnett and Bridges, 1981) compared with that during continuous cardiac and ventilatory activity.

Analysing discontinuous heart-beat data using standard statistical methods is difficult as the minute-counts ("heart rate" in beats per minute) are bi-modally rather than normally distributed, they are not random, and successive counts are not independent of one another. The demands of aerobic metabolism and haemolymph circulation mean that cardiac activity cannot be truly random; a beat-rate at any instant will depend in part on the previous rate and will in turn influence successive rates. Time-series analysis is most appropriate in this instance as observations do not need to be normally distributed and dependence through time is an underlying assumption of the method.

The general strategy of the analysis follows that recommended by Broom (1979), and Anderson & Grenfell (1984) for the treatment of biological data. Use was made of time-plots, auto-correlations, and correlograms for the description of periodicity within the data. Chatfield (1980) considered that auto-correlation techniques are "fairly simple" and will often detect the main properties of a given series. In the study reported here once periodicity was detected from the auto-correlations then more precise analysis of the predominant frequencies present was carried out using spectral or "power spectrum" techniques. The fitting of auto-regressive, moving average, or mixed higher order models to the data was used sparingly and kept to simple forms where such models were deemed appropriate.

In the time domain, obvious areas of interest were the periodicity in 24 hour patterns of cardiac, metabolic, and locomotor activity. In the frequency domain, the predominant frequencies present in a pulse of cardiac activity were of interest in terms of the haemodynamics and energetic efficiency of the cardiac system.

#### MATERIALS AND METHODS

The apparatus used for the laboratory observation of crab behaviour, oxygen consumption and heart-beat consisted of:

(i) The respirometer; a clear perspex cylindrical chamber, 460 mm in diameter and 510 mm high. Water temperature was controlled at 21°C and a 100 mm deep sand substratum was provided to allow the crab to bury. Oxygen depletion was measured using a Clark electrode connected to an oxygen, temperature, and salinity Analyser (TPS model 2052A). The respirometer operated as a multiple cycle "closed bottle", with oxygen automatically replenished when the concentration fell below a preset minimum. It was found that the oxygen concentration could fall to 70% of full saturation with no effect on crab behaviour or heart-beat rate.

(ii) Closed circuit video was used to monitor crab behaviour. The chamber was continuously illuminated by a single PHILLIPS Red fluorescent tube, and illuminated on a Light:Dark 12.5:11.5 hour regime by two ceiling-mounted 40 watt PHILLIPS white fluorescent tubes (with diffusers). The light level at the outside wall of the chamber was 5 Lux during the "night" and 10 Lux (approximately 0.2  $\mu\text{mol}/\text{m}^2/\text{sec}$  photon flux) during the "day" measured by a GOSSEN lightmeter. (See Gribble (1989) for the details of the spectra emitted by these tubes).

(iii) A NARCO BIOSYSTEMS Physiograph (Mk III) fitted with an impedance coupler (Type 7212) provided a chart-paper record of the crab heart-beat. Impedance coupler output was also fed to a signal conditioning circuit which converted each beat to a digital pulse. A micro-computer (OHIO SCIENTIFIC SBII) was used to count the pulses, integrate the count over 1 minute and to dump the results to magnetic tape every 30 minutes.

To calculate the oxygen consumption of the crab it was necessary to perform a calibration run, with the crab removed, at the end of each observation run. The depletion due to the sandbed micro-organisms was subtracted from the combined (crab + sandbed) depletion record.

The apparatus used for the observation of crab locomotor activity consisted of: (i) The observation aquarium; a cylindrical tank, 3 m diameter by 0.6 m high with a 100 mm deep, washed beach sand substratum (after Eales (1972).) Filtered, aerated sea water was forced up through the substratum to ensure that it did not become anaerobic.

(ii) A ceiling mounted video camera connected to a remote Time Lapse Video Recorder. The aquarium was continuously illuminated by six red 80 watt "SYLVANIA PORTA FLOOD" lights, and illuminated on a Light:Dark 12.5:11.5 hour regime by four ceiling mounted 40 watt PHILLIPS white fluorescent tubes.

#### Method of external electrode placement (impedance cardiogram)

The active electrode consisted of a 3 mm spiral of bared copper wire coated with ECG electrode gel and sealed to the surface of the dorsal carapace, directly above the anterior ventricle. The innominate electrode consisted of a 20 to 30 mm diameter loop of stainless steel wire bent to conform to the longitudinal groove in the ventral thorax and to fit between the groove and the abdominal flap. Electrodes were connected to the impedance coupler via light-weight insulated copper wires and a loose string harness restricted the chelae, preventing the crab from cutting the wires.

#### Experimental protocol

Heart beat recordings were made from 16 buried (undisturbed) *P. pelagicus* to establish baseline heart beat patterns. Continuous data were collected on heart

beat rate, oxygen consumption and the corresponding behaviour of 4 crabs observed for 48 hours. These observation runs were representative of the observations made throughout the study. In order to minimise individual variation the crabs were matched in terms of size, sex and moult stage.

Time-lapse video recording of 4 crabs in the large aquarium for 48 hours provided a "continuous variable" measure of locomotor activity which could be related directly to field conditions (see Eales, 1972; Gribble, 1989).

#### Analysis and presentation of data

Extensive use was made of the Econometrics and Time-series (ETS) procedure library of the SAS (v.4) statistical analysis package, SAS Institute Inc. In particular the IDENTIFY, and to a lesser extent the ESTIMATE routines of the ARIMA procedure were used to analyse the 24 hour data. The outputs from these routines were a correlogram, sample partial auto-correlation, sample inverse auto-correlation and a cross-correlation if two time series were specified. A chi-square test was automatically applied, testing the hypothesis that the data were different from a random distribution of data points within the time series (SA ETS manual, Anon. 1984). Heart-beat rate and oxygen consumption data were log transformed and each series was centred by the subtraction of its sample mean (following Anderson and Grenfell, 1984, p 590). In both laboratory and large aquarium data sets, observations were standardised to 30 minute means; making 96 data points per 48 hours (and therefore 95 possible lags). Interpretation of the auto-correlations was according to Chatfield (1980, p 25-30 and p 63-64).

Spectral or "power spectrum" analysis was performed with the aid of the SAS SPECTRA procedure which tabulates: the frequency, period, sine and cosine coefficients, as well as the spectral density and periodogram co-ordinates (SA ETS manual, Anon. 1984). Plots of spectral density against frequency and periodogram against either period or frequency were produced. Periodogram ordinates were smoothed using a moving average routine with weighting that was symmetrical and scaled such that weights added  $1/4$ ,  $1/2$ ,  $1/4$ , with the middle weight applied to the current periodogram ordinate. Appropriate weights were selected by trial and error following the selection process used by Anderson and Grenfell (1984). The integrity of the input data was tested within the SPECTRA procedure by Fisher's Kappa and Bartlett's Kolmogorov-Smirnov test statistics.

Comparison between patterns of cardiac activity and oxygen consumption observed in the laboratory and patterns of locomotor activity seen in the large aquarium provided a check for laboratory induced artefacts.

## RESULTS

### (i) Basic patterns in heart-beat

All 16 buried crabs showed a characteristic and regular pattern of "pausing" (bradycardia) followed by "pulsing" (tachycardia) (see examples Fig. 1). A slightly different pattern of pausing and pulsing was observed from each buried

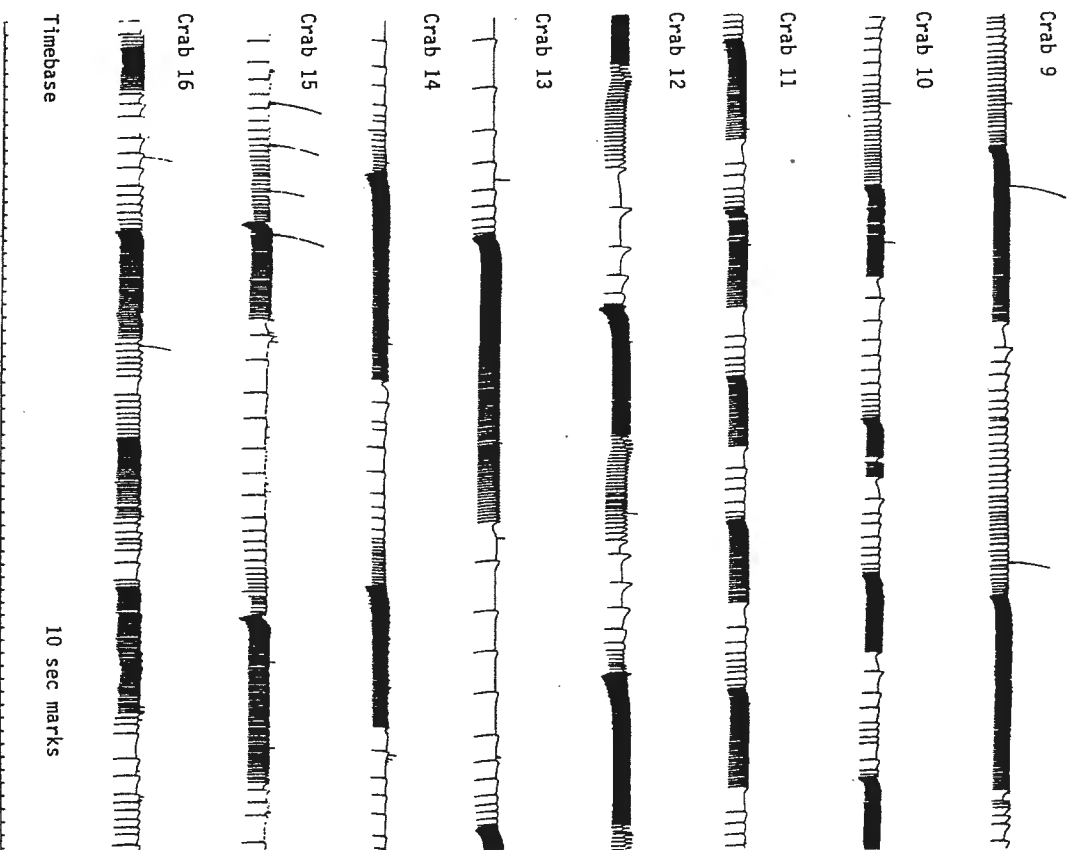


Fig. 1. Examples of the heart-beat of individual *P. pelagicus*, recorded by impedance cardiography, showing the regular pattern of "pausing" (bradycardia) followed by "pulsing" (tachycardia).

individual. The heart beat records of routinely active crabs also showed pauses but the pattern was irregular with a wide variation in the duration of both pauses and pulses. Auto-correlation was performed on 150 minute sections of the heart-

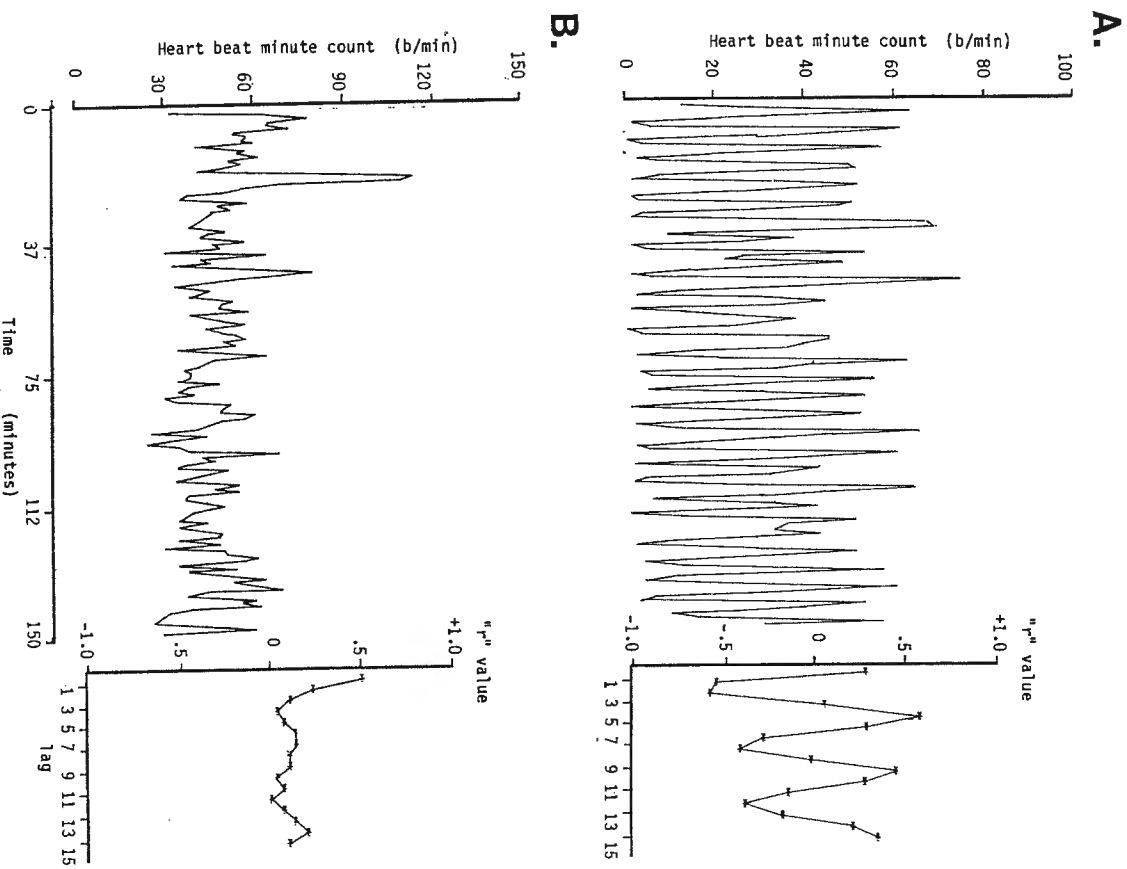


Fig. 2a. Heart-beat minute count of a quiescent (buried) *P. pelagicus*, with insert showing the correlogram derived from an auto-correlation of the data at 1 minute lag intervals.  
Fig. 2b. Heart-beat minute count of a non-quiescent (emerged) *P. pelagicus*, with insert showing the correlogram derived from an auto-correlation of the data at 1 minute lag intervals.

beat record characteristic of crabs when buried and routinely active. Minute-count time-plots and correlograms highlight the regularity in the pattern of pausing and pulsing while buried compared with the irregularity observed when routinely active (see Fig. 2a & b).

#### (ii) 24 hour patterns; Laboratory observations

The cross-correlations of heart-beat rate against oxygen consumption (example Fig. 3) showed a strong positive correlation at zero lag and at approximately 24 hours before and after zero lag, with a peak negative correlation at approximately 12 hours before and after zero lag. The accompanying time-plots give a visual confirmation of the close relationship of heart-beat rate, oxygen consumption and crab behaviour (emerged versus buried) through the diel cycle.

As with the cross-correlations, auto-correlations displayed a tendency for blocks of coefficients to occur on alternate sides of the overall mean at approximately 12 hour intervals; i.e., following a roughly sinusoidal oscillation, centred

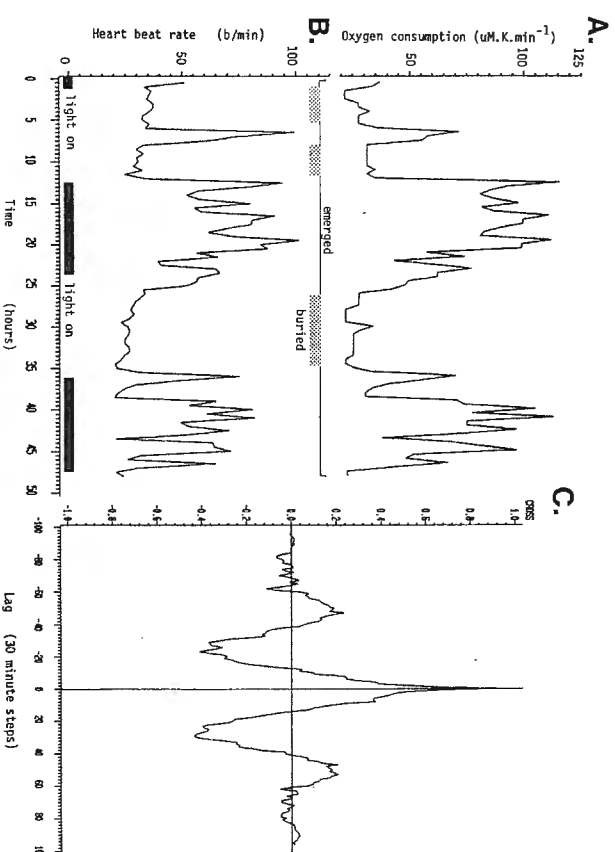


Fig. 3. a. A typical 48 hour record of oxygen consumption and activity of *P. pelagicus*:  
b. the corresponding 48 hour record of heart-beat rate;  
c. the cross-correlogram derived from the cross-correlation of oxygen consumption with heart-beat rate.  
Note: Crab activity (emerged or buried) and light regime is shown as horizontal bars across the base of the plots.

around zero correlation, which became damped with increasing lags. The general form of each correlogram was similar and all followed the form of an "alternating time series" as defined by Chatfield (1980).

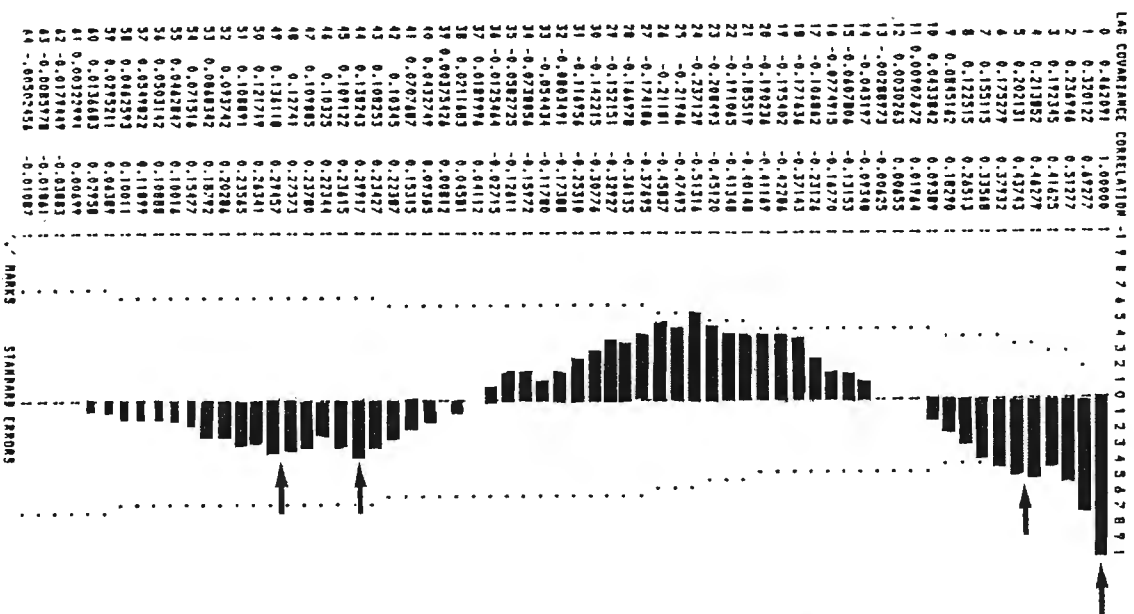


Fig. 4. A typical correlogram derived from the auto-correlation of 24 hours of heart-beat data from *P. pelagicus*. (Arrows point to the double peak observed within the main correlation peak).

An interesting aspect of the correlograms was the presence of an apparent double peak within the main correlation peak in each crab record (Fig. 4). A possible explanation is the presence of two slow oscillations of the same frequency but slightly out of phase.

### (iii) 24 hour patterns; Time lapse video

Locomotor activity data was scored as average distance moved per 30 minute interval and auto-correlations were performed on the resultant data set. Data were log transformed and centred in the same way as the heart-beat and oxygen consumption data.

An approximate 24 hour periodicity was apparent in the correlograms, however the correlation coefficients were relatively weak (less than  $r = .21$ ). Furthermore, the spectral and periodogram plots were not conclusive for these data. What they did illustrate was the variability in 24 hour behaviour patterns that can occur between individual crabs and within the record of a single crab.

### (iv) Spectral analysis and modelling of a cardiac activity pulse

The impedance record from Crab 4 was typical of a buried crab and spectral analysis was applied to its heart-beat "minute counts" (see similarity of basic patterns in Fig. 1.). A 350 minute section of data was selected and preliminary auto-correlation analysis was carried out. Data were centred about the mean and smoothed (see methods) for ease of computation but were not otherwise transformed (see Fig. 7 for raw data).

It was apparent from the periodogram plots (Fig. 5) that the underlying waveform in the data had at least two components, shown as two major spectral peaks at 0.14 cycle min<sup>-1</sup> (period = 7 minute 8 sec) and 0.15 cycle min<sup>-1</sup> (period = 6 minute 32 sec) respectively. A pause/pulse cycle could be modelled descriptively, therefore, as a "squared-up" sine wave with a "filtered" period of approximately 7 minutes, peak to peak.

The major peaks were composed of a complex of 5 narrow frequency bands the coefficients of which were substituted into an expansion of the Fourier transform equation such that:

$$\begin{aligned}
 X_T = & 35 + 20.42 * \text{COS}((T-1) * .8976) + 7.22 * \text{SIN}((T-1) * .8976) + \\
 & 2.54 * \text{COS}((T-1) * .8796) - 18.61 * \text{SIN}((T-1) * .8796) + \\
 & 11.43 * \text{COS}((T-1) * .9515) - 7.08 * \text{SIN}((T-1) * .9515) + \\
 & 10.36 * \text{COS}((T-1) * .9335) + 7.33 * \text{SIN}((T-1) * .9335) - \\
 & 6.39 * \text{COS}((T-1) * .9694) - 10.14 * \text{SIN}((T-1) * .9694),
 \end{aligned}$$

where  $T = 1, 2, \dots, 350$  minutes, and

$X$  is in beats min<sup>-1</sup>.

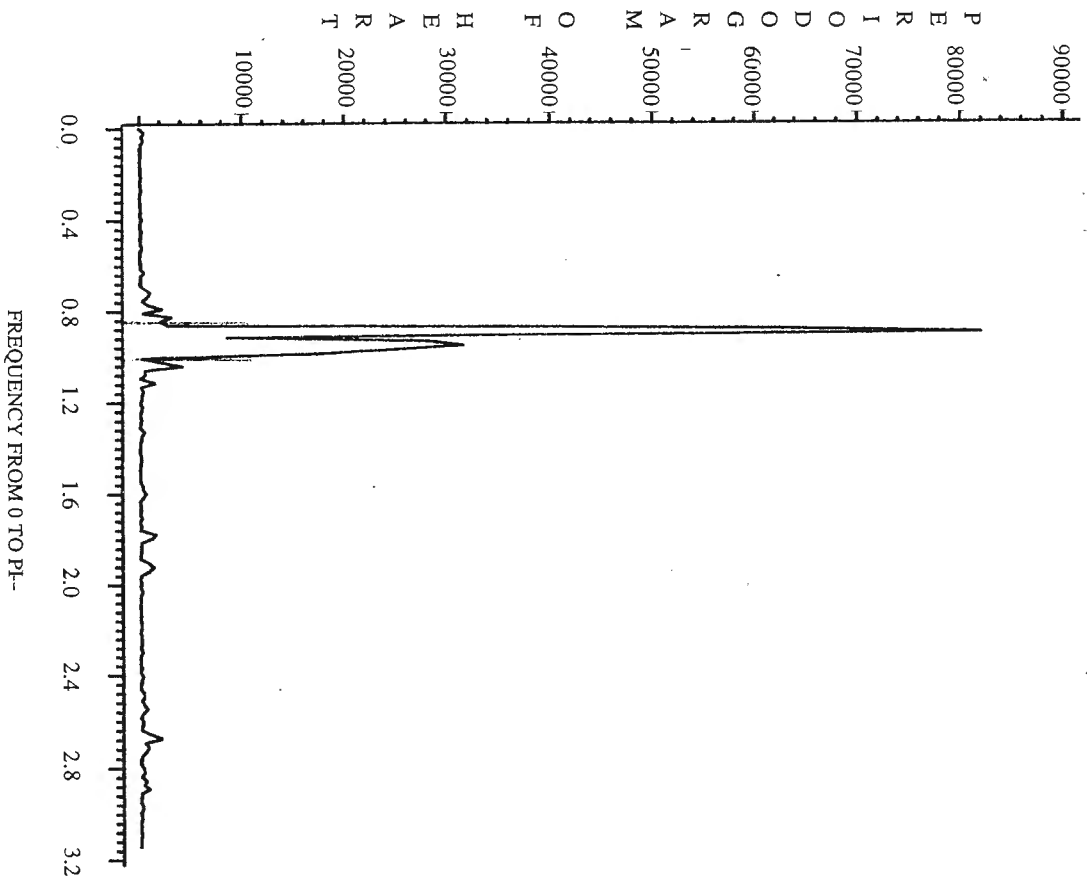


Fig. 5. A typical periodogram of 350 continuous heart-beat minute counts from a quiescent *P. pelagicus*. Note: To convert frequency to period  $P = 2\pi/\text{freq}$ .

(v) Simulation of a pulse "train"

The Fourier model was used to simulate 350 minutes of heart-beat data sampled at minute intervals which were plotted and are presented, together with the original observed data, in Figs. 6a & b. The similarity between the simulated and

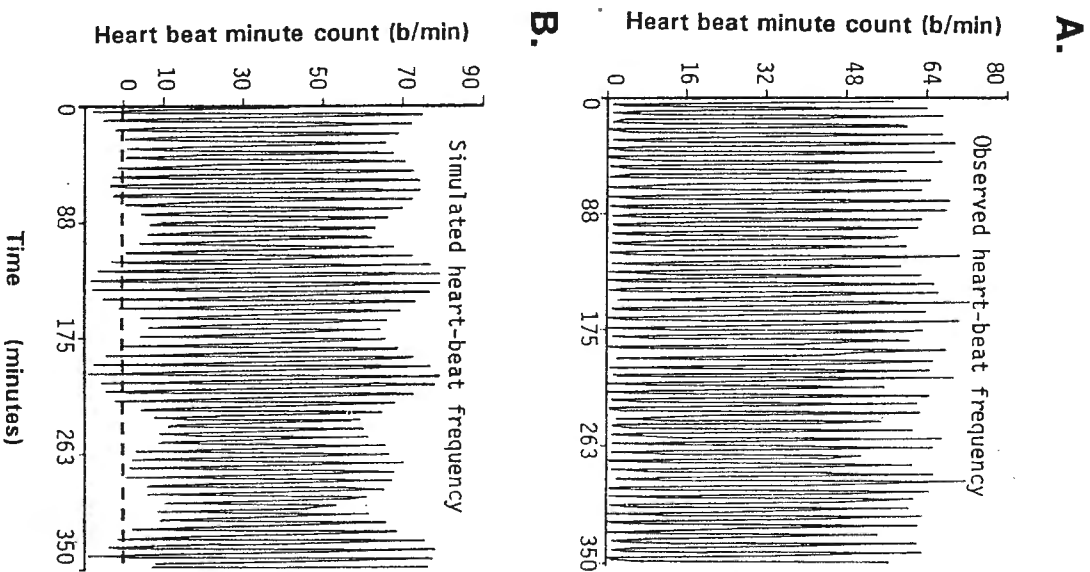


Fig. 6a. Heart-beat minute count of a quiescent *P. pelagicus*, showing a regular pattern of pulsing (tachycardia) followed by pausing (bradycardia).

Fig. 6b. Simulated train of pulses of cardiac activity using the Fourier transform model described in the text.

observed data was apparent, with almost identical heart-beat rate amplitudes and with exactly 51 "pauses" of cardiac activity seen in both. The simulation under-shoots the observed frequencies slightly. However, if the range of simulated

values is truncated at zero, which is reasonable given that the heart cannot exhibit "negative beats", then the model appears to be a good description of the original data.

An interesting aspect of the traces was that a slower oscillation became apparent in the amplitude of the simulated train of pulses of cardiac activity; a similar amplitude modulation could be seen in the observed data but was less clearly defined. The sampling rate was 1 per minute and the pulse rate was approximately 1 per 7 minutes, therefore at every 7th or 8th pulse the start of a pulse corresponded exactly with the time of sampling. Given that the heart-beat rate was highest at the start of a pulse (see Fig. 1), then every 17th pulse would appear to be a local maximum rate, due simply to the sampling regime. This phase relationship represents a cycle time of between 49 and 56 minutes, peak to peak, as observed in the simulation. The variation in the maximal pulse heart-beat rates predicted by the simulation was between 8% and 12%, and represents the theoretical confidence limits of values obtained using minute samples.

#### (vi) Simulation of a single pulse

To simulate a single pulse the Fourier transform model (above) was used to generate 21 minutes of data, mimicking data sampled at 1 minute intervals (Fig. 7b), and thus producing at least two complete 7 minute pause/pulse cycles. The profile of simulated pulses were compared heuristically with the shape of actual pulses, typical of buried crabs (Fig. 7a, and minute-count plots in Fig. 2a).

There was a striking resemblance between the forms of the simulated pulse and the observed pulse based on minute samples. Both displayed a rapid initial increase in heart-beat rate which peaked then dropped back to a plateau that was followed by a rapid drop in rate at the end of the pulse. Maximum heart-beat rate and the period of the pause and pulse were within realistic limits.

### GENERAL DISCUSSION AND CONCLUSIONS

Auto-correlation shows that the heart-beat of quiescent crabs follows a stable short-term "alternating" time-series, yet cross-correlations over 24 hours show strong temporal linking between the heart-beat rate, oxygen consumption and, by inference, with locomotor activity. Although the mode of cardiac activity is discontinuous, displaying pronounced pauses, the analysis shows there is a longer-term matching between perfusion and oxygen consumption (respiration). A mechanism must exist that allows the crab metabolism to coast over the pauses between the pulses of cardiac and ventilatory activity (Taylor 1984; Gribble, 1994, 1995). The current study provides a further example and statistical sub-

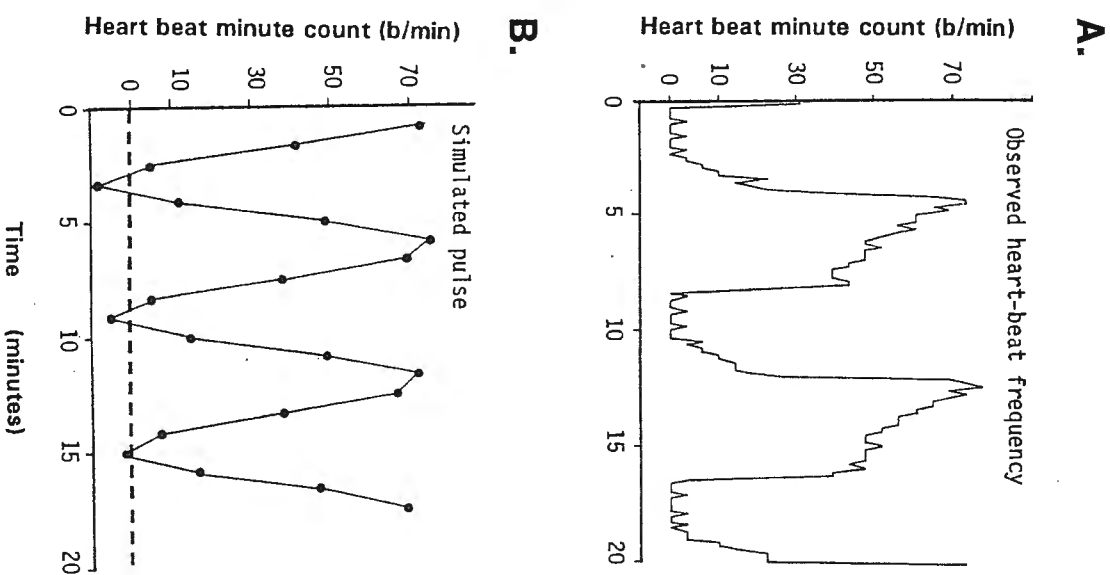


Fig. 7a. Heart-beat frequency record of *P. pelagicus* showing the typical profile of a pulse of cardiac activity. (Note. Data points were sampled at 10 second intervals to give a higher resolution in the profile.)

Fig. 7b. Simulated pulse of cardiac activity at 1 minute intervals using the Fourier transform model described in the text.

stantiation of similar observations made from other large decapods (see Ansell, 1973; McMahon and Wilkens, 1977; Bradford and Taylor, 1982; McMahon and Wilkens, 1982; Gribble, 1989; review McMahon and Burnett, 1990).

Correlograms derived from the laboratory and large aquarium data sets were of the same general form as were their periodicities, but the coefficients were weak and the timing did not match completely. In both sets of observations, crabs emerged during the dark and tended to be inactive during periods of light, but possible effects of laboratory-induced artefacts could not be fully excluded. The bi-modal peaks observed in the laboratory generated correlograms, however, may have parallels in field collected data. Sub-tidal crabs were observed to be mainly nocturnal to crepuscular, displaying two peaks of activity (catchability), one just after dusk and the other in the early morning (see Williams, 1979; Gribble, 1989; also Chatterton and Williams, 1994). This suggests that under constant conditions, either in the laboratory or in the field, the underlying pattern of activity was similar but that the expression of that pattern was subject to individual variation (review DeCoursey, 1983).

In the case of *P. pelagicus* there was the added factor of behaviour patterns that could change depending on habitat. In sub-tidal habitats, activity was essentially nocturnal, while in submerged inter-tidal habitats activity could occur throughout the 24 hour cycle and was linked to tidally influenced opportunistic foraging (Gribble, 1989; Gribble in press). Tide related activity in intertidal habitats illustrates the possible interaction of both exogenous (tidal and light stimuli) and endogenous (nocturnal rhythm) factors in the timing of activity. Given the complexity of the habitats and flexibility in behavioural response of *P. pelagicus* an "adaptable" diel rhythm could be expected.

The control and linking of the heart-beat rate and respiration in decapods has been the subject of considerable research, both from a cell biology and animal physiology viewpoint (e.g., Av-Ron et al., 1991; Burnett and Bridges, 1981; review McMahon and Burnett, 1990). Exogenous stimuli, such as light or tidal cues, can effect and/or entrain the pattern of an endogenous cardiac "pacemaker" oscillator via the central nervous system. The concept of a "local oscillator" pacemaker is one of a neuronal mechanism that can generate an endogenous pattern of stimulations controlling both the rate and pattern of ventricular contractions. Tazaki and Cooke (1983) describe such a cell-driven oscillator present in the cardiac ganglion of *Portunus sanguinolentus* (a portunid closely related and anatomically similar to *P. pelagicus*). Neuronal modulation of the cardiac oscillator network has been reviewed for crustaceans by Field and Larimer (1975 a & b), Young (1978) and neatly summarised by McMahon and Wilkens (1982), Wilkens (1987), and McMahon and Burnett (1990). Av-Ron et al. (1991) put forward a "local oscillator" bio-physical model that can explain a change in excitable states at a cellular level and by varying parameters can describe differ-

ent duration's of oscillation and quiescence; i.e., by extension, a possible mechanism for CNS control of the cardiac pausing.

The "clock-like" regularity of the pause/pulse pattern of cardiac activity observed in quiescent *P. pelagicus* argues for an endogenous control mechanism, such as a cellular "local oscillator" pacemaker. The longer term matching of heart-beat rate, oxygen consumption, and behaviour suggests that this endogenous mechanism can be modulated in response to external or exogenous influence. This conclusion is supported by the presence of non-periodic (irregular) pause/pulse patterns present in cardiac activity of routinely active crabs. The control mechanism was still operating but was adjusting the pause/pulse pattern dynamically in response to external changes (Gribble, 1994).

The co-ordination of heart-beat to scaphognathite-beat (gill irrigation) in *P. pelagicus* can be described by a quote from McMahon and Wilkens (1982) for crustaceans which display simultaneous bradycardia and apnoea, "the direct relationship between organ pumping rates and the partial pressure of oxygen (ambient) may passively serve to match the two systems so as to maximise the efficiency of the gas exchange across the gills." From the current study it appears that the relationship between the cardiac activity and oxygen consumption is more complex than a simple "direct" relationship, at least in the short term. The "passive matching mechanism" between cardiac and scaphognathite activity in the longer term may be the relationship between oxygen consumption and the required pumping rates to match this consumption, given short-term lag times to cover the simultaneous pausing of both organs.

For *P. pelagicus* in a steady-state environment the duration of pulse and pause were relatively stable, as shown by auto-correlogram and the periodogram. The two major peaks seen in the periodogram of the heart-beat rate (the basis of the Fourier transform model of a pause/pulse train), can be explained simply in terms of:

- (a) the frequency components that make up the pulse waveform, overlaid with;
- (b) a component that describes the interaction between the sampling rate and the fundamental pulse rate in the data.

The regularity of the model's squared-up "sine" waveform suggests that form and timing of a pause/pulse cycle were stable, while the shape suggests that frequency components associated with the rapid rise and fall of the heart-beat rates have been smoothed by the sampling regime.

The Fourier transform model gave a reliable description of the observed pulse amplitude (heart-beat rate within a pulse), the number of pulses that occur within a given time, and the average cycle time of a pause/pulse combination. It also highlighted a cyclic artefact caused by the interaction of the sampling rate with



the pulse rate, and allowed the estimation of confidence intervals about the mean values observed. Furthermore, the analysis does not require violation of the usual assumptions of normally distributed, independent, randomly "sampled" observations, nor are extreme transformations required to make the data fit a normal distribution.

The drawback of this type of cyclic regression model is that it is based on data collected during a steady-state condition observed while the crab was quiescent (buried). The analysis cannot model dynamically changing data that do not behave periodically. An example of this was seen in the routine activity data where pausing/pulsing could be present without periodicity being detected by autocorrelation. To model the dynamic cardiac response displayed by *P. pelagicus* during exercise, a more sophisticated form of modelling may be required (Gribble 1994).

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