Integration of Hydrostatic Pressure Information by Identified Interneurones in the Crab *Carcinus maenas* (L.); Long-Term Recordings

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Migrating species may utilise hydrostatic pressure. In the aquatic environment, hydrostatic pressure changes much more rapidly than in air. In shallow water, tidal changes will impose larger percentage changes on organisms than those experienced in deep water. Small changes in pressure often cause locomotion (barokinesis) accompanied by orientation to light or gravity, often partially compensating for the equivalent depth change. Until recently, identification of hydrostatic pressure receptors without a gas phase has proved elusive, but it is now known that thread hair receptors in the statocyst of the shore crab *Carcinus maenas* respond to small changes in hydrostatic pressure. Using a tide machine, the responses of thread hairs to sinusoidally changing pressure cycles have been examined, and this paper reports progress monitoring this receptor and making long-term recordings from hydrostatic pressure sensitive pathways in the crab's nervous system.

KEY WORDS

1. Animal Navigation. 2. Hydrostatics. 3. Trials.

1. INTRODUCTION. Migrating species may utilise hydrostatic pressure. Even in air, where pressure changes only by the equivalent of 1 mm of H_2O per metre, birds have been shown to be sensitive. Using cardiac conditioning, Kreithen and Keeton (1974) found a threshold for pigeons of about 0·001 bar equivalent to 1 cm of H_2O. It has been pointed out by Blaxter (1978) in a review of baroreception, that the birds could monitor a 10 metre change in altitude or respond to a 1 millibar change in atmospheric pressure, allowing synchrony of migration to weather patterns. In the aquatic environment, hydrostatic pressure changes with depth much more rapidly, at approximately 0·1 bar/metre. In shallow water, tidal changes that may be up to 10 metres in amplitude will impose larger percentage changes on organisms than those experienced in deep water (Blaxter, 1978). Small changes in pressure often cause locomotion (barokinesis) accompanied by orientation to light or gravity, often partially compensating for the equivalent depth change (Rice, 1964; Knight-Jones and Morgan, 1966). Such depth-regulating behaviour is considered a necessary component of selective tidal transport.

Until recently, identification of hydrostatic pressure receptors without a gas phase has proved elusive, but it is now known that thread hair receptors in the statocyst of the shore crab *Carcinus maenas* respond to small changes in hydrostatic pressure (Fraser and Macdonald,1994). Using a tide machine, the responses of thread hairs to sinusoidally changing pressure cycles have been examined (Fraser, Macdonald and Gibson, 1995). We report here on progress monitoring this receptor and making long-term recordings from hydrostatic pressure sensitive pathways in the nervous system.

In common with most marine species, at all active stages in their life cycle, crabs utilise hydrostatic pressure to give information on depth and synchronise rhythms with the tidal cycle (Hardy and Bainbridge, 1951; Morgan, 1967; Reid and Naylor, 1990, 1993). Early studies used fairly rapid changes in pressure and a variety of behavioural effects were found in many species (review by Knight-Jones and Morgan, 1966). For example, Rice (1964) noted responses in 41 out of 53 planktonic organisms tested with 1 bar steps in 2–3 seconds. Few early workers used rates of change of pressure modelled on normal tidal rates. A recent study that does measure rates of change of pressure, seems to cast some doubt on the use by larvae of pressure information.

Blue crab megalopae (*Callinectes sapidus*) use selective tidal stream transport to move them up an estuary. They are abundant in the water column during nocturnal rising tides and absent at other times. Although they responded appropriately to changes of pressure (Figure 1), there was doubt about whether the ability to sense

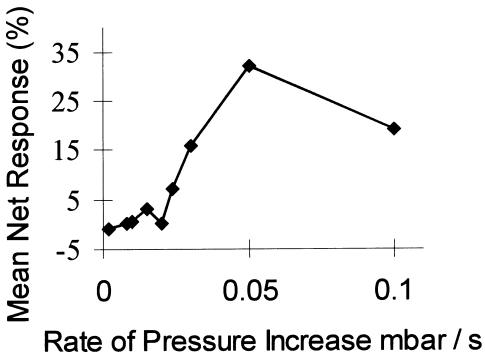

Figure 1. Mean change in the percentage megalopae in the upper subsection of a pressure chamber upon stimulation at different rates of pressure increase. Redrawn from Forward *et al.* (1995).

hydrostatic pressure is of primary importance in their normal estuarine orientation (Forward *et al.*, 1995).

Threshold rates of pressure increase for a significant ascent in the laboratory were 0·028 mb/second, whereas measurements in the field gave maximum rates between 0·00012 and 0·0064 mb/second. Forward *et al.* (1995) hence concluded that pressure was not used by the larvae. Thresholds for upwards movement to salinity change measured in the laboratory were less than the salinity changes found in the estuary during a rising tide. Hence from the threshold values, Forward concluded that salinity rather than pressure was the main depth cue involved in tidal transport in the estuary.

Nevertheless, pressure could still act along with salinity changes to make the overall system more sensitive. It is also worth pointing out that the field values for rates of change of pressure quoted in Forward *et al*. (1995) are extremely low. If the quoted maximum rates were sustained for the whole rising half of the tidal cycle, they would lead to maximum tidal heights of only 2·7 cm and 144 cm respectively. A sinusoidal pressure change of 3 metres would give maximum rates of just over 0·02 mb/sec, which is close to the threshold value of 0·028 mb/sec, and normally greater rates than those predicted from a simple sinusoidal model occur during the tidal cycle. Based on the sinusoidal model, the corresponding maximal tidal amplitudes would be 1·7 cm and 92 cm respectively. These values seem unrealistically low for a normal estuarine situation.

Adult shore crabs (*Carcinus maenas* (L.)) show free-running endogenous circatidal rhythms of locomotion that can be entrained to cycles of salinity, hydrostatic pressure and temperature (Naylor and Atkinson, 1972; Reid and Naylor, 1990). There is evidence for at least two functionally independent circatidal oscillators (Reid and Naylor, 1993). The crabs entrained to a sinusoidal pressure cycle of 0·5 bar (5 m H_2O), showing they could entrain to a cycle with maximum rates of change of pressure of 0·035 mb/sec (Figure 2).

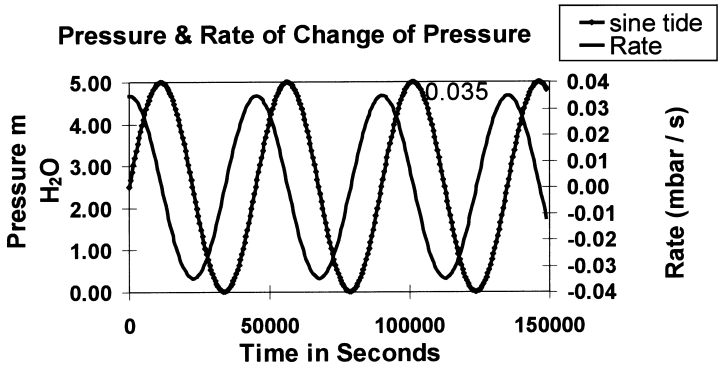

Figure 2. Sinusoidal pressure change with 5 m H_2O peak to peak amplitude, and the corresponding rates of change of pressure (maximum 0·035 mb/sec).

Whatever the exact role of hydrostatic pressure reception in the normal environment of the crab, it is clear that at various stages in their lives, crabs can monitor and alter their behaviour following small changes in hydrostatic pressure. The identity of the hydrostatic pressure receptor in animals lacking a gas-filled organ was a long-standing mystery in sensory physiology until Fraser and Macdonald (1994) reported that thread hair receptors in isolated statocysts of *Carcinus maenas* responded to step changes in hydrostatic pressure. They proposed a mechanism whereby the two mechanoreceptor neurones innervating each thread hair were activated by displacement of the chorda (a cuticular linking rod) following alteration of the volume of the cuticular hairs. On a simple model of the hair, they showed that a 1° displacement of the hair would lead to a chorda displacement of 0·0175 μm. If the whole of the change in volume of a hair (assuming a compressibility of $43{\cdot}9 \times 10^{-6}$ per bar for the hair interior contents) to a 1 bar pressure increase was translated into

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a chorda movement, then it would lead to a displacement of 0·0176 μm. Since Janse and Sandeman (1979a, b) had shown large responses from individual thread hair neurones to 2° displacements of the thread hairs, there seemed to be broad agreement between the sensitivity of the mechanoreceptor system and that required to translate a small movement of the chorda (produced by differential compression of cuticular and tissue elements of the hair system) into a signal. Enright (1963) had already shown that differential compression of different components of crustacea was likely. Tonic and phasotonic increases and decreases in firing frequency of statocyst thread hair receptors occurred following increasing and decreasing pressure steps of 0·22 to 1·1 bar above atmospheric pressure, with significant differences found in 50 out of 156 mixed units recorded (Fraser and Macdonald, 1994).

More recently, we have used tide machines to challenge the thread hair system in isolated statocysts of *Carcinus* with sinusoidally changing pressure regimes (Graham *et al*., 1987, Fraser *et al*., 1995, Cruickshank *et al*., 1997). Recordings of spontaneous activity from small numbers of thread hair units during pressure cycles showed clear modulation of responses with transient effects following the first cycle and both positive and negative going responses (Fraser *et al*., 1995). Plotting the spike frequency against the hydrostatic pressure values from such recordings gives a combination of positive going and negative going responses that can be resolved into two components thought to correspond to the two directional classes of thread hair units known to innervate each thread hair (Figure 3). The system hence uses the two

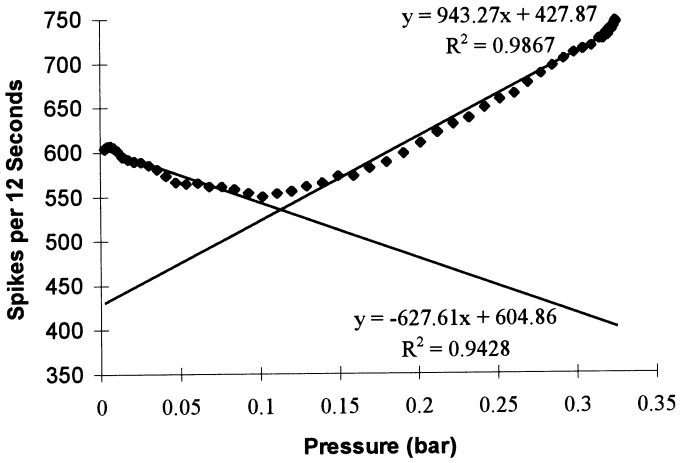

Figure 3. Intensity response function derived from a sinusoidal pressure change of 20 minute period and 3 m amplitude. The negative going and positive going components which may be fitted by linear regressions, correspond to spontaneous activity in the two directional classes of thread hair mechanoreceptors (see Fraser *et al*., 1995).

directional classes to monitor differences in pressure around a set point which is between 0·05 and 0·15 bar in the crabs studied. Fraser *et al*. (1995) concluded that the sensitivity of the system is more than adequate to account for known behavioural thresholds in crustacea of 5 mb. They did not calculate rates of change of pressure which, of course, are much higher for cycle periods of around 20 minutes (maximum rates 0·78 mb/sec) compared to 12·5 hours for a normal tidal cycle. Unpublished

results with cycle periods of between 160 minutes and 6·5 hours and amplitudes down to 0·1 m indicate that the thread hair system is sensitive enough to respond to rates of change of pressure below 0·02 mb/sec.

The thread hairs in the statocyst identified as responsive to hydrostatic pressure are perhaps better known as angular acceleration detectors (Sandeman and Okajima, 1972; Silvey, Dunn and Sandeman, 1976). They have been well characterised, and show a narrow range of peak sensitivity (see Fraser *et al.*, 1995). Furthermore, in a study of interneurones running between the brain and oesophageal connectives of *Carcinus*, four large interneurones have been well characterised anatomically and physiologically with input from these thread hairs (Fraser, 1974b; Fraser and Sandeman, 1975; Fraser, 1975a, b). Their role in walking and swimming has been elucidated by recording from these cells in free walking animals or tethered animals (Fraser, 1982; Fraser, Bevengut and Clarac, 1987). It has proved possible to record from these interneurones and others described by Fraser, (1974a) in crabs tethered in a pressure chamber subjected to various tidal cycles. Using computer controlled tide machines (Cruickshank *et al.*, 1997) and long-term recordings with implanted teflon-coated silver wire electrodes, recordings have been made for periods up to 43 days.

2. MATERIAL AND METHODS. A small hole (c. 0·5 mm diameter) is drilled through the carapace of the crab, above the point where the oesophageal connectives join the brain. Two teflon-coated electrodes are inserted through the hole and one is manipulated until large extracellular potentials indicate it is close to a nerve (Figure 4). The responses of the cells are then checked to a stimulus regime involving

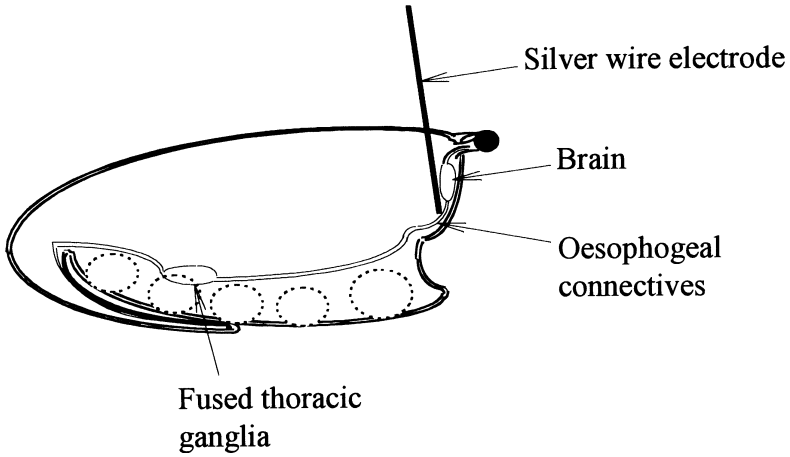

Figure 4. A 0·127 mm teflon-coated silver wire is glued close to the oesophageal connective near its insertion to the brain of the crab, allowing recordings from interneurones with axons running between brain and the thoracic ganglionic mass.

movement, and touch around the anterior carapace. The oesophageal connective is easily recognised by its characteristic response profile of large units. The electrode is then sealed in with cyanoacrylate adhesive, and the responses from statocyst interneurones noted to rotation in the planes of the statocyst horizontal and vertical canals. This allows unambiguous identification of left or right connective units (for

example, Cell A in the left connective is usually seen as a large unit responding on head-up rotation of the crab, or rotation in the plane of the right statocyst vertical canal (see Fraser *et al*., 1987; Fraser *et al*., 1995)). Spike potential recording, spike discrimination and counting of spikes was similar to that described by Fraser *et al*. (1995). The crab was usually tethered with a flexible coupling to a plastic pinboard support inside the chamber, since completely free crabs tended to destroy the insulation of the electrode wires.

Counts of spikes per 20-second or per 100-second period were transferred to a spreadsheet (Microsoft Office, Excel™) for plotting of sequences of up to 4000 values. Spectral analyses were carried out with SPSS for Windows software, allowing power spectra and spectral density to be calculated and plotted.

3. RESULTS. Many different interneurones in the oesophageal connectives show long quiescent periods with occasional periods of greatly increased activity. Most of the non-visual interneurones responded just before and during movement of the animals. Figure 5 shows the response of three sets of mixed units of different

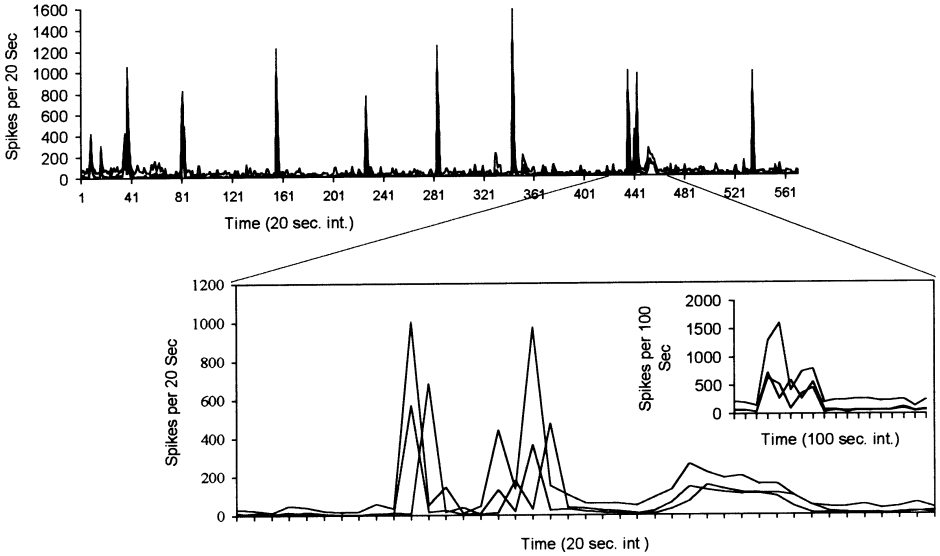

Figure 5. Recording from the left oesophageal connective in a free walking crab showing bouts of activity in several groups of mixed units. Spike triggers were set to record from cell A for one counter, and from smaller cells including cells C and D for two other counters.

heights in a free walking crab without any change in hydrostatic pressure. Although recordings with a short counting time showed that many of the interneurones fire at separate times, with longer counting times of around 100 seconds, all increases in different sets of units are extremely well synchronised.

Crabs tethered in the pressure chamber show similar bursts of elevated activity in the inter-neurones. Slower changes in spontaneous rates of firing are often superimposed, especially in the smaller cells. Figure 6 illustrates the sorts of recordings obtained for one set of mixed units recorded over 36 hours while applying a 30-minute period 0·3 bar amplitude pressure cycle. The bouts of activity seen in

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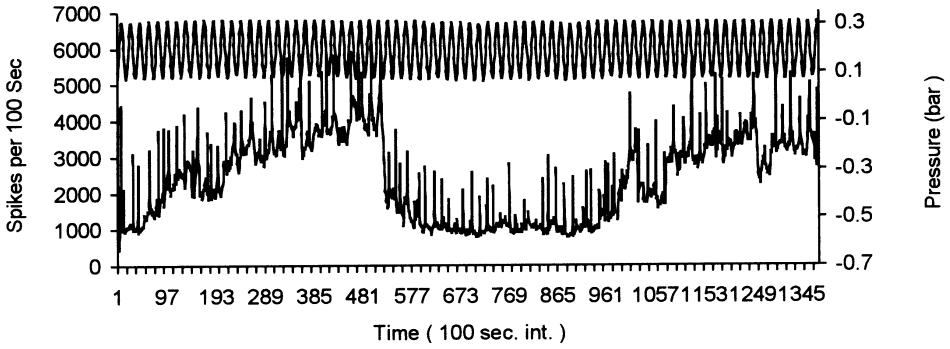

Figure 6. Recording from a mixed set of interneurones in the left oesophageal connective of a tethered *Carcinus* during the application of 30 minute, 0·3 bar cycles of hydrostatic pressure. There is no apparent modulation with the pressure cycle.

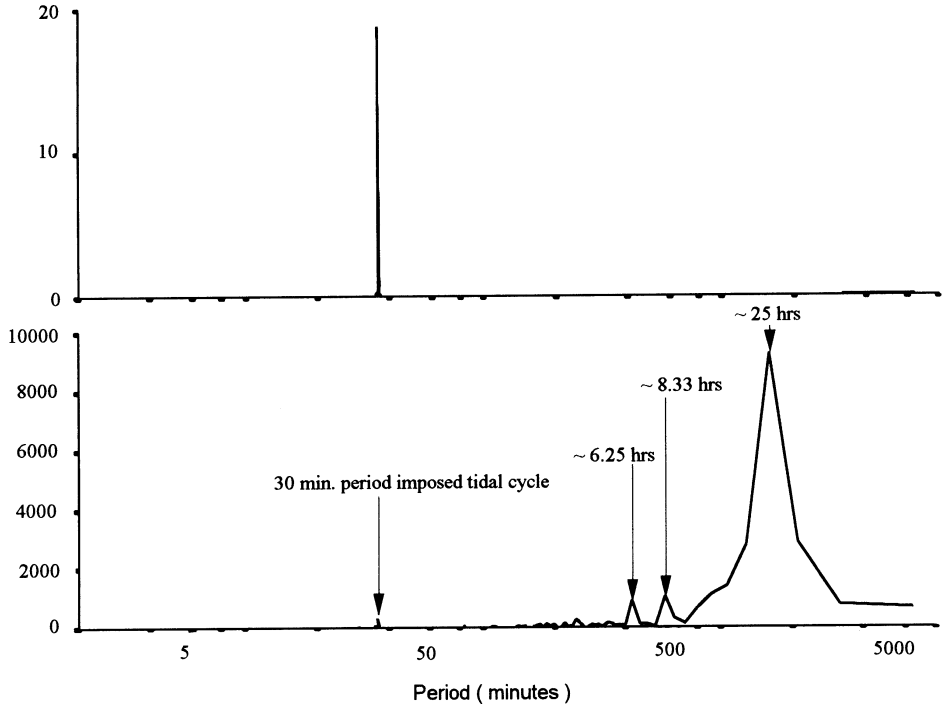

Figure 7. Spectral density of the pressure cycle (top) and interneurone spike activity (bottom) for a long sequence including that shown in Figure 6. Note that the interneurone shows large peaks at 25 hours, 8·33 hours and 6·25 hours, and a small peak at 30 minutes. The pressure cycle only shows a peak at the 30-minute period.

non-tidal un-tethered crabs are still apparent, but longer elevations in spontaneous activity are seen with abrupt changes in activity level.

Spectral analysis of a longer recording sequence (Figure 7) shows a small peak corresponding to the imposed tidal frequency, and much larger peaks at around 25 hours with elevated levels at 6·25 hours, 8·33 hours. In many such records, tidal period (12·5 hour) peaks are apparent. Analysis is continuing on a vast data set.

4. DISCUSSION. The elevation of interneurone activity at certain times, correlating with movements of appendages was first noted by Wiersma, and is known as the excited state (see Fraser, 1982). During studies on the role of the statocyst interneurones in swimming behaviour, the statocyst interneurones A, B, C and D fired before and during swimming, although their peak firing frequencies occurred at different times during the swimming bout (Fraser *et al*., 1987). We do not at present understand fully the factors underlying the excited state, although the output properties of the inter-neurones are well known. Clearly, the modulation seen in spontaneous levels of thread hairs is not translated directly into modulation of inter-neurone activity. Although a pressure cycle driven peak in activity of the inter-neurones occurs, much greater modulation occurs around circadian and circatidal periods, implying that these oscillators act at a different level, before affecting the statocyst inter neurones, so that an additional level of integration of thread hair activity must be taking place. Given the fixed relationship between the elevated inter neurone activity and loco-motor behaviour of the crabs, it is not surprising that the sorts of circadian and circatidal rhythms seen by Naylor and Atkinson, (1972) and Reid and Naylor (1990, 1993) are also apparent in the records of inter neurone spike frequency.

At present, we are working as far as possible at the level of clearly distinguishable single units to analyse further what has turned out to be an extremely complicated data set.

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REFERENCES

Blaxter, J. H. S. (1978). *Baroreception in* '*Sensory Ecology*'. Ed. M. A. Ali., Plenum Press, New York.

Cruickshank, S. F., Fraser, P. J., Macdonald, A. G. and Schraner, M. P. (1997). Tidal simulation during long-term recording from neurones in the crab *Carcinus maenas* (L.).

Enright, J. T. (1963). Estimates of the compressibility of some marine crustacea. *Limnol. Oceanogr*. **8**, pp. 382–387.

Forward, R. B. Jr., Tankersley, R. A., de Vries, M. C. and Rittschof, D. (1995). Sensory physiology and behaviour of blue crab (*Callinectes sapidus*) postlarvae during horizontal transport. *Mar. Fresh. Behav. Physiol*. **26**, pp. 233–248.

Fraser, P. J. (1974a). Interneurones in crab connectives (*Carcinus maenas* (L.)): giant fibres. *J. Exp Biol*. **61**, pp. 593–613.

Fraser, P. J. (1974b). Interneurones in crab connectives (*Carcinus maenas* L.): directional statocyst fibres. *J. Exp. Biol*. **61**, pp. 615–628.

Fraser, P. J. (1975a). Free hook hair and thread hair input to fibre 5 in the mud crab *Scylla serrata*, during antennule rotation. *J. Comp. Physiol*. **103**, pp. 291–313.

Fraser, P. J. (1975b). Three classes of input to a semicircular canal interneuron in the crab *Scylla serrata*, and a possible output. *J. Comp. Physiol*. **104**, pp. 261–271.

Fraser, P. J. (1981). Semicircular canal morphology and function in crabs. In Gualtierotti, T. (ed.) *The Vestibular System*: *Function And Morphology*. Springer-Verlag, New York, pp. 206–224.

Fraser, P. J. (1982). Views on the nervous control of complex behaviour. In: Sandeman, D. C. and Atwood, H. L. (eds.) *The Biology of Crustacea Vol* 4: '*Neural Integration and Behaviour*'. Academic Press, New York, pp. 293–319.

Fraser, P. J. (1990). Effects of temperature on statocyst afferents of the crab *Carcinus maenas*. *J. Thermal Biol*. **15**, pp. 25–32.

Fraser, P. J. and Sandeman, D. C. (1975). Effects of angular and linear accelerations on semicircular canal interneurones of the crab *Scylla serrata*. *J. Comp. Physiol.* **96**, pp. 205–221.

Fraser, P. J., Bevengut, M. and Clarac, F. (1987). Swimming patterns and the activity of identified equilibrium inter-neurones in the shore crab *Carcinus maenas*. *J. Exp. Biol.* **130**, pp. 305–330.

Fraser, P. J. and Macdonald, A. G. (1994). Statocyst hairs as pressure sensors in crabs. *Nature* **371**, pp. 383–384.

Fraser, P. J., Macdonald, A. G. and Gibson, R. N. (1995). Low pressure hydrostatic pressure receptors in the crab *Carcinus maenas* (L.). In: Rostain, J. C., Macdonald, A. G. and Marquis, R. E. (eds.) *Basic and Applied High Pressure Biology IV*. Medsubhyp Int. 5: pp. 59–68.

Graham, J. M., Bowers, R. and Gibson, R. N. (1987). A versatile tide machine and associated activity recorder. *J. Mar. Biol. Ass. UK*, **67**, pp. 709–716.

Hardy, A. C. and Bainbridge, R. (1951). Effect of pressure on the behaviour of decapod larvae. *Nature*, London **168**, pp. 327–328.

Janse, C. and Sandeman, D. C. (1979a). The role of the fluid filled balance organs in the induction of phase and gain in the compensatory eye reflex of the crab *Scylla serrata*. *J. Comp. Physiol.* **130**, pp. 95–100.

Janse, C. and Sandeman, D. C. (1979b). The significance of canal-receptor properties for the induction of phase and gain in the fluid filled balance organs of the crab *Scylla serrata*. *J. Comp. Physiol.* **130**, pp. 101–111.

Knight-Jones, E. W. and Morgan, E. (1966). Responses of marine animals to changes in hydrostatic pressure. Oceanogr. *Mar. Biol. Ann. Rev.* 4, pp. 267–299.

Kreithen M. L. and Keeton, W. T. (1974). Detection of changes in atmospheric pressure by the homing pigeon *Columba livia*. *J. Comp. Physiol.* **89**, pp. 73–82.

Morgan, E. (1967). The pressure sense of the swimming crab *Macropipus holsatus* (Fabricus), and its possible role in the migration of the species. *Crustaceana* **13**, pp. 275–280.

Naylor, E. and Atkinson, R. J. W. (1972). Pressure and rhythmic behaviour of inshore marine animals. *Symp. Soc. Exp. Biol.* **26**, pp. 395–416.

Rice, A. L. (1964). Observations on the effects of changes in hydrostatic pressure on the behaviour of some marine animals. *J. Mar. Biol. Ass.* U.K. **44**, pp. 163–175.

Reid, D. G. and Naylor, E. (1990). Entrainment of bimodal circatidal rhythms in the shore crab *Carcinus maenas*. *J. Biol. Rhythms* **5**, pp. 333–347.

Reid, D. G. and Naylor, E. (1993). Different free-running periods in split components of the circatidal rhythm in the shore crab *Carcinus maenas*. *Mar. Ecol. Prog. Ser.* **102**, pp. 295–302.

Sandeman, D. C. and Okajima, A. (1972). Statocyst induced eye movements in the crab *Scylla serrata*. I. The sensory input from the statocyst. *J. Exp. Biol.* **57**, pp. 187–204.

Silvey, G. E., Dunn, P. A. and Sandeman, D. C. (1976). Integration between statocyst sensory neurons and oculomotor neurons in the crab *Scylla serrata*. II. The thread hair sensory receptors. *J. Comp. Physiol.* **108**, pp. 45–52.