The effect of the Crustastun™ on nerve activity in two commercially important decapod crustaceans: the edible brown *Cancer pagurus* and the European lobster *Homarus gammarus*

A Scientific Report

by

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May 2012

INTRODUCTION

The Crustastun™ is a device designed to administer a lethal electric shock to shellfish such as crabs and lobsters before cooking, to avoid boiling a live shellfish (**www.crustastun.com**). It works by applying a 110 volt, 2-5 amp electrical charge to the shellfish. These parameters were determined by Robb (1999) and the effectiveness of the Crustastun in achieving the required stun currents was evaluated by Sparrey (2005). Crustastunning kills the animals instantaneously, and imposes no additional physiological stress as judged by indicative biochemical measures (Neil and Thompson, 2012).

A previous investigation (Neil, 2010) evaluated the effect of the Crustastun™ on nerve activity in a typical crab (the shore crab *Carcinus maenas*) and a typical clawed lobster (the Norway lobster or langoustine *Nephrops norvegicus*). The present report summarises the results obtained in a number of trials carried out to determine the effect of the Crustastun machine on activity in the nervous system of two other decapod crustaceans: the edible brown *Cancer pagurus* and the European lobster *Homarus gammarus.* These are important species that are commonly supplied live to processors and to the restaurant trade in the UK and other European countries. Moreover, the closely related species of crab, the Dungeness crab *Metacarcinus* (formerly *Cancer*) *magister*, and species of lobster, the American lobster *Homarus americanus*, are widely consumed seafood in North America. On the basis of the results obtained in this study, conclusions have been drawn about the effects of Crustastun usage on the neuronal functioning in these commercially important crustaceans.

Aims and objectives

The aims of this study were, as in the previous study (Neil, 2010), to use appropriate electrophysiological techniques to record from both the central nervous system and the peripheral nervous system of, in this case, the brown crab *Cancer pagurus* and the European lobster *Homarus gammarus*, in order to compare intact animals with those that have been subjected to 'Crustastunning'.

The specific objectives were:

1. To monitor intrinsic and evoked neuronal activity emerging from the anterior of the central nervous system, the 'brain' (supra-oesophageal ganglion) of crabs and lobsters, by making extracellular recordings in the circumoesophageal connectives, the main nerves conveying information to and from the brain. This would include making recordings in the "head" (cephalothorax) of the lobster after isolating it from the tail (abdomen)

2. To monitor intrinsic neuronal activity from the posterior of the central nervous system of lobsters by making extracellular recordings from neurones in the abdominal ventral nerve cord. This would include making recordings in the tail (abdomen) of the lobster after isolating it from the head (cephalothorax).

3. To record intrinsic activity from the peripheral nervous system, the motor nerves leaving the abdominal nerve cord of the lobster to supply the abdominal postural muscles, by making extracellular recordings from the appropriate motor nerves (3rd abdominal roots).

4. To demonstrate evoked motor activity in the peripheral nervous system of the crab by measuring the muscle forces produced by the activation of the motor neurones in the leg nerve supplying a muscle spanning a specific leg segment (the closer muscle of the dactylopodite) in crabs.

5. To demonstrate evoked sensory activity in the peripheral nervous system of crabs and lobsters by recordings from the sensory neurones in the leg nerve in response to stimulation of specific receptor types: mechanoreceptors in the cuticle (eg. cuticular hairs, campaniform sensillae) and proprioceptors spanning the leg segments internally (chordotonal organs).

These tests were designed to allow the following questions to be addressed, namely, after 'Crustastunning':

- Does any activity continue to be generated spontaneously in the central nervous systems of the brown crab and the European lobster, and if so are its characteristics altered from normal?

- Does any activity, either spontaneous or evoked, remain in the motor and neuromuscular systems of the animals, and if so are their characteristics altered from normal?

- Does any activity remain in the sensory nerves from peripheral mechanosensory organs of the animals, and if so are its characteristics altered from normal?

Anatomy

Decapod crustaceans, the taxonomic group to which crabs and lobsters belong, have nervous systems with the characteristic arthropod plan (Brusca and Brusca, 2002). This involves a ladder-like arrangement of paired nerve cords, with a dorsal brain (supraoeophageal ganglia) separate circumoesophageal connectives and segmental ganglia in the thorax and (if present) in the abdomen, from which nerves arise to supply the segmentally-arranged muscles and sense organs. Lobsters exemplify all these features (Figure 1) whereas in crabs a distinct abdomen has been lost and the thoracic ganglia are condensed into a single thoracic mass, from which all the peripheral nerve roots emerge (Figure 2).

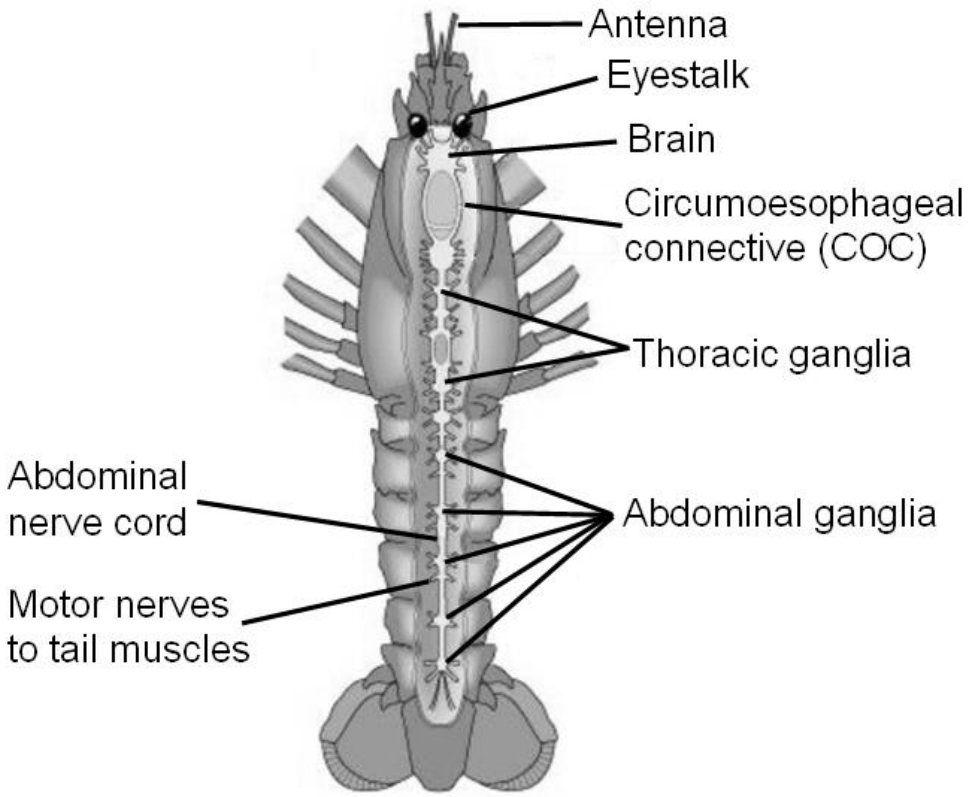

Figure 1. The arrangement of the nervous system in a clawed lobster such as the European lobster *Homarus gammarus*

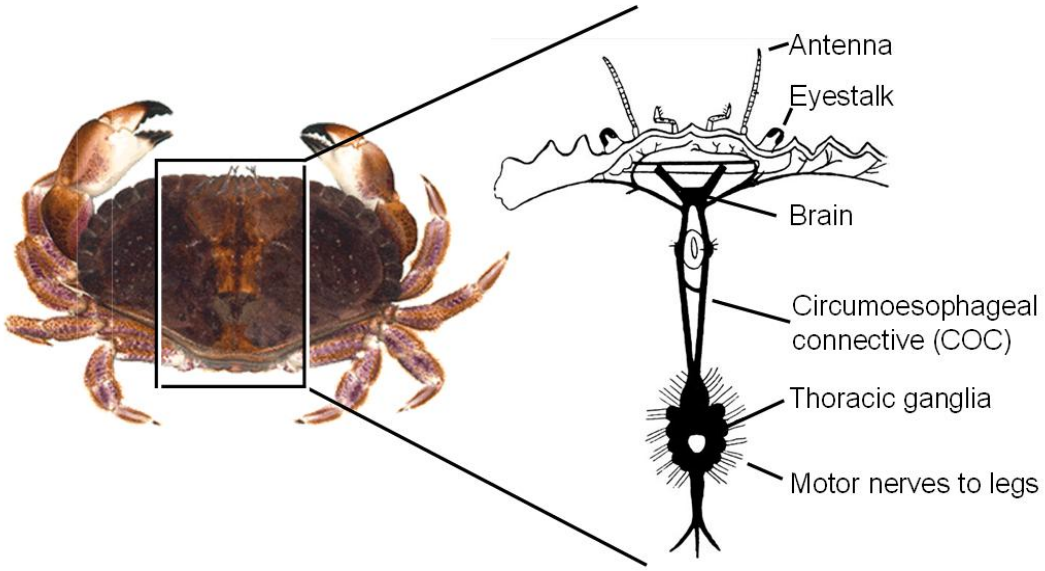

Figure 2. The arrangement of the nervous system in a crab such as the brown crab *Cancer pagurus*

Each of the four pairs of walking legs (pereiopods) of crabs and lobsters comprises a series of articulated segments, which are moved by paired muscles (Figure 3). A number of different mechanoreceptors are associated with the leg exoskeleton, including innervated cuticular sensory hairs which signal contact and water movement (Garm, 2005), and 'funnel canal organs' (a type of campaniform sensilla) which are pressure-sensitive (Libersat, 1987). In addition, a series of elastic strands span the various joints, into which are embedded sensory cells which detect joint flexion and extension (Bush, 1965). These so-called chordotonal organs thus act as proprioceptors monitoring the leg movements made by the crab (Hartman *et al.*, 1997). The chordotonal organ spanning the terminal leg segment, between the propopodite and the dactylopodite (the PD chordotonal organ) was selectively activated in this study. The branches (axons) of both the motor and the sensory nerves pass in a mixed leg nerve that travels through the centre of the leg segments.

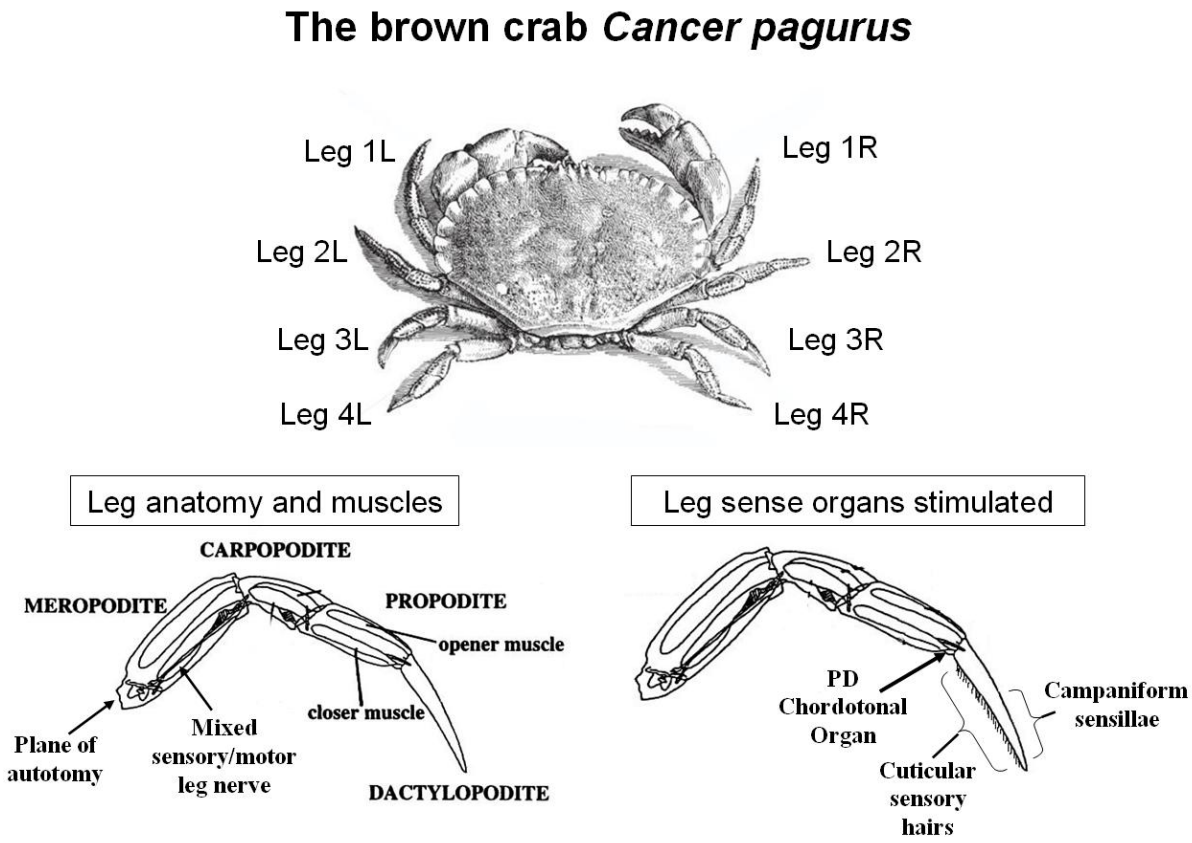

Figure 3. Schematic diagram of **t**he anatomy of the legs of the crab *Cancer pagurus*, including the arrangement of the muscles and sense organs.

MATERIALS AND METHODS

Ethical statement

The number of animals used in these trials was kept to the minimum necessary to obtain scientific results, considering that the gain in knowledge and long term benefit to the subject will be significant. All the live animals used were treated with proper care in order to minimize their discomfort and distress.

Animal supply and holding

Male brown crabs, *Cancer pagurus* of carapace width 120-140 mm, and male European lobsters, *Homarus gammarus* lobsters of carapace length 80-95 mm were used in these trials. All animals were in the intermoult stage with a hard exoskeleton. They were captured by commercial fishermen using baited traps (creels) laid offshore from St Abbs on the east coast of Scotland. After banding the claws of the lobsters, and nicking the tendons of the crab claws (both standard commercial practices) they were held initially in seawater tanks at the St Abbs Marine Station, and were then transferred in chilled containers to the University of Glasgow. Here they were retained individually in tanks within a closed seawater circulating system at 10^{o}C for at least two weeks before experimentation.

Crustastunning

The Crustastunning' procedure was applied without prior anaesthesia using a machine supplied by Studham Technologies Ltd., according to the manufacturer's operating instructions. The chamber was filled with a salt solution (~3g L^{-1}). Individual crabs or lobsters were removed from their holding tanks and placed into the Crustastun machine, the lid was closed and the animal was stunned by a 110 volt, 2-5 amp electrical charge for 10 s. The animal was then returned to its seawater container (water temperature 10^{o}C - 12^{o}C).

Exposing the nervous systems

In order to expose the central nervous system of the crab for recording, the carapace was removed and the preparation was submerged in a balanced salt solution corresponding in composition and osmolarity to crab haemolymph, at a temperature of 10^{o}C. The internal organs were then removed or displaced in order to expose the circumoesophageal connectives around the base of the stomach. A similar procedure was employed for the lobster, but prior to this the cephalothorax was separated from the abdomen.

To expose the abdominal ventral nerve cord of the lobster for recording, after separating the abdomen from the cephalothorax the dorsal skeletal plates (terga) were detached, and the bulk of the underlying deep flexor musculature was removed. The preparation was then submerged in a balanced salt solution corresponding in composition and osmolarity to lobster haemolymph, at a temperature of 10^{o}C. Selective removal of muscle blocks then revealed the motor roots emerging from the ventral nerve cord.

The leg nerves of crabs and lobsters were exposed for recording and stimulating using the following procedures. The intact animal was induced to shed a leg spontaneously (autotomy) by applying pressure to the basipodite segment (McVean, 1976, Smith and Hines, 1991). For the Crustastunned crabs and lobsters, which were effectively killed and did not express the autotomy reflex, a leg was detached by amputation. In either case the joint between the meropodite and carpopidite (M-C) was then disarticulated, and the muscle tendons spanning this joint were cut with fine scissors. The leg was separated gently at this point, revealing the leg nerve still attached to the distal portion. This isolated leg preparation was submerged in a balanced salt solution at a temperature of 10^{o}C until required, and remained viable for many hours.

Electrophysiological recordings

Electrophysiological recordings were made from the exposed nerves using various extracellular techniques. For recording from the circumoesophageal connectives of crabs and lobsters, and from the ventral nerve cord of lobsters, a suction electrode method was used. A fine-tipped polythene electrode containing salt solution was applied to the surface of the nerve, and a gentle suction was applied through attached tubing and a syringe. A silver wire positioned close to the tip of the electrode acted as the indifferent (reference) electrode. Such a recording configuration is termed ‘en passant’, as it involves attaching the suction electrode to an intact nerve, allowing both directions of nerve transmission to be recorded. However, in some cases the circumoesophageal connective was cut and the electrode was attached to either its anterior or posterior cut end. In this way the presence of active neurones transmitting information in ascending or descending directions could be ascertained.

For recording from the crab and lobster leg nerves, the isolated leg was clamped to a Perspex plate and the nerve was passed from an adjacent bath through a wall of petroleum jelly into a second small chamber, both of which contained balanced salt solution (Figure 4, upper panel). A bipolar electrode of two silver wires was used to make contact with the solutions in the inner and outer chambers respectively.

In each case the signals from the extracellular electrodes were passed to a differential pre-amplifier (A101, Isleworth Ltd.) for amplification and filtering. The amplifier output was then passed to an Analog/Digital converter (PowerLab, AD Instruments Ltd) and was both displayed and recorded on a standard PC computer using the associated software (Chart v7, AD Instruments Ltd.)

Stimulating the nerves

Motor responses in the legs were measured only in crabs, since their leg anatomy was more compatible with the force measuring procedure (see Figure 4, lower panel). To stimulate the motor axons in the crab leg nerve, the bipolar electrodes were connected to an isolated stimulator within the PowerLab (Figure 4, lower panel) and patterns of stimulating pulses at various amplitudes and frequencies were applied using a software

'stimulator control panel' within the Chart v7 software. Typically, stimulus trains of 3 s duration and 4 V amplitude were applied at a range of frequencies from 10 – 100 Hz.

Recording muscle force

Although stimulation of the crab leg nerve potentially activated motor neurons supplying all of the muscles located more distally in the leg, the forces produced by the closer muscle of the propopodite/dactylopopodite joint (P-D) were nevertheless recorded selectively. This was achieved by cutting the tendon of the antagonist muscle about that joint (the P-D opener muscle), and then attaching a thread from near the tip of the dactylopodite to the arm of a sensitive force transducer (FT-03, Grass Instruments Ltd.), mounted on a micromanipulator (Figure 4, lower panel). This selectively monitored the forces produced by the dactylopodite closer muscle. The output of the transducer was passed to a custom-built amplifier (x1000), and then fed to an input of the Powerlab A/D converter. The forces and the stimulus parameters were then both displayed and recorded on a standard PC computer using the Chart v7 software.

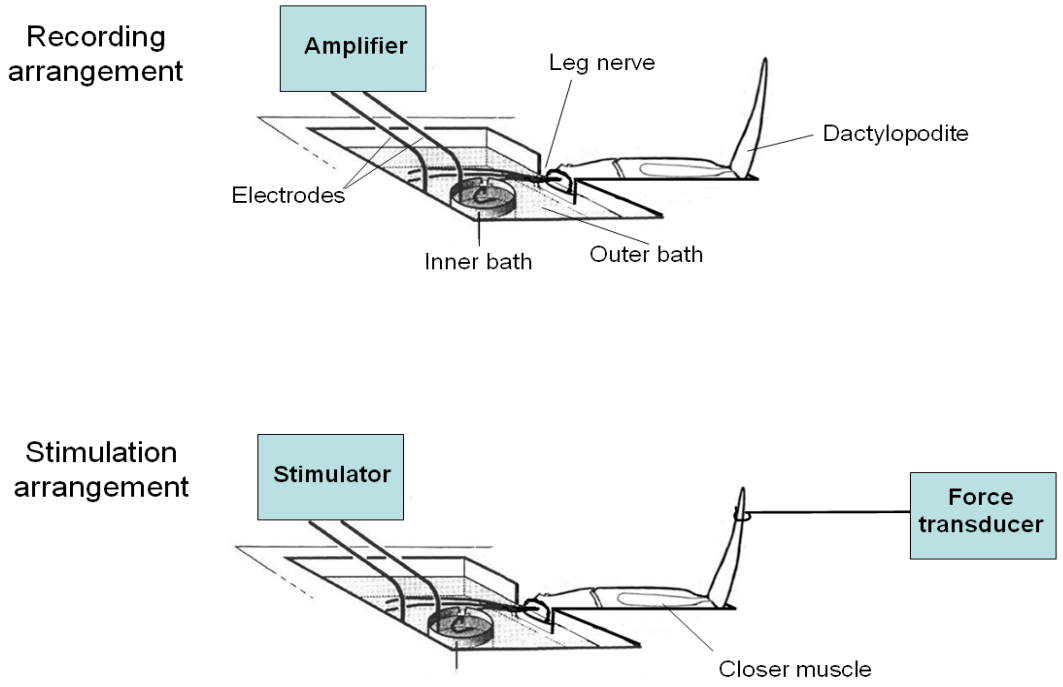

Figure 4. Experimental arrangements for recording from the nerve of an isolated crab or lobster leg (upper panel) and for stimulating the crab leg nerve while recording the forces produced by the dactylopodite closer muscle (lower panel).

RESULTS

For each species, *C. pagurus* and *H. gammarus*, the complete set of trials involved a total of 6 individual animals that were Crustastunned and the same number of intact animals as a control group. In the case of isolated legs, three legs per individual were tested. The neuronal data are presented as traces of the original electrophysiological recordings and where appropriate also as plots of the muscle forces produced in relation to stimulus parameters.

Activity in the central nervous system (CNS) of intact crabs and lobsters

Recordings made from one or both circumoesophageal connectives in intact *C. pagurus* crabs indicated that there was a high level of spontaneous neuronal activity passing along the axons of this nerve, even in the absence of any imposed stimulation (Figure 5 upper panel). Due to the variety of sizes of the extracellularly-recorded spikes, it can also be concluded that the signals arose from a large number of different individual nerve axons, of varying diameters.

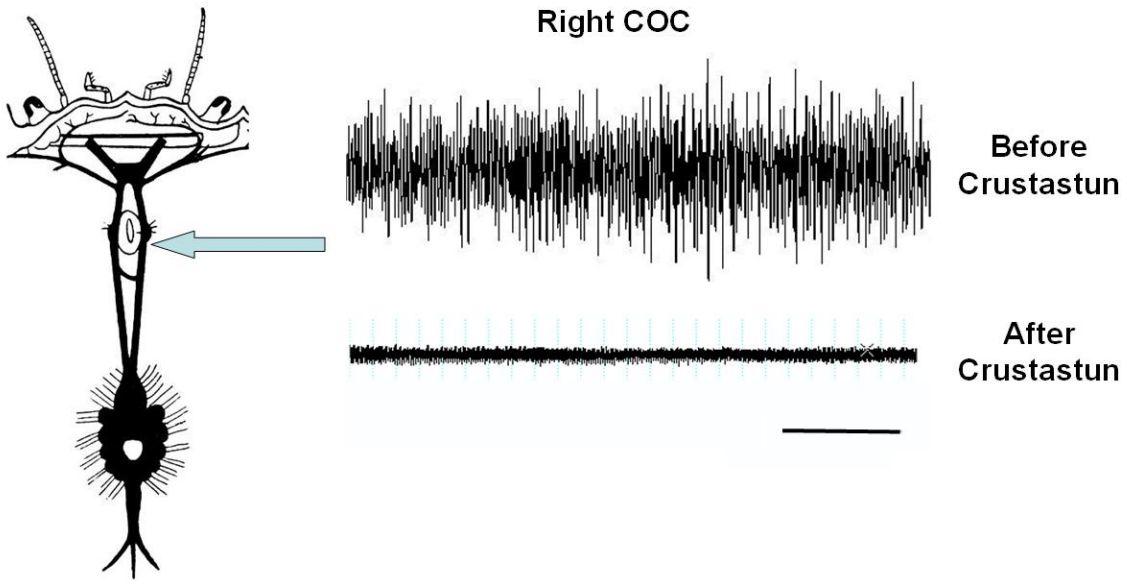

Figure 5. Spontaneous nerve activity recorded extracellularly in the right circumoesophageal connective (COC) of a brown crab, *Cancer pagurus*. Upper panel, intact animal; lower panel, animal after Crustastunning. Scale bar 1s.

When tactile stimuli were applied to the eyestalks or antennae, there were systematic changes in firing frequency in some of these axons, indicating that these were conveying descending activity from the brain. There were also high frequency bursts of activity that corresponded to the animal making struggling movements (fictive locomotion) (data not shown).

Recordings from the circumoesophageal connectives of intact *H. gammarus* lobsters provided essentially the same results, even when the cephalothorax was detached from the abdomen, with a high level of neuronal activity passing along the axons of this nerve (Figure 6, upper panel).

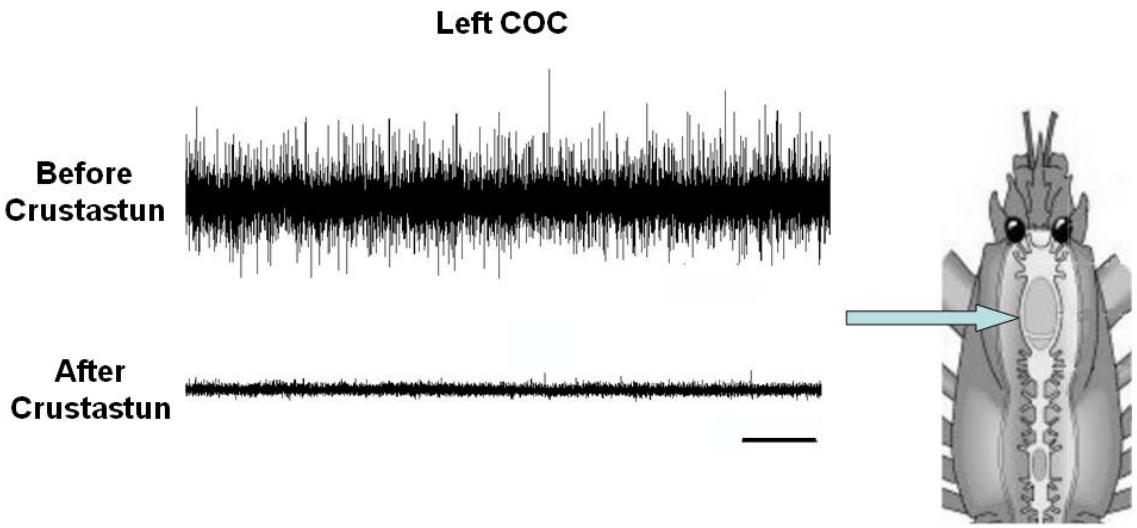

Figure 6. Spontaneous nerve activity recorded extracellularly in the left circumoesophageal connective (COC) of a *H. gammarus* lobster. Upper panel, intact animal; lower panel, animal after Crustastunning. Scale bar 1s.

Recordings from the abdominal nerve cord of the intact lobsters also encountered spontaneous nerve activity in all cases, even when the abdomen was detached from the cephalothorax (Figure 7 upper panel).

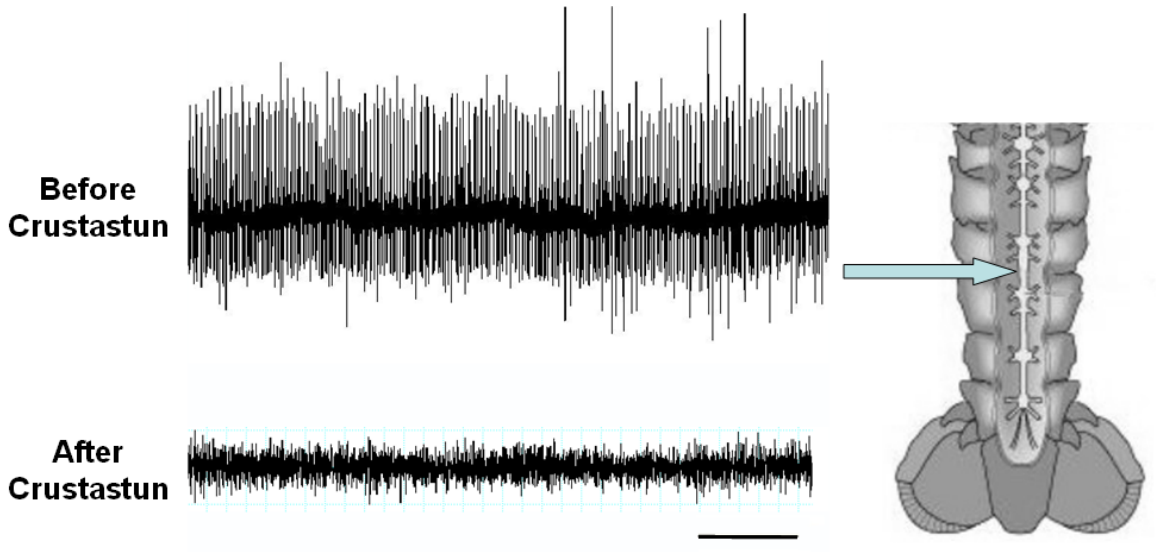

Figure 7. Spontaneous nerve activity recorded extracellularly between the 3rd and 4th ganglia in the abdominal nerve cord of a *H. gammarus* lobster. Upper panel, intact animal; lower panel, animal after Crustastunning. Scale bar 1s.

Activity in the peripheral nervous system of intact crabs and lobsters – motor responses

Patterned activity involving a number of motor neurons (represented by different spike sizes) was detectable in motor roots emerging from the ventral nerve cord of the lobster *H. gammarus* (Figure 8). This represents evidence for the action of the peripheral nervous system in intact animals, contributing to the generation of muscle tone in the abdominal muscles.

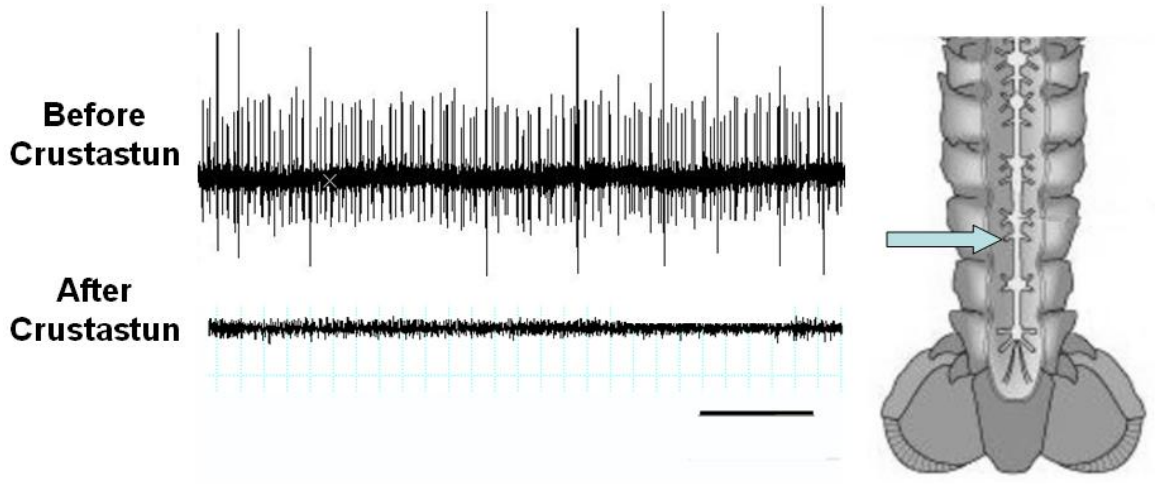

Figure 8. Spontaneous nerve activity recorded extracellularly from motor neurones in the 3rd motor root of the 4th abdominal ganglion of an intact *H. gammarus* lobster. Scale bar 1s.

Due to the relative inaccessibility of the motor roots emerging from the thoracic ganglia of crabs, equivalent recordings were not obtained from *C. pagurus*. However the normal operation of the motor pathways of the peripheral nervous system of this crab was demonstrated by stimulating the leg nerve of an autotomised leg at various frequencies while monitoring the force produced by the dactylopodite closer muscle. The force varied in a frequency-dependent manner typical of crustacean neuromuscular systems due to their synaptic properties of summation and facilitation (Figure 9).

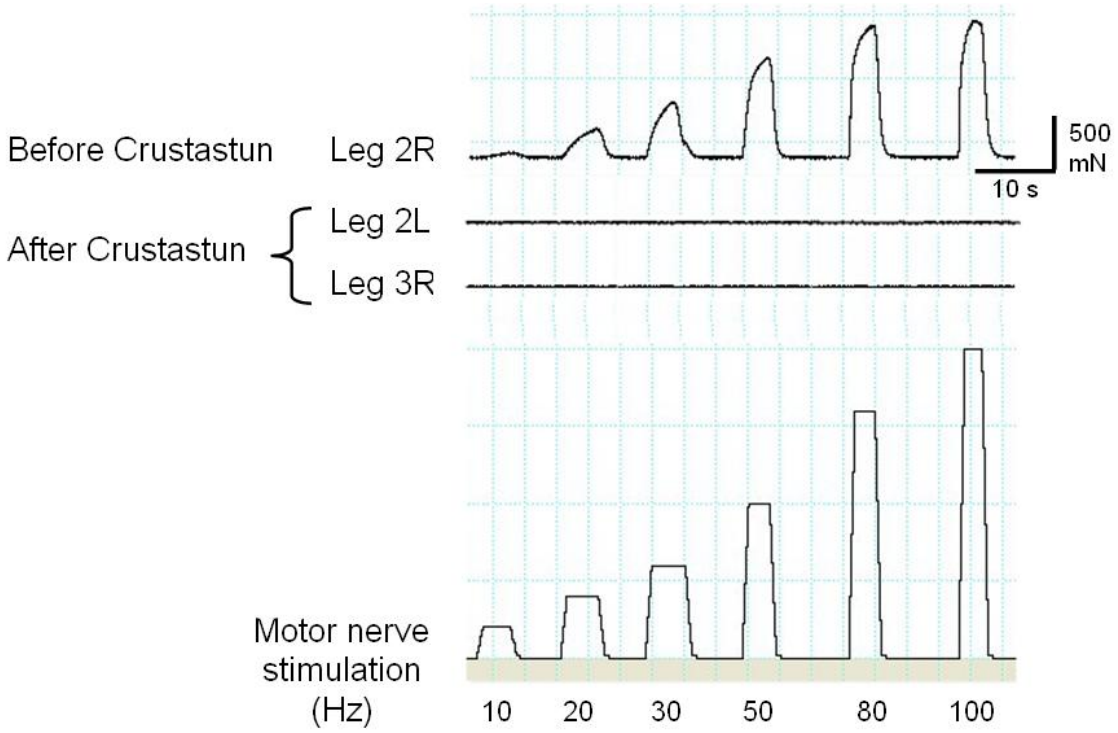

Figure 9. Forces produced by the dactylopodite closer muscle of the leg of *Cancer pagurus* in response to stimulation of the leg nerve at various frequencies. Top panels, leg autotomised from intact crab; lower two panels, legs amputated from the same crab after Crustastunning. Stimulus voltage 4V.

Activity in the peripheral nervous system of intact crabs and lobsters – sensory responses

Further evidence for activity in the peripheral nervous system in intact crabs and lobsters was obtained from the recordings of sensory activity made in their isolated legs, following autotomy. Examples from two lobsters are presented in Figures 10 and 11, and from two crabs in Figures 12 and 13.

The leg nerve of a crab or lobster contains a mixture of the axons of sensory and motor neurons, and the application of various stimuli to the distal part of the leg clearly elicited activity in a number of sensory neurons. These patterns of activity were typical for the various sense organs that were stimulated in each case. Thus brushing movements over the cuticle of the dactylopodite produced bursts of activity typical of the responses to displacement of cuticular sensory hairs (Figures 10-13, left panels). Compression (squeezing) of the cuticle of the dactylopodite elicited persistent tonic responses for the duration of the stimulus (Figures 10-13, centre panels). The responses to the movement and displacement phases of flexions and extensions applied at the P-D joint had characteristic phasic and tonic elements (Figures 10-13, right panels).

In order to test the persistence of activity in the nervous systems of intact crabs and lobsters, some preparations were re-tested at intervals of up to several hours. Activity persisted for up to the longest time tested (6 hours) in both their central nervous systems and in the nerves of autotomised legs (data not shown). A similar persistence was observed when a number of legs that were autotomised from an intact crab at the same time were held for differing periods of time before being prepared for recording. The sensory responses obtained at 4 h after autotomy were just as strong as those recorded immediately after autotomy.

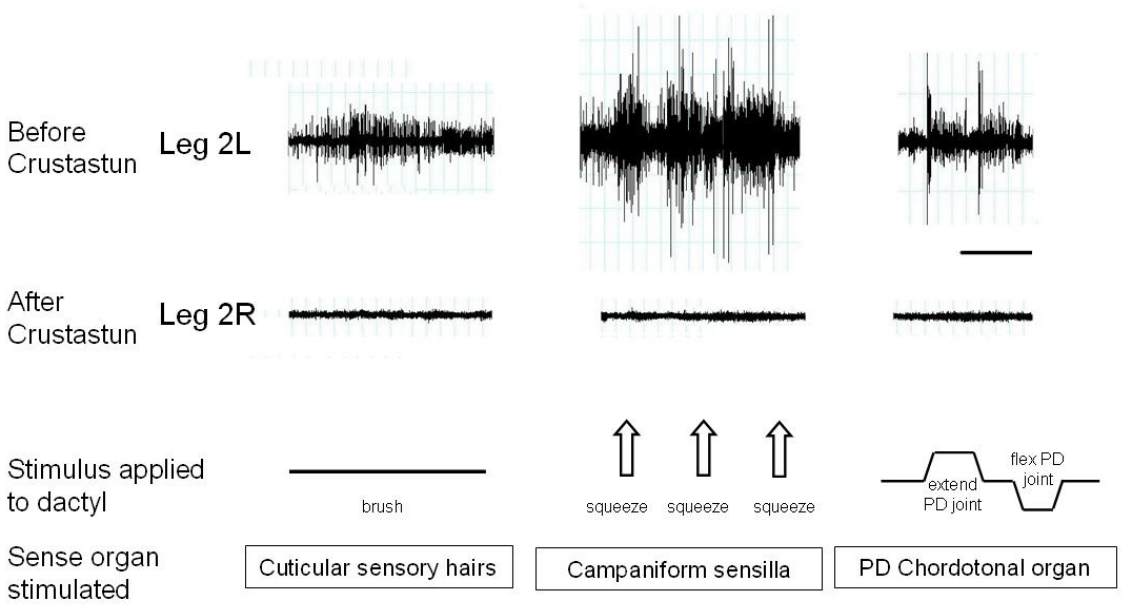

Figure 10. Responses of the leg nerve of the lobster *H. gammarus* to three forms of stimulation of the dactylopodite. Top panels, leg autotomised from intact animal; lower panels, leg amputated from an animal after Crustastunning. Scale bar 5 s.

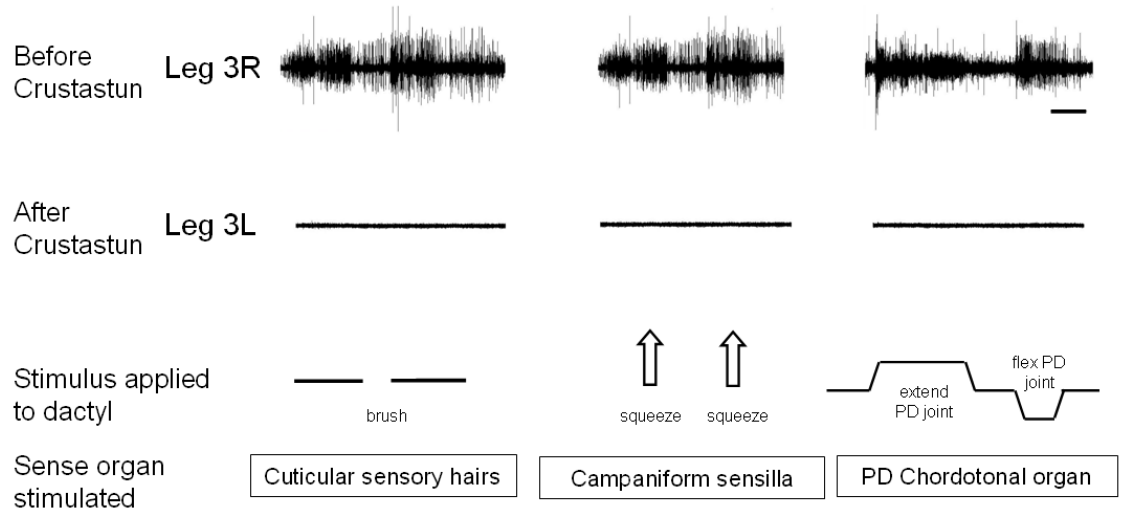

Figure 11. Responses of the leg nerve of a different lobster *H. gammarus* preparation to three forms of stimulation of the dactylopodite. Top panels, leg autotomised from intact animal; lower three panels, legs amputated from an animal after Crustastunning. Scale bar 5 s.

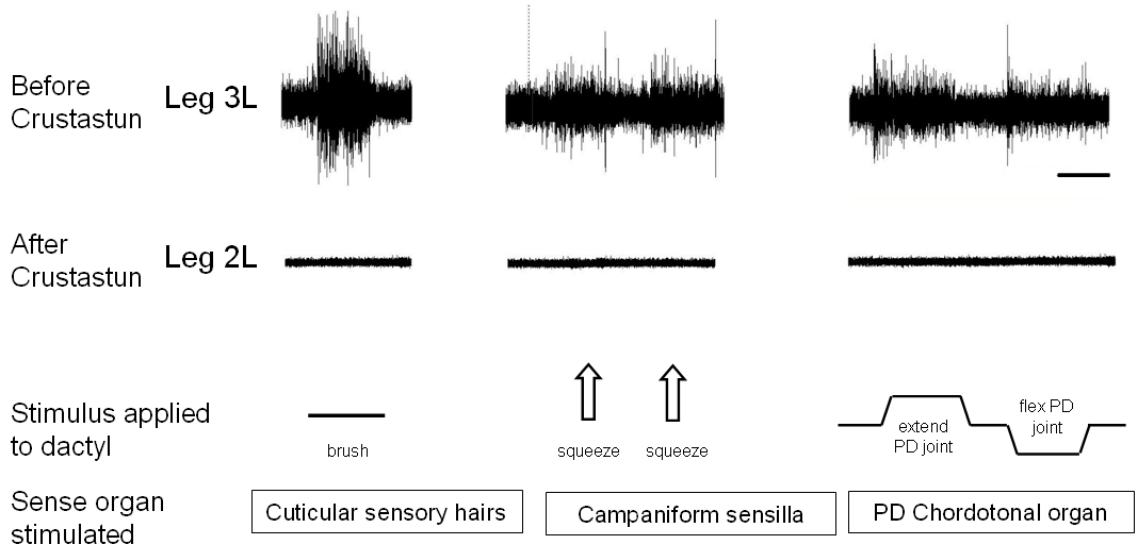

Figure 12. Responses of the leg nerve of the crab *C. pagurus* to three forms of stimulation of the dactylopodite. Top panels, leg autotomised from intact animal; lower panels, leg amputated from an animal after Crustastunning. Scale bar 5 s.

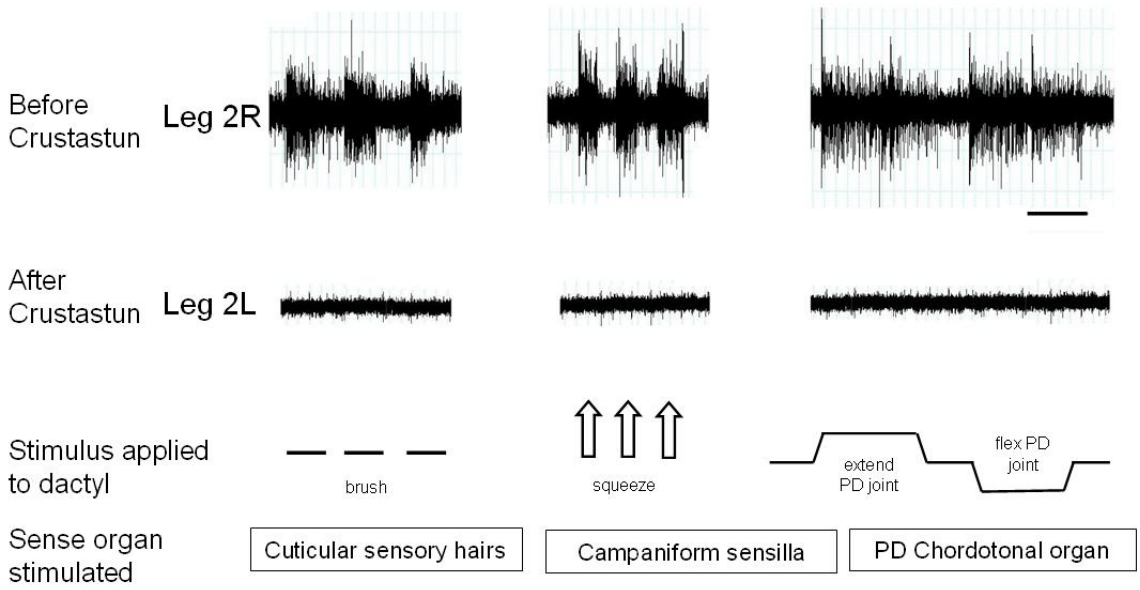

Figure 13. Responses of the leg nerve of a different crab *C. pagurus* preparation to three forms of stimulation of the dactylopodite. Top panels, leg autotomised from intact animal; lower three panels, legs amputated from an animal after Crustastunning. Scale bar 5 s.

Activity in the nervous system of crabs and lobsters following Crustastunning

After Crustastunning the stunned crabs showed no further visible movements (limb movement, antennule flicking, a ventilation current and eye retraction reflexes), and never recovered, i.e they were effectively killed. After Crustastunning the stunned lobsters showed either no further visible movements (limb movement, antennule flicking, a ventilation current, pleopod beating and eye retraction reflexes), or in a few cases showed some transient movements of the mouthpart exopodites and abdominal pleopods, lasting for a few seconds, and thereafter became immobile and never recovered, and were then effectively killed. One feature that was never observed in either the crabs or the lobsters as a result of Crustastunning was an evoked autotomy of either the claws or the walking legs (pereiopods).

Recordings from the central nervous systems of crabs and lobsters that had been subjected to Crustastunning indicated that no neuronal activity was detectable in the circumoesophageal connectives in any of the individual animals tested of either species (examples in Figures 5 and 6, lower panels). The abdominal nerve cords of the Crustastunned lobsters were also silent, with no indication of spontaneous neuronal activity (Figure 7, lower panel). As expected, due to this lack of central nervous system activity, there was no corresponding motor activity in the abdominal motor nerve roots of these Crustastunned lobsters (Figure 8, lower panel), which contrasts with the responses obtained in an intact lobster (see Figure 8, upper panel).

The recordings from the leg nerves of the Crustastunned crabs and lobsters provided a means of testing whether the peripheral system retained any ability to convey neuronal information, even though the central nervous system might be silent. However, in all the legs tested of either species there were no sensory responses to the three stimuli applied (Figures 10 and 11, lower panels for *H. gammarus*; Figures 12 and 13, lower panels for *C. pagurus*) and, in the tests performed on crabs, there was no muscle force development in response to stimulating motor nerves (Figure 9, lower panels).

DISCUSSION AND CONCLUSIONS

Activity in the nervous systems

The results obtained here from intact *Homarus gammarus* lobsters and *Cancer pagurus* crabs are very similar to those obtained previously on *Nephrops norvegicus* lobsters and *Carcinus maenas* crabs (Neil, 2010). They are also consistent with the literature on the neurophysiology of crustacean nervous systems (see, for example, the articles in Wiese, 2002) in showing that the central nervous systems of lobsters and crabs display continuous nerve activity, which in turn produces outputs in the motor nerves to the body and limb muscles. A large body of evidence, including studies conducted in this laboratory (Chachri et al., 1994; Holmes et al, 2002), indicates that this activity persists even when parts of the CNS are isolated from each other by severing the nerve cord at one or more levels (Larimer and Moore, 2003). Even isolated single ganglia of the abdominal nerve cord can produce patterned outputs (e.g. Chachri and Neil, 1993), and there is an extensive literature on the most-studied ganglion that can continue to operate in isolation, the stomatogastric ganglion (reviewed by Marder and Bucher, 2007).

It is therefore not surprising to have found in the present study that, as a result of dissection or of detaching the cephalothorax from the abdomen of the lobster, nerve activity continues to be recorded in the isolated anterior or posterior portions of the body, even though the nerve cord is transected at one or more levels. Also, as expected, this activity includes both descending signals from the brain and ascending signals from more posterior parts of the nervous system.

Although not attempted in these trials, it is without doubt that any procedure that attempted to make a sagittal section through a lobster or crab, in an attempt to destroy the entire nervous system, would inevitably leave small sections untouched and sufficiently intact to be able to continue generating patterned nerve activity, and to respond to sensory stimulation with reflex outputs localized to the muscles in the segments still innervated.

A characteristic feature that is common in these isolated parts of the nervous system is the long-lived nature of continued activity and signal conduction. It is widely reported that, provided the structures are bathed in an appropriate solution, activity can continue for many hours, and indeed, as in other lobsters and crabs (Neil, 2010), this was observed in the present study both with the central nervous preparations and with the isolated *H. gammarus* and *C. maenas* legs after autotomy. Such robustness makes it easier to interpret any loss of activity following a procedure such as Crustastunning as due to the intervention itself, rather than to any underlying decline in nervous system responsiveness.

The use of autotomised legs

Autotomy is a natural process for defence (Juanes and Smith, 1995), invoked by particular stimuli (Smith and Hines, 1991). In decapod crustaceans it involves a specific neuromuscular reflex acting across a fixed plane (McVean, 1976), although there may be a degree of voluntary control (Fleming *et al*., 2007). In addition to specific mechanosensory

stimuli, crabs show autotomy when an appendage has been subjected to various other stimuli such as heating, shock, wounding, acetic acid injection or minor electric shocks to the appendages (Elwood et al. (2009), or when cooled by placing the animals on ice.

Induced autotomy was used in the present study to obtain an isolated leg from an intact lobster or crab, since it has been found that this process has virtually no measurable effect on the stress levels in the animal, as indicated by the low levels of L-lactate circulating in the haemolymph (Patterson *et al*., 2007). Forced removal of a limb in the living animals was not employed, since it is known to induce a significantly greater stress, due to the greater extent of tissue damage imposed (Patterson et al., 2007). Limb amputation was only used on animals which had been killed by the Crustastunning process.

The effects of Crustastunning

The findings obtained on the effect of Crustastunning on nerve activity in lobsters and crabs are relatively conclusive. As far as can be determined from the extracellular recording method used, the various forms of spontaneous activity within the central nervous system were completely and almost instantaneously arrested. Consistent with this, there were no outputs produced in the motor nerves supplying the abdominal muscles of lobster, which are known to be synaptically driven from neurones within the CNS.

The recordings made on isolated lobster and crab legs allow some further conclusions to be drawn, namely that Crustastunning also has a specific effect on the functioning of the peripheral parts of the nervous system. There was both a loss of responsiveness to all three types of sensory stimulation, and also, as tested specifically in the crab, a failure in neuromuscular activation. The first of these effects would have rendered the animals insensitive to external stimuli, while the second would have rendered them paralysed and incapable of making movements.

Crustastunning did not induce autotomy in either of the species used in the present study, nor in the two other species studied previously (Neil, 2010). This finding, which is consistent with those made during routine commercial use of this instrument that autotomy of claws or legs never occurs, suggests that the observed inactivation of the sensory and motor divisions of the nervous system must have included the neuromuscular reflex pathways underlying autotomy. This result is especially striking since other attempts to electrically stun crabs using a low electrical field strength to the whole animal (Roth and Øines, 2010) failed to inactivate the animals, but actually caused extensive autotomy. Moreover, Elwood *et al.* (2009) found that weak electrical stimuli applied to the legs induced them to autotomise. A plausible interpretation of these different findings is that weak electrical stimuli artificially activate the sensory and/or motor pathways involved in the autotomy reflex, resulting in the shedding of the limb, whereas the Crustastunning inactivates these pathways more extensively and completely, so that no limb losses occur. Moreover, this would imply that neuronal inactivation by Crustastunning occurs very rapidly, before the reflex neuromuscular action underlying autotomy can be elicited. It is therefore possible that the inactivation of the central and peripheral nervous system found more widely in the present study following Crustastunning would also have occurred

almost instantaneously, although the methods used here were not appropriate to detect this directly.

Taken together these results indicate that as a result of Crustastunning the nervous system is incapacitated simultaneously at two levels, i.e. both centrally and peripherally, which completely prevents all normal neuronal functioning.

In terms of identifying the reasons for recording no sensory signals or inducing no motor activity in the peripheral nervous system, the recording method used does not allow definitive conclusions to be made. It is indeed possible that the conduction processes in the axons of both the sensory and motor neurones have been disrupted by the electrical currents generated by the Crustastun. However, it cannot be excluded that the Crustastunning has affected only the sensory transduction processes in the receptor endings of the sense organs, rather than the nerve transmission mechanism in the sensory nerves. Similarly, Crustastunning may have destroyed synaptic transmission at the neuromuscular junctions, and/or excitation-contraction coupling processes within the muscle fibres, rather than the nerve transmission mechanism in the motor nerves. It is of course possible that all of these processes have been affected simultaneously. To distinguish between these possibilities would require an examination of each of the contributing processes by using other, more appropriate, electrophysiological methods in a targeted approach.

Scope of conclusions

The results obtained in this study of the effects of Crustastunning on the brown crab, *Cancer pagurus* and the European lobster *Homarus gammarus* are consistent with those found previously in two other decapod crustacean species (the crab *Carcinus maenas* and the Norway lobster *Nephrops norvegicus*) (see Neil, 2010). This is to be expected considering the virtually identical anatomies and physiologies of the two species of each group. As such, the findings will also be applicable to species that are closely related to *C. pagurus* and to *H. gammarus*, namely the Dungeness crab *Metacarcinus* (formerly *Cancer*) *magister*, and the American lobster *Homarus americanus*, which are widely consumed seafood species in North America. In all these commercially important crustaceans, Crustastunning almost instantaneously arrests spontaneous activity within the central nervous system, with an accompanying loss of sensory responsiveness and a failure in neuromuscular activation.

Acknowledgements

This research has been conducted independently by DMN, using a Crustastun machine kindly loaned by Studham Technologies Ltd. Thanks to Dr Keith Todd of the St Abbs Marine Station and the fishermen of St Abbs for the supply of animals. The technical assistance of Mr Graham Adam is gratefully acknowledged.

References

Brusca, R.C and Brusca, G.J. (2002). Invertebrates. Sunderland, MA: Sinauer Associates Inc.

Bush, B.M.H. (1965). Proprioception by chordotonal organs in the mero-carpopodite and carpo-propodite joints of *Carcinus maenas* legs. Comparative Biochemistry and Physiology **14**, 185–199.

Chrachri, A. and Neil, D.M. (1993). Interaction and synchronization between two abdominal motor systems in crayfish. Journal of Neurophysiology **69**, 1373-1383.

Chrachri, A., Neil, D.M. and Mulloney, B. (l994). State-dependent responses of two motor systems in the crayfish *Pacifastacus leniusculus*. Journal of Comparative Physiology **A 175**, 371-380.

Elwood, R.W., Barr, S. and Patterson, L. (2009). Pain and stress in crustaceans? Applied Animal Behaviour Science **118**, 128-136.

Fleming, P.A., Muller, D., Bateman, P.W. (2007). Leave it all behind: a taxonomic perspective of autotomy in invertebrates. Biological Reviews **82**, 481–510.

Garm, A. (2005). Mechanosensory properties of the mouthpart setae of the European shore crab *Carcinus maenas.* Marine Biology **147**, 1179–1190.

Hartman, H.B., Johnston, E.E. and Storer, P.D. (1997). Manipulation by the crab claw is dependent upon chordotonal organ afference. Journal of Experimental Zoology **279**, 579–586.

Holmes, J.M., Neil, D.M., Galler, S. and Hilber, K. (2002). Correlation of Synaptic and Mechanical Properties of Two Slow Fibre Phenotypes in a Crustacean Muscle. In: (ed. K. Wiese) *The Crustacean Nervous System*, Birkhäuser Verlag, Basel, Switzerland pp. 341-352.

Juanes, F. and Smith, L. (1995). The ecological consequences of limb damage and loss in decapod crustaceans: a review and prospectus. Journal of Experimental Marine Biology and Ecology **193**, 197–223.

Larimer, J.L. and Moore, D. (2003). Neural basis of a simple behavior: abdominal positioning in crayfish. Microscopical Research Techniques **60**, 346–359.

Libersat, F., S.N. Zill, and F. Clarac (1987). Single-unit responses and reflex effects of force-sensitive mechanoreceptors of the dactyl of the crab. Journal of Neurophysiology **57**, 1601–1617.

Marder, E. and Bucher, D. (2007). Understanding Circuit Dynamics Using the Stomatogastric Nervous System of Lobsters and Crabs. Annual Reviews of Physiology **69**, 291–316.

McVean A. (1976). Autotomy: Mini Review. Comparative Biochemistry and Physiology **51A**, 497-505.

Neil, D.M. (2010). The effect of the Crustastun™ on nerve activity in crabs and lobsters. Scientific Report to Studham Technologies.

Neil, D.M. and Thompson, J. (2012). The stress induced by the Crustastun™ process in two commercially important decapod crustaceans: the edible brown *Cancer pagurus* and the European lobster *Homarus gammarus.* Scientific Report to Studham Technologies.

Patterson, L., Dick, J.T.A. and Elwood, R.W. (2007). Physiological stress responses in the edible crab, *Cancer pagurus*, to the fishery practice of de-clawing. Marine Biology **152**, 265–272.

Robb, D. (1999). The humane slaughter of Crustacea: Electrical stunning. Unpublished research report.

Roth, B. and Øines, S. (2010). Stunning and killing of edible crabs (*Cancer pagurus*). Animal Welfare **19**, 287-294

Smith, L.D. and Hines, A.H. (1991). The effect of cheliped loss on blue crab *Callinectes sapidus* Rathbun foraging rate on soft-shelled clams *Mya arenaria*. Journal of Experimental Marine Biology and Ecology **151**, 245–256.

Sparrey J. (2005). Testing of Crustastun single crab and lobster stunner. Unpublished research report.

Wiese, K. (2002). The Crustacean Nervous System. Basel, Switzerland: Birkhäuser Verlag.