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Electrical Stunning for Humane Slaughter of New Zealand Crustaceans - Final Report

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by Craig Johnson, Nikki Kells and Matt Perrott of Massey University

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Scientific Interpretative Summary

This SIS is prepared by MPI to provide context to the following report.

Electrical Stunning for Humane Slaughter of New Zealand Crustaceans - Final Report

This report describes research to investigate the effectiveness of a commercially available stunning device for two commercial New Zealand crustacean species *(*NZ rock lobster and freshwater crayfish/kōura*)*. Results are based on observations of the electroencephalogram (EEG) and behaviour of minimally anaesthetised crustaceans in response to electrical stunning. The research showed that, except for the largest rock lobster, there was no evidence of return of activity indicative of awareness post-stunning. All freshwater crayfish/kōura and a sub-set of rock lobster were killed outright by the stun. The use of the device appears to be an acceptable method of killing or stunning all but the largest rock lobsters. The results will contribute to the implementation of current animal welfare regulations, standards, and guidelines and the review and development of future animal welfare standards for crustacean slaughter.

406074 Electrical Stunning for Humane Slaughter of New Zealand Crustaceans

Final Report

Craig Johnson, Nikki Kells and Matt Perrott
School of Veterinary Science
Tāwharau Ora
Massey University

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Executive Summary

Crustacean species, including rock lobster, crabs and koura (freshwater crayfish) are covered by the Animal Welfare Act 1999 and are acknowledged as being sentient (able to feel pain and distress). Regulation 11 of the Animal Welfare Regulations 2018 came into force on 1 October 2018. It states that one must not kill any farmed or commercially caught animal of these species for commercial purposes unless it is rendered insensible first (for example by being stunned or chilled). This applies to primary processors as well as those slaughtering crustaceans at fish markets, and chefs and caterers slaughtering crustaceans in the kitchen.

The preferred method of making a crustacean insensible is by using electrical current. At least one device that is suitable for a kitchen setting is commercially available. It was specifically developed in the United Kingdom to stun North Atlantic/European species of lobster and crabs. Published results indicate that it is effective in stunning these species, ensuring no recovery before death. However, New Zealand species of rock lobster can be larger than North Atlantic/European species at harvesting and there are concerns that available devices may not stun them effectively due to differences in tissue depth / thickness, structure and neurological architecture. This project investigates the effectiveness of a commercially available stunning device for two commercial New Zealand crustacean species (*J. edwardsii* and *P. zealandicus*).

A review of the current literature found:

A growing consensus that decapod crustacea are able to perceive pain.

Evidence that Crustastun™ has been shown to be effective in abolishing the coordinated electrical activity of the nervous system in a number of decapod species.

There are no direct studies of Crustastun™ which utilise techniques of anaesthesia that would be considered appropriate in a New Zealand experimental context.

The design of this experimental study adapted the experimental studies used in other species and in particular adopted anaesthetic and electrode placement techniques so that the effect of the stun could be established.

A total of 10 *J. edwardsii* and 10 *P. zealandicus* were sourced from the southern fishery and were requested to be at the upper end of the size of commercially harvested animals. Animals were anaesthetised and instrumented to record the electrical activity of the nervous system. They were then stunned using the commercial stunning apparatus (Crustastun™) and the behavioural and electrical effects of a single electrical stun recorded.

With the exception of one *J. edwardsii* (a very large animal that did not fit properly into the stunner), all animals were delivered a successful stun (as indicated by the stunning device). Of the other nine, three appeared to be killed outright by the stun (no evidence of movement or muscle tone). Six exhibited some degree of muscle tone and/or occasional very slow uncoordinated movement of the limbs. Such movements or muscle tone did not resemble those seen prior to anaesthesia, or subsequent to anaesthesia pre-stunning. No recovery of spontaneous or evoked movement was observed in any animal subsequent to stunning. All ten *P. zealandicus* appeared to be killed outright by the stun.

All animals used in this study were anaesthetised throughout and were either killed by the stun or killed prior to the return of normal behaviour. The anaesthetic was considered necessary to guard the welfare of the animals because this device has not previously been used on the two species in question, but it meant that the duration of the stun in the *J. edwardsii* that were not killed outright could not be determined. Electrical activity was recorded for five minutes following the stun and there was no evidence of return of activity that might indicate awareness during this time. These results indicate that the maximum acceptable stun-to-kill time for animals that are not killed outright is at least five minutes, though it could be much longer.

Crustastun™ appears to be an acceptable method of killing or adequately stunning all but the largest *J. edwardsii*. No stun was applied to the largest animal, presumably

because the electrical load of this animal fell outside of the machine's programmed range.

Crustastun™ appears to be an acceptable method of killing *P. zealandicus*.

Further research would be necessary to accurately determine a safe maximum stun-to-kill time.

Background and Objectives

Crustacean species, including rock lobster, crabs and koura (freshwater crayfish) are covered by the Animal Welfare Act 1999 and are acknowledged as being sentient (able to feel pain and distress). The New Zealand common-name nomenclature of these animals is to some extent confusing and so this report will refer to the animals using their scientific names, *Jasus edwardsii* and *Paranephrops zealandicus*.

Regulation 11 of the Animal Welfare Regulations 2018 came into force on 1 October 2018. It states that one must not kill any farmed or commercially caught animal of these species for commercial purposes unless it is rendered insensible first (for example by being stunned or chilled). This applies to primary processors as well as those slaughtering crustaceans at fish markets, and chefs and caterers slaughtering crustaceans in the kitchen.

The preferred method of making a crustacean insensible is by using electrical current. At least one device that is suitable for a kitchen setting is commercially available (Crustastun™, Mitchell and Cooper Ltd, UK). It was specifically developed in the United Kingdom to stun North Atlantic/European species of lobster and crabs. Published results indicate that it is effective in stunning these species, ensuring no recovery before death.

However, New Zealand species of rock lobster, which include *J. edwardsii*, can be larger than North Atlantic/European species at harvesting and there are concerns that available devices may not stun them effectively due to differences in tissue depth / thickness, structure and neurological architecture, e.g. number and location of the heart and ganglia may vary between European and New Zealand species. Currently there are no data on the effectiveness of commercially available stunning devices on any New Zealand crustacean species.

This project will investigate the effectiveness of a commercially available stunning device for two commercial New Zealand crustacean species (*J. edwardsii* and *P. zealandicus*). Data will contribute to developing regulations, standards and guidelines and mitigating risks in compliance. Confirmation of efficacy will support MPI's implementation of current and future animal welfare standards for crustacean species and create certainty for operators who may wish to purchase and use these expensive electrical stunning devices. The outcomes will also be used to ensure that New Zealand animal welfare requirements remain in line with international science and best practice.

Review of Previous Literature

There is a small amount of literature concerning the welfare of crustacea in the supply chain and more containing evidence that these animals are able to perceive pain. An investigation of the conditions under which lobster were kept in retail outlets in the UK (Carder 2017) found that most lobsters experience welfare compromise with the majority having banded claws, high stocking density, no dim or dark lighting, and no separate shelter away from other lobsters. Gardner (2004) reviews the welfare of crustacea after capture and during a variety of killing methods. The more specialised literature relevant to this project will be discussed under the following headings:

Pain in crustacea
Slaughter and Stunning of Crustacea
Electrophysiology and Evaluation of Crustatun™
Anaesthesia of Crustacea

Pain in Crustacea

There is an increasing amount of evidence in the literature that decapod crustacea are able to perceive pain in a similar cognitive manner and with similar consequences to vertebrates. These studies have been extensively reviewed by Elwood (2019) who draws the conclusion that crustacea show complex responses to noxious stimulation that are consistent with an ability to perceive pain. These responses include:

Avoidance learning (Sherwin 2001): The ability to learn to avoid a noxious stimulus.

Motivational trade off (Appel and Elwood 2009): Hermit crabs evacuate shells when they receive an electric shock, but a greater shock is required to cause evacuation from a highly desirable shell.

Protective grooming behaviour (Barr et al. 2008): Shrimp pay attention to and extensively groom an antenna following a noxious stimulus to that antenna.

Autotomy (Magee and Elwood 2013): Crabs can be induced to shed a limb by a noxious stimulus that does not cause tissue damage.

Long-term motivational change (Elwood and Stewart 1985; Fossat et al. 2014): Crayfish are motivated to stay in dark areas, but can change

their motivation to prefer well-lit areas if they are subject to noxious stimuli when in the dark.

Sensitisation of touch nociceptors – note in squid, not yet demonstrated in crustacea (Crook et al. 2011)

Cognitive effects on physiological changes (McCambridge et al. 2016): Crabs with tissue damage become more defensive and submissive.

Complex responses to analgesics (Maldonado and Miralto 1982): Morphine reduces the response to electric shocks in a dose-dependent manner.

Self-administration of analgesics – note in honeybees, not yet demonstrated in crustacea (Groening et al. 2017)

It is worth noting that some of these studies are not recent, but they have only recently been assembled to form a compelling case for pain perception in crustacea. Whilst this evidence has now resulted in a wide acceptance that crustacea are capable of pain perception, this view is not universally accepted (Rose et al. 2014).

Overall, widening acceptance of the ability of crustacea to perceive pain and the inclusion of these creatures in the definition of 'animal' in the Animal Welfare Act acknowledges that crustacea should be treated as sentient. As such it is imperative that processes such as slaughter that have noxious potential should be undertaken using techniques that minimise the welfare impact to these animals.

Slaughter and Stunning of Crustacea

A number of methods of stunning and killing have been investigated in crustacea and they are reviewed by Yue (2008). In general methods that are associated with poor welfare outcomes include:

any procedures whereby the abdomen is separated from the thorax (Yue 2008)

the removal of tissue, flesh, or limbs while the crustacean is alive and fully conscious (Yue 2008)

placing crustaceans in slowly heated water to the boiling point (Gardner 1997; Adams et al. 2019)

placing crustaceans directly into boiling water (Yue 2008)

carbon dioxide narcosis (Gardner 2004)

placing marine crustaceans in fresh water (Gardner 1997)

the unfocused microwaving of the body as opposed to focal application to the head (Yue 2008)

Methods associated with better welfare include:

chilling in air or ice slurry (Weineck et al. 2018)

chemical anaesthesia (Gardner 1997)

electrical stunning (Weineck et al. 2018)

spiking (for crabs, but not lobster) or splitting (for lobster, but not crabs) when carried out by a skilled technician (Yue 2008; Roth & Oines 2010)

Recently the Crustastun™ (Buckhaven 2000) has been developed and assessed for commercial use in a number of species of crustacea:

American lobster (Fregin & Bickmeyer 2016)
American crayfish (Fregin & Bickmeyer 2016)
European lobster (Fregin & Bickmeyer 2016)
Brazilian spiny lobster (Ogawa et al. 2007)
Langoustines (Albalat et al. 2008; Neil 2010)
Shore crab (Neil 2010)
Edible brown crab (Neil 2012; Neil & Thompson 2012)
European lobster (Neil 2012; Neil & Thompson 2012)

To date no studies have been performed assessing the effectiveness of the Crustastun™ on New Zealand species of lobster.

Electrophysiology and Evaluation of Crustatun™

There is a considerable literature concerning crustacean electrophysiology, much of which concerns the stomatogastric system (McGaw & Curtis 2013). This is of scientific

interest because it forms a small sensorimotor system (Blitz 2011) which is accessible, and so amenable to study compared to those of the central nervous systems of vertebrates.

Very few studies have investigated the role of electrophysiology related to processes of awareness. Fregin & Bickmeyer (2006) and Neil (2010, 2012) have both used electrophysiological methods to investigate the Crustastun™ as a stunning/killing device in northern hemisphere lobster. These studies use recording methods developed by Gruhn & Rathmayer (2002) to record far-field neuronal activity from multiple segments along the body of the lobster. Fregin & Bickmeyer (2006) demonstrated the effects of a number of methods of anaesthesia including electrical stunning. They also validated the recording technique by undertaking control studies on dead animals. They demonstrated that electrical stunning resulted in changes to the electrical rhythm of the nervous system which appeared to have a short tonic phase followed by a much longer relaxation phase. These changes appear to be broadly similar to the epileptiform changes induced by electrical stunning in mammals (Farouk et al. 2017). Neil (2010, 2012) investigated the effects of electrical stunning on exposed nerve activity and found that the nerves recorded from demonstrated immediate cessation of activity following application of the Crustatun™. Other studies utilising the Crustastun™ evaluate physiological stress arising from its use (Neil & Thompson 2012) and the meat quality of animals stunned using the device (Albalat et al. 2008). These studies indicate that the use of the Crustastun™ is not associated with increased stress and meat quality is improved compared to animals killed by chilling on ice.

Anaesthesia of Crustacea

A number of studies have investigated techniques for anaesthetising crustaceans. Many of these utilise clove oil derivatives because they are considered to be safe for human consumption and so can be used immediately prior to killing and still allow consumption of the meat (Waterstrat & Pinkham 2005, Tuan & Chan 2018). Mosley & Lewbart (2014) review other suitable anaesthetic techniques in crustacea. Previous studies evaluating the electrophysiological effects of the Crustatun™ do not mention the use of any anaesthetic technique other than chilling with ice (Neil 2010, Fregin 2016).

Key findings

This review discusses the literature that exists on the welfare of decapod crustacea with a focus on previous studies that have investigated the use of the Crustastun™ to stun/slaughter these animals. Whilst the literature is somewhat patchy in its coverage of these areas, a number of conclusions can be drawn:

There is a growing consensus that decapod crustacea are able to perceive pain.

Crustastun™ has been investigated as a method of stun/slaughter in several species of decapod and has been shown to be effective in abolishing the coordinated electrical activity of the nervous system.

There are no direct studies of Crustastun™ which utilise techniques of anaesthesia that would be considered appropriate in a New Zealand experimental context.

The design of the proposed experimental study to investigate the effectiveness of Crustastun™ in New Zealand commercial decapods (*J. edwardsii* and *P. zealandicus*) will be able to adapt the experimental studies used in other species, but will need to ensure that the anaesthetic and electrode placement techniques used enable suitable pre-stun electrophysiological data to be recorded so that the effect of the stun itself can be established.

Methods

This study was undertaken with the approval of the Massey University Animal Ethics Committee (protocol # 20/41), and carried out in accordance with the Massey University Code of Ethical Conduct for the Use of Animals in Research, Teaching and Testing.

In order to record nervous system activity from intact animals in a minimally invasive manner that did not require prolonged recovery between instrumentation and testing, we developed a method to record spontaneous electrical activity using electrodes inserted into the musculature through naturally occurring gaps between adjacent carapace segments. To safeguard welfare, all animals were anaesthetised prior to electrode placement and subsequent stunning.

Animals

J. edwardsii were sourced from the southern fishery (Whitecaps Fishing Company in Stewart Island) and were requested to be at the upper end of the size of commercially harvested animals. They were caught off the coast of Fjordland, transported to Massey from Stewart Island in two batches and transported in polystyrene boxes. Boxes were lined with ice packs and the animals separated from the ice packs and each other by wet paper and sacking. The first six animals arrived on 6th October and the remaining four on 18th November. *P. zealandicus* were sourced commercially (Ernslaw One Ltd in Tapanui) and were transported in a polystyrene box lined with ice packs and were separated by wet sacking and wood shavings. All ten animals arrived on 10th November.

All animals were judged to be healthy on arrival and were acclimatised for between one and two weeks prior to use in the study. Both species were held in circulating cold water systems. *J. edwardsii* were held in individual 40-litre holding tanks filled with natural seawater and topped up with artificial seawater, located in the Ecology department at Massey University (Figure 1a). Aerated seawater was circulated at a constant rate of 1–2 litres per minute. The main reticulated supply contained an in-tank biofilter. The holding room was maintained at 14°C and external windows provided a natural light/dark cycle. Animals were fed green lipped muscles daily or every second day, based on consumption rate. Tanks were vacuumed twice weekly to remove faeces and detritus. *P. zealandicus* were held together in a single 50-litre aquarium filled with stream water and topped up with RO water, located in the neurophysiology laboratory at Massey University (Figure 1b). The tank contained stones and logs to provide shelter and separation of individuals. Tank water was chilled to approximately 13°C and aerated. Animals were fed dried meal worms and/or blood worms daily or every second day. The tank was vacuumed weekly

along with a partial water change to remove nitrogenous wastes. The size and weight of all animals used in the study and the dose of lidocaine anaesthesia is recorded in Table 1.

Pilot Study

Pilot experimental studies were undertaken at Massey on 14th and 15th of October utilising two *J. edwardsii*. Results from the pilot study were used to inform the decision to proceed with the main study and also to optimise the experimental protocol in terms of anaesthesia and electrode placement.

The first animal (*J. edwardsii* 1) was given 5 mL of 2% lignocaine (100 mg) as a single dose. Within five minutes of administration, the animal was deemed effectively anaesthetised, based on relaxation of the tail muscle, absence of muscle tone in the legs, and loss of righting reflex. In the second animal (*J. edwardsii* 2) lidocaine was given incrementally in 1 mL doses (20 mg). After each 20 mg dose, the animal was returned to the holding box for 10 minutes, after which time sedation was assessed. After the third dose (60 mg total), the animal showed loss of muscle tone in the tail and legs and minimal righting reflex, so was instrumented and stunned.

Examination of electrophysiological recordings from the two pilot animals revealed no observable differences in the amplitude of baseline (pre-stunning) data, suggesting that the higher dose of lignocaine given to animal 2 did not interfere with data acquisition. In mammals, increasing depth of anaesthesia is known to influence brain electrical activity, therefore the minimum dose required for effective anaesthesia is adopted for studies of brain activity. Based on the apparent lack of impact on electrophysiological recordings, an anaesthetic dose of 100 mg/kg lidocaine (animal 1) was adopted for the main study (Table 1).

Electrode positions for these two animals is recorded in Table 2 and illustrated in Fig 8. The electrode placement used in animal 1 was adopted for the main study because that used in animal 2 was associated with more obvious burning of the carapace during stunning. Both electrode positions allowed adequate electrical activity to be recorded.

The results of this study were compatible with the main study and so these two animals were included in the final dataset.

Experimental Protocol (J. edwardsii)

On the day of the study, the animals were transported to the Neuroscience Laboratory in thermally insulated containers with shallow water. They were anaesthetised by intramuscular injection of 2% lidocaine hydrochloride (Lopaine, Ethical Agents, NZ), injected into the musculature of the first abdominal segment. See Table 1 for weight and size of crayfish and dose of anaesthetic.

Once anaesthetised a four-electrode montage of recording electrodes was applied to the animals to allow two channels of electrical activity to be recorded. Electrodes and leads were secured to the animal and electrodes isolated from the environment by application of cyanoacrylate adhesive (Super 'T' gap filling, Satellite City, California, USA) and accelerant (Zip Kicker, Zapglue, Ilinois, USA). Electrical activity was recorded using IsoDAM signal amplifiers (WPI Instruments, Florida, USA), digitised using an analogue to digital converter (Powerlab, ADI, New Zealand) and recorded to computer file (Chart software, ADI, New Zealand).

Following placement of electrodes, a three-minute baseline was recorded after which animals were placed in the stunning device (Crustastun, Mitchell and Cooper, UK). The chamber of the Crustastun™ measured 440x360mm and was 130mm deep. The stun was delivered using setting 2 (large crustacean). Delivery of a successful stun current was illustrated by the stun current indicator on the front panel of the device. Where the stun current was too low or high, this was also illustrated by the stun current indicator and accompanied by an audible alarm.

Electrical data were continuously recorded during the delivery of the stun and for a five-minute period afterwards. The animal was then removed from the stunning device and placed in a container for further observations (20-30 minutes) to determine if it was stunned or dead. Stunning was deemed successful if the animal was quiescent upon removal from the stunning device. Death was ascertained by permanent loss of muscle tone and absence of spontaneous or evoked movement of the body or appendages (including mouthparts and swimmarets).

Any animals for which death by stunning was not confirmed were subsequently euthanased via intramuscular injection of 2.5 g of pentobarbital potassium (Provet NZ Pty Ltd, New Zealand), followed by midline separation.

Experimental Protocol (P. zealandicus)

Animals were removed individually from the home tank, weighed and lightly anaesthetised via intramuscular injection of lidocaine. The animal was then covered

with a freshwater-dampened towel and left undisturbed for 10 minutes to allow anaesthesia to take effect. A dose rate of approximately 200 mg/kg (see Table 1) was required to achieve adequate anaesthesia (cessation of purposeful movement and reduced or absent muscle tone in the tail). Animals were then instrumented for electrical data recording as described for *J. edwardsii* above (refer to Table 2 for electrode placement).

Data Analysis

Raw data were subjected to Fast Fourier transformation, yielding the summary variables median frequency (F50), 95% spectral edge frequency (F95) and total power (PTOT) for consecutive 1-second epochs. The mean F50, F95 and PTOT were calculated for the 60-seconds immediately prior to stunning and 60-seconds immediately after stun application for each channel in each individual. From this, the mean changes in F50, F95 and PTOT were determined for the entire cohort. Any data contaminated with movement artefact (e.g., spontaneous tail or limb movements, closure of stunner lid) were excluded from analysis. This meant that some data sets included less than 60 EEG epochs per period.

Paired t-tests were performed to compare post stunning means for each frequency variable with pre-stunning values. Statistical analyses were conducted in SAS v9.4 (SAS Institute Inc, Cary, NC).

Results

With the exception of one *J. edwardsii* (#6, weighing 1760 grams), all animals were delivered a successful stun (as indicated by the stunning device). In the case of animal #6, three attempts to deliver a successful stun resulted in short bursts of current (~2 seconds per attempt) followed by a stun failure alarm. When the lid was opened, the animal was exhibiting slow, uncoordinated movement of the appendages similar to those observed prior to being placed in the stunner. Of the other nine, three appeared to be killed outright by the stun (no evidence of movement or muscle tone). Six exhibited some degree of muscle tone and/or occasional very slow uncoordinated movement of the limbs. Such movements or muscle tone did not resemble those seen prior to anaesthesia, or subsequent to anaesthesia pre-stunning. No recovery of spontaneous or evoked movement (reflex response to eye stalk touch) was observed in any animal subsequent to stunning. As a precaution, the animals for which death was not confirmed were euthanased with pentobarbital.

All ten *P. zealandicus* appeared to be killed outright by the stun (no evidence of spontaneous or evoked movement, or muscle tone after removal from the stunner).

Electrophysiological data (J. edwardsii)

Data from 9 animals were included in the analyses. Of note, there appeared to be more individual variability in the electrical responses of *J. edwardsii* to stunning, when compared with *P. zealandicus*. This may have been due to the larger variability in size and weight of individuals.

Data from Channel 1 demonstrated a consistent pattern of decreases in F50 and F95 and an increase in PTOT following stunning (Figure 2). Of the three variables, only F50 was significantly different post stunning (Table 3).

Data from Channel 2 were less consistent, with some individuals showing the same pattern of decreases in F50 and an increase in PTOT as seen in Channel 1, whereas little to no change was observed in others. Whilst a similar trend was observed overall, high individual variability meant these changes were less marked (Figure 3) and did not reach statistical significance at $p<0.05$ (Table 4).

A typical example of changes in the frequency spectra after stunning is shown in Figure 4, where a marked increase in power in the lower frequencies is clearly visible.

Electrophysiological data (P. zealandicus)

Data from Channel 1 demonstrated a consistent pattern of decreases in F50 and F95 and an increase in PTOT following stunning (Figure 5). All three variables were significantly different post stunning (Table 5).

Data from Channel 2 were less consistent, with some individuals showing the same pattern of decreases in F50 and an increase in PTOT as seen in Channel 1, whereas little to no change was observed in others. Whilst a similar trend was observed overall, high individual variability meant these changes were less marked (Figure 6). Despite this, both F50 and F95 differed significantly post stunning (Table 6).

A typical example of changes in the frequency spectra after stunning is shown in Figure 7, where a marked increase in power in the lower frequencies is clearly visible.

Discussion

This study aimed to evaluate the ability of the Crustastun™ to adequately stun and/or slaughter *J. edwardsii* and *P. zealandicus* for commercial purposes. The criteria for a successful stun were that insensibility would be achieved rapidly and maintained for long enough to allow killing by conventional means if the animals were not killed by the Crustastun™ itself. Since this was the first formal use of the Crustastun™ in these species, all animals were anaesthetised prior to instrumentation and stunning and so the duration of the stun in those animals that were not killed outright could not be measured.

Of the nine *J. edwardsii* to which a stun was applied, stunning with the Crustastun™ resulted in instantaneous changes to the electrical activity of the nervous system that qualitatively resembled EEG changes seen in mammals during application of stunning techniques (Johnson et al. 2012, Rault et al. 2014, Sabow et al. 2017). Five of these animals appeared to have been killed outright by the stun with no sign of return of behavioural function following the stun. Those that were not killed outright showed no sign of return of normal behaviour following the stun, but it is not possible to determine if this was due to the stun or to continuation of the effects of the anaesthetic.

The largest animal in the *J. edwardsii* group was 30% longer and 96% heavier than the mean of the other 9 animals, weighing 1,760g (Table 1). It was physically difficult to fit into the Crustastun™, needing to have its tail folded underneath it rather than being placed flat on the plate (the total length of this animal with antennae folded back was 470mm). During stunning, the Crustastun™ detected that the animal was unsuitable and did not deliver a stun. This may have been due to the position of the animal resulting in an electrical load that was outside the range of the device and may have been due to poor contact between the animal and the electrodes or to displacement of the water in the stunner raising the level and shorting the electrodes. Information provided from Daryl Sykes of the New Zealand Rock Lobster Industry Council (personal communication) indicates that the New Zealand restaurant trade typically deals with lobster live weights in the range of 600–1000 grams.

In all ten *P. zealandicus* used in this study, stunning with the Crustastun™ resulted in instantaneous changes to the electrical activity of the nervous system that qualitatively resembled EEG changes seen in mammals during application of stunning techniques (Johnson et al. 2012, Rault et al. 2014, Sabow et al. 2017). All ten animals appeared to have been killed outright by the stun with no sign of return of behavioural function following the stun.

All animals used in this study were anaesthetised throughout and were either killed by the stun or killed prior to the return of normal behaviour. The anaesthetic was considered necessary to guard the welfare of the animals because this device has not previously been used on the two species in question, but it meant that the duration of the stun in those *J. edwardsii* that were not killed outright could not be determined. Electrical activity was recorded for five minutes following the stun and there was no evidence of return of activity that might indicate awareness during this time. These results indicate that the maximum stun-to-kill time for animals that are not killed outright is at least five minutes, though it could be much longer. Further research would be necessary to accurately determine a safe maximum stun-to-kill time.

Conclusions

Crustastun™ appears to be an acceptable method of killing or adequately stunning all but the largest *J. edwardsii*. No stun was applied to the largest animal, presumably because the electrical load of this animal fell outside of the machine's programmed range. Our study is not able to specify a cut-off point beyond a liveweight of 1160 g for effective stunning of *J. Edwardsii*. The results do however show the need for the tail to lie flat and for the animal to be fully enclosed In order for the crustastun™ to operate reliably.

Crustastun™ appears to be an acceptable method of killing *P. zealandicus*.

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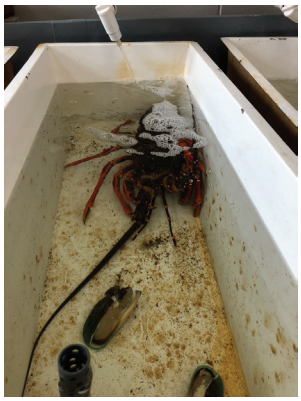
Figure 1

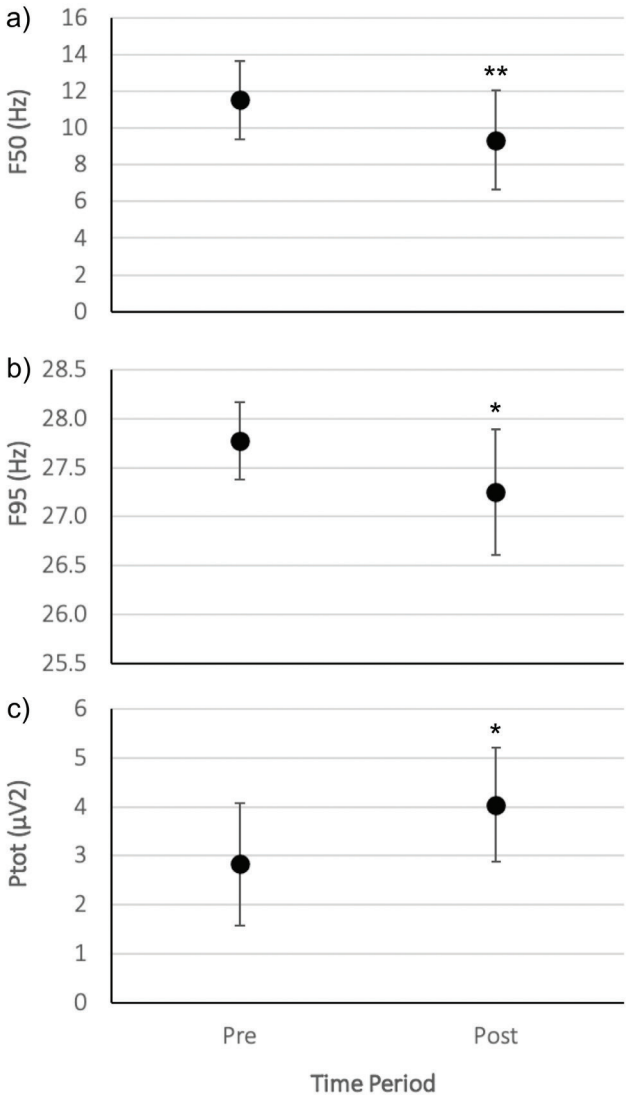
Acclimatisation systems for *P. zealandicus* and *J. edwardsii*.


a


b

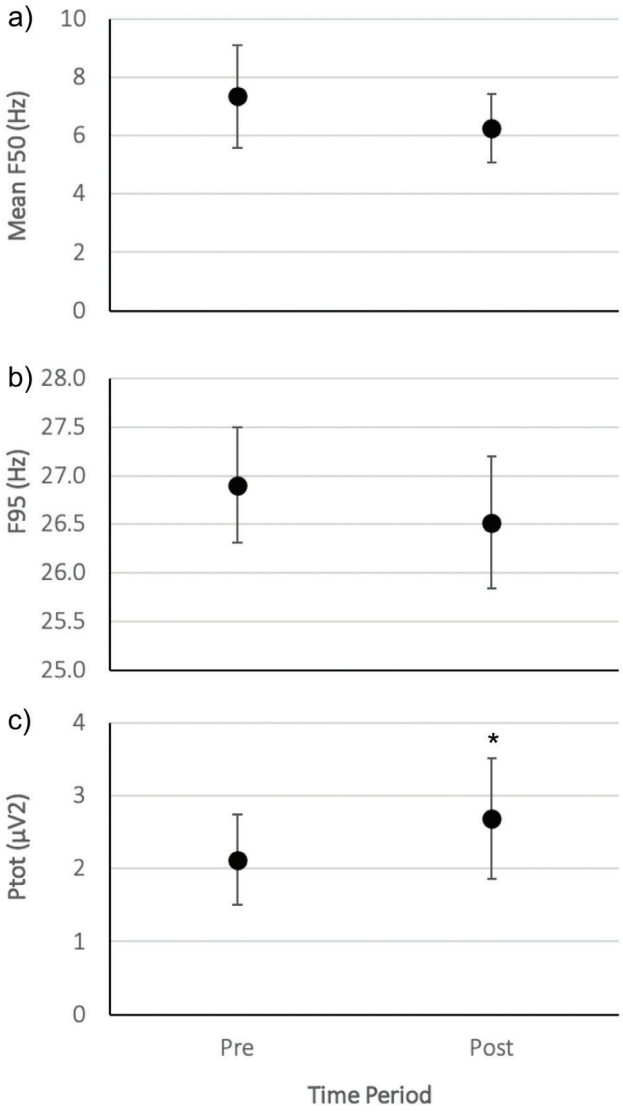
a) *P. zealandicus* on arrival with acclimatisation tank in background.
b) *J. edwardsii* in acclimatisation tank with mussels in foreground.

Figure 2



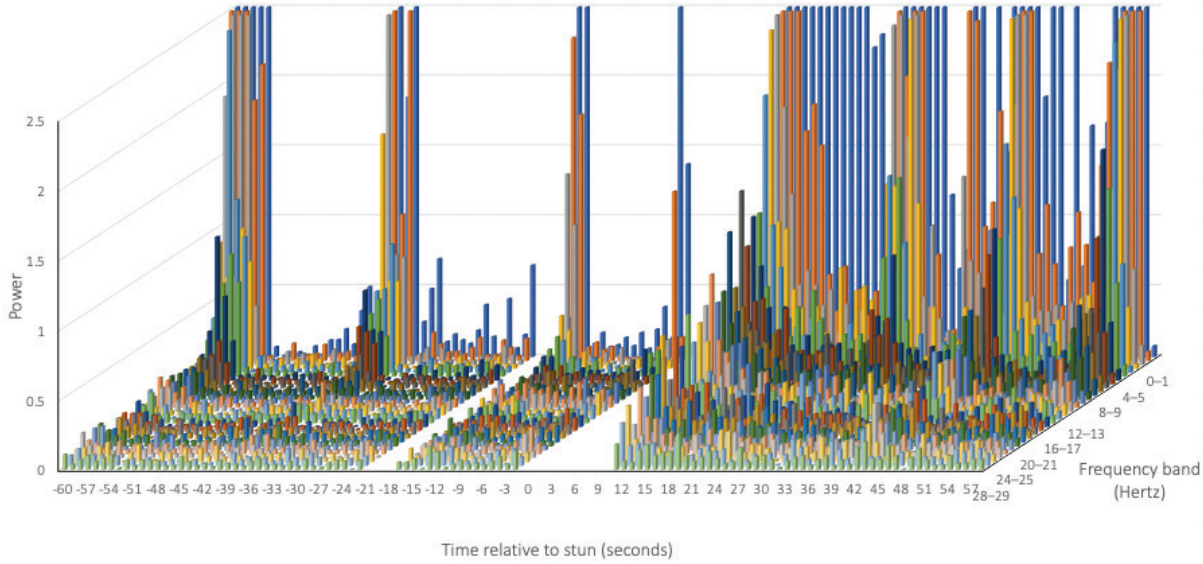
Mean a) F50, b) F95 and c) PTOT of Channel 1 (head to cranial abdomen) in *J. edwardsii*, n=9 in the 60-seconds prior (Pre) and 60-seconds immediately after (Post) electrical stunning using the Crustastun™ commercial benchtop stunner. Asterisks indicate statistical significance as follows: ***p<0.01; ** p<0.05; * p≤0.1

Figure 3



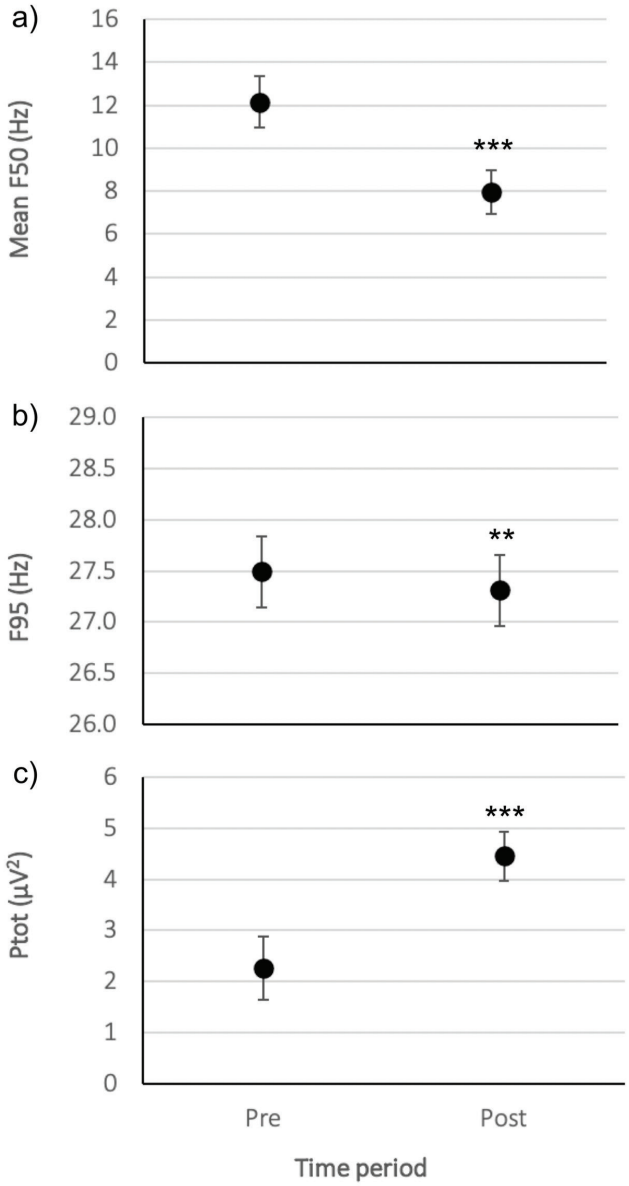
Mean a) F50, b) F95 and c) PTOT of Channel 2 (head to caudal abdomen) in *J. edwardsii*, n=9 in the 60-seconds prior (Pre) and 60-seconds immediately after (Post) electrical stunning using the Crustastun™ commercial benchtop stunner. Asterisks indicate statistical significance as follows: ***p<0.01; ** p<0.05; * p≤0.1

Figure 4



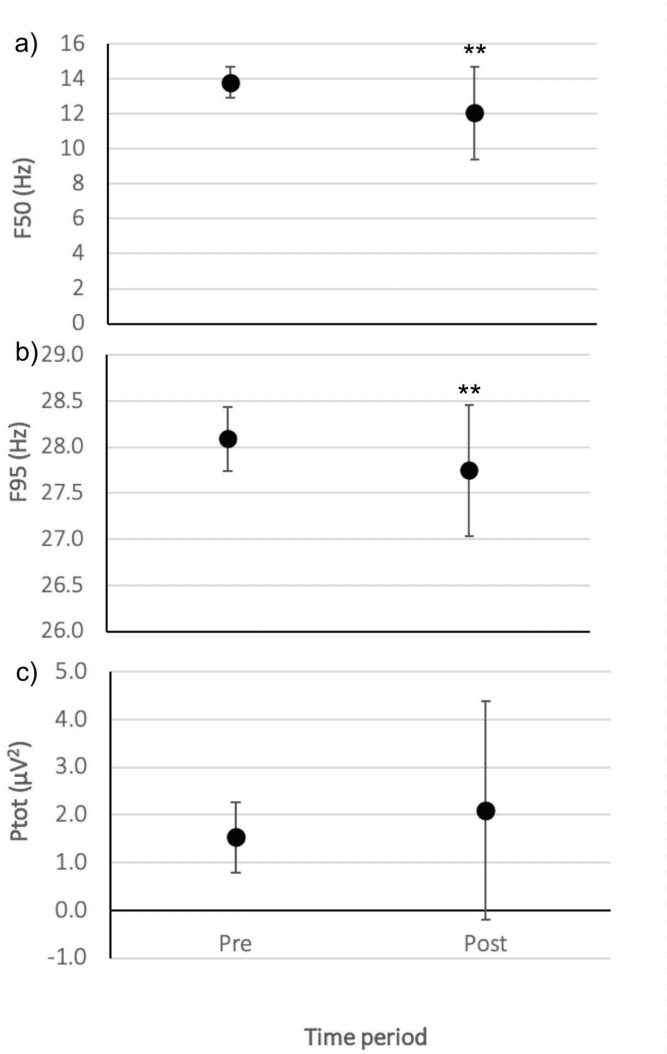
Example of a compressed spectral array from an individual *J. edwardsii* before and after electrical stunning (Time 0) using the Crustastun™ commercial benchtop stunner. Recorded from electrodes spanning the head to cranial abdomen. Missing data are where artefact induced by transfer to the stunner and/or stun application have been removed.

Figure 5



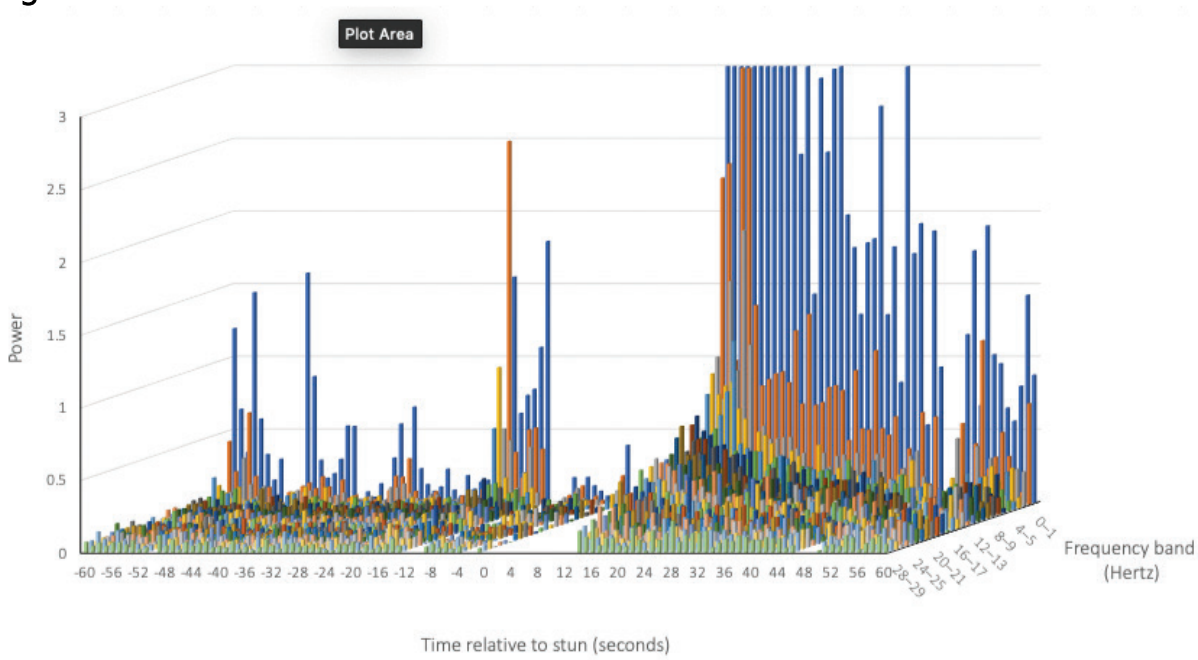
Mean a) F50, b) F95 and c) PTOT of Channel 1 (head to cranial abdomen) in *P. zealandicus*, n=10 in the 60-seconds prior (Pre) and 60-seconds immediately after (Post) electrical stunning using the Crustastun™ commercial benchtop stunner. Asterisks indicate statistical significance as follows: ***p<0.01; ** p<0.05; * p≤0.1

Figure 6



Mean a) F50, b) F95 and c) PTOT of Channel 2 (head to caudal abdomen) in *P. zealandicus*, n=10 in the 60-seconds prior (Pre) and 60-seconds immediately after (Post) electrical stunning using the Crustastun™ commercial benchtop stunner. Asterisks indicate statistical significance as follows: ***p<0.01; ** p<0.05; * p≤0.1

Figure 7



Example of a compressed spectral array from an individual *P. zealandicus* before and after electrical stunning (Time 0) using the Crustastun™ commercial benchtop stunner. Recorded from electrodes spanning the head to cranial abdomen. Missing data are where artefact induced by transfer to the stunner and/or stun application have been removed.

Figure 8

J. edwardsii following anaesthesia and instrumentation, prior to placement in Crustastun™ illustrating electrode placement. Yellow electrode (not easily visible) – common non-inverting; blue electrode – channel 1 inverting; white electrode – channel 2 inverting; black electrode – ground.

Table 1

Sizes, weights and anaesthetic doses for *J. edwardsii* and *P. zealandicus*.

Animal	Weight (g)	Carapace Length	Tail Width* (mm)	Lidocaine dose (mg)	Outcome
J. edwardsii 1	776	113	64	100	Stunned
J.edwardsii 2	723	111	60	40	Killed
J.edwardsii 3	1,163	117	72	116	Killed
J.edwardsii 4	899	116	65	90	Stunned
J.edwardsii 5	814	115	65	81	Stunned
J.edwardsii 6	1,760	153	81	176	Failed to stun†
J.edwardsii 7	943	120	65	94	Killed
J.edwardsii 8	877	121	66	88	Stunned
J.edwardsii 9	721	110	65	72	Stunned
J.edwardsii 10	1,130	131	76	112	Stunned
P. zealandicus 1	44	51	29	5	Killed
P. zealandicus 2	43	52	25	15	Killed
P. zealandicus 3	28	47	24	8	Killed
P. zealandicus 4	48	56	29	10	Killed
P. zealandicus 5	51	58	32	10	Killed
P. zealandicus 6	44	53	29	9	Killed
P. zealandicus 7	61	63	34	12	Killed
P. zealandicus 8	28	46	23	6	Killed
P. zealandicus 9	54	54	31	11	Killed
P. zealandicus 10	47	55	31	10	Killed

* Distance between primary spines on second abdominal segment

† Animal was too large to place properly into stunner

Table 2

Electrode placement.

Animal	Common non-inverting	Channel 1 inverting	Channel 2 inverting	Ground
J.edwardsii 1	Articulation of left antenna with head	Lateral under carapace of first abdominal segment on left	Lateral under carapace of fourth abdominal segment on left	Lateral under carapace of fourth abdominal segment on right
J.edwardsii 2	Articulation of left antenna with head	Dorsal midline between first and second abdominal segment	Dorsal midline between fourth and fifth abdominal segment	Lateral under carapace of fourth abdominal segment on right

Table 3

Summary of statistical analyses of Channel 1 data from *J. edwardsii*, n=9 before (Pre) and after (Post) electrical stunning using the Crustastun™ commercial benchtop stunner. LSM – least square mean, DF – degrees of freedom.

Variable	Period	LSM	DF	t-value	P-value
F50	Pre	11.56	8		
	Post	9.36	8	2.32	0.049
F95	Pre	27.78	8		
	Post	27.25	8	2.06	0.073
Ptot	Pre	2.83	8		
	Post	4.04	8	-1.94	0.088

Table 4

Summary of statistical analyses of Channel 2 data from *J. edwardsii*, n=9 before (Pre) and after (Post) electrical stunning using the Crustastun™ commercial benchtop stunner. LSM – least square mean, DF – degrees of freedom.

Variable	Period	LSM	DF	t-value	P-value
F50	Pre	7.36	8		
	Post	6.28	8	1.25	0.278
F95	Pre	26.91	8		
	Post	26.52	8	0.89	0.422
Ptot	Pre	2.13	8		
	Post	2.7	8	-2.09	0.104

Table 5

Summary of statistical analyses of Channel 1 data from *P. zealandicus*, n=10 before (Pre) and after (Post) electrical stunning using the Crustastun™ commercial benchtop stunner. LSM – least square mean, DF – degrees of freedom.

Variable	Period	LSM	DF	t-value	P-value
F50	Pre	12.15	9		
	Post	7.96	9	9.06	<0.001
F95	Pre	27.86	9		
	Post	27.24	9	4.06	0.0029
Ptot	Pre	2.26	9		
	Post	4.46	9	-9.03	<0.001

Table 6

Summary of statistical analyses of Channel 2 data from *P. zealandicus*, n=10 before (Pre) and after (Post) electrical stunning using the Crustastun™ commercial benchtop stunner. LSM – least square mean, DF – degrees of freedom.

Variable	Period	LSM	DF	t-value	P-value
F50	Pre	13.807	9		
	Post	12.073	9	2.72	0.024
F95	Pre	28.089	9		
	Post	27.746	9	2.58	0.03
Ptot	Pre	1.531	9		
	Post	2.099	9	-1.17	0.272