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Effectiveness and quality evaluation of electrical stunning versus chilling in Norway lobsters (*Nephrops norvegicus*)

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ABSTRACT

In the last decade, public interest in the welfare of decapod crustaceans has increased in many parts of the world. This has led to changes in legislation on methods for slaughter in a number of countries, while in others pressure for regulation changes is growing. Electro-stunning may have the potential for reducing noxious stimuli experienced by crustaceans during slaughter. However, data on activity in the central nervous system (CNS) and product quality-related data (indicative of consumer acceptability) are not available for most of the important decapod crustaceans, including the commercially valuable Norway lobster, *Nephrops norvegicus*. In this study, recordings of nerve activity showed that electro-stunning can render *N. norvegicus* rapidly insensible qualifying it as a potential humane slaughter procedure. In contrast, placing lobsters on ice for 30 min did not suppress neural activity. In terms of subsequent shelf life, results at day 7 based on QIM, total bacteria counts, H_2S-producing bacteria, muscle pH, TMA and biogenic amines indicate no significant differences between the methods of stunning. From a quality perspective, electro-stunning did shorten the period that the product would be considered fresh (higher K-values up to day 5) and triggered faster melanosis development in the cephalothorax, an effect possibly linked to the increased temperature recorded in this area due to the imposed electrical current. However, no significant differences were detected on the cooked products by a trained sensory taste panel. While shelf life is not affected by the electro-stunning process, care should nevertheless be taken to avoid melanosis development, and the consumer perception of freshness in electro-stunned product should be studied further.

1. Introduction

There is growing public concern in many parts of the world about the welfare of decapod crustaceans that are retailed as seafood. This is partly stimulated by the debate over whether decapod crustaceans have the capacity to feel pain or suffering, with some arguments being made in support of this view (Passantino et al., 2021; Conte et al., 2021) while others contend that the evidence for this is not yet well developed (Diggles, 2019). Nevertheless, in some countries this has led to the introduction of legislation for ethical methods of slaughter (Rowe, 2018) while other countries are considering including all decapod crustaceans in welfare legislation, e.g. the Animal Welfare (Sentience) Bill in the UK.[1] To improve crustacean welfare during slaughter, research has been undertaken to investigate various methods of stunning prior to slaughter. However, for any such procedure to be viable for commercial use it must also be the case that it has no detrimental effect on either the quality or the shelf life of these products (Neil, 2012a).

Electro-stunning may have potential as an effective stunning method for some crustacean groups, as it can be faster in inducing insensibility (within a few seconds) compared with other methods such as chilling, freezing, gradual heating, boiling, piercing of ganglia, salt baths and gas (CO_2) (Adams et al., 2019; Fregin & Bickmeyer, 2016; Neil, 2012b; Roth & Oines, 2010; Weineck et al., 2018). However, there is empirical evidence that electrical stunning may not be 100% effective when applied to some species of crustaceans (see Fregin & Bickmeyer, 2016; Weineck et al., 2018), and the parameters of electro-stunning must be optimised if they are to prevent unnecessary suffering and yet be sufficient to kill the animal (irreversible stunning). Even if not sufficient for slaughter,

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electrostunning may nevertheless maintain animals in a stunned state for sufficient time that another method of dispatch can be applied, before sensibility returns (Fregin & Bickmeyer, 2016; Weineck et al., 2018). Recording nerve activity before and after electro-stunning provides an indication of whether this technique is completely effective, as previously demonstrated for the commercially important species, the European lobster *Homarus gammarus* and the edible brown crab *Cancer pagurus* (Neil, 2012b).

The electric shock may also pose a number of potential risks to the quality of the product. Reported quality-related challenges include the loss of claws and legs through autotomy (Roth & Grimsbo, 2016), which may affect product acceptability. Also, the electrical current can cause overheating, either localised to the shell surface, or if prolonged, through the whole animal (Roth & Grimsbo, 2016), which could impart tissue damage and accelerate bacterial growth. Finally, electro-stunning currents induce a brief but gross contracture in the musculature (i.e. epilepsy-like seizures or spasms, see Fregin & Bickmeyer, 2016) which may cause irreversible structural damage to and deplete the energy reserves of the muscles that represent the saleable meat product (Atsushi et al., 1990).

Nevertheless, it remains to be investigated whether electro-stunning has any other effects on the decapod post-mortem metabolism, and hence also on the shelf life of seafood products derived from them. Standardised quality evaluations, based on measures such as muscle pH, muscle nucleotides and the derived K-value, bacterial load and trimethylamine (TMA) concentration, combined with organoleptic assessments, have been published for decapod crustaceans when stored on ice (Zhang et al., 2021). The results of these measures are already available for the Norway lobster *Nephrops norvegicus*, a decapod crustacean also known as langoustine, (Albalat et al., 2011; Bekaert et al., 2015; Lopez-Caballero et al., 2006), which is a species that supports a valuable fishery in the EU with yearly landings of round 59,000 tonnes (FAO, 2021). This species is caught by both trawling and creel-trapping, and there are markets for both live-traded and processed product (Leocadio et al., 2012; Morello et al., 2009; Neil, 2012a). It therefore represents an important target species to be included in any legislation for ethical slaughter that includes decapod crustacean species. For these reasons, in the present study we have investigated electro-stunning of *N. norvegicus* as a humane slaughter method and compared it with the most commonly used slaughter technique, chilling on ice, in terms of a range of quality measures.

2. Material and methods

2.1. Experimental design

Norway lobsters (*Nephrops norvegicus*) were caught by otter trawl in the Clyde Sea area, Scotland UK, on three fishing trips that took place in May, August and October 2007 using the research vessel Aplysia from the University Marine Biological Station Millport (UMBSM). For all trips, once on board, animals were kept alive in running seawater and landed to the UMBSM facilities within 4 h. Animals used in the three trials had a carapace length (CL) range between 38 and 42 mm.

2.1.1. First trial: electrophysiological recordings

Animals collected during the May fishing trip were transported in seawater containers to the aquarium facilities at the University of Glasgow and placed within a closed seawater circulating system for at least one week before experimentation. Only intact and vigorous specimens, as defined by (Albalat et al., 2017), were selected for use. Electrical stunning was applied to 6 individuals without prior anaesthesia using a commercially available device (Crustastun™; Studham Technologies Ltd, Cambridge, UK) (Fig. S1A) following the manufacturer's operating procedures. The chamber of the stunning device was filled with a salt solution (NaCl 3 g/L) and a stunning cycle of 110 V, 2–5 amp electrical charge for 10 s was applied. Another 6 individuals were held on crushed ice for 30 min to impose an ice-stun prior to the experimental procedures. A further 6 animals were used untreated controls.

Animals were prepared so that electrophysiological recording could be made with or without stunning. In order to expose the central nervous system, the carapace was removed and the cephalothorax was separated from the abdomen. Internal organs were removed or displaced to expose the circumoesophageal connectives and the preparation was then submerged in a balanced salt solution corresponding to the osmolarity to *N. norvegicus* haemolymph (1010 mOsm kg^{-1}) at a temperature of 10 °C. To expose the abdominal ventral nerve cord the dorsal skeletal plates were detached and muscle bundles were removed to reveal the ventral nerve cord and also the motor roots that emerge from it.

To make extracellular recordings from the circumoesophageal connectives and from the ventral nerve cord and its motor roots a suction electrode method was used (Johnson et al., 2007), using an '*en passant*' recording configuration to allow both directions of nerve transmission to be monitored. In three cases, the circumoesophageal connective was cut and the electrode was then attached to either its anterior or posterior cut end, to selectively record ascending or descending neuronal traffic. Evoked sensory activity was recorded from the leg nerves of autotomized legs (pre-stunning) or amputated legs (post-stunning) using bipolar electrodes. In all cases signals from the electrodes were passed to a differential pre-amplifier (A101, Isleworth Ltd, UK) for amplification and filtering, digitized by an A/D converter (PowerLab, AD Instruments Ltd, UK) and displayed and recorded on a standard PC computer using associated software (Chart v7, AD Instruments Ltd., UK).

2.1.2. Second trial: quality and sensory evaluation

Once animals were landed at UMBSM, those from the August fishing trip were separated into 2 groups (n = 240 animals). Animals from one group (n = 120) were electrically stunned by Crustastun™ (details section 2.1.1), while animals from the other group (n = 120) were placed directly into a container of crushed ice for 30 min. Thereafter, both groups of animals were transported on ice to the University of Glasgow where they were kept at a temperature of 0–2 °C for up to 7 days. Animals from both experimental groups were taken on days 1, 3, 5 and 7 of ice storage (n = 10 animals/group/time point) and a visual assessment using a Quality Index Method (QIM) and a melanosis score index were performed on whole animals (details section 2.2). Furthermore, sample portions of the muscle from individual tails (n = 10 animals/group/time point in total) were taken for the measurement of muscle pH (n = 10), for the analysis of ATP and its breakdown products (K-values) (n = 3), total and H_2S producing bacteria load (n = 3), TMA (n = 4) and biogenic amine levels (n = 3). Other samples of tail meat from animals from both groups (n = 60/group) on day 1 of storage were frozen and subsequently analysed by an independent sensory panel trained for the sensory evaluation of *N. norvegicus*.

2.1.3. Third trial: temperature recordings

Animals from the October fishing trip were electrically stunned using the Crustastun™ device. In this trial, seven stun cycles were performed with 4–5 animals per cycle (n = 35 animals in total). Observations on animal posture and temperature were recorded by taking photographs and thermal images (Fluke Ti20 thermal imager) before and after the stunning procedure and the temperature of the brine (salt solution; 3 g/L) in the Crustastun™ device was also recorded.

2.2. Sensory assessment

2.2.1. Sensory assessment of raw samples (quality index method, QIM)

QIM was based on scoring five different attributes (general appearance of claws, cephalothorax, upper tail, under tail and odour) all shown to increase linearly with storage time on ice using four demerit scores (from 0 to 3) per attribute (Suppl. Table 1) as described in (Gornik et al., 2013). Three assessors scored each animal (n = 10/group) independently on days 1, 3, 5 and 7 of storage time on ice. Prior to assessment,

Table 1
Sensory panel scores recorded in cooked tail meat from *N. norvegicus* that had been electrical or ice-stunned. Data on each group represents the mean of n = 10 assessors ± SEM. (*) indicates differences are significantly different $p < 0.05$.

	Stunning method	
	Electrical	Ice
Smell character	5.63 ± 0.39	5.01 ± 1.71
Smell strength	5.07 ± 1.22	3.89 ± 2.20
Springiness	6.09 ± 2.87	5.91 ± 1.44
Firmness	6.09 ± 1.09	7.01 ± 2.13
Chewiness	5.8 ± 1.12	7.46 ± 2.43
Moistness	5.73 ± 0.86	5.81 ± 1.09
Flavour	6.51 ± 1.28	6.57 ± 1.45
Aftertaste	6.11 ± 1.27	6.20 ± 1.65

samples were equilibrated to room temperature for 15 min and to avoid bias sample group identity was not disclosed.

2.2.2. Melanosis

In a similar way to the QIM scoring, melanosis development was assessed visually by three assessors. Each animal was scored (n = 10/group) independently on days 1, 3, 5 and 7 of storage time on ice. Melanosis was scored in six different body parts (1- dorsal cephalothorax, 2- claws, 3- ventral cephalothorax, 4- pleopod appendages, 5- dorsal abdomen and 6- tail fan) using a numeric scale from 1 to 4, where 1 = complete absence of black spots; 2 = a few back spots; 3 = considerable spotting/blackening and 4 = substantial spotting/blackening as in (Martinez-Alvarez et al., 2007).

2.2.3. Sensory testing panel

Descriptive analysis of sensory attributes of cooked samples (around 60 tails for each treatment group) was carried out by an independent trained panel recruited by the Food Innovation Institute, Queen Margaret University in Edinburgh, consisting of ten experienced judges. The panel used a derivative of a quantitative descriptive analysis method (QDA) adapted for use with *N. norvegicus* as described in (Albalat et al., 2011). For each of the sensory attributes a two-anchored linear scale (0–10) was used in which the score of five is the midpoint (Suppl. Table 2). Samples from animals stunned electrically using the Crustastun™ or ice-stunned were frozen on day 1 and then stored at −22 °C for 4 weeks until they were sent on dry ice to the Sensory Testing Laboratory. Sample preparation involved thawing the animals, and boiling *N. norvegicus* tail samples for 3 min to ensure a core temperature of 75 °C, to comply with EU regulations, and then peeled. To increase the reliability of the findings and to reduce any bias because of sample presentation order, samples were given three-digit random code numbers and were presented to the panel in a random manner. Two independent taste sessions were performed on the same day with no significant differences on the scoring of the different attributes obtained between sessions. Sensory evaluation sessions were carried out in a computerised sensory room, and the data gathered were analysed using computer software FIZZ (http://www.biosystemes.com).

2.3. Tail muscle pH

Portions of around 1 g of tissue from the abdominal muscle from different animals (n = 10/group/time point) were homogenised in distilled water in a ratio 1:10 (w:v) according to (Chiou & Huang, 2004) and measurements were taken with a standard glass-bodied pH electrode (Model FB68788; Fisher Scientific, Manchester, UK).

2.4. ATP and breakdown products (K-values) in N. norvegicus tail muscle

Nucleotide extracts from muscle portions from different animals (n = 3/group/time point) were prepared as described in (Ryder, 1985) Briefly, frozen abdominal muscle samples were weighted and homogenised on ice with 5 × volume (w/v) of chilled 0.6 M perchloric acid using and Ultra Turrax T25 homogeniser. Homogenates were centrifuged at 16,000 g for 10 min at 4 °C and supernatants were neutralised to pH 6.5–6.7 using 1M KOH. Potassium percholate was removed using sintered glass and filtrates were diluted with phosphate buffer. Extracts were analysed using high-performance liquid chromatography (HPLC) coupled to a PDA detector as described in (Albalat et al., 2009). Standard curves were prepared from adenosine 5′-triphosphate (ATP), adenosine 5′-diphosphate (ADP), adenosine 5′-monophosphate (AMP), inosine 5′-monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx) all sourced from Sigma-Aldrich (Dorset, UK). K-values were calculated according to (Saito et al., 1959).

2.5. Microbiological analysis in N. norvegicus tail muscle

To quantify total viable counts (TVCs) muscle portions from different animals (n = 3/group/time point) were surface-sterilised by immersion for 2 min in 0.1% benzalkonium chloride made up in sterile sea water (SSW). Abdominal muscle was dissected, and a small piece (0.4–1.0 g) was placed aseptically into a stomacher bag. According to the muscle weight SSW containing 0.1% bacterial peptone (Difco) was added in a ratio 1/10 (w/v) and the sample was homogenised in a Seward stomacher (Biomaster 80) 2 cycles each of 2 min on high-speed setting. The homogenised material was transferred into sterile plastic universals and an appropriate dilution series was set up using SSW as diluent. A 100 μl volume of these dilutions were spread inoculated in tetraplicates onto marine agar (MA) plates.

In order to quantify key spoilage bacteria within the total bacterial load, H_2S producing bacteria were isolated from abdominal muscle using marine iron agar (MIA) plates prepared as described in (Gram et al., 1987) with minor modifications. In this case, instead of iron agar (IA) a marine both (Difco/DB, Oxford, UK) was used and supplemented with 0.04% (w/v) L-cystein (Sigma-Aldrich, Dorset, UK) and 0.03% (w/v) sodium thiosulfate (both from Sigma-Aldrich, Dorset, UK). In all cases, plates (tetraplicates/sample) were incubated for 48 h at 20 °C and total bacterial numbers were recorded as colony forming units per gram of muscle (cfu/g). Using rankit plots, results were found to follow a non-normal distribution and so data were normalised by conversion into logarithmic values (LOG_{10} cfu/g).

2.6. Determination of trimethylamine (TMA) concentration in N. norvegicus tail muscle

TMA was determined following the picrate method (Dyer, 1945) with some minor modifications introduced by (Stroud et al., 2007). To this end, muscle portions from different animals (n = 4 group/time point) were homogenised with trichloroacetic acid, TCA (7.5%), in a ratio 1:2 (w / v). TMA was extracted with toluene which was made to react with picric acid.

2.7. Determination of biogenic amines in N. norvegicus tail muscle

Biogenic amines (histamine, tyramine, cadaverine, agmatine, putrescine, spermidine and tryptamine) were determined according to (Veciana-Nogues et al., 2020) with slight modifications. Muscle portions from different animals (n = 3/group) of 1 g were homogenised on ice in 5× volume of 0.6 M perchloric acid (PCA) using an Ultra Turrax Homogenizer. Immediately after homogenization, 5 mL the whole homogenate was centrifuged at 2,800 g for 10 min at 4 °C. PCA extracts were filtered through a 0,45 μm filter and stored at −80 °C until analysis. Biogenic amines were analysed by HPLC with post-column

derivatization. A SpectraSystem P2000 HPLC pump was used coupled to a fluorescence detector (FP-920) from Jasco. Separations were carried out using a Thermo Hypersil Gold column 250 × 4.60 mm, with an internal particle diameter of 5 μm. The mobile phase was composed of: solvent A (0.1 M sodium acetate and 10 mM sodium octanesulfonate, adjusted to pH 5.20 with acetic acid) and solvent B (a solution of 0.2 M sodium acetate and 10 mM sodium octane sulfonate adjusted to pH 4.50 with acetic acid mixed with acetonitrile in a proportion 6.6/3.4, v/v). Biogenic amines were eluted with a gradient of solvent A (starting at 70%) and decreasing to 10% over 56 min. Post-column derivatization reagent consisted in 15.5 g of boric acid and 13.1 potassium hydroxide in 500 mL of H_2O (pH adjusted to 10.5–11 with 30% of KOH). Then 1.5 mL of Brij-35 (30%) and 1.5 mL of mercaptoethanol were added and finally 0.1 g of o-phthalaldehyde (OPT) dissolved in 2.5 mL of ethanol were all mixed. Flow rate of mobile phase was 1.0 mL/min and flow rate of derivatization reagent was 0.5 mL/min. Spectrofluorometric detector was set at excitation wavelength of 340 nm and emission wavelength of 445 nm. Standard curves were prepared from histamine dihydrochloride, tyramine freebase, tryptamine hydrochloride, cadaverine hydrochloride, putrescine hydrochloride, agmatine sulphate and spermidine trihydrochloride all from Sigma Aldrich (Dorset, England, UK).

2.8. Statistical analysis

Differences between electro-stunned and iced-stunned groups at each sampling time were analysed by independent sample t-tests. Data were tested for normality using the Levene's test. P-values lower than 0.05 were considered statistically significant (SPSS, Statistics, version 23). The unit of replication (n) refers to the number of animals.

2.9. Ethical statement

Ethical approval for procedures on decapod crustaceans is currently not required in the UK. Nevertheless, all the live Norway lobsters used were treated with proper care in order to minimise their discomfort and distress. There was no practical alternative to the use of live animals. The total number of lobsters used (n = 18 for trial 1 neuronal study; and n = 240 for trial 2 and n = 35 animals for trial 3 quality studies) was the minimum required to obtain scientific results and a reasonable threshold of certainty for statistical validation, considering that the gain in knowledge and long term benefit to the subject will be significant. Experimental design considered the 3R's (Replacement, Reduction and Refinement), and suffering and distress were minimised by electrical stunning for no more than 10 s, which rendered the lobsters insensible. No lobsters had to be euthanased; all were killed by the electrical stunning treatment while insensible. Therefore, the pre-defined humane endpoint (consistent behavioural indications of consciousness recovery post stunning) was never invoked.

3. Results

3.1. The effectiveness of electro-stunning as a stunning and slaughter procedure: trial 1

In untreated animals neuronal traffic was recorded in the central nervous system both anteriorly in the circumoesophageal connective (COC) (Fig. 1A; upper panel) and posteriorly in the abdominal nerve cord (ANC) (Fig. 1B; upper panel). In addition spontaneous activity was recorded in the segmental motor nerves that serve to maintain tone in the tail musculature (Fig. S2; upper panels), and evoked activity was stimulated in the leg sensory nerves by mechanical stimulation of cuticular receptors (Fig. S3; upper panels). Consistent results were obtained from the 6 animals tested, and representative traces from one of these animals are shown.

When first measured following electrical stunning (~2 min) there was a total absence of activity at all levels within the CNS (COC and ANC), and also in both the motor and sensory nerves (Fig. 1, S2 and S3, lower panels). Again this was found to be the case in all 6 animals tested, indicating that all these animals were effectively stunned by this

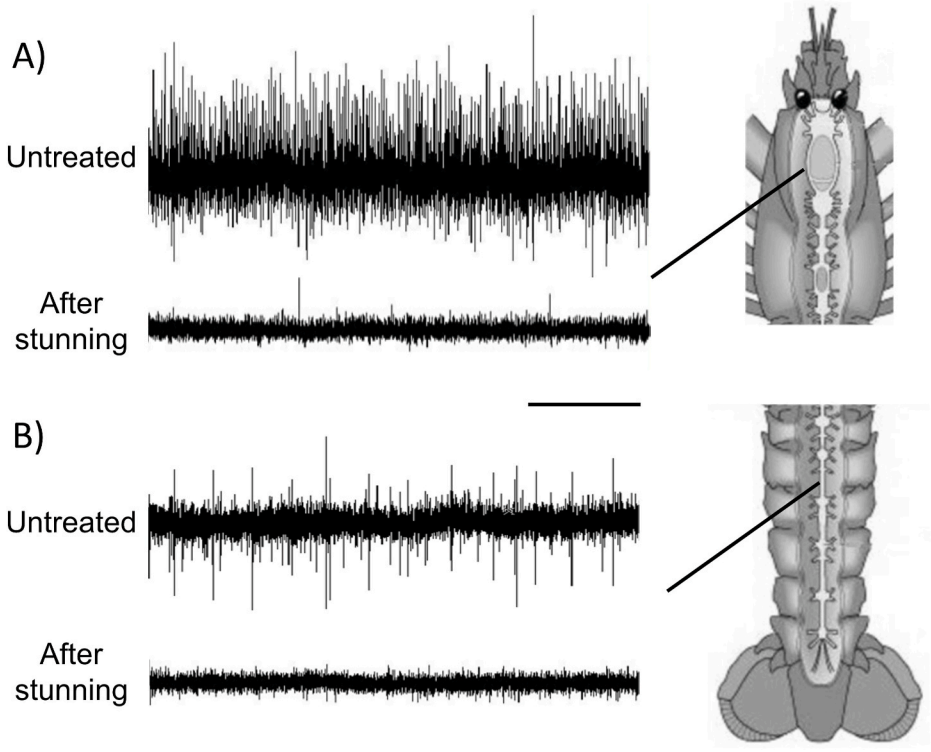

Fig. 1. Spontaneous nerve activity recorded extracellularly in A) the left circumoesophageal connective and B) the abdominal nerve cord of a *N. norvegicus*. Upper panels, an untreated animal; lower panel, another animal after electrical stunning. Scale bar 1s.

procedure. No recovery of nerve activity (nor any body movements) could be observed even 4 h later, indicating that the animals were also effectively killed by the electro-stunning.

In contrast, after placing on ice for 30 min, recordings from the central and peripheral nervous systems of all the 6 animals tested were virtually the same, in terms of the number, amplitude and rate of nerve impulses, as found in untreated animals (Fig. 1).

3.2. Effects of electro-stunning on quality-related parameters: trial 2

3.2.1. Visual assessment

QIM scores were very similar in *N. norvegicus* irrespective of the stunning method if animals were kept on ice for up to 7 days (Fig. 2A). However, melanosis development in the dorsal cephalothorax (Fig. 2B) was significantly higher in animals where electrical stunning had been applied. This increased melanosis development was significantly different from day 3 of ice storage ($t = -2.14$; $df = 18$; $p = 0.04$ on day 3; $t = -2.97$; $df = 18$; $p = 0.007$ on day 5 and $t = -2.71$; $df = 18$; $p = 0.022$ on day 7) and it was only apparent on this body part as no significant differences were seen in any of the other body parts except for ventral cephalothorax on day 7, which similarly, was higher in animals that had been electrically stunned ($t = -2.71$; $df = 18$; $p = 0.02$) (Fig. S4). Sensory evaluations of cooked tail meat after 1 day on ice storage performed by a trained professional independent panel were not significantly different for any of the parameters evaluated (Table 1).

3.2.2. Freshness

K-values, which are based on the relative concentrations of ATP and its breakdown products, were measured as freshness indicator. As shown in Fig. 3A, K-values were significantly higher in *N. norvegicus* that had been electro- compared to ice-stunned ($t = -3.13$; $df = 4$; $p = 0.02$ on day 0; $t = -2.78$; $df = 4$; $p = 0.049$ on day 1 and $t = -5.46$; $df = 4$; $p = 0.005$ on day 3). This difference, which was apparent after the stunning process was completed, was no longer detected from day 5 onwards. The profiles of ATP and its breakdown products are shown in Fig. S5. From the individual nucleotide data, it is clear that the drop of AMP in post-mortem muscle is faster in electro-stunned animals and that means IMP increases at earlier post-mortem stage compared to ice-stunned animals. Further, the concentrations of Hx, which have been related to bitter-off taste, were similar among groups (0.33 ± 0.09 μmol/g in electrically and 0.34 ± 0.01 μmol/g in ice-stunned group) at the end of the 7 day storage period ($t = 0.079$; $df = 4$; $p = 0.941$). Finally, pH values in the tail meat were <7 to start with but increased in a linear fashion up to values of around 7.6–7.7 in both groups with storage time, with no statistically significant differences detected between the stunning groups (Fig. 3B).

3.2.3. Microbiological data

Tail meat from electrostunned *N. norvegicus* had significantly lower TVCs than ice-stunned samples on day 1 ($t = -4.46$; $df = 4$; $p = 0.011$) of storage. However, no differences in TVCs were observed from day 3 onwards (Fig. 4A). Specific determinations of the numbers of H_2S producing bacteria produced no statistically significant differences between electro- and ice-stunned groups (Fig. 4B).

3.2.4. Bacteria-related metabolites

TMA levels remained low up to day 7 of storage on ice (Fig. 5A). TMA concentrations were similar in electro- and ice-stunned groups over the whole period of storage. Results for biogenic amines show how on day 7 of ice storage the main amine in *N. norvegicus* was agmatine (Fig. 5B). Other biogenic amines detected in lower concentrations were putrescine, cadaverine and tyramine. In relation to the stunning method, no statistically significant differences were detected.

3.3. Temperature in electrostunned N. norvegicus: trial 3

The electro-stunning process was found in most cases to induce a flexure of the tail (Fig. S1B), presumably due the occurrence of tetanic contraction of the abdominal flexor muscles. Before electrical-stunning, animals presented body temperatures of 12.5–13.0 °C (Fig. S6A), and the brine in the Crustastun™ chamber had a temperature between 15 and 16 °C. Immediately after electrical stunning, the temperature of the animals increased, especially in the region of dorsal cephalothorax (Fig. S6B). The temperature of the dorsal surface of the cephalothorax reached around 24–27 °C on average (max temp. around 30 °C). Thermal images of the transverse section of the cephalothorax (Fig. S6C) reveal a thermal gradient from the dorsal surface to the ventral surface after electrical stunning (Fig. S6D).

4. Discussion

4.1. Activity in the nervous systems

The results obtained here are consistent with the literature on the neurophysiology of crustacean nervous systems (Wiese, 2002) in showing that the central nervous system of intact *N. norvegicus* displays continuous nerve activity, and in turn receives inputs from sensory nerves in the periphery and produces outputs in the motor nerves to the body and limb muscles. It is also well established that this activity persists even when parts of the central nervous system are isolated from

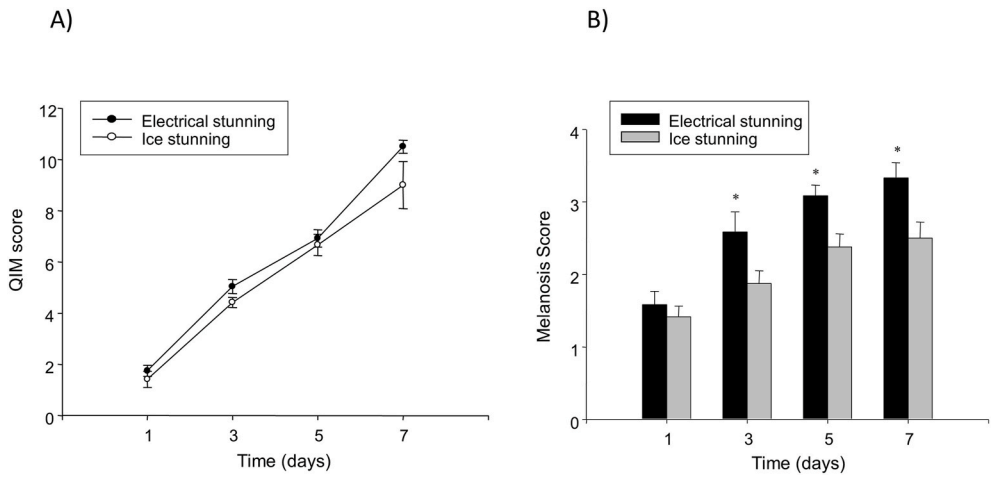

Fig. 2. A) QIM and B) Melanosis score in the dorsal cephalothorax of *N. norvegicus* electrically or ice-stunned and kept on ice for up to 7 days. Data represents the mean of n = 10 animals/group/time point ± SEM. (*) indicates differences are significantly different $p < 0.05$.

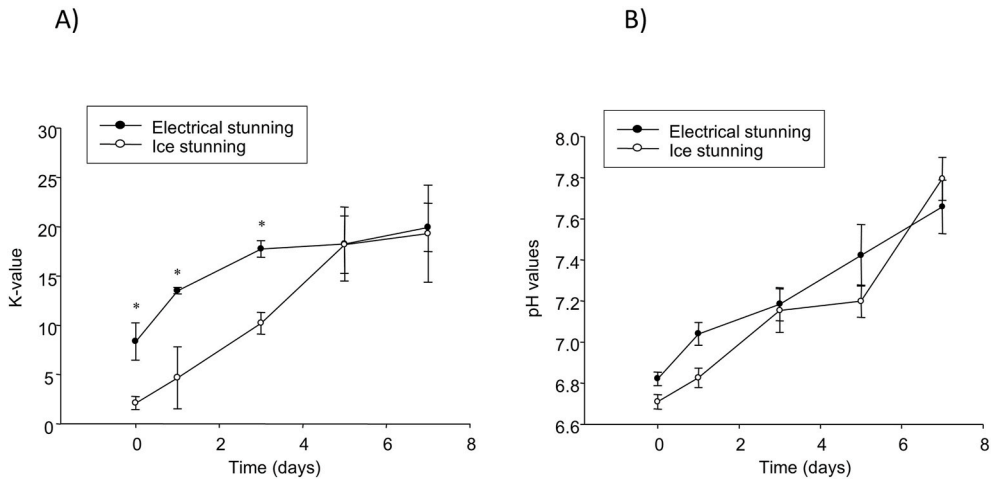

Fig. 3. A) K-value and B) abdominal muscle pH of *N. norvegicus* electrically or ice-stunned and kept on ice for up to 7 days. Data represents the mean of n = 3 animals/condition/time point for K-values and n = 10 animals/condition/time point for muscle pH ± SEM. (*) indicates differences are significantly different $p < 0.05$.

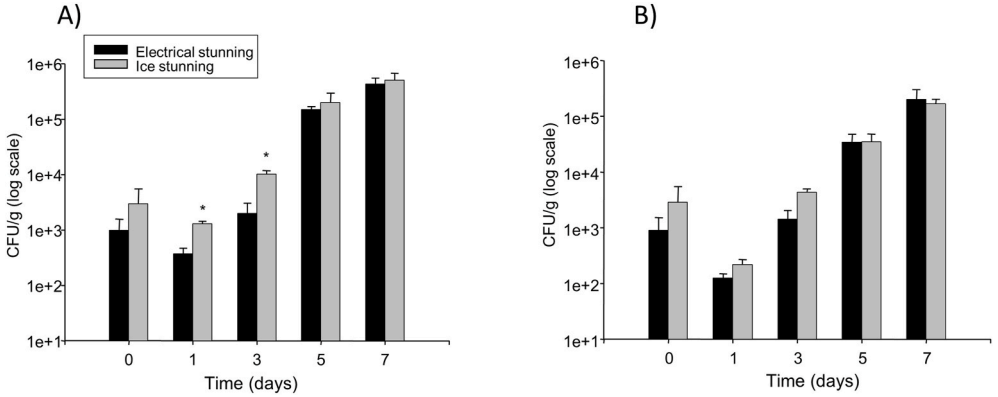

Fig. 4. A) Total viable counts and B) H_2S producing bacteria producers in abdominal muscle of *N. norvegicus* electrically or ice-stunned and kept on ice for up to 7 days. Data represents the mean of n = 3 animals/condition/time point ± SEM. (*) indicates differences are significantly different $p < 0.05$.

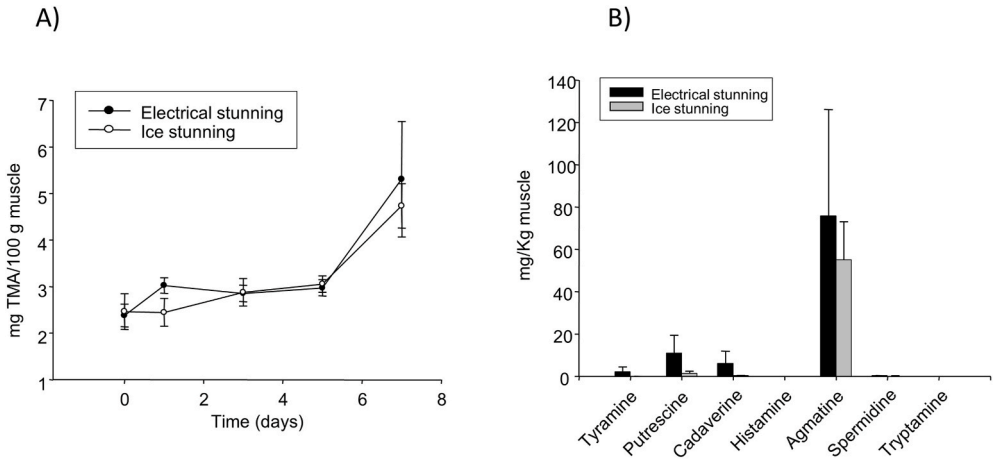

Fig. 5. A) TMA and B) biogenic amines concentrations in abdominal muscle of *N. norvegicus* electrically or ice-stunned and kept on ice for up to 7 days. Data represents the mean of n = 4 animals/condition/time point for TMA and n = 3 animals/condition/time point for biogenic amines ± SEM. (*) indicates differences are significantly different $p < 0.05$.

each other by severing the nerve cord at one or more levels (Marder & Bucher, 2007). It is for this reason that any attempt to arrest neuronal activity by carving or spiking the animal would fail as a humane slaughter method as it would inevitably leave small sections sufficiently intact to be able to continue functioning.

We found that following electro-stunning all normal neuronal

functioning ceases, both centrally and peripherally. According to the manufacturer, the target stunning current of the Crustastun™ (1.3 amp) is achieved within 0.5 s and is maintained throughout the stun cycle. It is impossible to know with certainty if nerve conduction fails at this point, as all electrical signals are completely masked by the artefact generated by the stun current. However it is a reasonable assumption that this occurs almost instantaneously, within the stun cycle (10 s). After electro-stunning we found neither central nervous activity, sensory responsiveness nor motor action in all the 6 lobsters examined. This demonstrates that this method may be effective in rendering *N. norvegicus* rapidly insensible under the conditions used in this study, although more data are required from individuals of various sizes to confirm this. In contrast, and in agreement with previous research performed on other large cold water decapod crustaceans (Roth & Oines, 2010) we found that ice-stunning induces torpor or paralysis (defined as an absence of movements but not necessarily an absence of activity in the nervous system), but even after 30 min does not completely suppress neural activity, so no true anaesthesia (defined as the absence of neural function that mediates the perception or elaboration of sensory stimuli) is established. It therefore cannot be regarded as a humane method of slaughter for these large cold-water crustaceans. In contrast, ice-stunning does seem to be more effective in small, warm-water decapod species such as the whiteleg shrimp P*enaeus vannamei* (Weineck et al., 2018) or the giant tiger prawn *Penaeus monodon* (Diggles, 2019).

These results have fundamental relevance from an animal welfare perspective. Although currently decapod crustaceans in the UK are not protected, animal welfare recommendations for vertebrate aquatic species such as farmed fish dictate that animals should be rapidly rendered insensible prior to death (EFSA Panel on Animal Health and Welfare AHAW, 2013). Our limited data suggest that electrical stunning can meet these criteria for being an acceptable humane method of both causing insensibility and also killing a decapod crustacean such as *N. norvegicus*.

4.2. Effect of electro-stunning on the quality of N. norvegicus

From a sensory perspective, a key difference between the stunning methods was the fact that melanosis development was triggered at a faster rate in animals that had been electrostunned. This activation of post-harvest melanin production (Coates & Albalat, 2014) was an issue in the dorsal cephalothorax, with melanosis scores on day 3 for electrostunned lobsters being higher than those recorded on day 7 in animals that had been ice-stunned. The increase in melanosis development could be due to the increased temperature experienced during the electro-stunning process, a phenomenon seen in this study and others (Roth & Grimsbo, 2016), which albeit brief in nature could nevertheless trigger the activation of the polyphenol oxidase enzyme (Giménez et al., 2010). In addition to increased temperature, the physical pressure exerted by the electro-stunning apparatus per se might have contributed to cytoarchitecture disruption, which has been shown to be a key factor in triggering the activation of the polyphenol oxidase system (Xu et al., 2020). However, while the use of antimelanotics was not tested, it is possible that an anti-melanotic treatment before electro-stunning could delay this process (Aubourg et al., 2007; Goncalves & de Oliveira, 2016) although this itself might cause an unacceptable accumulation of residues in the edibles tissues (Coates & Albalat, 2014).

Nevertheless, the faster melanosis development was not sufficient to impact overall QIM evaluation, which is based not only the visual appearance of the cephalothorax but also on the appearance of the chelipeds, dorsal and ventral abdomen and the odour characteristics (Gornik et al., 2013). Therefore, from a sensory perspective the animals were very similar after the two stunning methods. The QIM values are consistent with those previously reported for this species when *N. norvegicus* are stored on ice (Gornik et al., 2013). QIM values above 8 have been reported to be unacceptable from a sensory perspective, a value that was reached in both groups at around day 6. The QIM reflected similar odour characteristics between groups, and also similarities in TMA and biogenic amines levels, metabolites that are mainly driven by the proliferation of spoilage bacteria metabolism (Biji et al., 2016; Nevigato et al., 2018). While both TMA and biogenic amines such as agmatine were present in samples on day 7 of ice storage, proliferation of bacteria (TVCs) was already substantial on day 5 ($\log10^5$). TVCs in this invertebrate species have been reported to be particularly high even in fresh samples and numbers tend to decrease when the animals are placed on ice (Gornik et al., 2013). However, with both stunning protocols, an increase in TVCs was detected from day 1 onwards, and reached $\log10^6$ on day 7. The fact that no differences were obtained indicates that the increased temperature recorded due to the electro-stunning was not long enough to trigger a faster microbial growth and activity in contrast to the effects reported when this product is stored at different temperatures (Gornik et al., 2011). Therefore, from a spoilage and microbiological safety perspective the method of stunning did not have a clear significant effect. However, differences were obtained in terms of freshness, in this case the speed of nucleotide interconversions was faster in electro-stunned compared to ice-stunned samples. While no other studies for comparison in decapods are available, studies in fish have shown that electricity leads to earlier rigor mortis onset and resolution compared to cold treatment (slurry ice) (Knowles et al., 2007), an effect triggered by a faster initial rate of nucleotides interconversion, which includes faster ATP degradation (Scherer et al., 2005). Decapod crustaceans, such as *N. norvegicus*, do not exhibit rigor mortis per se due to proteolysis overtaking the rigor mortis process (Gornik et al., 2009) but in any case, the intercoversions especially in the first 4 days were more advanced in electro-stunned muscle compared to ice-stunned samples. Therefore, from this perspective samples were in a fresher postmortem biochemical state when animals were placed on ice, a difference that was apparent up to day 5.

The consequences from a consumer perspective are difficult to ascertain. From the sensory assessment of cooked product, no major differences could be detected by the trained panel.

5. Conclusions

From a quality perspective, our results indicate that electro-stunning delivers a product of largely similar shelf life compared to ice-stunned animals, when they are subsequently stored on ice. However, electro-stunning has a small detrimental effect on the freshness of the product and can trigger faster melanosis development in the cephalothorax area, factors that will affect animals destined to the whole fresh and frozen markets. It therefore has to be recognised that such possible detriments, although minor, would be a consequence of compliance with any legislation that mandates electro-stunning.

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CRediT authorship contribution statement

Amaya Albalat: Writing – original draft, Formal analysis, Visualization. **Sebastian G. Gornik:** Formal analysis, Visualization. **Chonchanok Muangnapoh:** Formal analysis, Visualization. **Douglas M. Neil:** Conceptualization, Formal analysis, Supervision, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.108930.

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