Article

Effectiveness of the Food-Safe Anaesthetic Isobutanol in the Live Transport of Tropical Spiny Lobster Species

Jayagopal Pozhoth [1] and Andrew Jeffs [2,*]

1   The Marine Products Export Development Authority-SRD, Nagapattinam 611001, India; jayagopal@mpeda.gov.in
2   Leigh Marine Laboratory, Institute of Marine Science, University of Auckland, P.O. Box 349, Warkworth 0941, New Zealand
*   Correspondence: a.jeffs@auckland.ac.nz; Tel.: +64-21-256-3303

Abstract: The strong demand for live spiny lobsters in Asian markets is being met by long-distance airfreight. Holding lobsters out of water during transportation often results in mortalities due to the accumulation of metabolites, especially ammonia. This study examined the potential to improve the survival of tropical lobster species exported from India through the use of the food-safe aquatic anaesthetic isobutanol, both with and without cold stunning, typically used prior to live lobster transportation. The results of the study indicate that treatment with 50 ppm isobutanol in ambient seawater temperature (i.e., 28 °C) prior to simulated live transport for 22 h significantly reduces ammonia levels in the haemolymph in all four lobster species (*Panulirus homarus*, *P. ornatus*, *P. versicolor* and *P. polyphagus*) compared to lobsters treated with cold stunning (i.e., 16.5 °C) with and without 10 ppm isobutanol. Cold stunning at 16.5 °C combined with 10 ppm isobutanol reduced ammonia levels compared to cold stunning alone only in *P. ornatus*. All experimental lobsters were returned to ambient seawater after simulated transport and were alive after 48 h. These results indicate that isobutanol has the potential to be used to suppress metabolism during the live transport of tropical lobsters and to reduce mortalities during live transport.

Keywords: spiny lobster; live transport; anaesthetic; ammonia; *Panulirus*; emersion

1. Introduction

Spiny lobsters are one of the most valuable seafood species in the world, with well over USD 1 billion of harvest each year and extensive global trading [1–3]. With the strong growth in a number of Asian economies, where spiny lobsters are highly prized, the market demand and prices in the Asian region for spiny lobsters have increased dramatically in the past 20 years. For example, the total imports of lobster by China grew from 3600 t to almost 18,000 t in the five years from 2009 [3]. As a consequence of the market demand, spiny lobsters are now being shipped to Asia from fisheries in many distant locations, including South Africa, Australia, New Zealand, India and Cuba, as well as from parts of North, Central and South America. The Asian consumer market has a very strong preference for live lobsters, with holding tanks of live lobsters a frequent sight outside seafood restaurants and markets, with consumers paying premium prices for the live product. For example, in China, the average import price of live New Zealand lobsters for the year of 2020 was USD 71.18 kg^{-1}, whereas frozen whole lobsters from the same source had a much lower value of USD 48.18 kg^{-1} [4]. Therefore, there is a significant advantage to global lobster traders in being able to transport live lobsters long distances by airfreight with minimum mortality, because the losses in transport are borne by the seller [3,5].

India has commercial fisheries for eight species of spiny lobsters and two species of slipper lobsters [6–9]. The most important spiny lobster species are *Panulirus polyphagus*, *P. homarus*, *P. ornatus* and *Puerulus sewelli*, whereas only minor fisheries exist for *P. versicolor*, *P. penicillatus*, *P. longipes* and *Linuparus somniosus*. During 2019, a total of 1490 t of lobsters

worth USD 23.1 million were exported from India. The mortality of lobsters during the typical live transport of lobsters to market is estimated to be about 5% for normal shipments, but much higher levels of mortality are frequently encountered and are mostly related to the stress caused by post-harvest handling and live transport procedures [5,10]. The overall losses in transit may be as high as 10%, representing a total loss of about USD 2.3 million to India's export trade in lobsters, and much higher when all live trade in spiny lobsters into Asia is considered.

Lobsters show a considerable ability to survive out of water without oxygen exchange over their gills by switching to anaerobic metabolism, for which they have considerable capacity [11–13]. As a result, this physiological trait is exploited for the long-distance live transport of spiny lobsters into Asian markets [3,5]. The physiology of a variety of species of lobsters from cool and temperate waters undergoing transport in air has been the subject of a number of studies, with the different species showing similar physiological responses [10–15]. Lobsters are usually tolerant to a relatively wide range of temperatures, and because they are poikilothermic, their metabolism can be suppressed through a reduction in their body temperature [16,17]. Consequently, it is common practice among live lobster traders to use "cold anaesthesia" to reduce physical movement, metabolic rate and oxygen demand, by cooling down lobsters to near the lower limit of their temperature tolerance prior to shipment to produce a resulting increase in survival during shipping [11,18–20]. For example, the survival of *Panulirus japonicus* after 24 h emersion at 5, 15 and 25 °C was 100, 83, and 60%, respectively [21]. Similarly, the survival of *Panulirus cygnus* during emersion was significantly negatively correlated to air temperature [18].

Spiny lobsters are ammonotelic, metabolising protein and producing ammonia as the primary waste product, which is mainly excreted by diffusion from the haemolymph into seawater across the surface of the gills [14,21]. Upon emersion, the excretion of ammonia into seawater via the gills of the lobster is curtailed, leading to ammonia accumulating in the haemolymph, which is toxic and causes morbidity and, ultimately, death in lobsters [21–23]. Besides ammonia, other changes in haemolymph chemistry from normal baseline concentrations, such as lactic acid, pH and glucose, are considered important indicators of physiological stress in spiny lobsters [18,22]. Changes in respiratory variables and haemolymph composition, including acutely toxic levels of ammonia, during exposure to air have been observed in the American clawed lobster, *Homarus americanus* [23]; the European clawed lobster, *H. gammarus* [24–26]; the southern red rock lobster, *Jasus edwardsii* [27]; and the Caribbean spiny lobster, *Panulirus argus* [28].

Anaesthetics have the potential to artificially depress the metabolic demands of live marine organisms through the suppression of stress responses by dulling stress receptors, reducing physical activity by immobilising nerve signals to muscles and by directly suppressing metabolic rate. All of these effects of anaesthetics are likely to be advantageous during the live transport of marine organisms in an oxygen-limited environment, reducing the risk of mortality due to metabolic stress [29]. Despite the potential usefulness of anaesthetics for the live transport of seafood species, there has only been a limited number of studies that have examined the potential of using anaesthetics in live crustacean transport [30–34]. Consequently, anaesthetics are not currently widely applied commercially for extending the live transport of crustaceans.

Crustaceans respond differently to anaesthesia than fish, and many commonly used fish anaesthetics, e.g., tricaine methane-sulphonate, quinaldine and magnesium sulphate, are ineffective on crustaceans, require excessively large dosages or are not considered to be food safe [29,32]. Research on a variety of crustacean species has shown that eugenol is a safe and effective anaesthetic [33–35]. For example, Malaysian river prawns, *Machrobrachium rosenbergii*, anaesthetised with eugenol were found to have significantly lower oxygen consumption than un-anaesthetised prawns [31]. However, anaesthesia was maintained in these experiments by holding the animals in a dosed solution of the anaesthetic and moving them to clean water for recovery. Isoeugenol, the derivative of eugenol, which

is commercially available as AQUI-S®, (AQUI-S NZ Ltd., New Zealand), was only found to assist in the transport of the European crab, *Cancer pagurus*, when the crabs were maintained in a dosed solution of seawater during transport for 48 h [30]. Attempts to transport crabs in air after treatment with AQUI-S® resulted in 100% mortality. However, low doses of AQUI-S are used to reduce stress in live spiny lobsters while handling in preparation for live transport [34]. However, experimental attempts to use AQUI-S at higher doses to provide deeper anaesthesia and metabolic suppression throughout the period of live transport of lobsters were unsuccessful due to the rapid clearance and recovery of the AQUI-S by the lobsters [34]. The anaesthetics methyl pentynol and isobutanol have previously been identified as effective general anaesthetics for the American lobster, although methyl pentynol was found to be toxic at higher concentrations, i.e., in excess of 6000 ppm in clean aerated seawater at 12 °C [36]. Isobutanol readily induced anaesthesia in adult American lobsters held at concentrations ranging between 500 and 14,400 ppm in 10 °C seawater, and lobsters treated with the highest dose recovered in clean seawater after spending an hour out of water. Isobutanol is considered to be safe for human consumption, being a widely approved human food additive [37] and occurring naturally in a variety of fermented alcoholic beverages where it imparts a winey flavour. Being food safe, low cost and readily available, isobutanol would make a useful aquatic anaesthetic for the live shipment of tropical lobsters if it proved effective.

Given the significant financial losses due to the mortality of live lobsters exported from India, the aim of the present study was to determine whether isobutanol could be used as an aquatic anaesthetic to improve the survival of four of the most valuable live lobster species exported from India (*P. homarus*, *P. versicolour*, *P. ornatus* and *P. polyphagus*), with isobutanol either used on its own or in conjunction with the typical chill-stunning methods used for the live transport of spiny lobsters from India.

2. Materials and Methods

2.1. Experimental Animals

Given that the aim of this initial study was to assess the potential of isobutanol to be used as an aquatic anaesthetic to improve the survival of four of the most valuable live lobster species exported from India, a simultaneous experiment was conducted on all four species (*P. homarus*, *P. versicolour*, *P. ornatus* and *P. polyphagus*) to avoid the potential of experimental artefacts arising from serial experimentation with individual species. Consequently, live spiny lobsters (100–300 g body weight, BW) of all four species were collected from local commercial fishers near Chennai, India, immediately upon landing their vessels to shore. Lobsters that had low vitality, were close to moulting, had missing appendages, had signs of disease or had any physical injuries on the body were discarded. Moult stage was assessed using the pleopod method developed for *P. ornatus* [38], and standard behavioural reflex tests for live crustaceans were used for the identification of lobsters of poor vitality [15]. The 100 selected lobsters were given a quarantine treatment of 100 ppm formalin dip for 30 min before being acclimatised for 10 days in a 2000 L recirculating fibre glass tank. Seawater was filtered to 20 μm through a biological filter and was kept at ambient temperature (28 °C). The tank was kept covered so that the lobsters only experienced dim light during daylight hours, and shelters were provided for the animals. Lobsters were fed to satiation three times a day with a combination of fresh green mussels (*Perna viridis*) and clams (*Donax cuneatus*). Lobsters were fasted for 24 h on day 10 prior to the start of the experiment, as wild-caught lobsters are normally starved by live seafood exporters for at least 24 h before shipping. On day 11, preliminary isobutanol dosage ranging trials were conducted with groups of 20 lobsters at seawater temperatures of both 16.5 and 28 °C, representing the temperature of chill stunning used for commercial lobster export and ambient seawater temperature, respectively. Lobsters were assessed as being sufficiently anaesthetised when their appendages became immobilised, including during subsequent handling, which would otherwise normally elicit an active escape response [15]. A concentration of 10 ppm isobutanol was determined as the lowest

sufficient dose to induce rapid (i.e., within 25 min) immobilisation at 16.5 °C and 50 ppm at 28 °C.

On day 11, nine lobsters of each of the four species (*P. homarus*, *P. versicolor*, *P. ornatus* and *P. polyphagus*) were randomly selected for the experiment. The lobsters were closely examined, and any lobster in a pre-moult stage was excluded from the experiment. Three lobsters per species were randomly assigned to one of the following three experimental treatments:

(1) Standard chill-stunning transport: Lobsters were immersed in chilled seawater at 16.5 °C for 25 min and then tightly wrapped in paper and transferred to 70 × 40 × 40 cm polystyrene boxes, which are typically used for air freight shipping of live lobsters. This is a standard method used by live lobster exporters in Chennai, India.

(2) Chill-stunning transport with anaesthetic: Lobsters were immersed in chilled seawater at 16.5 °C with 10 ppm isobutanol for 25 min and then tightly wrapped in paper and transferred to polystyrene boxes. The concentration of 10 ppm isobutanol was based on the results of the preliminary anaesthetic dosage ranging trials at this temperature.

(3) Ambient transport with anaesthetic: Lobsters were immersed in ambient seawater at 28 °C with 50 ppm isobutanol for 25 min and then tightly wrapped in paper and transferred to polystyrene boxes. The concentration of 50 ppm isobutanol was based on the results of the preliminary anaesthetic dosage ranging trials at this temperature.

The three treatments were prepared simultaneously and held for 22 h, which is a period that is typical of air freight transport times from Indian lobster processors to key markets in Asia, such as Singapore and Hong Kong. In all treatments, two 500 mL bottles of frozen seawater were added to the polystyrene boxes to help maintain a lower temperature within the box. Temperature and humidity within the boxes were recorded every 60 min with a hand-held digital combined thermistor/hygrometer probe poked through a small hole into an internal air space in the polystyrene box. They were found to remain relatively stable over the 22 h period, with an average temperature of 20 °C ± 1 S.E. and humidity of 90% ± 5 S.E. After 22 h, the boxes were opened, and 0.5 mL of haemolymph was extracted from the base of the fifth walking leg in each lobster with a disposable syringe and accurately measured into a glass vial, and an aliquot of 0.5 mL of sodium oxalate was immediately added to the haemolymph sample to prevent it from coagulating, and the stoppered vials were vortexed briefly and immediately placed on ice. The lobsters were returned to the acclimatisation tank and observed for 48 h for any sign or morbidity or mortality.

2.2. Determination of Ammonia in Haemolymph

The haemolymph samples were immediately analysed in the laboratory for ammonia using a colorimetric method, which is based on the chemical conversion of ammonia to indophenol [39]. An aliquot of 2 mL of 80% ethanol was added to 0.4 mL of the lobster haemolymph sample (containing 0.2 mL of haemolymph). The sample was centrifuged at 5000 rpm for 5 min. An aliquot of 1 mL of the supernatant was collected and combined with 2.5 mL of reagent A (10 g of phenol and 50 mg of sodium nitroprusside in 500 mL of distilled water). For each haemolymph sample, a negative control or blank was run simultaneously, which consisted of 0.8 mL of 80% ethanol, 0.2 mL of 10% sodium oxalate solution and 2.5 mL of reagent A. After 5 min, an aliquot of 2.5 mL of a solution prepared by dissolving 5 g of sodium hydroxide in 10 mL of sodium hypochlorite and diluted with 500 mL of distilled water was added to the sample and blank tubes. After a further 5 min, the tubes were incubated in a hot bath at 37 °C for 20 min and then cooled to room temperature over 30 min before measuring their optical density at 625 nm. The optical density of the blank was deducted from that of the sample, and the concentration of ammonia in the haemolymph was calculated from a standard curve derived from analysing a set of ammonia standards prepared by dissolving anhydrous ammonium chloride in distilled water and analysing in the same manner as the haemolymph samples.

2.3. Statistical Analyses

Data for the ammonia concentrations in the haemolymph were tested to confirm normality using the Kolmogorov–Smirnov test and heterogeneity of variances using Bartlett's test. Means of haemolymph ammonia for the three treatments for the four lobster species were compared with a two-way analysis of variance (ANOVA). If the ANOVA indicated significant ($p < 0.05$) treatment effects, then the individual means were compared using Tukey's Honestly Significant Difference (HSD) test [40,41].

3. Results

All lobsters were alive after 22 h of simulated transport, and 48 h after being returned to the water, all lobsters were active and showed no obvious signs of morbidity, such as lethargy or a disinterest in feeding. There were significant differences in the ammonia concentration of the haemolymph taken from the lobsters subjected to the three treatments ($F_{2, 24} = 134.39$, $p < 0.0001$) and among the four lobster species ($F_{3, 24} = 134.4$, $p < 0.0001$) (Figure 1). Tukey's HSD comparisons showed that, for all four lobster species, the lobsters treated with 50 ppm isobutanol in seawater at ambient temperature had significantly lower haemolymph ammonia than chill-stunned lobsters ($p < 0.05$) or chill-stunned lobsters treated with 10 ppm isobutanol ($p < 0.05$). The mean haemolymph ammonia was between 35 and 55% less in lobsters treated with 50 ppm isobutanol in seawater at ambient temperature compared to the other two treatments among all four lobster species. *Panulirus ornatus*, which were chill stunned and treated with isobutanol, also had significantly lower haemolymph ammonia than lobsters that were only chill stunned ($p < 0.05$). However, for the other three species of lobster, there was no significant difference in the production of haemolymph ammonia in the chill-stunned lobsters or the chill-stunned and isobutanol-treated lobsters (Figure 1).
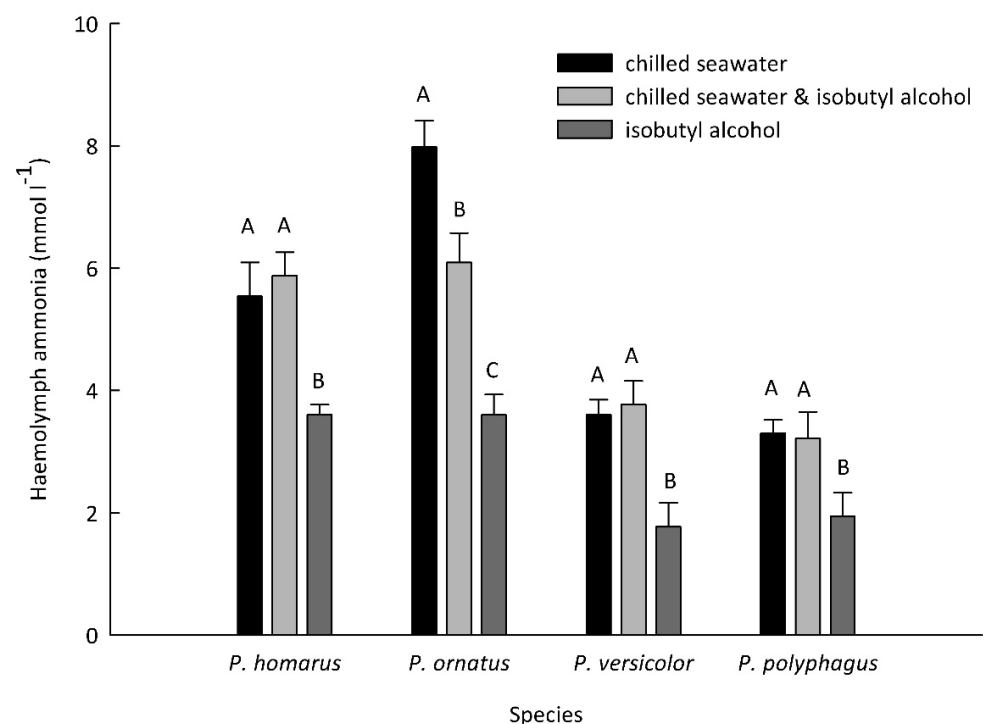

Figure 1. Mean haemolymph ammonia (±S.D.) for four species of tropical spiny lobsters after being subjected to three simulated live shipping treatments over 22 h; (1) chill stunned in 16.5 °C seawater, (2) chill stunned and 10 ppm isobutanol and (3) 50 ppm seawater with isobutanol at ambient temperature (28 °C) (n = 3 per species per treatment). Different superscript letters represent significant differences ($p < 0.05$) between the treatments within a species.

4. Discussion

Although all lobsters from the three treatments survived the 22 h of simulated shipping, the anaesthetic treatment of the four species of tropical spiny lobsters in ambient seawater (i.e., 28 °C) with 50 ppm isobutanol consistently reduced the haemolymph ammonia concentrations compared to chill stunning, or the combination of chill stunning and 10 ppm isobutanol. The mean haemolymph ammonia concentrations in the four species of lobsters treated with isobutanol and subjected to simulated live transport were between one-third and one-half less than the mean haemolymph ammonia concentrations of chill-stunned lobsters, which is the current method presently used by exporters of live lobsters from India. However, at the conclusion of the simulated shipment, all lobsters had elevated haemolymph ammonia concentrations, which are otherwise typically around 0.5–2 mmol L^{-1} in spiny lobsters under ambient conditions [21,28,42,43], which is slightly higher than for most reports from other decapod crustaceans [44] (Figure 1). Elevated haemolymph ammonia concentrations in lobsters are the result of periods of emersion, with much higher levels than those observed in this study being able to be tolerated without mortality in spiny lobsters. For example, 24 h of emersion in chill-stunned southern red rock lobster caused haemolymph ammonia levels to increase to over 16 mmol L^{-1}, but the levels recovered to within the normal range by 12 h after the lobsters were returned to seawater [43]. Likewise, in the temperate Japanese spiny lobster *P. japonicus*, haemolymph ammonia levels increased to 39 mmol L^{-1} after chill stunning to 5 °C and simulated live transport for 48 h with 100% survival [21]. Even higher levels of haemolymph ammonia (i.e., 57 mmol L^{-1}) were recorded in Japanese spiny lobsters that were chill stunned to 15 °C and subjected to simulated live transport for 48 h, but these lobsters experienced nearly 20% mortality. The only previous study investigating emersion in tropical spiny lobsters, *P. argus*, found that haemolymph ammonia levels doubled in 2 h from 0.42 to 0.8 mmol L^{-1} and rapidly returned to normal after re-immersion in seawater [28]. While the toxicity of accumulated ammonia is thought to be a primary cause of morbidity and mortality in the transport of live crustaceans, the overall physiological shift from aerobic homeostasis in emersed crustaceans is multifactorial [10,12,15]. While it would have been possible to run further biochemical assays on biochemical indicators from the haemolymph in this study (e.g., lactic acid and glucose), this would have required the removal of a greater volume of the haemolymph from the lobsters, potentially compromising their subsequent survival. This is an opportunity for future research, particularly as the anaesthetic may have differential effects on physiological pathways, which may be revealed through monitoring a variety of biochemical indicators in the haemolymph.

There has been little previous research on the use of isobutanol as an anaesthetic for crustaceans. Isobutanol was found to be an effective anaesthetic for *H. americanus*, with concentrations of 500–14,400 ppm applied at an ambient temperature of 10 °C inducing anaesthesia rapidly in lobsters in as little as 1 min, with no detectable adverse effects for anaesthetised lobsters after they were emersed for an hour and allowed to recover for up to several hours [36]. These were much higher doses of isobutanol than those used in the current study, suggesting that there is the potential to apply higher doses to these tropical lobster species to extend metabolic suppression during live transport with a low risk of overdose. For example, the current results indicate that, while lobsters treated with 50 ppm isobutanol at 28 °C consistently had lower haemolymph ammonia after 22 h of emersion, three lobster species dosed with 10 ppm together with chill stunning mostly showed no difference in ammonia levels to lobsters in the chill-stunning-only treatment. *Panulirus ornatus* was the only species to show a small but significant reduction in haemolymph ammonia when treated with chill stunning together with 10 ppm isobutanol, suggesting that this species may be more sensitive to isobutanol than the other three species tested. It is also possible that the efficacy of isobutanol increases with a rise in the temperature, as the anaesthetic potency of many aquatic anaesthetics is affected by temperature [29,45]. For example, the potency of ethanol on the crustacean *Daphnia magna* increases markedly with water temperatures between 5 and 30 °C [45].

While only 10 ppm isobutanol was required to produce an anaesthetic effect when used in combination with chill stunning in seawater of 16.5 °C in this study, it is likely that the majority of the observed effects on the lobsters were due to the temperature, which is considered to be around the lower thermal limit for tropical lobster species. However, the physiological effects of lowered temperatures on tropical lobsters are not well described, and it is possible that, while the chill stunning of tropical lobsters may suppress physical activity and stress during handling whilst packing and live shipping, it may result in delayed physiological impacts caused by acute thermal stress. Delayed physiological effects from chill stunning have previously been identified as an issue for the live shipping of spiny lobsters from temperate waters [14,34,46]. The cost of physiological recovery from acute thermal stress could be another possible explanation for the general trend of higher ammonia levels recorded in the haemolymph of lobsters exposed to chill-stunning treatments in this current study. Further investigation is required to ascertain the physiological effects and practical advantages of using manipulated temperatures in the live transport of lobsters. Likewise, given the promising results of this preliminary study, further research is necessary to determine the optimal concentrations of isobutanol required for different lobster species and for different combinations of temperature and transport duration.

5. Conclusions

In summary, the results of this study indicate that treatment with 50 ppm isobutanol at ambient temperatures significantly lowers metabolic stress as measured by the haemolymph ammonia levels of tropical spiny lobster species subjected to live transportation methods compared to the currently employed method of chill stunning used in India. An effective, non-toxic anaesthetic for crustaceans would greatly benefit the live transport industry, reducing the stress and mortality of animals during the transport procedure. Economic losses due to mortality during live transport are estimated to be well over USD 1 million per annum for the Indian live lobster export industry. Isobutanol is currently accepted by the U.S. Food and Drug Authority as a food additive, which is commonly used for flavouring [37], and shows good potential to be used as an anaesthetic for crustaceans, especially spiny lobsters, during live transport. Further research is warranted to more precisely determine the appropriate dosage and treatment protocol for the use of isobutanol in live lobster shipment, either alone or in combination with temperature manipulation, and to determine if there are any resulting organoleptic changes in lobsters resulting from the use of this food flavouring additive.

References

1. Spanier, E.; Lavalli, K.L.; Goldstein, J.S.; Groeneveld, J.C.; Jordaan, G.L.; Jones, C.M.; Phillips, B.F.; Bianchini, M.L.; Kibler, R.D.; Díaz, D.; et al. A concise review of lobster utilization by worldwide human populations from pre-history to the modern era. *ICES Mar. Sci.* **2015**, *72*, i7–i21. [CrossRef]
2. Jeffs, A.G. Status and challenges for advancing lobster aquaculture. *J. Mar. Biol. Assoc. India* **2010**, *52*, 320–326.
3. Pereira, G.; Josupeit, H. The world lobster market. In *Globefish Research Programme*; Food and Agricultural Organization: Rome, Italy, 2017; Volume 123, 41p.
4. New Zealand Seafood Industry Council. Seafood New Zealand Export Database. 2021. Available online: https://seafoodnewzealand.org.nz/publications/export-information/export-statistics/ (accessed on 25 December 2021).
5. Hart, G. *Assessing the South-East Asian Tropical Lobster Supply and Major Market Demands*; Report# FR2009-06; Australian Centre for International Agricultural Research: Canberra, Australia, 2009.
6. Food and Agriculture Organization. *World Fishery and Aquaculture Production Statistics Database*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2020.
7. The Marine Products Export Development Authority. *Seafood Export Database*; The Marine Products Export Development Authority of India: Kerala, India, 2020.
8. Vijayakumaran, M.; Radhakrishnan, E.V. Live transport and marketing of spiny lobsters in India. *Mar. Freshw. Res.* **1997**, *48*, 823–828. [CrossRef]
9. World Bank. World Integrated Trade Solutions. 2021. Available online: https://wits.worldbank.org/ (accessed on 25 December 2021).
10. Fotedar, S.; Evans, L. Health management during handling and live transport of crustaceans: A review. *J. Invertebr. Pathol.* **2011**, *106*, 143–152. [CrossRef] [PubMed]
11. Morris, S.; Oliver, S. Circulatory, respiratory and metabolic response to emersion and low temperature of *Jasus edwardsii*: Simulation studies of commercial shipping methods. *Comp. Biochem. Physiol. A* **1999**, *122*, 299–308. [CrossRef]
12. Morris, S.; Oliver, S. Respiratory gas transport, haemocyanin function and acid-base balance in *Jasus edwardsii* during emersion and chilling: Simulation studies of commercial shipping methods. *Comp. Biochem. Physiol. A* **1999**, *122*, 309–321. [CrossRef]
13. Ozbay, G.; Riley, J.G. The effects of calcium carbonate buffering on the haemolymph acid-base level of the American lobster (*Homarus americanus*): A pre-shipment conditioning technique. *J. Aquat. Food Prod. Technol.* **1999**, *8*, 21–32. [CrossRef]
14. Taylor, H.H.; Paterson, B.D.; Wong, R.J.; Wells, R.M.G. Physiology and live transport of lobsters: Report from a workshop. *Mar. Freshw. Res.* **1997**, *48*, 817–822. [CrossRef]
15. Stoner, A.W. Assessing stress and predicting mortality in economically significant crustaceans. *Rev. Fish. Sci.* **2012**, *20*, 111–135. [CrossRef]
16. McLeese, D.W. Effects of temperature, salinity and oxygen on the survival of the American lobster. *J. Fish. Res. Board Can.* **1956**, *13*, 247–272. [CrossRef]
17. Robohm, R.A.; Draxler, A.F.J.; Wieczorek, D.; Kapareiko, D.; Ritchford, S. Effects of environmental stressors on disease susceptibility in American lobsters: A controlled laboratory study. *J. Shellfish Res.* **2005**, *24*, 773–779.
18. Spanoghe, P.T.; Bourne, P.K. Relative influence of environmental factors and processing techniques on *Panulirus cygnus* morbidity and mortality during simulated live shipments. *Mar. Freshw. Res.* **1997**, *48*, 839–844. [CrossRef]
19. Lorenzon, S.; Giulianini, P.G.; Martinis, M.; Ferrero, E.A. Stress effects of different temperatures and air exposure during transport on physiological profiles in the American lobster *Homarus americanus*. *Comp. Biochem. Physiol. A* **2007**, *147*, 94–102. [CrossRef] [PubMed]
20. Jussila, J.; Tiitinen, V.; Fotedar, R.; Kokko, H. A simple and efficient cooling method for postharvest transport of the commercial crayfish catch. *Freshw Crayfish* **2013**, *19*, 15–19. [CrossRef]
21. Huang, C.-Y.; Chen, J.-C. Effects of emersion on the haemolymph metabolites of the Japanese lobster, *Panulirus japonicus* (Decapoda, Panuliridea). *Crustaceana* **2001**, *71*, 1041–1058. [CrossRef]
22. Hunter, D.A.; Uglow, R.F. Handling-induced changes in haemolymph ammonia concentration and ammonia excretion rate of *Crangon crangon* (L). *Opheila* **1993**, *38*, 137–147. [CrossRef]
23. Young-Lai, W.; Charmantier-Daures, M.; Charmantier, G. Effect of ammonia on survival and osmoregulation in different life stages of the lobster *Homarus americanus*. *Mar. Biol.* **1991**, *110*, 293–300. [CrossRef]
24. Taylor, E.W.; Whiteley, N.M. Oxygen transport and acid base balance in haemolymph of the lobster, *Homarus gammarus*, during aerial exposure and resubmersion. *J. Exp. Biol.* **1989**, *144*, 417–463. [CrossRef]
25. Whiteley, N.M.; Taylor, E.W. The acid-base consequences of aerial exposure in the lobster, *Homarus gammarus* (L.) at 10 and 20 °C. *J. Therm. Biol.* **1990**, *15*, 47–56. [CrossRef]
26. Whiteley, N.M.; Taylor, E.W. Oxygen and acid-base disturbances in the haemolymph of the lobster *Homarus gammarus* during commercial transport and storage. *J. Crust. Biol.* **1990**, *12*, 19–30. [CrossRef]
27. Taylor, H.H.; Waldron, F.M. Respiratory responses to air-exposure in the southern rock lobster, *Jasus edwardsii* (Hutton) (Decapoda: Palinuridae). *Mar. Freshw. Res.* **1997**, *48*, 889–897. [CrossRef]
28. Vermeer, G.K. Effects of air exposure on desiccation rate, hemolymph chemistry, and escape behavours of the spiny lobster, *Panulirus argus*. *Fish. Bull.* **1987**, *85*, 45–51.
29. Ross, L.; Ross, B. *Anaesthetic and Sedative Techniques for Aquatic Animals*; Blackwell Publishing: Oxford, UK, 2008.

30. Barrento, S.; Marques, A.; Vaz-Pires, P.; Leonor Nunes, M. *Cancer pagurus* (Linnaeus, 1758) physiological responses to simulated live transport: Influence of temperature, air exposure and AQUI-S®. *J. Therm. Biol.* **2011**, *36*, 128–137. [CrossRef]

31. Coyle, S.D.; Dasgupta, S.; Tidwell, J.H.; Beavers, T.; Bright, L.A.; Yasharian, D.K. Comparative efficacy of anesthetics for the freshwater prawn *Macrobrachium rosenbergii*. *J. World Aquac. Soc.* **2005**, *36*, 282–290. [CrossRef]

32. Coyle, S.D.; Durborow, R.M.; Tidwell, J.H. *Anesthetics in Aquaculture*; SRAC Publication No. 3900; Southern Regional Aquaculture Center: Stoneville, MS, USA, 2004.

33. Saydmohammed, M.; Pal, A.K. Anesthetic effect of eugenol and menthol on handling stress in *Macrobrachium rosenbergii*. *Aquaculture* **2009**, *298*, 162–167. [CrossRef]

34. Robertson, J.D.; Delorme, N.J.; Hickey, A.; Jeffs, A.G. Assessment of the potential of the anesthetic AQUI-S for live transportation of the southern rock lobster, *Jasus edwardsii*. *Bull. Mar. Sci.* **2018**, *94*, 1137–1151. [CrossRef]

35. Waterstrat, P.R.; Pinkham, L. Evaluation of eugenol as an anesthetic for the American lobster *Homarus americanus*. *J. World Aquac. Soc.* **2005**, *36*, 420–424. [CrossRef]

36. Foley, D.M.; Stewart, J.E.; Holley, R.A. Isobutyl alcohol and methyl pentynol as general anesthetics for the lobster, *Homarus americanus* Milne Edwards. *Can. J. Zool.* **1966**, *44*, 141–143. [CrossRef]

37. Food and Drug Authority. Code of Federal Regulations Title 21 Food and Drugs. Available online: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-172/subpart-F/section-172.515 (accessed on 25 December 2021).

38. Turnbull, C.T. Pleopod cuticular morphology as an index of moult stage in the ornate rock lobster, *Panulirus ornatus* (Fabricius 1789). *Aust. J. Mar. Freshwat. Res.* **1989**, *40*, 285–293. [CrossRef]

39. Boltz, D.F.; Howell, J.A. *Colorimetric Determination of Non-Metals*; Wiley-Interscience: New York, NY, USA, 1978.

40. Underwood, A.J. *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*; Blackwell: London, UK, 1997.

41. Zar, J.H. *Biostatistical Analysis*; Prentice Hall: Hoboken, NJ, USA, 1999.

42. Paterson, B.D.; Grauf, S.G.; Smith, R.A. Haemolymph chemistry of tropical rock lobsters (*Panulirus ornatus*) brought onto a mother ship from a catching dinghy in Torres Strait. *Mar. Freshw. Res.* **1997**, *48*, 835–838. [CrossRef]

43. Speed, S.R.; Baldwin, J.; Wong, R.J.; Wells, R.M.G. Metabolic characteristics of muscles in the spiny lobster, *Jasus edwardsii*, and responses to emersion during simulated live transport. *Comp. Biochem. Physiol. B* **2001**, *128*, 435–444. [CrossRef]

44. Florkin, M. Blood chemistry. In *The Physiology of Crustacea*; Waterman, T.H., Ed.; Academic Press: New York, NY, USA, 1960; Volume 1, pp. 141–159.

45. McKenzie, J.D.; Calow, P.; Clyde, J.; Miles, A.; Dickinson, R.; Lieb, W.R.; Franks, N.P. Effects of temperature on the anaesthetic potency of halothane, enflurane and ethanol in *Daphnia magna* (Cladocera: Crustacea). *Comp. Biochem. Physiol. C* **1992**, *101*, 15–19. [CrossRef]

46. Forgan, L.G.; Tuckey, N.P.L.; Cook, D.; Jerret, A. Temperature effects on metabolic rate and cardiorespiratory physiology of the spiny rock lobster (*Jasus edwardsii*) during rest, emersion and recovery. *J. Comp. Physiol. B* **2014**, *184*, 437–447. [CrossRef] [PubMed]