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Investigating clove oil and its derivatives as anaesthetic agents for decapod crustaceans to improve welfare commercially and at slaughter

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Decapods have been recently classified as sentient beings in UK policy and therefore the establishment of humane methods for the live transportation and slaughter of commercially valuable shellfish as well as for decapods used in research is critical. Formerly overlooked, the use of anaesthetics provides a promising avenue for improving welfare standards for husbandry and slaughter for decapod crustaceans destined for human consumption or research. In particular, clove oil and its derivatives (eugenol and isoeugenol) have been trialled and recommended in literature as naturally-derived and effective, reversible anaesthetic compounds for a variety of decapods, including two commercially important British shellfish, brown crab (*Cancer pagurus*) and Norway lobster (*Nephrops norvegicus*). Further investigations should be undertaken to confirm the use of such anaesthetics is suitable for improving welfare standards in the British shellfish sector and in research to ensure that when the legislation changes, humane solutions are present.

KEYWORDS

clove oil, eugenol, decapod, crustacean, anaesthesia, sentience, humane slaughter, welfare

Introduction

Under the former UK Animal Sentience Bill, decapod crustaceans were not covered, by definition, as 'animals' (DEFRA, 2021a). In 2021, the UK Government commissioned independent research into the sentience of decapods and cephalopod molluscs for their inclusion in the Bill (DEFRA, 2021b). To decide how welfare standards are applied to certain animal groups, the capacity for those animals to feel, also known as *sentience*, is often sought. This embodies felt experiences, including positive and negative feelings (e.g. pleasure, comfort, distress, hunger etc.) as well as sensory experiences (including tactile, olfactory, auditory and visual) (Crump et al., 2022a). For decisions regarding animal welfare, typically

scientists look for evidence of negatively perceived experiences including pain, distress, fear and awareness (European Food Safety Authority, 2005). In response to the UK Government's call, the London School of Economics published their review of the evidence in November 2021, using a framework centred around neural and behavioural responses to pain and considered the threshold for sentience to have been met (Birch, 2017; Birch et al., 2021; Crump et al., 2022a). This included the presence of nociceptors, presence of integrative brain regions and that their responses are impacted by analgesics or local anaesthetics (Birch et al., 2021). This subject is, however, controversial, and has sparked debate over the inferences of pain from indicators of stress (Stevens et al., 2016) and scientific rigour in defining sentience and consciousness in the context of animal welfare (Dawkins, 2017; Briffa, 2022). Dawkins argues that the science and politics of sentience should be kept separate with the benefit of doubt given in policy making (Dawkins, 2022). Birch states that sentience cannot be empirically proven therefore the 'precautionary principle' should apply; scientific certainty should not be a pre-requisite to legal protection (Birch, 2017). The Animal Welfare (Sentience) Act 2022, recognising decapods as sentient animals, passed Royal Assent on the 28th April 2022 (DEFRA, 2022).

With this development, there is growing interest to consider methods for the humane slaughter of decapod crustaceans. A humane method of slaughter seeks to minimise animal suffering, pain and distress during their killing and related operations (EU Council, 2009). Commonly practised methods include bisecting from the head down the mid-line for lobsters ('splitting'), inserting a knife or dowl into the nerve centres ('spiking') and dropping them into boiling water. Other methods include slowly heating the water, placing the animal in a hypercapnic seawater bath, inducing osmotic shock by 'drowning' in freshwater and electrical stunning using the 'Crustastun'TM (Gardner, 1997; Yue, 2008).

The desired product quality does not necessarily match the desirable welfare standards (Gardner, 2004). Schmidt-Nielsen (1962) argued that it is unethical to subject an animal to conditions it would seek to avoid, particularly if death is slow. In UK slaughterhouses, stunning before dispatch is a compulsory procedure; standard operating procedures ensure that strict instructions are followed for rendering protected animals insensible (DEFRA, 2015). Schedule 1 of the Animals (Scientific Procedure) Act 1986 also specifies the humane dispatch of vertebrates and cephalopods used in research (UK Government, 2014).

In comparison, many of the methods used for decapods may cause pain and distress and would be deemed inhumane in protected animals, causing autotomy, muscle spasms, rigidity and tearing of appendages or abdomens (Gardner, 1997; European Food Safety Authority, 2005). Chilling, either in cold air or in ice slurry, is often used to induce a hypometabolic, torpor-like state, suggested to render the animal insensible before slaughter (Yue, 2008). Whilst sufficiently cooling an animal can induce hypometabolic paralysis via a Q_{10} effect (where muscle performance decreases with decreasing temperature), it does not completely suppress neural activity and may not produce an anaesthetic effect for temperate, cold-adapted species (Fregin and Bickmeyer, 2016; Weineck et al., 2018; Albalat et al., 2022). Melting ice can cause osmotic shock for marine species (Yue, 2008). When used for non-lethal stunning, the

CrustastunTM initiates seizures in lobsters which potentially mask the responses to external stimuli rather than truly removing them (Fregin and Bickmeyer, 2016). Albalat et al. (2022) found that electro-stunning impacts product freshness, initiating a more rapid melanosis in *Nephrops norvegicus*. When the methods of slaughter were reviewed by Conte et al. (2021), only electrical stunning, splitting and spiking were deemed humane and only when the animal is handled by trained personnel. Conte et al. (2021) did not discuss the use of anaesthetic compounds to humanely render insensibility before dispatch or induce a swift and painless death.

Anaesthesia as an alternative

The use of anaesthetics on crustaceans is not new. Anaesthetics are used in prawn and shrimp aquaculture to improve survival rates during transport and handling. They often display territorial and cannibalistic tendencies and swim with fast movements (Coyle et al., 2004; Coyle et al., 2005; Vartak and Singh, 2006). Various anaesthetics are widely available for such use; products include Aqui-S[®] and Aqua Life TMS (also known as Tricaine Methanesulfonate or MS 222) (Syndel Canada, 2015; AQUI-S New Zealand Ltd, 2022). Commonplace surgical procedures in crustacean aquaculture, such as eyestalk ablation (removal of eye contents to induce gonadal maturity), benefit from anaesthesia by reducing the trauma experienced by the animal (Taylor et al., 2004). For lobsters [and crabs], anaesthetics allow humane procedures to be performed, whilst minimising injury to the handlers (Waterstrat and Pinkham, 2005). Controlled overdoses of anaesthetic compounds may enable humane euthanasia for cooking or necropsy. In his experiments on Australian giant crabs (*Pseudocarcinus gigas* (Lamarck)), Gardner (1997) found only Aqui-S[®], clove oil and chloroform to initiate a relaxed, reversible paralysis, with chloroform and clove oil both being successfully used as a method of euthanasia with no apparent distress. Clove oil was recommended as the superior choice as chloroform is hepatotoxic. For food preparation, anaesthetic compounds would need to be approved as safe for use in food animals. It was observed that the eggs of gravid females appeared to be unharmed by the clove oil treatment with embryos continuing their development and later hatching (Gardner, 1997). In her paper, de Souza Valente (2022) suggested that eugenol and isoeugenol are amongst the most effective natural compound-based anaesthetics for decapods.

Clove oil, eugenol and isoeugenol

Clove oil, isolated from clove (*Syzygium aromaticum*) is well known for its medicinal properties and as a flavouring agent. Often associated with toothache because of its analgesic effect, it is also known to be antibiotic, antiviral, antifungal, insecticidal, anaesthetic, antioxidant and even cytotoxic towards cancerous cells (Chaieb et al., 2007). Chaieb et al. (2007) found clove oil to be comprised of predominantly eugenol (4-allyl-2-methoxyphenol, 88.59%), followed by eugenyl acetate (5.62%). Isoeugenol (4-propenyl-2-methoxyphenol) is derived from eugenol, with some

comparable properties, it is the active ingredient in the anaesthetic AQUI-S® (AQUI-S New Zealand Ltd, 2022). Clove oil is becoming a popular alternative as an anaesthetic for fish due to its low cost, being naturally occurring and ‘not unpleasant to work with’ (Gardner, 1997; Coyle et al., 2004; Waterstrat and Pinkham, 2005). In crustaceans, eugenol and isoeugenol are thought to be absorbed through the gills and inhibit the transmission of neural signals including the blocking of voltage-gated sodium and calcium channels (Zahl et al., 2011; Cowing et al., 2015; Wycoff et al., 2018; de Souza Valente, 2022). Whilst clove oil is widely available, eugenol concentrations vary as a result of production area, extraction method, and the material used (Hu et al., 2018). As a result, the use of clove oil as an approved anaesthetic would require that the quality is known, and the eugenol concentration is consistent.

In 1983, the US National Toxicology Program conducted experiments on Eugenol to test for carcinogenic properties in rats and mice. No evidence for carcinogenicity was determined in rats and only equivocal evidence in mice (National Toxicology Program, 1983). Similar experiments were conducted for isoeugenol in 2010 and findings showed evidence of carcinogenicity in male mice, and equivocal evidence in male rats and female mice (National Toxicology Program, 2010).

According to the World Health Organization (WHO), Eugenol has an acceptable human daily intake (ADI) of 0–2.5 mg/kg of body weight (WHO, 2006). Isoeugenol is not present on their list. Allergic reactions to both eugenol and isoeugenol were documented in fragrance patch tests (Frosch et al., 1995). Api et al. (2016) found isoeugenol to be a moderate skin sensitizer in humans. In terms of pharmacokinetics, eugenol has been tested on Pacific white shrimp (*Penaeus vannamei*). Eugenol, administered at 300 mg/L via anaesthetic bath for 5 minutes had a terminal elimination half-life ($T_{1/2}$) of 1.3 hours for the hepatopancreas and 11 hours for the muscle (Tang et al., 2022). Of note, the concentration of eugenol in the muscle after 2 hours is 1.60 mg/kg and lower than the WHO ADI recommendation of 2.5 mg/kg.

The pharmacokinetics of isoeugenol have not been tested on crustaceans but were tested on Fischer 344 rats in 2002. Badger et al. (2002) administered isoeugenol both intravenously and as an oral dose. For oral doses, more than 85% of the isoeugenol was excreted in the urine within 72 hours as metabolites and none was found in the blood. When administered intravenously, isoeugenol had a mean systemic clearance of 1.9 L/min/kg, with a $T_{1/2}$ of 12.1 mins, suggesting that it is rapidly removed from rat blood (Badger et al., 2002). More recently, isoeugenol has been shown to suppress mitochondrial respiration in the heart tissues of Australian red spiny lobsters (*Jasus edwardsii*) (Robertson et al., 2022). Similar studies should be conducted to document the depletion of eugenol and isoeugenol from the tissues of other decapod crustaceans if they are to be considered as anaesthetics for food animals.

Kildea et al. (2004) investigated the accumulation and clearance of clove oil (eugenol-based) and AQUI-S® (isoeugenol-based) from the flesh of silver perch (*Bidyanus bidyanus*). They found that for a perch killed through a controlled overdose of clove oil (450 mg/L), the flesh retained a mean concentration of 26.34 mg/kg of eugenol (Kildea et al., 2004). This initially appears high considering the WHO ADI limit of 2.5 mg/kg. However, for a person weighing

approximately 50 kg, they calculated that 125 mg of eugenol could be safely consumed, equating to roughly 5 kg of overdosed perch (Kildea et al., 2004). It should be noted that the eugenol concentration of the dose was unknown. Kildea et al., state that the eugenol concentration of clove oil varies between 70%–90%, highlighting a flaw in the way clove oil is used.

Evidence of clove oil, eugenol and isoeugenol as anaesthetics for decapod crustaceans

Table 1 below shows all the accessible literature found where clove oil, eugenol or isoeugenol were used to experimentally anaesthetize decapod crustaceans via an anaesthetic bath (for marine species, a seawater tank containing a set dose of anaesthetic per litre into which the decapod is submerged). Limited literature exists on clove oil as an injectable anaesthetic. The purposes range from identifying appropriate transport sedatives, surgical anaesthesia, and agents for euthanasia. Interspecies responses are highly variable and seemingly unrelated to interspecies size with intertidal species potentially showing more resilience (Morgan et al., 2001; Aréchiga-Palomera et al., 2016). Efficacy is affected by dosage, temperature and possibly intraspecies size (Li et al., 2018; Ghanawi et al., 2019).

Determining the stages of anaesthesia in decapod crustaceans is more challenging than for vertebrates as the usual clinical indicators (such as blood pressure, and respiratory rate) are difficult to assess (Zahl et al., 2012). To measure the stages, researchers look for evidence of anaesthesia through behavioural changes, responses to tactile and visual stimuli, loss of equilibrium, loss of righting behaviour, changes to heart rate and various water quality parameters. These vital signs are often used in combination, yet currently, there is no universally agreed suite of indicators. In decapods, the stages can be broken down similarly to those given by Zahl et al. (2012); Sneddon (2012) and Coyle et al. (2004) for fish. It was suggested by de Souza Valente (2022) that stage I is sedation with partial loss of righting reflex and equilibrium but still displaying responsive and defensive behaviour. Stage II is a loss of equilibrium, righting reflex, and defensive behaviour yet still some responsiveness to stimuli. Stage III is surgical anaesthesia with complete immobility and unresponsiveness, reduced heart rate but preserved ventilatory function. In vertebrates, stage III is often broken down further into light anaesthesia, surgical anaesthesia and deep narcosis; however, this has not yet been published for decapods (Sneddon, 2012; Zahl et al., 2012; de Souza Valente, 2022). By stage IV, the animal is moribund, displaying cardiac and respiratory arrest with possibility of death (de Souza Valente, 2022). Although the stages are clearly defined, in practice, they are less discrete and often blend together (e.g. Hewer, 1937). Some authors have instead defined their own stages of anaesthesia for their decapod study species (Vartak and Singh, 2006; Cowing et al., 2015; Ghanawi et al., 2019).

In terms of behaviour, levels of mobility and aggression can indicate the presence of anaesthesia. For example, Jensen et al. (2013) determined anaesthesia when spiny lobsters (*Sagmariasus verreauxi*)

TABLE 1 Existing, accessible literature arranged alphabetically by first author, concerning the use of clove oil, eugenol and isoeugenol via anaesthetic bath on decapod crustaceans for the purpose of transport, anaesthesia and euthanasia.

Species	Purpose	Individual Mass	Effective Dose [Induction Time]	Reference
<i>Fenneropenaeus indicus</i> †	Transport Sedative	NA	Eugenol 1.3 mg/L	(Akbari et al., 2010)
<i>Macrobrachium tenellum</i> ‡	Anaesthesia	1.1 g (± 0.03)	Clove oil 300-900 mg/L [17-8 mins]	(Aréchiga-Palomera et al., 2016)
<i>Cancer pagurus</i>	Transport Sedative	667.37 g (± 93.2)	Aqui-S® (50% Isoeugenol) 300 mg/L [10-15 mins] + 40 mg/L	(Barrento et al., 2011)
<i>Nephrops norvegicus</i>	Transport Sedative	NA	Eugenol 600-900 µl/L [4.6-7.3 min]	(Cowing et al., 2015)
<i>Macrobrachium rosenbergii</i> ‡	Transport Sedative	2.1 g (± 0.1)	Clove oil 100 mg/L [100% lightly sedated in 15 mins] Aqui-S® (50% Isoeugenol) 100 mg/L [100% light-fully sedated in 45 mins]	(Coyle et al., 2005)
<i>Pseudocarcinus gigas</i>	Anaesthesia Euthanasia	1-7 kg	Clove oil anaesthesia: 0.03-1 ml/L [85-16 mins] Euthanasia: 0.06-1.0 ml/L [180-28 mins] Aqui-S® (50% Isoeugenol) anaesthesia: 0.125-1 ml/L [70-20 mins]	(Gardner, 1997)
<i>Cherax quadricarinatus</i> ‡	Anaesthesia	<5-37 g	Clove oil 500 µl/L [313.18 ± 21.52 s]	(Ghanawi et al., 2019)
<i>Eriocheir sinensis</i>	Anaesthesia	185.8 g (± 55.5)	Clove oil 20 ml/L. Not recommended.*	(Hajek et al., 2009)
<i>Penaeus monodon</i>	Anaesthesia	3.21 g (± 0.18)	Eugenol 60–210 mg/L [25.5-2.9 mins]	(Jiang et al., 2020)
<i>Palaemonetes sinensis</i>	Anaesthesia	NA	Eugenol 100-300 µl/L (in water <16°C)	(Li et al., 2018)
<i>Cancer magister</i>	Anaesthesia	662 g	Clove oil 0.5-1.5 ml/L [68-16 mins]	(Morgan et al., 2001)
<i>Pugettia producta</i>	Anaesthesia	60 g	Clove oil 0.015-0.25 ml/L [54-2 mins]	
<i>Hemigrapsus oregonensis</i>	Anaesthesia	1.6 g	Clove oil 1.0-3.0 ml/L [188-87 mins]	
<i>Penaeus vannamei</i> †‡	Transport sedative Anaesthesia	0.1 g (± 0.7)† 15 g (± 0.1)‡	Eugenol 20 µl/L† & 20–50 µl/L‡ for transport 175 µl/L [4.1 mins]† & 400 µl/L [2.6 mins]‡ for anaesthesia	(Parodi et al., 2012)
<i>Jasus edwardsii</i>	Transport sedative	NA	Aqui-S® 40 ppm- 200 ppm [10-20 mins]	(Robertson et al., 2018)
<i>Macrobrachium rosenbergii</i>	Anaesthesia	32 g (± 2.1)	Eugenol 200 µl/L [31.3 mins ±1.3]	(Saydmohammed and Pal, 2009)
<i>Penaeus semisulcatus</i>	Anaesthesia	1.8-2.1 g	Clove oil 100-150 mg/L [5-3 mins]	(Soltani et al., 2004)
<i>Neohelice granulata</i>	Anaesthesia	10.2 g (± 0.35)	Eugenol 8000 µl/L [24 min] Not recommended.*	(Souza et al., 2018)
<i>Penaeus vannamei</i>	Anaesthesia	12 g (± 2.0)	Eugenol 300 mg/L [5 mins]	(Tang et al., 2022)
<i>Macrobrachium rosenbergii</i> †‡	Transport sedative Anaesthesia	NA	Clove oil 30-75 mg/L [14.0-3.3 mins]† for anaesthesia & 15 mg/L (<=3hrs)† for transport. Not recommended for juveniles.*	(Vartak and Singh, 2006)
<i>Homerus americanus</i>	Anaesthesia	NA	Eugenol 75-100 ppm [8.0-8.4 mins]	(Waterstrat and Pinkham, 2005)
<i>Penaeus vannamei</i>	Anaesthesia	NA	Eugenol 200 ppm [6-20 mins]	(Wycoff et al., 2018)

†=post larvae, ‡=juvenile.

* Hajek et al. trialled one dose and used clove oil with unknown eugenol content. Souza et al. found eugenol to be effective at the highest concentration tested and average recovery time was below 30 minutes. It was outperformed by different injectable compounds. Vartak and Singh found that the doses trialled for juveniles, whilst effective, had unsatisfactory induction and recovery times when clove oil of unknown eugenol concentration was used as an anaesthetic.

no longer avoided capture and the tail-flapping reflex was absent. In their study on *Nephrops norvegicus*, Cowing et al. (2015) were able to use a response to tactile stimuli to classify which behaviours indicated which stage of anaesthesia when sedated with eugenol. In this instance, the external stimuli were presented by brushing the antennae or rostrum with a pipette (Cowing et al., 2015). Partial and complete losses of equilibrium determined stage I and II of clove oil-induced anaesthesia in redclaw crayfish (*Cherax quadricarinatus*) respectively, with a complete loss of equilibrium determined when the animal was unable to right itself from recumbency (Ghanawi et al., 2019). Whilst not conclusive, water quality parameters may indicate the presence of anaesthesia. Eugenol-induced anaesthesia may reduce respiration rates at higher anaesthetic concentrations. Akbari et al. (2010) found that dissolved oxygen levels in the eugenol baths of Indian shrimp (*Fenneropenaeus indicus*) were significantly higher in those with higher eugenol doses. Finally, Wycoff et al. (2018) measured the heart rate (HR) of decapod crustaceans, using electrocardiograms as a response to a eugenol anaesthetic, along with other physiological parameters. Before the administration of eugenol, HR was shown to pause for a beat or two when the animal was tapped on the head with a glass rod. HR remained constant for 40 minutes after eugenol was administered despite disturbance by external stimuli (Wycoff et al., 2018).

Ensuring that eugenol is properly dissolved in the water has the benefit of more efficient use of the active compound. Eugenol is immiscible in cold water (<15°C) therefore ethanol is frequently used as a solvent (Vartak and Singh, 2006). Ethanol is reported as having no anaesthetic effect on decapod crustaceans in doses up to 10 times the eugenol dose (Morgan et al., 2001; Vartak and Singh, 2006; Cowing et al., 2015; Aréchiga-Palomera et al., 2016), and therefore makes an appropriate solvent for anaesthetic experiments.

Other considerations

Coyle et al. (2004) believe that a suitable anaesthetic should induce anaesthesia rapidly, preserve the state of anaesthesia for the appropriate length of time and allow for a rapid recovery once the anaesthetic is removed. The anaesthetic compound should be effective in small doses and the toxic dose should greatly exceed the effective dose to observe a wide safety margin. An anaesthetic with no withdrawal time i.e. time needed to assure that treatment residues in the marketable product are below a determined maximum residue limit, is also advantageous for approval by regulating bodies (Coyle et al., 2005). Many studies use induction and recovery times as a way of justifying their recommended doses (Morgan et al., 2001; Coyle et al., 2005; Vartak and Singh, 2006; Saydmohammed and Pal, 2009; Parodi et al., 2012; Ghanawi et al., 2019). Soltani et al. (2004) defined the most effective concentration as one that minimizes induction and recovery time. Increasing concentrations of clove oil caused induction times to fall and recovery times to increase (Soltani et al., 2004). Identifying the appropriate dosage for any given crustacean may prove challenging as the optimal dose may involve a trade-off and could also depend on the research objectives. Clove oil may also have variable results if the eugenol concentration varies.

It is worth also considering existing regulations in destination countries. For example, as of 2018, eugenol and isoeugenol, which are approved food additives in China, were not registered as anaesthetic compounds for fish destined for human consumption in Chinese markets (Ke et al., 2018). When evaluated, 55 samples of market fish contained eugenol in concentrations spanning 3.11 – 30,690 µg/kg, of which 43 samples would have exceeded the maximum residual limit of 50 µg/kg as imposed in Japanese law (Ke et al., 2018). Those authors further estimated the daily intake of eugenol via fish consumption in China. Concern was expressed over the unauthorized use of eugenol and isoeugenol as anaesthetics. It was found that for both women and men, the estimated daily intake (EDI) from eating fish was far below WHO's ADI of 2.5 mg/kg eugenol, yielding negligible risk to health (Ke et al., 2018). A substantial proportion of Scottish-caught shellfish are exported live to European or Asian markets (Mesquita et al., 2017). Any anaesthetics used either for anaesthesia or slaughter should adhere to the importer's legislation.

Discussion

Whilst there is potential for the improvement of welfare standards for crustaceans before slaughter, much research is still required. This includes establishing definitive indicators of the stages of anaesthesia and euthanasia, recognition of pain, and the pharmacokinetics of the compounds in more decapod crustaceans (de Souza Valente, 2022). Eugenol is approved as a food additive; however, in the UK it is not yet recognized by veterinary medicine as an anaesthetic compound. In 2017, Liderfeed®, a zootechnical additive for fattening chickens and containing eugenol (5%), was found to be safe for chickens at a dose of 100 mg/kg of complete feed (Rychen et al., 2017). Whilst this is far removed from studies on decapod crustaceans, it is noteworthy that a food additive containing eugenol has been recommended for a meat animal. It is unclear if using these compounds for slaughter would affect the smell or flavour of the meat; this can be addressed with an expert tasting panel as with Albalat et al., 2022.

The classification of decapods as sentient beings in UK policy brings the opportunity to improve the welfare of shellfish species caught in UK waters. European lobster (*Homarus gammarus*), brown crab (*Cancer pagurus*), velvet swimming crab (*Necora puber*) and Norway lobster (*Nephrops norvegicus*) are all frequently caught and, to date, no research exists on the impact of clove-oil-derived anaesthetics on lobster or velvet swimming crabs. Aside from slaughter methods, commonplace procedures in the industry, such as declawing or 'nicking' (cutting the tendons in the claws) and live export may potentially undermine UK welfare standards (Crump et al., 2022b). Eugenol makes a promising candidate for European lobsters after having been successfully trialled as an anaesthetic in *H. americanus* (Waterstrat and Pinkham, 2005), with which it shares a genus. Calls have been made for the law to be amended to include decapod crustaceans under the Animal Welfare Act (UK Government, 2006) and 1986 Animals in Scientific Procedures Act (Crump et al., 2022b). If this were to happen, studies into anaesthetic compounds for decapod crustaceans will become essential,

particularly from a veterinary and research perspective. Whilst further research is required before clove oil or its derivatives could be used commercially to improve decapod welfare, the use of these compounds highlights a promising avenue for further study and should be fully investigated in anticipation of legislative change.

Author contributions

NK and MJ conceived the idea for the literature review. FS researched and wrote the original manuscript. Critical review was provided by NK, MJ, TM, E-MB and J.CM. All authors contributed to the article and approved the submitted version.

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References

- Akbari, S., Khoshnod, M. J., Rajaian, H., and Afsharnasab, M. (2010). The use of eugenol as an anesthetic in transportation of with Indian shrimp (*Fenneropenaeus indicus*) post larvae. *Turkish J. Fish. Aquat. Sci.* 10, 423–429. doi: 10.4194/trjfas.2010.0317
- Albalat, A., Gornik, S. G., Muangnapoh, C., and Neil, D. M. (2022). Effectiveness and quality evaluation of electrical stunning versus chilling in Norway lobsters (*Nephrops norvegicus*). *Food Control* 138, 108930. doi: 10.1016/j.foodcont.2022.108930
- Api, A. M., Belsito, D., Bhatia, S., Bruze, M., Calow, P., Dagli, M. L., et al. (2016). RIFM fragrance ingredient safety assessment, isoeugenol, CAS registry number 97-54-1. *Food Chem. Toxicol.* 97, S49–S56. doi: 10.1016/j.fct.2015.12.021
- AQUI-S New Zealand Ltd (2022) *AQUI-s® safety data sheet*. Available at: https://www.aqui-s.com/images/aquis/PDF/SDS/HSD002_AQUI-S_SDS_NZ_2022.pdf.
- Aréchiga-Palomera, M. A., Zaragoza, O. B. D. R., Galván, S. R. G., and Villasante, F. V. (2016). Evaluation of natural extracts with anesthetic properties in juveniles macrobrachium tenellum. *Panam. J. Aquat. Sci.* 11, 251–257.
- Badger, D. A., Smith, R. L., Bao, J., Kuester, R. K., and Sipes, I. G. (2002). Disposition and metabolism of isoeugenol in the male Fischer 344 rat. *Food Chem. Toxicol.* 40, 1757–1765. doi: 10.1016/S0278-6915(02)00183-7
- Barrento, S., Marques, A., Vaz-Pires, P., and Leonor Nunes, M. (2011). Cancer pagurus (Linnaeus 1758) physiological responses to simulated live transport: influence of temperature, air exposure and AQUI-s®. *J. Therm. Biol.* 36, 128–137. doi: 10.1016/j.jtherbio.2010.12.006
- Birch, J. (2017). Animal sentience and the precautionary principle. *Anim. Sentience* 2, 1–16. doi: 10.51291/2377-7478.1200
- Birch, J., Burn, C., Schnell, A., Browning, H., and Crump, A. (2021). *Review of the evidence of sentience in cephalopod molluscs and decapod crustaceans* (London: LSE Consulting).
- Briffa, M. (2022). Sentience in decapods: an open question. *Anim. Sentience* 7, 1–5. doi: 10.51291/2377-7478.1740
- Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-nakbi, A., Rouabhi, M., Mahdouani, K., et al. (2007). The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*syzygium aromaticum* l. myrtaceae): a short review. *Phyther. Res.* 21, 501–506. doi: 10.1002/ptr
- Conte, F., Voslarova, E., Vecerek, V., Elwood, R. W., Coluccio, P., Pugliese, M., et al. (2021). Humane slaughter of edible decapod crustaceans. *Animals* 11, 1–13. doi: 10.3390/ani11041089
- Cowing, D., Powell, A., and Johnson, M. (2015). Evaluation of different concentration doses of eugenol on the behaviour of nephrops norvegicus. *Aquaculture* 442, 78–85. doi: 10.1016/j.aquaculture.2015.02.039
- Coyle, S. D., Dasgupta, S., Tidwell, J. H., Beavers, T., Bright, L. A., and Yasharian, D. K. (2005). Comparative efficacy of anesthetics for the freshwater prawn macrobrachium rosenbergii. *J. World Aquac. Soc* 36, 282–290. doi: 10.1111/j.1749-7345.2005.tb00332.x
- Coyle, S. D., Durborow, R. M., and Tidwell, J. H. (2004) *Anesthetics in aquaculture*. Available at: <http://fisheries.tamu.edu/files/2013/09/SRAC-Publication-No.-3900-Anesthetics-in-Aquaculture.pdf>.
- Crump, A., Browning, H., Schnell, A., Burn, C., and Birch, J. (2022a). Sentience in decapod crustaceans: a general framework and review of the evidence. *Anim. Sentience* 32, 1–34. doi: 10.51291/2377-7478.1691
- Crump, A., Browning, H., Schnell, A. K., Burn, C., and Birch, J. (2022b). Invertebrate sentience and sustainable seafood. *Nat. Food* 3, 884–886. doi: 10.1038/s43016-022-00632-6
- Dawkins, M. S. (2017). Animal welfare with and without consciousness. *J. Zool.* 301, 1–10. doi: 10.1111/jzo.12434
- Dawkins, M. S. (2022). The science of animal sentience and the politics of animal welfare should be kept separate. *Anim. Sentience* 6, 1–4. doi: 10.51291/2377-7478.1708
- DEFRA. (2015). *Guidance: red and white meat slaughterhouses: standard operating procedures* (United Kingdom: UK Government). Available at: <https://www.gov.uk/guidance/red-and-white-meat-slaughterhouses-standard-operating-procedures>.
- DEFRA. (2021a). *Animal welfare (sentience) act 2021* (London: House of Lords). Available at: <https://bills.parliament.uk/bills/2867>.
- DEFRA. (2021b). *Our action plan for animal welfare*. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/985332/Action_Plan_for_Animal_Welfare.pdf.
- DEFRA. (2022). *Animal welfare (sentience) act 2022* (UK Government). Available at: <https://bills.parliament.uk/bills/2867>.
- de Souza Valente, C. (2022). Anaesthesia of decapod crustaceans. *Vet. Anim. Sci.* 16, 100252. doi: 10.1016/j.vas.2022.100252
- EU Council (2009). COUNCIL REGULATION (EC) no 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. *Off. J. Eur. Union* 52, 1–30.
- European Food Safety Authority (2005). Aspects of the biology and welfare of animals used for experimental and other scientific purposes. *Asp. Biol. Welf. Anim. used Exp. other Sci. Purp.* 1–136.
- Fregin, T., and Bickmeyer, U. (2016). Electrophysiological investigation of different methods of anesthesia in lobster and crayfish. *PLoS One* 11, 1–19. doi: 10.1371/journal.pone.0162894
- Frosch, P. J., Pilz, B., Andersen, K. E., Burrows, D., Camarasa, J. G., Dooms-Goossens, A., et al. (1995). Patch testing with fragrances: results of a multicenter study of the European environmental and contact dermatitis research group with 48 frequently used constituents of perfumes. *Contact Dermatitis* 33, 333–342. doi: 10.1111/J.1600-0536.1995.tb02048.x
- Gardner, C. (1997). Options for humanely immobilizing and killing crabs. *J. Shellfish Res.* 16, 219–224.
- Gardner, C. (2004). “Treating the prawn well on its way to the barbie: welfare of aquatic crustaceans,” in *Welfare underwater: issues with aquatic animals*. Ed. B. Jones (RSPCA Australia Inc: Canberra), 21–24.

- Ghanawi, J., Saoud, G., Zakher, C., Monzer, S., and Saoud, I. P. (2019). Clove oil as an anaesthetic for Australian redclaw crayfish *Cherax quadricarinatus*. *Aquac. Res.* 50, 3628–3632. doi: 10.1111/are.14319
- Hajek, G. J., Choczewski, M., Dziaman, R., and Klyszejko, B. (2009). Evaluation of immobilizing methods for the Chinese mitten crab, *Eriocheir sinensis* (Milne-Edwards). *Electron. J. Polish Agric. Univ.* 12, 16–21.
- Hewer, C. L. (1937). The stages and signs of general anaesthesia. *Br. Med. J.* 2, 274–276. doi: 10.1136/bmj.2.3996.274
- Hu, Q., Zhou, M., and Wei, S. (2018). Progress on the antimicrobial activity research of clove oil and eugenol in the food antiseptic field. *J. Food Sci.* 83, 1476–1483. doi: 10.1111/1750-3841.14180
- Jensen, M. A., Fitzgibbon, Q. P., Carter, C. G., and Adams, L. R. (2013). Recovery periods of cultured spiny lobster, *Sagmariasus verreauxi* juveniles: effects of handling, force feeding, exercising to exhaustion and anaesthesia on oxygen consumption and ammonia excretion rates. *Aquaculture* 410–411, 114–121. doi: 10.1016/j.aquaculture.2013.06.020
- Jiang, S., Zhou, F., Yang, W., Wu, Z., Le, Y., Yang, Q., et al. (2020). Anaesthetic effect of eugenol at different concentrations and temperatures on black tiger shrimp (*Penaeus monodon*). *Aquac. Res.* 51, 3268–3273. doi: 10.1111/are.14662
- Ke, C., Liu, Q., Li, L., Chen, J., Zhao, C., Xu, J., et al. (2018). Residual levels and risk assessment of eugenol and its isomers in fish from China markets. *Aquaculture* 484, 338–342. doi: 10.1016/j.aquaculture.2017.07.034
- Kildea, M. A., Allan, G. L., and Kearney, R. E. (2004). Accumulation and clearance of the anaesthetics clove oil and AQUI-STM from the edible tissue of silver perch (*Bidyanus bidyanus*). *Aquaculture* 232, 265–277. doi: 10.1016/s0044-8486(03)00483-6
- Li, Y., She, Q., Han, Z., Sun, N., Liu, X., and Li, X. (2018). Anaesthetic effects of eugenol on grass shrimp (*Palaemonetes sinensis*) of different sizes at different concentrations and temperatures. *Sci. Rep.* 8, 1–9. doi: 10.1038/s41598-018-28975-w
- Mesquita, C., Miethé, T., Dobby, H., and McLay, A. (2017). Crab and lobster fisheries in Scotland: results of stock assessments 2013–2015. *Scottish Mar. Freshw. Sci.* 8, 1–90. doi: 10.7489/1990-1
- Morgan, J., Cargill, C., and Groot, E. (2001). The efficacy of clove oil as an anesthetic for decapod crustaceans. *Bull. Aquac. Assoc. Canada* 101, 27–31.
- National Toxicology Program. (1983). Carcinogenesis studies of eugenol (CAS no. 97-53-0) in F344/N rats and B6C3F1 mice (Feed studies). *Natl. Toxicol. Program Tech. Rep. Ser.* 223, 1–159.
- National Toxicology Program. (2010). Toxicology and carcinogenesis studies of isoeugenol (CAS no. 97-54-1) in F344/N rats and B6C3F1 mice (gavage studies). *Natl. Toxicol. Program Tech. Rep. Ser.* 551, 1–178.
- Parodi, T. V., Cunha, M. A., Heldwein, C. G., De Souza, D. M., Martins, Á. C., Garcia, L. D. O., et al. (2012). The anesthetic efficacy of eugenol and the essential oils of *Lippia alba* and *Aloysia triphylla* in post-larvae and sub-adults of *Litopenaeus vannamei* (Crustacea, penaeidae). *Comp. Biochem. Physiol. - C Toxicol. Pharmacol.* 155, 462–468. doi: 10.1016/j.cbpc.2011.12.003
- Robertson, J., Delorme, N., Hickey, A., and Jeffs, A. (2018). Assessment of the potential of the anesthetic AQUI-s for live transportation of the southern rock lobster, *jasus edwardsii*. *Bull. Mar. Sci.* 94, 1137–1151. doi: 10.5343/bms.2017.1111
- Robertson, J., Jeffs, A., Hedges, C., and Hickey, A. J. R. (2022). Cardiac mitochondrial energetics of the Australasian red spiny lobster, *jasus edwardsii*, when exposed to isoeugenol within the commercial anaesthetic AQUI-s. *J. Exp. Biol.* 225, 1–6. doi: 10.1242/jeb.242771
- Rychen, G., Aquilina, G., Azimonti, G., Bampidis, V., Bastos, M., de, L., et al. (2017). Scientific opinion on efficacy of lidenfeed® (eugenol) for chickens for fattening. *EFSA J.* 15, 1–7. doi: 10.2903/j.efsa.2017.4931
- Saydmohammed, M., and Pal, A. K. (2009). Anesthetic effect of eugenol and menthol on handling stress in *Macrobrachium rosenbergii*. *Aquaculture* 298, 162–167. doi: 10.1016/j.aquaculture.2009.10.020
- Schmidt-Nielsen, K. (1962). Humane killing of crustaceans. *Sci. (80-)*. 135, 587–588. doi: 10.1126/science.135.3503.587.c
- Sneddon, L. U. (2012). Clinical anesthesia and analgesia in fish. *J. Exot. Pet Med.* 21, 32–43. doi: 10.1053/j.jepm.2011.11.009
- Soltani, M., Marmari, G., and Mehrabi, M. R. (2004). Acute toxicity and anesthetic effects of clove oil in *Penaeus semisulcatus* under various water quality. *Aquac. Int.* 12, 457–466. doi: 10.1023/B:AQUI.0000042137.26705.7b
- Souza, C. F., Lima, T., Baldissera, M. D., Geihs, M. A., Maciel, F. E., Nery, L. E. M., et al. (2018). Nanoencapsulated *Melaleuca alternifolia* essential oil exerts anesthetic effects in the brachyuran crab using *Neohelice granulata*. *An. Acad. Bras. Cienc.* 90, 2855–2864. doi: 10.1590/0001-3765201820170930
- Stevens, E. D., Arlinghaus, R., Browman, H. I., Cooke, S. J., Cowx, I. G., Diggles, B. K., et al. (2016). Stress is not pain. comment on Elwood and Adams, (2015) 'Electric shock causes physiological stress responses in shore crabs, consistent with prediction of pain'. *Biol. Lett.* 12, 2. doi: 10.1111/faf.12010
- Syndel Canada (2015) *Aqua life TMS safety data sheet*. Available at: <https://syndel.com/wp-content/uploads/2019/01/Aqua-Life-TMS-CDN-SDS-09-15-Rev.08-17.pdf>.
- Tang, Y., Zhang, H., Yang, G., Fang, C., Kong, C., Tian, L., et al. (2022). Pharmacokinetics studies of eugenol in Pacific white shrimp (*Litopenaeus vannamei*) after immersion bath. *BMC Vet. Res.* 18, 1–13. doi: 10.1186/s12917-022-03145-3
- Taylor, J., Vinaterra, L., Ozorio, R., Scheweitzer, R., and Andreatta, E. R. (2004). Minimizing the effects of stress during eyestalk ablation of *Litopenaeus vannamei* females with topical anesthetic and a coagulating agent. *Aquaculture* 233, 173–179. doi: 10.1016/j.aquaculture.2003.09.034
- UK Government (2006). *Animal welfare act 2006* (United Kingdom: UK Government). Available at: <https://www.legislation.gov.uk/ukpga/2006/45/data.pdf>.
- UK Government (2014). *Animals (Scientific procedures) act 1986 (Consolidated version)* (London: UK Government). Available at: <https://www.gov.uk/government/publications/consolidated-version-of-aspa-1986>.
- Vartak, V., and Singh, R. K. (2006). Anesthetic effects of clove oil during handling and transportation of the freshwater prawn, *Macrobrachium rosenbergii* (de man). *Isr. J. Aquac. - Bamidgah* 58, 46–54. doi: 10.46989/001c.20426
- Waterstrat, P. R., and Pinkham, L. (2005). Evaluation of eugenol as an anesthetic for the American lobster *Homarus americanus*. *J. World Aquac. Soc.* 36, 420–424. doi: 10.1111/j.1749-7345.2005.tb00347.x
- Weineck, K., Ray, A. J., Fleckenstein, L. J., Medley, M., Dzibuk, N., Piana, E., et al. (2018). Physiological changes as a measure of crustacean welfare under different standardized stunning techniques: cooling and electroshock. *Animals* 8, 1–21. doi: 10.3390/ani8090158
- WHO. (2006). *Evaluation of certain food additives* (Geneva: World Health Organization). doi: 10.1017/cbo9780511622670.019
- Wycoff, S., Wineck, K., Conlin, S., Suryadevara, C., Grau, E., Bradley, A., et al. (2018). Effects of clove oil (eugenol) on proprioceptive neurons, heart rate, and behavior in model crustaceans. *Impulse (Sydney)*. 15, 1–21.
- Yue, S. (2008). The welfare of crustaceans at slaughter. *Agribus. Rep.* 2008, 1–10. Available at: <https://www.wellbeingintlstudiesrepository.org/agreports/vol2008/iss2008/5>.
- Zahl, I. H., Kiessling, A., Samuelsen, O. B., and Hansen, M. K. (2011). Anaesthesia of Atlantic halibut (*Hippoglossus hippoglossus*) effect of pre-anaesthetic sedation, and importance of body weight and water temperature. *Aquac. Res.* 42, 1235–1245. doi: 10.1111/j.1365-2109.2010.02711.x
- Zahl, I. H., Samuelsen, O., and Kiessling, A. (2012). Anaesthesia of farmed fish: implications for welfare. *Fish Physiol. Biochem.* 38, 201–218. doi: 10.1007/s10695-011-9565-1