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THE LONDON SCHOOL
OF ECONOMICS AND
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Jonathan Birch, Charlotte Burn, Alexandra Schnell, Heather Browning and Andrew Crump

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FOREWORD

By Professor Nicola S. Clayton FRS FSB FSPS CPsychol FBPsS

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Birch and colleagues have developed a highly important and extremely useful framework for evaluating the evidence for sentience, the capacity to experience pain, distress and/or harm, in cephalopod molluscs (including cuttlefish, octopods and squid) and decapod crustaceans (including crabs, crayfish, lobsters, prawns, shrimps). Birch and colleagues develop eight criteria in their framework for evaluation, which they use to assess the evidence from over 300 publications of scientific research as well as investigating the potential welfare implications of current commercial practices.

The framework combines and integrates the authors' empirical and theoretical expertise in animal behaviour, comparative cognition, sensory ecology, neuroscience, animal welfare and philosophy. The eight criteria are as follows: the possession of (1) nociceptors, (2) integrative brain regions and (3) the connections between the two, (4) responses affected by potential local anaesthetics or analgesics, (5) motivational trade-offs between the cost of threat and the potential benefit of obtaining resources; (6) flexible self-protective tactics used in response to injury and threat; (7) associative learning (in other words, learning that goes beyond mere habituation and sensitisation) and finally (8) behaviour that shows the animal values analgesics when injured.

In reviewing the relevant evidence, there are inevitably challenges, especially juxtaposing evidence from the field of comparative cognition, where the emphasis lies in ruling out simpler explanations for a given behaviour, response or performance on various problem-solving tasks, with evidence from animal welfare, where the question revolves around potential capacities (such as the potential to experience pain). Furthermore, it may be the case that some of the criteria are more convincing by themselves than others. For example, behaviour that shows the animal values analgesics when injured would seem convincing evidence in its own right, and evidence of goal-directed actions is also persuasive, whereas associative stimulus-response learning could potentially be achieved without sentience, so would not be enough by itself.

Birch and colleagues' approach to this conundrum is to evaluate the evidence in terms of a confidence level per criterion for each species in question, ranging from no confidence to very high confidence. They suggest that very strong evidence of sentience should be assumed if the animal in question satisfies at least seven of the eight criteria, whereas a high confidence level for five or more criteria would be classified as strong evidence, and a high confidence level for three or more criteria amounts to substantial evidence of sentience.

Using this approach, the authors conclude that there is very strong evidence of sentience in octopods, because there is either high or very high confidence that octopods satisfy criteria 1, 2, 3, 4, 6, 7 and 8, and medium confidence for criterion 5. It would be interesting to know whether certain criteria are more likely to co-correlate than others (for example criteria 4 and 8, both of which concern responses to analgesics). For squid and cuttlefish, the evidence was less strong but nonetheless substantial.

For the decapods, the authors found strong evidence in true crabs, with high or very high confidence that the crabs satisfy criteria 1, 2, 4, 6 and 7. They also found substantial evidence in anomuran crabs, astacid lobsters and crayfish, and in caridean shrimps. In interpreting these findings the authors are clear to point out that the evidence of sentience is dependent on how much scientific research has been conducted on the various species and taxa in question and that absence of evidence is not evidence of absence.

In the light of these evaluations, the authors make a strong recommendation that all cephalopod molluscs and decapod crustaceans should be regarded as sentient animals for the purposes of UK animal welfare law. They do not recommend restricting to just some groups, e.g. octopods and true crabs, and provide clear justifications as to why. They also provide very helpful recommendations regarding commercial practices. They recommend against declawing, nicking, eyestalk ablation and the sale of live decapod crustaceans to untrained, non-expert handlers, and they include suggestions for best practices for transport, stunning and slaughter.

This is an excellent report which argues that the cephalopod molluscs and decapod crustaceans should be included in the UK animal welfare law in an explicit way, based on a detailed and important scientific and philosophical framework and evaluation, coupled with extremely helpful suggestions for improving best practice and welfare, and for regulating existing practices that currently raise widespread concerns about the welfare of these animals.

EXECUTIVE SUMMARY

Sentience is the capacity to have feelings, such as feelings of pain, pleasure, hunger, thirst, warmth, joy, comfort and excitement. It is not simply the capacity to feel pain, but feelings of pain, distress or harm, broadly understood, have a special significance for animal welfare law.

Drawing on over 300 scientific studies, we have evaluated the evidence of sentience in two groups of invertebrate animals: the **cephalopod molluscs** or, for short, **cephalopods** (including octopods, squid and cuttlefish) and the **decapod crustaceans** or, for short, **decapods** (including crabs, lobsters and crayfish). We have also evaluated the potential welfare implications of current commercial practices involving these animals.

Our framework

We have developed a rigorous framework for evaluating scientific evidence of sentience based on eight criteria. In short, these are:

1) possession of nociceptors;
2) possession of integrative brain regions;
3) connections between nociceptors and integrative brain regions;
4) responses affected by potential local anaesthetics or analgesics;
5) motivational trade-offs that show a balancing of threat against opportunity for reward;
6) flexible self-protective behaviours in response to injury and threat;
7) associative learning that goes beyond habituation and sensitisation;
8) behaviour that shows the animal values local anaesthetics or analgesics when injured.

To be clear, no single criterion provides conclusive evidence of sentience by itself. No single criterion is intended as a “smoking gun”. This is especially true for criterion 1, which (although relevant as the first part of the pain pathway) could easily be satisfied by a non-sentient animal. Nonetheless, we consider all these criteria to be relevant to the overall case.

After reviewing all relevant evidence, we have arrived at a **confidence level** for each criterion, describing our level of confidence that the animals in question satisfy or fail the criterion. The possible confidence levels are very high confidence, high confidence, medium confidence, low confidence, very low confidence, and no confidence.

Our confidence level takes into account both the amount of evidence and the reliability and quality of the scientific work. We only use “very high confidence” when there is a large amount of high quality, reliable evidence, removing any room for reasonable doubt. We use “high confidence” in cases where we are convinced, after carefully considering all the evidence, that the animals satisfy/fail the criterion, even though some room for reasonable doubt remains. We use “medium confidence” in cases where we have some concerns about the reliability of the evidence that prevent us from having high confidence. We use “low confidence” for cases where there is little evidence that an animal satisfies or fails the criterion, and “very low” or “no confidence” when the evidence is either seriously inadequate or non-existent.

To be clear, when we say we have low confidence that a criterion is satisfied, this does not mean that we think sentience is unlikely or disproven. What it means is that the evidence one way or the other is thin, low-quality, or both.

To move from the individual criteria to an overall judgement, we use an approximate grading scheme. On our scheme, high or very high confidence that an animal satisfies 7 or more of the criteria amounts to **very strong evidence** of sentience. High or very high confidence that an animal satisfies 5 or more criteria amounts to **strong evidence** of sentience, and high or very high confidence that an animal satisfies 3 or more criteria amounts to **substantial evidence** of sentience.

Our findings regarding cephalopods

There is very strong evidence of sentience in octopods. We have either high or very high confidence that octopods satisfy criteria 1, 2, 3, 4, 6, 7 and 8, and medium confidence that they satisfy criterion 5. There is somewhat less evidence concerning other coleoid cephalopods (squid and cuttlefish). However, the evidence is still substantial. We have high confidence that other coleoid cephalopods satisfy criteria 1, 2, 3, and 7. See **Table 1** for a summary.

Our findings regarding decapods

There is strong evidence of sentience in true crabs (infraorder Brachyura). We have either high or very high confidence that true crabs satisfy criteria 1, 2, 4, 6 and 7. There is somewhat less evidence concerning other decapods. There is substantial evidence of sentience in anomuran crabs (infraorder Anomura). We have high confidence that they satisfy criteria 1, 2 and 6, and medium confidence that they satisfy criterion 5. There is also substantial evidence of sentience in astacid lobsters/crayfish (infraorder Astacidea). We have either high or very high confidence that these animals satisfy criteria 1, 2 and 4. See **Table 1** for a summary.

Comparative remarks

For both cephalopods and decapods, in cases where we are not able to have high or very high confidence that a criterion is satisfied, this is invariably because of a lack of positive evidence, rather than because of clear evidence that the animals fail the criterion. There are no cases in which we have very high/high confidence that a taxon *fails* a criterion.

While this may seem surprising, it should be noted that cephalopods and decapods were selected for scrutiny precisely because they seem like plausible candidates for sentience. If we had reviewed evidence for other invertebrate animals (e.g. jellyfish), we might well have ended up with very high confidence that the criteria are failed.

The amount of evidence of sentience for a given biological taxon is limited by how much scientific attention the question of sentience in that taxon has received. Octopods and true crabs have received sustained scientific attention, whereas (for example) nautiloids and penaeid shrimps have barely been studied. Various other taxa (e.g. squid, cuttlefish, anomurans) have received an intermediate level of attention in relation to sentience, resulting in an intermediate amount of evidence.

There is no dramatic difference in the quality or volume of evidence regarding cephalopods as opposed to decapods. There is more evidence for sentience in octopods than in true crabs, but the difference is not vast, and the evidence for sentience in true crabs is slightly more substantial than the evidence for sentience in other, less-studied cephalopods. This leads us to recommend that, if cephalopods are to be included in the scope of animal welfare laws, decapods should also be included.

Our central recommendation

We recommend that **all cephalopod molluscs and decapod crustaceans** be regarded as sentient animals for the purposes of UK animal welfare law. They should be counted as “animals” for the purposes of the Animal Welfare Act 2006 and included in the scope of any future legislation relating to animal sentience.

The Animal Welfare Act 2006 states that the power to extend the scope of the Act “may only be exercised if the appropriate national authority is satisfied, on the basis of scientific evidence, that animals of the kind concerned are capable of experiencing pain or suffering.” We recommend that Defra considers this threshold to have been satisfied by both cephalopods and decapods.

We do not recommend any attempt to restrict the scope of protection to just some cephalopods (e.g. the octopods) or to some decapods (e.g. the true crabs), particularly not in a way that privileges the most intensively studied laboratory species. Extending protection to all vertebrates (as existing legislation does) involves making evidence-based generalizations from intensively studied laboratory species (such as lab rats) to other relevant species, and it would be consistent to do the same for invertebrate taxa, within reason.

A better approach, in our view, would be to protect all cephalopods and decapods in general legislation, while also developing enforceable best-practice guidance and regulations that are specific to the welfare needs of commercially important species.

Recommendations relating to specific commercial practices

Declawing. We have high confidence that declawing (removing one or both of the claws from a crab before returning it back to the water) causes suffering in crabs. Declawing was banned in the UK from 1986 until 2000, when the relevant legislation was overridden by a European Union regulation. Reinstating the ban on declawing in the UK would be an effective intervention to improve the welfare of decapods.

Nicking. We also have high confidence that the practice of nicking (cutting the tendon of a crab's claw) causes suffering and is a health risk to the animals. We encourage the development and implementation of practical alternatives to nicking.

Wholesale and retail. We recommend a ban on the sale of live decapod crustaceans to untrained, non-expert handlers. For example, live decapod crustaceans can be ordered from online retailers. This practice inherently creates a risk of poor handling and inappropriate storage and slaughter methods. Ending this practice would be an effective intervention to improve the welfare of decapods.

Storage and transport. We have high confidence that, for decapods, good welfare during transport and storage requires access to dark shelters and cool temperatures (for damp storage, no more than 8°C; the minimum suitable temperature is yet to be established but may be around 3-4°C) and an appropriate stocking density. The government may wish to consider adding legal force to the existing recommendations for the transport of crustaceans drawn up by Seafish or developing new guidelines.

Stunning. Current evidence indicates that electrical stunning with appropriate parameters for the species can induce a seizure-like state in relatively large decapods, and that stunning diminishes, without wholly abolishing, the nervous system's response to boiling water. We interpret this as evidence that electrical stunning is better than nothing. We recommend more research on the question of how to achieve effective electrical stunning, especially for small animals, and on the question of how electrical stunning may be implemented when decapods are slaughtered at sea.

Slaughter (decapods). We recommend that the following slaughter methods are banned in all cases in which a more humane slaughter method is available, unless preceded by effective electrical stunning: boiling alive, slowly raising the temperature of water, tailing (separation of the abdomen from the thorax, or separation of the head from the thorax), any other form of live dismemberment, and freshwater immersion (osmotic shock). On current evidence, the most reasonable slaughter methods are double spiking (crabs), whole-body splitting (lobsters), and electrocution using a specialist device on a setting that is designed and validated to kill the animal quickly after initially stunning it.

Slaughter (cephalopods). Various different slaughter methods are currently used on fishing vessels in European waters, including clubbing, slicing the brain, reversing the mantle and asphyxiation in a suspended net bag. We are not able to recommend any of these methods as humane. On current evidence, there is no slaughter method for cephalopods that is both humane and commercially viable on a large scale. We recommend the development of codes of best practice in this area, and we encourage further research on the question of how to implement more humane slaughter methods at sea for both cephalopods and fish.

Eyestalk ablation. In shrimp aquaculture globally, it is a common practice to sever the eyestalks of breeding females to accelerate breeding ("eyestalk ablation"). We suspect this does not currently happen at the UK's two penaeid shrimp hatcheries, because they import hatchlings from overseas. Assuming this to be the case, a ban on eyestalk ablation in the UK would be a reasonable precautionary measure but might not generate an immediate welfare benefit.

Octopus farming. Although there is no octopus farming in the UK, there is some interest in it elsewhere in the world. However, octopuses are solitary animals that are often aggressive towards each other in confined spaces. We are convinced that high-welfare octopus farming is impossible. The government could consider a ban on imported farmed octopus. A pre-emptive ban on octopus farming in the UK could be considered but would have no immediate welfare benefit.

In sum, the time has come to include cephalopod molluscs and decapod crustaceans in UK animal welfare law in an explicit way, and to take proportionate steps to regulate practices that are a source of reasonable and widespread animal welfare concerns.

Table 1. A summary of confidence levels regarding the evidence of sentience in cephalopods and decapods. *The colours and letters represent our confidence level that the criterion in question (column) is satisfied by the taxon in question (row). VH (dark green) indicates very high confidence, H (light green) indicates high confidence, M (dark yellow) indicates medium confidence, L (light yellow) represents low confidence, and VL (light grey) represents very low confidence. For descriptions of the criteria, see the main text. Importantly, low/very low confidence implies only that the scientific evidence one way or the other is weak, not that the animal fails or is likely to fail the criterion.*

	Criterion 1	Criterion 2	Criterion 3	Criterion 4	Criterion 5	Criterion 6	Criterion 7	Criterion 8
Octopods (Octopoda)	**VH**	**VH**	**H**	**H**	**M**	**VH**	**VH**	**H**
Cuttlefish (Sepiida)	**H**	**VH**	**H**	**L**	**M**	**M**	**VH**	**L**
Other coleoids (squid, all orders)	**H**	**VH**	**H**	**L**	**M**	**L**	**H**	**L**
Nautiloids	**H**	**L**	**L**	**L**	**L**	**L**	**M**	**VL**
True crabs (Brachyura)	**H**	**VH**	**L**	**VH**	**L**	**VH**	**H**	**VL**
Anomuran crabs (Anomura)	**H**	**VH**	**L**	**L**	**M**	**H**	**L**	**VL**
Astacid lobsters/crayfish (Astacidea)	**H**	**VH**	**L**	**VH**	**L**	**L**	**M**	**VL**
Spiny lobsters (Achelata)	**H**	**VH**	**L**	**L**	**L**	**L**	**M**	**VL**
Caridean shrimps (Caridea)	**H**	**VH**	**L**	**M**	**L**	**M**	**L**	**VL**
Penaeid shrimps (Penaeidae)	**H**	**L**	**L**	**M**	**L**	**L**	**L**	**VL**

PART I. A FRAMEWORK FOR EVALUATING EVIDENCE OF SENTIENCE

1.1 Defining sentience

Sentience (from the Latin *sentire*, to feel) is the **capacity to have feelings**. Feelings may include, for example, feelings of pain, distress, anxiety, boredom, hunger, thirst, pleasure, warmth, joy, comfort, and excitement. We humans are sentient beings, and we are all familiar with such feelings from our own lives. A sentient being is “conscious” in the most elemental, basic sense of the word. It need not be able to consciously *reflect* on its feelings, as we do, or to understand the feelings of others: to be sentient is simply to *have* feelings.

In discussions about animal welfare, sentience is sometimes defined in a narrower way, as specifically referring to the capacity to have negative, aversive feelings. The UK’s Animal Welfare Committee (formerly the Farm Animal Welfare Committee) has defined sentience as the capacity to experience pain, distress, or harm (AWC, 2018). A disadvantage of this narrower definition is that it leaves out the positive side of subjective experience: feelings of warmth, joy, comfort, and so on. An advantage is that it draws our attention specifically to the type of feeling that raises the most severe type of ethical concern. In this report, we will define sentience as the capacity to have feelings, including both positive and negative feelings. However, we will focus in practice on the negative side of sentience, owing to the special significance of feelings of pain, distress or harm for animal welfare law (as emphasized, for example, in the Animal Welfare Act 2006).

Sentience is distinct from **nociception**. Nociception is the **detection by a nervous system of actually or potentially noxious stimuli** (such as extreme heat, extreme acidity or alkalinity, toxins, or breaks to the skin), achieved by means of specialised receptors called **nociceptors**. A nociceptor is “a high-threshold sensory receptor of the peripheral somatosensory nervous system that is capable of transducing and encoding noxious stimuli” (International Association for the Study of Pain, 2017). The detection of a noxious stimulus does not necessarily require sentience. It is possible in principle for a noxious stimulus to be detected without any experience or feeling on the part of the system that detects it.

Yet sentience and nociception are not unrelated. In humans, feelings of pain, distress or harm are often part of the response to noxious stimuli, as initially detected by nociceptors. For example, touching a hot stove or cutting your finger on a knife will activate nociceptors, these nociceptive signals will be processed by the brain, and the result will be an experience of pain. Not all pain experiences are the result of the activation of nociceptors, but many are. One of the subtleties to bear in mind here is that other responses to the activation of nociceptors, such as reflex withdrawal, can still be independent of the experience of pain.

In humans, feelings of pain have two main aspects: a sensory aspect (an injury or potential injury is perceived) and an affective aspect (the feeling is unpleasant, aversive, negative). These two aspects of pain are widely recognised in human pain research (Auvray et al., 2010). It is the affective, negatively valenced aspect of pain that is the main source of ethical concern. Put simply, pain *feels bad*—the urge to do something to alleviate it is typically strong—and this affective side of pain is what we seek to control with analgesics (painkillers) such as morphine (Price et al., 1985; Caputi et al., 2019).

Pain is one example within a broader category of **negatively valenced affective states**, a category which also includes states of anxiety, fear, hunger, thirst, coldness, discomfort and boredom (Burn, 2017). All of these states feel bad, and they all motivate behaviours aimed at removing their causes. All negatively valenced feelings have the potential to contribute to poor welfare. As a result, they are all sources of legitimate ethical concern. We regard all negative feelings as forms of “distress or harm”, and we will regard all of them as relevant to questions of sentience.

1.2 The question of invertebrate sentience

Which animals, other than humans, are sentient? The progress of neuroscience and biology in the late twentieth and early twenty-first centuries gradually rendered untenable the suggestion that sentience might be uniquely human, resulting in the widespread acceptance within the scientific community of the sentience of mammals and birds (Boly et al., 2013). In recent years, bestselling books (Montgomery, 2015; Godfrey-Smith, 2016) have popularised the idea that octopods may be sentient.

This is an idea that had already been taken seriously by scientists for several decades. The UK led the way on this issue in 1993 by bringing the common octopus (**Octopus vulgaris**) within the scope of the Animals (Scientific Procedures) Act 1986 (**ASPA**). In 2012, following the 2010 EU directive on the use of animals for scientific purposes, the scope of the Act was extended to all cephalopod molluscs.

In 2012, the Cambridge Declaration on Consciousness (Low et al., 2012) crystallised a scientific consensus that humans are not the only conscious beings. It added that "non-human animals, including all mammals and birds, and many other creatures, including octopuses" possess neurological substrates complex enough to support conscious experiences. Although this statement was phrased in terms of consciousness rather than sentience, a capacity for conscious experience and a capacity for sentience are closely linked, because feelings are conscious experiences in the most basic, elemental sense of "conscious". The reference to "octopuses" highlights a growing recognition within the international scientific community that at least some invertebrates may be sentient.

The primary aim of this report is to evaluate the evidence of sentience in two invertebrate taxa: the **cephalopod molluscs (for short: cephalopods)** (**Figure 1**) and the **decapod crustaceans (for short: decapods)** (**Figure 2**). The cephalopods are a class of around 750 species in the mollusc phylum, including all species of octopus, squid, cuttlefish, and nautilus (Tanner et al., 2017). The decapods are an order of invertebrate animals of the crustacean subphylum containing around 15,000 species, including the true crabs, lobsters, crayfish, and true shrimps (De Grave et al., 2009; Wolfe et al., 2019).

These taxa have been selected by Defra because there has been a substantial amount of recent debate surrounding their potential inclusion in animal welfare law. Although this report will focus on the cephalopods and the decapods, we intend the framework we develop to be general enough to facilitate future evaluations of the evidence of sentience in other taxa.

Figure 1. Cephalopod molluscs. From top to bottom: squid, octopus, cuttlefish. *Photographs by Alexandra Schnell.*

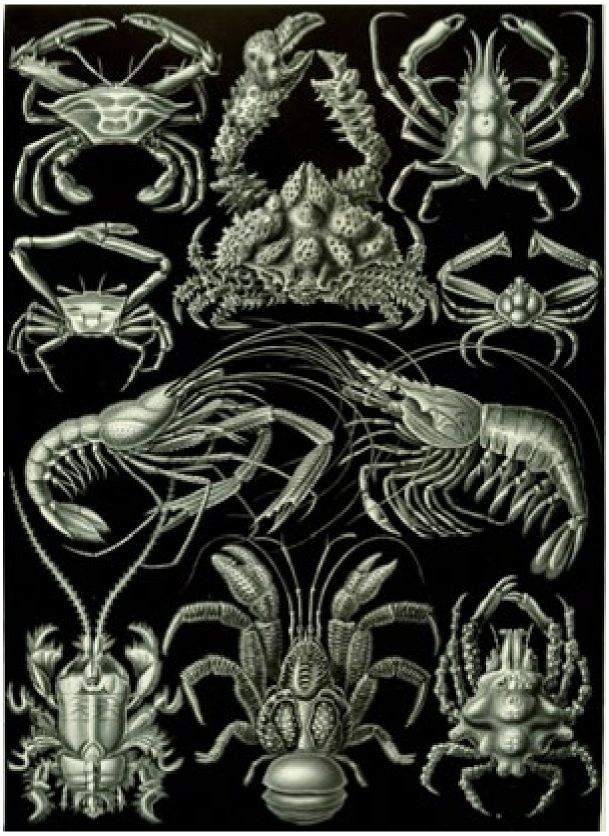

Figure 2. Decapod crustaceans. Plate from Ernst Haeckel, *Kunstformen der Natur*, 1904.

1.3 Why the question matters

The question of invertebrate sentience matters both ethically and legally. It matters ethically because, if a being is sentient, there are limits on what a human can ethically do to that being. A sentient being has interests, and it is unethical to act in a way that shows inadequate consideration, or no consideration at all, for these interests. This idea lies at the heart of existing animal welfare protections. Everyone agrees, for example, that it is wrong to treat a dog as if it had no interest in shelter, food, water, and comfort. If some invertebrates are sentient, then it is also wrong to treat them in a way that shows inadequate consideration for their interests.

Sentience matters legally in the UK for several reasons. First, no invertebrate was included within the scope of the UK's Animal Welfare Act 2006 (**AWA**), but the Act gives the Secretary of State the power to expand the scope of the Act if new scientific evidence of the capacity for pain and suffering in invertebrates comes to light. Since pain and suffering are components of sentience, evaluating evidence of sentience in invertebrates is crucial for setting the scope of AWA.

Second, the Welfare of Animals (Transport) (England) Order 2006 (**WATEO**) already includes all "cold-blooded invertebrate animals" and requires that their transport should not cause injury or unnecessary suffering. Since suffering requires sentience, sentience is relevant to the scope of WATEO.

Third, Schedule 4 of the Welfare at the Time of Killing (England) Regulations 2015 (**WATOK**) requires that all animals not otherwise protected are still required to be killed humanely, i.e. without avoidable pain, distress, or suffering. However, there remains a great deal of uncertainty as to which methods of killing (if any) cause avoidable pain, distress and suffering to invertebrates and which do not. Again, the question of which invertebrates are sentient is crucial to the proper application of these regulations.

Fourth, different legislation applies to scientific procedures, and the concept of sentience plays a crucial role in that legislation. As noted above, the common octopus (**O. vulgaris**) was brought within the scope of ASPA in 1993. In the European Union (EU), all cephalopods (including octopods, squid, cuttlefish, and nautiloids) were included within the scope of EU Directive 2010/63/EU on the protection of animals used for scientific purposes, and ASPA was amended accordingly in 2012.

Fifth, in recent years, a debate has arisen as to how the UK will enshrine in law a commitment to recognising animal sentience following the UK's exit from the EU. The government has pledged to introduce new legislation that achieves this task. One crucial issue to be resolved is the scope of the new legislation.

1.4 The difficulty of answering the question

There are major obstacles to answering the question of invertebrate sentience with certainty, or beyond all reasonable doubt. Feelings, such as feelings of pain, cannot be directly observed. The best evidence we have of sentience in other human beings is that they can report their experiences—they can tell us what they are feeling. Even for other mammals, we do not have this type of evidence.

What we do have for other mammals is evidence of substantial similarity to humans in brain organisation, brain function, cognition, affect and behaviour. The part of the brain most closely linked to subjective experiences in humans is the neocortex, a structure in the cerebral cortex consisting of six richly organised layers of neural tissue. In humans, the neocortex is about 2-4mm thick and forms the strikingly crinkled outer layer of the brain. In non-primate mammals, it is much smoother, but still present. The presence of a neocortex in other mammals, with the same six-layered organisation, means it is a point of near-total scientific consensus that other mammals are sentient.

This strategy of looking for neural mechanisms and structures that are shared with the human brain also works, but to a lesser extent, for birds. Birds have a structure called the dorsal pallium that resembles the mammalian neocortex in striking ways. Although the architecture is different (the structure is nucleated with six clusters rather than laminated with six layers) the patterns of connectivity are similar (Clayton & Emery, 2015; Güntürkün & Bugnyar, 2016). It is generally considered implausible that the differences in brain organisation between mammals and birds could make the difference between the presence and absence of sentience. So, there is wide agreement that birds too are sentient (Boly et al., 2013).

Yet this strategy starts to break down when we look at vertebrates that are more distantly related to humans, such as fish. The brains of fish differ substantially from those of mammals. There is no neocortex and no structure that closely resembles the neocortex. The result is that, even for fish, scepticism about their sentience is sometimes expressed (Key, 2016), though these expressions of scepticism are met with vigorous resistance (e.g. Sneddon et al., 2018). The brains of invertebrates differ from those of humans much more radically than those of fish. Invertebrates and humans are separated by over 500 million years of evolution. Even the basic overarching structure of the vertebrate brain (which consists of a forebrain, a midbrain and a hindbrain) is not present in invertebrates (Feinberg & Mallatt, 2016).

We cannot, however, conclude with any confidence that sentience is absent in an invertebrate simply because its brain is differently organised from a vertebrate brain. By way of analogy, the eye of a cephalopod is organised in a very different way from a mammalian eye, but we cannot conclude from this that cephalopods cannot see. There may be multiple neurological routes to the same result. We have no reason to think that sentience could not be achieved by systems that are structurally different from vertebrate brains (e.g. Feinberg & Mallatt, 2016; Ginsburg & Jablonka, 2019).

This raises the question: What constitutes evidence of sentience in a species that is so evolutionarily distant from humans that we cannot expect similarities of brain organisation to resolve the issue? The answer is that we must rely, at least partly, on behavioural and cognitive signatures of sentience. We need to characterise carefully the type of behaviours and cognitive abilities that imply a clear risk of pain, distress, or harm in the animal, and integrate this behavioural and cognitive evidence with what we know about the animal's nervous system. Researchers have grappled for a long time with the task of finding the most relevant indicators (e.g. Smith & Boyd 1991; Bateson 1991; AHAW 2005; Varner 2012; Sneddon et al. 2014; Broom 2014), and we will draw on this past work in this report, while also using a set of criteria that we believe improve on past attempts.

It will always be conceivable, for any set of behavioural, cognitive and neuroscientific signatures, that these signatures could be achieved without sentience. This is why we cannot resolve the question of invertebrate sentience with certainty or put it beyond reasonable doubt. But that level of proof is too much to demand in this context. In the presence of severe welfare risks, it is sometimes necessary to act on the basis of evidence that does not deliver complete certainty. This is a generally accepted principle in the field of animal welfare science (Bateson 1992; Bradshaw, 1998; Birch, 2017) and was explicitly given as the rationale for the inclusion of **O. vulgaris** in the scope of ASPA in 1993. The Chairman of the Animal Procedures Committee (now the Animals in

Science Committee) wrote that “the scientific evidence currently available [at that time] is insufficient to conclude with any certainty that cephalopods can experience pain and suffering” but emphasized the importance of giving the benefit of the doubt to the common octopus despite this uncertainty (APC 1992, Section 3). At the same time, we should not automatically assume sentience in animals that have been repeatedly and meticulously investigated for evidence of sentience with little or no convincing evidence being found.

1.5 The Smith & Boyd (1991) criteria

In 1991, a Working Party of the Institute of Medical Ethics produced a list of seven criteria for sentience that have been influential on subsequent animal welfare policy (Smith & Boyd, 1991). For example, these criteria were applied in 2005 by the Animal Health and Animal Welfare Panel of the European Food Standards Agency in a scientific report that shaped the 2010 EU directive on the use of animals for scientific purposes (AHAW, 2005). The list was as follows:

1) Possession of receptors sensitive to noxious stimuli, located in functionally useful positions on or in the body, and connected by nervous pathways to the lower parts of a central nervous system.
2) Possession of brain centres which are higher in the sense of level of integration of brain processing (especially a structure analogous to the human cerebral cortex).
3) Possession of nervous pathways connecting the nociceptive system to the higher brain centres.
4) Receptors for opioid substances found in the central nervous system, especially the brain.
5) Analgesics modify an animal's response to stimuli that would be painful for a human.
6) An animal's response to stimuli that would be painful for a human is functionally similar to the human response (that is, the animal responds so as to avoid or minimise damage to its body).
7) An animal's behavioural response persists, and it shows an unwillingness to resubmit to a painful procedure; the animal can learn to associate apparently non-painful with apparently painful events.

We think these criteria provide a good starting point. However, they were designed with the assessment of vertebrate animals in mind. They are not ideal criteria for our purposes in this report. There are two main issues that create a need for modified and updated criteria.

First, the criteria (especially the neurobiological criteria) are in some respects too narrow. For example, the reference to opioids in criterion 4 is making a particular assumption about the type of neurotransmitters that modulate aversive experiences (they are assumed to be opioids), and this assumption may not be valid for invertebrates. There are many other endogenous neurotransmitters that may potentially modulate aversive experiences. What matters, in our view, is that the animal’s decision-making in response to threatened or actual noxious stimuli can be modulated by neurotransmitters in a way consistent with the experience of pain, distress or harm. The Smith and Boyd criteria give too much significance to the question of whether the relevant neurotransmitter is an opioid.

Second, the criteria are in some respects too vague and too easy to satisfy. This is especially true of the behavioural criteria, 6 and 7. Regarding criterion 6, it is far too vague to talk of a response that is “functionally similar to the human response”. When we touch a hot stove, we withdraw our hand immediately, but this is just a reflex. Even though we also experience pain, the pain does not cause the withdrawal of the hand: the pain is felt after the hand has begun to withdraw. So, finding a similar reflex in an animal would not be convincing evidence of pain. We need much more refined criteria than this in order to pinpoint the precise behavioural/cognitive functions that do provide evidence of negative affective states. These functions must go beyond mere reflexes and must implicate centralised, integrative processing of information about threatened or actual noxious stimuli.

Regarding criterion 7, persistent responses and an unwillingness to resubmit to a procedure may be indicative of sensitisation (whereby an animal becomes more sensitive in future to a stimulus it has encountered before) rather than associative learning. But sensitisation is found in animals with no central nervous system, such as cnidarians (jellyfish and sea anemones) (Ginsburg & Jablonka, 2019, pp. 279-287). It does not require centralised, integrative processing. A rigorous set of behavioural/cognitive criteria for sentience needs to identify abilities that require centralised, integrative processing. Criteria that can be satisfied by a system with no central nervous system will not command widespread support from the scientific community and will not be robust enough to forge a consensus.

1.6 Our criteria

We will apply the following set of criteria for sentience:

1) The animal possesses receptors sensitive to noxious stimuli (**nociceptors**).
2) The animal possesses **integrative brain regions** capable of integrating information from different sensory sources.
3) The animal possesses neural pathways **connecting** the nociceptors to the integrative brain regions.
4) The animal's behavioural response to a noxious stimulus is modulated by chemical compounds affecting the nervous system in either or both of the following ways:
    a. The animal possesses an **endogenous neurotransmitter system** that modulates (in a way consistent with the experience of pain, distress or harm) its responses to threatened or actual noxious stimuli.
    b. **Putative local anaesthetics, analgesics (such as opioids), anxiolytics or anti-depressants** modify an animal's responses to threatened or actual noxious stimuli in a way consistent with the hypothesis that these compounds attenuate the experience of pain, distress or harm.
5) The animal shows **motivational trade-offs**, in which the disvalue of a noxious or threatening stimulus is weighed (traded-off) against the value of an opportunity for reward, leading to flexible decision-making. Enough flexibility must be shown to indicate centralized, integrative processing of information involving an evaluative common currency.
6) The animal shows **flexible self-protective behaviour** (e.g. wound-tending, guarding, grooming, rubbing) of a type likely to involve representing the bodily location of a noxious stimulus.
7) The animal shows **associative learning** in which noxious stimuli become associated with neutral stimuli, and/or in which novel ways of avoiding noxious stimuli are learned through reinforcement. Note: habituation and sensitisation are not sufficient to meet this criterion.
8) The animal shows that it **values a putative analgesic or anaesthetic when injured** in one or more of the following ways:
    a. The animal learns to self-administer putative analgesics or anaesthetics when injured.
    b. The animal learns to prefer, when injured, a location at which analgesics or anaesthetics can be accessed.
    c. The animal prioritises obtaining these compounds over other needs (such as food) when injured.

Our criteria revise and update the Smith and Boyd (1991) criteria in light of the problems we have identified. Although behavioural and cognitive criteria (criteria 5-8) are especially important in the case of invertebrates, we have still included neurobiological criteria (criteria 1-4) so that the overall picture has a balance of neurobiological and cognitive/behavioural evidence.

To be clear, no single criterion provides conclusive evidence of sentience by itself. No single criterion is intended as a "smoking gun". This is especially true for criterion 1, which could easily be satisfied by a non-sentient animal. Nonetheless, we consider all these criteria to be relevant to the overall case. We discuss in Section 1.7 how to evaluate that overall case.

Criteria 1-3 are based on the Smith and Boyd criteria, with some changes to replace the emphasis on "higher" and "lower" brain regions with an emphasis on integrative brain regions. Instead of a narrow focus on opioids, our criterion 4 allows various forms of responsiveness to endogenous compounds or drugs to count as evidence of sentience, if they modulate the animal's behaviour in a way consistent with the hypothesis that these compounds are altering the animal's experiences of pain, distress or harm.

Smith and Boyd's criteria 4 and 5 are closely related, since analgesics normally work by substituting for endogenous neurotransmitters, exploiting the same mechanisms. For this reason, we have replaced them with a single criterion that can be satisfied in two different ways (our criterion 4).

We have replaced Smith and Boyd's vague behavioural criteria (6 and 7) with a much more detailed and rigorous set of cognitive and behavioural criteria (our criteria 5-8). These criteria identify four main types of behavioural and cognitive abilities that are likely to involve negatively valenced affective states: motivational trade-offs, flexible self-protective behaviour, associative learning, and the valuing (as shown by self-administration, conditioned place preference or prioritisation) of analgesics or anaesthetics when injured.

In each case, the criterion leaves soom room for interpretation. Rather than attempting to deal with all possible ambiguities in this section, we will explain as we go along how we are testing each criterion against the scientific evidence. We will, however, clarify two important points. The first concerns flexibility. "Flexibility" is not intended to imply a capacity for planning ahead or for reflection. In general, it implies only that the animal shows an ability to respond adaptively to the same noxious stimulus in different ways, depending on other aspects of its situation. Flexibility in this sense can be contrasted with fixed, reflexive behaviour that is context-specific.

A difficulty here is that even animals without a central nervous system, such as sea anemones, show *some* degree of flexibility: they have reflexes that can be inhibited by another stimulus, such as the presence of a conspecific (Haag and Dyson, 2014). Accordingly, criteria 5 and 6 emphasize specific *types* of flexibility that are likely to implicate centralized, integrative processing of information. Criterion 5 highlights the valuing and disvaluing of threat and reward in a common currency. As will become clear later, we are looking here for a level of sophistication that cannot be explained as the inhibition of a reflex by another stimulus. Criterion 6 emphasizes self-protective behaviour that is location specific, and likely to be guided by an internal representation of where on the body an aversive stimulus is located. Here, we are looking for a level of sophistication that goes beyond a reflex response to injury.

The second point concerns associative learning (criterion 7). Simple forms of associative learning appear to occur unconsciously in humans (Greenwald and De Houwer, 2017), and this has led to ongoing debate and inquiry as to which kinds of associative learning are linked most strongly to sentience and why (Birch et al., 2020). Instrumental learning (Skora et al., 2021), reversal learning (Travers et al., 2017), learning "incongruent" spatial relationships (Ben-Haim et al., 2021), and learning across temporal gaps between stimuli ("trace conditioning"; Clark et al., 2002) are more complex and more strongly linked to sentience than classical conditioning involving two stimuli presented at the same time. However, given the ongoing debate on this issue, we will regard all evidence of associative learning as relevant to the overall evidential picture. We stress, however, that it is only one part of that picture.

Our criteria are not unreasonably demanding (they are not demands for absolute certainty). This can be seen by noting that well-researched mammals, such as lab rats (**Rattus norvegicus**), would satisfy all of them (Navratilova et al., 2013). At the same time, the criteria are also rigorous and robust. This can be seen by noting that cnidarians (jellyfish and sea anemones) would not convincingly satisfy any of the criteria on the basis of current evidence of which we are aware. We have found two reports of associative learning in sea anemones (Ross, 1961; Hodgson, 1981), and one detailed study (Haralson et al., 1975), but nothing that could allow more than medium confidence. There is some behavioural flexibility in sea anemones (Haag & Dyson, 2014) but not of a type that satisfies criterion 5. Because our criteria are rigorous and robust, without being unreasonably demanding, we believe they provide a framework for evaluating evidence of sentience that can command widespread support.

1.7 Our grading scheme

How can we move from our eight criteria to a judgement about the overall strength of the evidence? We have to be pragmatic. It would not be reasonable to demand unequivocal satisfaction of all eight criteria before we are willing to attribute sentience to an animal. It is clear that, if we are highly confident that a substantial number of these criteria are satisfied by an animal, then the possibility that the animal is sentient should be taken seriously and risks to its welfare should be considered. What is needed here is a simple, practical grading scheme that relates the number of criteria satisfied to the strength of evidence for sentience.

A grading scheme can only ever provide *approximate* guidance, and evaluations must be sensitive to the particular details of particular cases. For example, extra caution may be warranted if many indicators are uncertain rather than shown to be absent. Extra caution may also be warranted if the animal goes *beyond* what is minimally necessary to display the indicator (e.g. by satisfying criterion 4 or criterion 8 in more than one way). Moreover, the criteria are not exactly equal in their significance. Criterion 8 provides particularly compelling evidence in its own right, whereas criterion 1 (by contrast) could only ever form a small part of a wider case for sentience, due to the difference between sentience and nociception highlighted in Section 1.1. Nonetheless, we think a grading scheme still provides a helpful framework for organising our thinking about sentience.

For each criterion, we will use confidence levels to communicate the strength of the evidence that the animals under discussion satisfy or fail the criterion. The possible confidence levels are **very high confidence**, **high confidence**, **medium confidence**, **low confidence**, **very low confidence** and **no confidence**. Confidence levels take into account both the amount of evidence for a claim and the reliability and quality of the scientific work.

We will use the category of “very high confidence” only when we judge that the weight of scientific evidence leaves no room for reasonable doubt. Sometimes, for specific criteria, this very high standard of evidence can be met. We will use the category of “high confidence” in cases where we are convinced, after carefully considering all the evidence, that the animals satisfy/fail the criterion, even though some room for reasonable doubt remains. We will use the category of “medium confidence” in cases where we have some concerns about the reliability of the evidence that prevent us from having high confidence. We will use “low confidence” for cases where there is little evidence that an animal satisfies or fails the criterion, and “very low” or “no confidence” when the evidence is either seriously inadequate or non-existent.

To be clear, when we say we have “low confidence” that a criterion is satisfied, this does not mean that we think sentience is unlikely or disproven. What it means is that the evidence one way or the other is thin, low-quality, or both.

With this in mind, we propose the following *approximate* grading scheme:

> **High or very high confidence that 7-8 criteria are satisfied: Very strong evidence of sentience.**
>
> Welfare protection clearly merited. No urgent need for further research into sentience in this taxon.
>
> **High or very high confidence that 5-6 criteria are satisfied: Strong evidence of sentience.**
>
> If remaining indicators are uncertain rather than shown absent, further research into the question of sentience is advisable. However, these animals should be regarded as sentient in the context of animal welfare legislation.
>
> **High or very high confidence that 3-4 criteria are satisfied: Substantial evidence of sentience.**
>
> If remaining indicators are uncertain rather than shown absent, further research is strongly recommended to provide more insight. Despite the scientific uncertainty regarding these animals, it may still be reasonable to include them within the scope of animal welfare legislation, e.g. if they are closely related to animals that have been more extensively studied and for which the evidence is stronger.
>
> **High or very high confidence that 2 criteria are satisfied: Some evidence of sentience.**
>
> Sentience should not be ruled out. If remaining indicators are uncertain rather than shown absent, further research may provide insight into the question.
>
> **High or very high confidence that 0-1 criteria are satisfied: Sentience unknown or unlikely.**
>
> If remaining indicators are uncertain rather than shown absent, the right conclusion is that sentience is simply unknown. However, if the other indicators are shown to be absent by high-quality scientific work, we can conclude that sentience is unlikely.

This scheme is not intended to give the final word on the strength of evidence. It is a rule of thumb. In applying it, one has to be sensitive to the overall evidential picture, taken as a whole, and to the differences between the criteria. We think it is ultimately more helpful to have an approximate grading scheme than to attempt a scoring scheme in which each criterion is given a numerical weight, since these weights would have an element of arbitrariness.

When using this grading scheme, it is crucial to not to demand a separate assessment of the evidence for every individual species. For example, very few of the roughly 15,000 species of decapod have been studied scientifically in relation to any of these indicators of sentience. However, the same can be said of vertebrates. We need to be willing to consider evidence from multiple decapod species in order to reach a general judgement about infraorders of the decapods, rather than insisting on separate species-by-species evaluations. If we were to grade all 15,000 species separately, most species would end up in the “sentience unknown or unlikely” category due to never having been studied, but this would be a misapplication of our framework. This species-by-species approach has never been taken with vertebrates. Many mammalian species have never been studied in relation to sentience (a great deal of the evidence for mammals comes from the lab rat, **R. norvegicus**), but it would be inaccurate to declare on that basis that their sentience is unknown when there is copious relevant evidence from other mammals that can provide a basis for sound inferences.

To organise our thinking about higher taxa in the decapods, we will use the taxonomy of De Grave et al. (2009), in which the decapods are subdivided in two suborders (Dendrobrachiata, Pleocyemata) and the Pleocyemata further subdivided into ten infraorders. This way of classifying decapods is supported by molecular evidence (Wolfe et al., 2019). Scientific attention in relation to sentience has focussed on the Brachyura (true crabs), with some work on the Anomura (anomuran crabs, including hermit crabs), the Astacidea (astacid lobsters and crayfish), the Achelata (spiny lobsters) and the Caridea (caridean shrimps), with very little work on other infraorders, including the commercially farmed penaeid shrimps. The question of how to manage our uncertainty when scientific attention to different infraorders has been so uneven is one we will revisit in Section VII.

Much the same can be said of the cephalopods: there are around 750 species (with their phylogenetic relationships described in Tanner et al., 2017), but very few have been studied in relation to these indicators of sentience. Here too, we need to be willing to generalise across species. We need to consider evidence from multiple species within an order (e.g. the octopods) to be relevant to the question of whether sentience should be attributed to species of that order.

In this case, we will work with four main categories: octopods (order Octopoda), cuttlefish (order Sepiida), other coleoids (including all squid) and nautiloids. The category of “other coleoids” is a relatively broad one, including, for example, both myopsid squid (Myopsida) and the cuttlefish-like bobtail squid (Sepiolida). We will refer to more specific taxonomic categories when describing the experimental evidence itself, but we need to generalize in order to draw general conclusions, as has always been the case with vertebrates. This simply highlights another important sense in which the grading scheme provides *approximate* guidance, not an algorithm for attributing sentience.

We will now apply our criteria and our grading scheme to evaluate the evidence of sentience in cephalopods and decapods.

PART II. EVALUATING THE EVIDENCE OF SENTIENCE: CEPHALOPODS

SUMMARY OF PART II

- There is very strong evidence of sentience in octopods. We have either high or very high confidence that octopods satisfy criteria 1, 2, 3, 4, 6, 7 and 8, and medium confidence that they satisfy criterion 5.
- There is somewhat less evidence concerning other coleoid cephalopods (squid, cuttlefish). However, the evidence is still substantial. We have high confidence that other coleoid cephalopods satisfy criteria 1, 2, 3, and 7.
- There is little evidence, one way or the other, concerning nautiloids, although we have high confidence that they satisfy criterion 1 and medium confidence that they satisfy criterion 7.
- In cases where we are not able to have high or very high confidence that a criterion is satisfied, this is invariably because of a lack of positive evidence, rather than because of clear evidence that the animals fail the criterion.

In this section, we review all evidence from cephalopods that bears on our eight criteria for sentience. Relevant past reviews on this topic since 2000 include AHAW (2005), Andrews et al. (2013), Sneddon et al. (2014), Broom (2014), della Rocca et al. (2015), Sneddon (2015) and Fiorito et al. (2015). Although these are all high-quality reviews, new evidence has come to light since they were written, and they do not apply the framework we have set out in Part I. Rather than relying on past reviews, we have revisited all of the original evidence in order to produce a fresh review. Our conclusions, summarised above, are also summarised in **Table 2**.

Table 2. A summary of the evidence of sentience in cephalopods. *The colours and letters represent our confidence level that the criterion in question (column) is satisfied by the order (or orders) of animals in question (row). VH (dark green) indicates very high confidence, H (light green) indicates high confidence, M (dark yellow) indicates medium confidence, L (light yellow) represents low confidence, and VL (light grey) represents very low confidence. We have not had reason to use the category of no confidence. For descriptions of the criteria, see the main text. Importantly, low/very low confidence implies only that the scientific evidence one way or the other is weak, not that the animal fails or is likely to fail the criterion.*

	Criterion 1	Criterion 2	Criterion 3	Criterion 4	Criterion 5	Criterion 6	Criterion 7	Criterion 8
Octopods (Octopoda)	VH	VH	H	H	M	VH	VH	H
Cuttlefish (Sepiida)	H	VH	H	L	M	M	VH	L
Other coleoids (squid, all orders)	H	VH	H	L	M	L	H	L
Nautiloids	H	L	L	L	L	L	M	VL

2.1 Criterion 1: The animal possesses receptors sensitive to noxious stimuli (nociceptors)

CONFIDENCE LEVEL

We have very high confidence that octopods (order Octopoda), myopsid squid (Myopsida) and bobtail squid (Sepiolida) satisfy criterion 1. We have high confidence, based on evolutionary considerations and evidence from other molluscs with much simpler nervous systems, that other cephalopods, including other squid, cuttlefish (order Sepiida) and nautiloids (Nautilida) also satisfy criterion 1.

SUMMARY OF EVIDENCE

There is high quality evidence that squid and octopods possess afferent sensory neurons that respond differentially to noxious stimuli, and which undergo sensitisation and show spontaneous activation following exposure to noxious stimuli. Octopods also possess molecular markers of nociceptors in their arms. This evidence currently relies heavily on octopus studies (particularly O. vulgaris), with a few newer studies on squid.

Full review of evidence: As noted in Section 1, a **nociceptor** is "a high-threshold sensory receptor of the peripheral somatosensory nervous system that is capable of transducing and encoding noxious stimuli" (International Association for the Study of Pain, 2017). Unlike other sensory receptors, nociceptors have relatively high thresholds before they fire, meaning that they are only activated by extreme stimuli, such as those that are intense, prolonged, or repeated, thus representing an actual or potential threat of tissue damage. Some nociceptors cannot be activated by any stimuli, unless they are sensitised by inflammatory molecules, which are released when tissue is damaged (Smith & Lewin, 2009). There are different types of nociceptors. Some respond to extreme mechanical, heat, cold, chemical, or light stimulation, whilst others are polymodal, meaning that they respond to two or more classes of stimuli (Sneddon et al., 2014; Walters, 2018). Nociceptors can also vary in how quickly they respond to stimuli, with some responding only when stimulation is prolonged. Several other earlier reviews have concluded that the presence of nociceptors in cephalopods is "likely, but not proven" (Andrews et al., 2013; della Rocca et al., 2015; Fiorito et al., 2015), but these appear to pre-date some of the more recent experimental work described below.

Hague et al. (2013) found that severed arms of **Octopus vulgaris** would show rapid reflex withdrawal responses to noxious stimuli (forcep pinches, fresh water and acetic acid) but not innocuous stimuli (gentle touch and seawater). These were severed arms and thus not connected to the central nervous system (**CNS**). Clearly, the presence of nociceptors in a severed arm, while not irrelevant to questions of sentience, could only ever be a small part of the picture. However, they also found that severing the axial nerve cord in the arm would eliminate the response, which suggests a connection to more central pathways.

These results complement early findings by Rowell (1963) who noted that severed arms showed immediate reflexive full withdrawal when encountering noxious stimuli, as compared to merely skin flinching and orientation of the suckers in response to lighter pricking. Altman (1971) also observed that amputated and denervated octopus arms would withdraw from food pieces treated with quinine hydrochloride. An early study on neural firing in octopus (**O. vulgaris**) arms found some neurons that fired only in response to forcefully applied mechanical stimuli such as blows or pinches (Rowell ,1966).

Several more recent studies have looked directly at neural firing in response to tissue damage or noxious stimuli, in both octopus and squid. Crook et al. (2013) demonstrated the presence of mechanosensitive nociceptors in the fin of squid (**Doryteuthis pealeii** also known as **Loligo pealeii**) that activated only in response to filaments that produced tissue damage, and which were sensitised by both these stimuli and by crush injuries to the fin, an effect that was suppressed by injection of local anaesthetic. Sensitisation was seen across the whole body, rather than just a localised response, which may suggest induction of a general cautious state rather than specific wound-tending (see criterion 6).

These tests were performed on both attached and excised fins. When the fin was attached, squid showed behavioural sensitisation (increased escape response) after crush injury. Long lasting spontaneous neuronal activity was observed for at least 24 hours following injury, but only in attached fins, suggesting necessary engagement with other parts of the body or nervous system. Measurements were taken at the fin nerve, which connects the fin nerve branches to the brain, suggesting connecting pathways from the peripheral nociceptors to the CNS.

These findings were supported by a recent study by Howard et al. (2019) on the bobtail squid **Euprymna scolopes** (order Sepiolida), which found sensitisation of peripheral nerves after crush injury; as well as lasting lifetime neural excitability in animals that received injuries in their early life.

Similar results in octopus have been demonstrated by Alupay et al. (2014) (**Abdopus aculeatus**) and Perez et al. (2017) (**Octopus bocki**). Alupay et al. applied a crush injury to the arms and observed an immediate behavioural response, as well as a decreased sensory threshold for response to subsequent stimuli on both these arms (as well as nearby arms) and in whole-body responses for the 24 hours following injury. The arms were then removed to test neural firing. They were able to identify neurons that fired only in response to noxious stimuli, as well as increased sensitisation on injured arms and those nearby (they found increased neural firing in response to the 'damaging' but not the 'light' filaments). Measurements were taken at the axial nerve cord, implying that information from arm mechanosensors was being passed through to at least this part of the CNS.

Similarly, Perez et al. (2017) again found that octopus possess neurons that show short-term sensitisation and spontaneous firing after crush injury in the mantle. Their measurements were taken at the pallial nerve, which is the primary nerve connecting the mantle to the brain. In a study of the Hawaiian bobtail squid, **Euprymna scolopes** (order Sepiolida), Bazarini & Crook (2020) found increased firing rates in the pallial nerve in response to noxious stimuli in their studies.

Recently, Crook (2021) took electrophysiological measures of the brachial connectives (which connect arm nerve cords to brain) in Bock's pygmy octopus (**O. bocki**) and showed that there was ongoing activity after application of a noxious stimulus (injected acetic acid) which was silenced by use of an anaesthetic (lidocaine). This is strong evidence that these signals are being sent from the arms to the CNS.

There is also molecular evidence of the presence of nociceptors in octopus arms. In a detailed study of **O. vulgaris**, di Cristina (2017) found a number of markers associated with detection of noxious stimuli in the arm tips. Di Cristina observed "putative nociceptive fibres" running along the axial nerve of the arm. These results suggest the presence of peripheral nociceptors and their connection to the CNS. We note, however, that these results are reported in a PhD thesis rather than a peer-reviewed journal.

The presence of nociceptors in other related species can also serve as evidence of nociception, via evolutionary/phylogenetic reasoning (Andrews et al., 2013), given that nociceptive processes appear highly conserved across a range of taxa, including many other molluscs. Crook & Walters (2011) and Walters (2018) describe evidence for nociception in a range of molluscs, primarily gastropods. For example, the gastropod mollusc *Aplysia* has nociceptors. The presence of nociceptors in other molluscs makes their presence in cephalopods more likely. Ecological considerations also speak in favour of the presence of nociceptors in cephalopods. As soft-bodied, mobile animals, cephalopods are at great risk of damage and predation, but they also have the capacity to avoid or escape, so nociception would be highly beneficial to these animals.

Finally, indirect behavioural evidence of the presence of nociceptors comes from the fact that octopus are able to learn avoidance of noxious stimuli, suggesting they can differentially detect and process these inputs (see criterion 7). For example, Ross (1971) observed that octopus (**O. vulgaris**) would learn to avoid hermit crabs with sea anemones on their shells. Contact with the stinging anemones would trigger retreat behaviour and the octopus would not eat these crabs. However, behavioural evidence will be considered later, under other headings, and here we want to focus on neurophysiological evidence.

2.2 Criterion 2: The animal possesses integrative brain regions capable of integrating information from different sensory sources

CONFIDENCE LEVEL	*We have very high confidence that coleoid cephalopods (octopods, squid, cuttlefish) satisfy criterion 2.*
SUMMARY OF EVIDENCE	*There is extremely strong evidence that coleoid cephalopods possess complex, centralised brains capable of integrating different types of information, including nociceptive. Although there is no structure identified as a direct analogue to the mammalian cerebral cortex, the vertical lobe is the brain centre responsible for learning and memory. These structures are not present in nautiloids.*

Full review of evidence: The complex structure and hierarchical organisation of the coleoid cephalopod brain is well documented (Andrews et al., 2013; Budelmann, 1995; della Rocca et al., 2015; Fiorito et al., 2015; Hochner, 2012; Hochner et al., 2006; Shigeno et al., 2018; Zarrella et al., 2015; Zullo et al., 2009; Zullo & Hochner, 2011).

Coleoid cephalopods have a brain to body ratio higher than most fish and reptiles (Packard, 1972). Early studies on **Octopus vulgaris** (Young, 1963a; Wells, 1978), squid of the **Loligo** genus (Young, 1974, 1976, 1977, 1979; Messenger, 1979) and cuttlefish of the **Sepia** genus (Sanders & Young, 1940; Boycott, 1961) provide detailed outlines of the structure of the cephalopod nervous system and central brain, on which most subsequent work rests. From this work we know that the octopus brain contains ~170 million nerve cells, of which 130 million are found in the optic lobes and 40 million in the central brain. The brain has a complex structure, made up primarily of the sub- and supra-oesophageal masses (both containing numerous lobes, around 30 in total; Nixon & Young, 2003), as well as the optic lobes. The brain shows clear hierarchical organisation and high connectivity between centres. While the sub-oesophageal mass (SUB) is primarily a lower motor control centre, the supra-oesophageal mass (SEM) contains intermediate/higher motor control centres, as well as memory/learning centres. The SEM is likely to play a role in resolving potential conflicts between input and action patterns on each side of the body. The higher motor centres connect to the lower for input and output.

Shigeno et al. (2018) draw structural and functional analogies between regions of the cephalopod brain and the vertebrate brain. The SUB is roughly equivalent to the vertebrate spinal cord, and other regions of the SEM to the hypothalamus, thalamus, basal ganglia and cerebellum. Of greatest interest is the frontal-vertical lobe as an analog to the cerebral cortex, hippocampus and amygdaloid complex. This lobe plays a role in learning and memory as well as a likely role in evaluation and decision-making (Young, 1963b, 1991).

The vertical lobe is often described as the ‘highest’ brain centre, analogous to the mammalian hippocampus (Fiorito et al., 2015; Hochner et al., 2006; Nixon & Young, 2003; Shomrat et al., 2015). It contains ~25 million of the brain’s 40 million cells (Shomrat et al., 2015) and these regions also appear to contain a distinct cell type: small cells which are hypothesised to have an inhibitory function (Young 1963a). Brown and Piscopo (2013) found that there is distinct synaptic plasticity within the vertical lobe of cephalopods, a feature associated with the learning and memory centres of vertebrates.

The vertical lobe system receives a wide variety of inputs from the entire body, including eyes, arms, mouth and mantle (Young, 1979). There is evidence for integration across senses, since **O. vulgaris** can combine peripheral arm information with visual information to guide movement in a maze task (Gutnick et al., 2001).

Most of this evidence is about the octopus, though similar findings have been seen across taxa. The primary differences are that octopus brains are more centralised, while cuttlefish and squid have larger optic lobes (Budelmann, 1995; Boycott, 1961; Packard, 1972). Squid and cuttlefish also show a reduced inferior frontal lobe system and lower tactile discrimination and learning (Young, 1991), and the vertical lobe complex is structurally different (Young, 1979). Nautiloids appear to have more simple brains which, though still quite complex structures containing multiple lobes, lack the ‘higher’ brain structures associated with learning and memory (Budelmann, 1995), although Nixon & Young (2003) suggest that the cerebral cord may function as a ‘higher’ integrative centre.

An unusual feature of cephalopod neuroanatomy is the peripheral distribution of processing. The peripheral nervous system makes up almost two-thirds of the total number of neurons, with ~300 million cells in the arm cords (Young 1963a). There is relatively low connectivity between the brain and the periphery, suggesting that a lot of processing occurs peripherally, while the central brain plays a role primarily for co-ordination of information and decisionmaking (Hochner, 2012). The arm cords appear to act as reflex centres for the individual arms, in some sense elaborating on orders received from the brain (Wells, 1978). However, the central brain is still highly sophisticated.

2.3 Criterion 3: The animal possesses neural pathways connecting the nociceptors to the integrative brain regions

CONFIDENCE LEVEL	*We have high confidence that coleoid cephalopods (octopods, squid, cuttlefish) satisfy criterion 3. More neurophysiological evidence would be required for us to have very high confidence.*
SUMMARY OF EVIDENCE	*There is indirect evidence regarding connections between the nociceptors and integrative brain regions in cephalopods. There is high connectivity between the peripheral nervous system and the central brain, as well as between the different lobes of the brain, and these pathways could relay nociceptive signals to integrative brain regions, but this has not yet been demonstrated beyond all doubt.*

Full review of evidence: In multiple studies already reviewed under criterion 1, electrophysiological measurements were taken at the nerve cords linking peripheral nerves to the central brain and found to show increased activity in response to noxious stimuli (Crook et al., 2013; Alupay et al., 2014, Perez et al., 2017; Bazarini & Crook, 2020; Crook, 2021). This shows compellingly that signals from nociceptors are reaching the brain, but it does not show that they are reaching the vertical lobe system. Past research has documented many connections between the peripheral nervous system and the vertical lobe, but it has tended to assume (rather than explicitly demonstrating) that these connections are involved in transmitting nociceptive information.

When discussing the functions of the lobes of the brain, Young (1963a) refers to an input to the brain which is “presumed to be of nocifensor (pain) fibres”, but this is hypothesised based on functional rather than structural considerations. Young (1979) describes several afferent pathways to the vertical lobe system as possibly conveying nociceptive signals, and Nixon & Young (2003) similarly assume that the vertical lobe system processes pain signals from the body. Young (1991) describes the connectivity of the nervous system, including connections of afferent fibres from the arms to the lateral inferior frontal lobe, which then progress through to the superior frontal and vertical lobe system. Although this is not directly related to nociceptors, he takes it as presumed that pain/trauma signals are part of this pathway. Budelmann & Young (1985) found that afferent fibres from the arms pass through to the frontal and subvertical lobes (though not the vertical lobe; information is taken to be passed to there from these lobes) and speculate that they could be related to nociception. There is high connectivity between regions of the brain, particularly between the ‘lower’ control regions of the sub-oesophageal mass and the ‘higher’ supra-oesophageal mass (e.g. Shigeno et al., 2018), but this is not direct evidence of the transfer of nociceptive signals.

The picture is further complicated by the distributed nature of the cephalopod nervous system. Many of the peripheral afferent nerves (particularly in the arms) do not connect directly to the central nervous system (CNS), but instead to central ganglia within the arms, which then pass on reduced information to the brain (di Cristina, 2017). There are around 140,000 afferent neurons connecting the arms to the central brain (Hochner, 2012; Levy & Hochner, 2017), and many of these input into the frontal lobe system (Nixon & Young, 2003). However, what type of information is lost in this ‘compiling’ and what is transmitted is still unknown.

One potential source of information is from studies on anaesthesia (see also criterion 4). Local and general anaesthetics are shown to shut down both afferent and efferent neural signals to/from the brain (Butler-Struben et al., 2018). Given that the stimuli used to test this were forcep pinches that could be considered noxious, this is suggestive of cessation of nociceptive transmission. The lack of response to other surgical procedures while under anaesthetic is also suggestive, though care must be taken to separate immobility effects from true anaesthesia and loss of sensation.

There is also behavioural evidence that suggests information about noxious stimuli must be processed within central brain regions. For example, as a result of sophisticated behavioural responses to noxious stimuli in their tests, Alupay et al. (2014) infer that perception of noxious stimuli in the arms and mantle was conveyed to "higher processing centres". However, this evidence is discussed under other headings, and (as in Section 2.1) we want to focus on neurophysiological evidence in this section.

Past reviews of the evidence for the connections between nociceptors and the vertical lobe conclude it is "uncertain" (Andrews et al., 2013) or "likely, but not proven" (Fiorito et al., 2015; Zarrella et al., 2015). We agree with these assessments. In our framework, we have high confidence that there are such connections, but not very high confidence.

2.4 Criterion 4: The animal's behavioural response to a noxious stimulus is modulated by chemical compounds affecting the nervous system in either or both of the following ways: (a) The animal possesses an endogenous neurotransmitter system that modulates (in a way consistent with the experience of pain, distress or harm) its responses to threatened or actual noxious stimuli; or (b) putative local anaesthetics, analgesics (such as opioids), anxiolytics or anti-depressants modify an animal's responses to threatened or actual noxious stimuli in a way consistent with the hypothesis that these compounds attenuate the experience of pain, distress or harm

CONFIDENCE LEVEL	*We have high confidence that octopods satisfy criterion 4. There is not enough evidence at present for us to have medium or high confidence that other cephalopods satisfy criterion 4.*
SUMMARY OF EVIDENCE	*A notable 2021 study provides evidence of the modification of responses to noxious stimuli by a local anaesthetic (lidocaine) in octopods. At present, there is some evidence that magnesium chloride can also act as a local anaesthetic in octopods. There is also evidence for the presence of relevant endogenous neurotransmitters and receptors (including enkephalins, oestrogen and serotonin) in cephalopods, but these have not been directly linked to activity in nociceptive pathways. Further studies, particularly on the effects of analgesics and similar drugs, are important to provide this information.*

Full review of evidence: Regarding the presence of an endogenous neurotransmitter system, as well as response to analgesia, past reviews have concluded that the presence of such a system is likely, but that there is insufficient data available (Andrews et al., 2013; Fiorito et al., 2015; Zarrella et al., 2015). Although there are a large number of identified neurotransmitters in cephalopod brains (reviewed in Messenger, 1996), none has yet been identified as playing a role in responses to noxious stimuli.

There is some evidence for the presence of opioids and similar compounds (enkephalin-like peptides), in the brains and bodies of octopus as well as leucine-enkephalin and delta opioid receptors in the peripheral nervous system (Sha et al., 2012). Martin et al. (1979) used antibodies to identify Met-enkephalin-like proteins in the octopus vena cava, but the action of these was not affected by the application of the opioid antagonist naloxone (Voight et al., 1981). Stefano et al. (1981) found that opioids (morphine and met-enkephalin) suppressed dopamine release in brain tissue of octopus (**Octopus bimaculatus**). The effect was reversed with naloxone, implying mediation by opioid receptors. However, Frazier et al. (1973) found that opioids and antagonists both played the same inhibitory role on the squid axon (Loligo pealei), which suggested that the opioids were not acting as analgesics.

In a PhD thesis, Di Cristina (2017) found the presence of transcripts designated as opioid receptors and opioid-like peptides in the sub-oesophageal mass and optic lobe in the brain of Octopus vulgaris, suggesting the possibility of a pain-modulating system. However, as these molecules can play multiple roles apart from modulating responses to noxious stimuli, further work is needed on the effects of these compounds, including the effects of opioid-antagonists such as naloxone.

Through phylogenetic reasoning, the fact that the presence of opioid receptors is widespread and highly conserved through many vertebrate and invertebrate taxa is reason to think it is present in cephalopods (Andrews et al., 2013, though cf. Crook & Walters, 2011). However, even if this were the case, we would still need further evidence to support the claim that the system modulates nociceptive pathways.

Although the focus is typically on opioids, other compounds such as cannabinoids or steroids may function as endogenous modulators for nociceptive processing (Andrews et al., 2013). From studies on other molluscs, although enkephalins were not promising, FMRFamide may instead be a good candidate for nociceptive signalling (Crook & Walters, 2011). Loi & Tublitz (1997) identified FRMFamide-like proteins in the brains of cuttlefish (**Sepia officinalis**), but only in the role of chromatophore regulation. Wollensen et al. (2008) found FMRFamide-like immunoreactivity throughout the brain of pygmy squid (**Idiosepius notoides**). Di Cristina (2017) found transcripts of genes for FMRFamide receptors in brain and body tissues of **O. vulgaris**.

Endogenous oestrogens modulate nociceptive processing in mammals, and there is some evidence for a similar phenomenon in cephalopods. Bazarini & Crook (2020) examined the role of oestrogens in processing and responding to noxious stimuli in Hawaiian bobtail squid (**E. scolopes**). They found that environmental oestrogen exposure altered behavioural responses to noxious (fin crush) and potentially threatening (vibration) stimuli by lowering responsiveness to the former and creating hypersensitivity to the latter. Oestrogen exposure also impaired sensitisation of neural firing in response to injury. These results suggest that oestrogens play a role in modulation of nociceptive responses in this species. However, we do not see this result alone as enough to conclude that squid satisfy criterion 4.

Serotonin plays a role in mechanism of nociceptive sensitisation following noxious stimulus in molluscs, and modulation of nociceptive signals in vertebrates (Perez et al., 2017). Octopus (**Octopus bimaculoides**) possess serotonin transporter binding sites that are orthologs to those found in humans (Edsinger & Dölen, 2018). Perez et al. (2017) tested the effect of fluoxetine (a serotonin reuptake inhibitor that increases the concentration of serotonin) on neural nociceptive responses in Bock’s pygmy octopus (**O. bocki**). They found that fluoxetine treatment increased rates of spontaneous firing after injury, though there was no effect on neural sensitisation. They suggest that elevated serotonin levels may enhance neural and behavioural responses to tissue injury and that spontaneous firing may play a role in injury guarding and escape behaviours. However, as these tests were done on prepared tissue samples from euthanised animals, they only show change in afferent firing, not changes in the brain. We cannot take this as evidence that fluoxetine attenuates an experience of pain, distress, or harm in a live animal.

Serotonin also appears to play a role in modulating learning in octopus, as it is active in the vertical lobe (Shomrat et al., 2010). It may do so through modulating signals for reward/punishment (Shomrat et al., 2015), which could signal involvement in nociceptive pathways and decision-making, but we cannot yet be confident of this.

Zarrella et al. (2015) describe a range of genes that show increased or decreased expression in response to fear conditioning (e.g. genes for stathmin, tyrosine hydroxylase, dopamine transporter, octopressin, cephalotocin). In particular, they suggest that an increase in stathmin under innate and learned fear responses demonstrates that it plays a similar role to that played in the vertebrate amygdala in formation of fear memory and expression of fear responses.

One recent study (Butler-Struben et al., 2018) investigated local and general anaesthesia in cephalopods. Of particular relevance to our criterion 4 was the result that lidocaine and magnesium chloride were effective local anaesthetics, suppressing activity in the peripheral nervous system as measured by electrodes. However, this study did not link the local anaesthetic to behavioural responses to injury.

Very recent evidence (Crook, 2021), discussed in greater detail under criterion 8 (Section 2.8), provides this missing piece of the puzzle, showing that lidocaine abolishes injury-directed grooming behaviour directed at the site of a noxious stimulus in Bock's pygmy octopus (**O. bocki**). We regard this as a convincing demonstration of the effectiveness of lidocaine in modulating responses to noxious stimuli in octopods, satisfying criterion 4b

We have found no work exploring the effects of analgesics, anxiolytics or anti-depressants in cephalopods. Regarding other compounds, Edsinger & Dölen (2018) found that octopus (**Octopus bimaculoides**) respond to MDMA with increased social behaviour; but no work was done on decision-making effects or changes in response to noxious stimuli.

2.5 Criterion 5: The animal shows motivational trade-offs, in which the disvalue of a noxious or threatening stimulus is weighed (traded-off) against the value of an opportunity for reward, leading to flexible decision-making. Enough flexibility must be shown to indicate centralized, integrative processing of information involving an evaluative common currency.

CONFIDENCE LEVEL	*There is not enough evidence for us to have high confidence that any cephalopod mollusc satisfies criterion 5. However, indirect evidence from coleoid cephalopods is suggestive of motivational trade-offs, allowing medium confidence.*
SUMMARY OF EVIDENCE	*We have found no study that directly tests for motivational trade-offs in cephalopods. There are various studies showing that injury produces sustained behavioural change. The results are compatible with the hypothesis that cephalopods are aware of their injuries and change their priorities when injured, but they are also compatible with the hypothesis that injury directly produces increased sensitivity to threat.*

Full review of evidence: What we are looking for here is robust evidence that an animal is motivated to avoid a noxious stimulus, and that this motivation is *weighed* (*traded off*) against other motivations in a flexible decision-making system.

A study by Wilson et al. (2018) on the common cuttlefish **S. officinalis** showed that, when cuttlefish are exposed to infrasonic pulses which mimic the central hydrodynamic signatures of predatory attacks, they abandon an opportunity to hunt and instead exhibit defensive behaviour. Juvenile cuttlefish (*n* = 9, i.e. 9 individual animals) were presented with a simulated predatory attack by way of graded infrasonic particle acceleration (3, 5, and 9Hz) at the same time as they were shown a short video sequence of live decapod prey. Behavioural responses were tested in light *versus* dark conditions and after 24 hours of food deprivation. The results showed that cuttlefish attempted to hunt the moving prey in the video sequence, but they shifted their attention to defensive behaviours as the threatening stimulus became more threatening. At the lowest acceleration intensity, the cuttlefish changed their body patterning. At the higher acceleration intensity, simulating a larger or nearby predator, the cuttlefish blanched their skin, exhibited jet-escape behaviour and sometimes combined this with releasing ink.

The study showed an effect of hunger on the responses: when cuttlefish were food deprived, their escape thresholds were significantly higher at 3 Hz but not at 9 Hz. One possible explanation for this hunger-dependence is a motivational trade-off, in which the value of the food opportunity to the animal (which is greater when it is hungry) is weighed against the disvalue of exposure to threat. However, an alternative explanation is that hunger simply inhibits threat detection, a simple phenomenon also found in the nematode worm **Caenorhabditis elegans** (Ghosh et al., 2016). To provide evidence against the alternative explanation, more data would be needed. Ideally, an experiment would hold fixed the hunger level, the threat level and the signal strength, and investigate whether an opportunity for a higher quality reward (e.g. a more desirable food item) increases tolerance of threat.

In a different study, Bedore et al. (2015) studied defensive responses in cuttlefish (**S. officinalis**). Cuttlefish are well known for their predator avoidance behaviour, particularly their dynamic camouflage abilities, which involve rapid changes in colour, pattern and texture (Hanlon & Messenger, 2018). Camouflage patterns can be combined with a freeze response, with mantle compression (by at least 5%), ventilation rate reduction, and the covering of siphons, funnel or mantle cavity to decrease bioelectric cues (Bedore et al., 2015). In this study, cuttlefish were placed in a tank and presented with an approaching predator on an iPad screen. Cuttlefish (*n* = 11; the electric potential was recorded for *n* = 7) were presented with 7 videos in randomised order (control *versus* silhouette of looming predator). Cuttlefish exhibited freeze responses to approaching fish stimuli in 80% of the trials.

This study does not directly test for motivational trade-offs. The results suggest that the need to minimise detection by an approaching predator is prioritised over normal respiration behaviour, but they do not show a trade-off against opportunity for reward. It is conceivable that the animal is deciding to tolerate one aversive experience (oxygen deprivation) in order to prevent a worse one (predation), but we cannot be sure that the freeze response actually leads to oxygen deprivation. Octopuses can survive out of water for short periods with their siphon and mantle cavity occluded, ‘breathing’ from the water trapped in their mantle, so it is possible that cuttlefish might also be storing water in their mantle cavity during the freeze response.

In a study by Ross (1971), octopods (**O. vulgaris**, *n* = 12) were presented with hermit crabs, a common prey item. There were two types of hermit crabs, crabs with a clean shell and crabs with an anemone on their shell. Ross (1971) found that the octopuses attacked all hermit crabs, ingesting those with a clean shell (no anemone) but retreating within seconds from the hermit crabs armed with anemones. Most octopuses repeated the attack several times over a period of a few hours but eventually the attacks ceased, and the octopuses only approached cautiously. When an octopus arm came into contact with the anemone, it would pull it back sharply. After 24 h, no interactions were observed between the octopus and the hermit crabs with anemones, and the octopus would move to the top of the tank when the hermit crab approached.

The results from this study suggest that **O. vulgaris** is sensitive to anemone stings, will abandon hunting opportunities that repeatedly lead to stings, and will move away from hermit crabs that bear stinging anemones. However, the study does not test whether this behaviour involves a motivational trade-off. To do this, it would be necessary to vary the quality of the opportunity for reward and investigate whether octopods will incur higher risks of stinging to access higher quality rewards.

Another study on cuttlefish shows a similar pattern, whereby cuttlefish avoid the claws of their prey (crabs) after being pinched and learn to attack the crab from behind, in an apparent display of trial-and-error learning (Boal et al., 2000). Several other studies in octopuses also demonstrate that they cease to interact with other objects in their tank when presented with noxious stimuli (e.g. electric shock) (Boycott & Young, 1957; Mackintosh, 1964; Fiorito & Scotto, 1992; Wells, 1978). These electric shocks were clearly aversive: one study showed that octopuses learn rapidly when electric shocks are used as negative reinforcement (Sutherland et al., 1963). However, like the Ross (1971) study, these studies do not directly test for motivational trade-offs in decision-making. It is possible that the suppression of interaction with desirable items (i.e. balls or prey) is due to the physiological effect of the aversive stimulus itself, rather than by a centralised evaluation system.

Crook et al. (2011) investigated how injury affects the behaviour of squid **D. pealeii** (*n* = 18; 8 injured; 10 sham treated). Shortly after injury, squid use crypsis, a defensive behaviour commonly observed in cephalopods to avoid detection, rather than escape jetting behaviour in response to a visual threat. However, between 1-48 hours after injury, squid escape earlier and continue escape behaviours for longer. The results from this study suggest a strong effect of injury on visual responsiveness. Significant differences in response to touch between injured and sham-treated squid indicate that tactile sensitisation also occurs. Strikingly, arm injury caused little or no interference with effective hunting behaviour several hours after injury. One possible explanation for this pattern is that injured squid are aware of their injuries and attach greater value to the need to escape, relative to their other needs. But an alternative explanation is that visual and tactile receptors are sensitised, and we are not regarding sensitisation as evidence of sentience in this report.

A different study by Crook et al. (2014) provides further evidence that, following injury, squid (*n* = 72), **D. pealeii**, increase responsiveness to threats. In this study the arms of squid were injured (*n* = 20, injured without anaesthetic; *n* = 16 injured without anaesthetic; *n* = 20 uninjured; *n* = 16 uninjured treated with anaesthetic) and behaviours were recorded for 6 hours after injury. The study found that minor injury produced no effects on spontaneous swimming or other detectable behaviours (to the human observer). However, black seabass (predatory fish) selectively targeted injured squid. Squid in the injured group (without anaesthetic) had longer alert distances and alert behaviours at earlier stages of predation encounters than squid from the other groups. This suggests that injured squid had earlier initiation of defensive responses. Injured squid also had longer flight initiation distances compared with squid in the other treatment groups. Here too, the evidence does not distinguish between an explanation based on centralised decision-making and an explanation based on sensitisation of receptors.

Another study on squid demonstrates that minor injury affects schooling decisions (Oshima et al., 2016). In this study, adult squid (*n* = 29), **D. pealeii**, received three closely spaced crushes with serrated forceps to the fin (either left or right). Control squid (*n* = 13) were handled in the same manner but received no injuries. Following treatment, schooling behaviour of groups of squid was recorded for 24 h. Results show that injured squid were more likely to school shortly after injury (0.5–2h), but no differences were found compared with sham-treated squid at long time points (6–24h). The position of injured squid within the school was flexible and differed depending on whether the threatening stimulus was visual or olfactory. When an olfactory predator cue was presented, the injured individuals were more likely to school on the outside of the group, to potentially engage in predator inspection behaviour. By contrast, when a visual predator was presented (fish model), injured individuals were more likely to school in the centre of the group, suggesting that once the predator is approaching, injured squid are highly motivated to reduce risk by positioning themselves in the centre of the group.

The study demonstrates that squid with fin injuries make schooling decisions that differ from uninjured squid. One possible explanation is that the injured squid are aware of their injuries and attach greater value to the protection afforded by being at the centre of the group. However, an alternative explanation based on increased sensitivity to threat, rather than centralised decision-making, is not ruled out.

Finally, another study on a different squid species, the Hawaiian bobtail squid (*n* = 68), **E. scolopes**, shows that injury in early life produces permanent changes to defensive behaviour and short-term memory (Howard et al., 2019). Although this study does not directly test for motivational trade-offs, it demonstrates that injury can result in long-term effects. Squid that were injured in early life were more cautious in the presence of predators but were unable to learn to inhibit behaviour when a prey item was present.

2.6 Criterion 6: The animal shows flexible self-protective behaviour (e.g. wound-tending, guarding, grooming, rubbing) of a type likely to involve representing the bodily location of an injury or noxious stimulus

CONFIDENCE LEVEL	*We have very high confidence that octopods satisfy criterion 6. We have medium confidence that cuttlefish satisfy criterion 6.*
SUMMARY OF EVIDENCE	*The strongest evidence of wound-grooming and guarding is shown in octopods, where injured individuals have been shown to curl their adjacent arms around the injured site or attempt to scrape away a noxious stimulus. There is evidence based on personal observation of wound-tending in cuttlefish, allowing medium confidence, but there is a lack of peer-reviewed evidence. In squid, there is evidence of widespread nociceptive sensitisation following injury, but no evidence of protective behaviour directed specifically at the site of a wound.*

Full review of evidence: What we are looking for here is robust evidence of self-protective behaviours that go beyond reflexes: to meet this criterion, the animal should be able to vary its response in a targeted way, according to where on the body the noxious stimulus is administered.

Alupay et al. (2014) provides strong evidence to support criterion 6 in octopods, demonstrating that algae octopus, **Abdopus aculeatus**, ($n = 9$) exhibit flexible self-protective behaviours to an injured site. Injured octopuses received a crush to one arm with serrated forceps ($n = 5$) and sham-treated octopuses ($n = 4$) received a light arm touch. Behaviours were recorded prior to injury or sham treatment, and at 10 min, 6 h and 24 h after treatment. Four out of 5 injured octopuses induced autotomy (i.e. voluntary amputation) of the injured arm. All injured octopuses inked and jetted at the onset of stimulation and showed immediate wound-grooming behaviour. Specifically, injured subjects held the arm stump or wound site in their beak for at least 10 mins. At 6 h, octopuses did not exhibit ongoing grooming, and mechanical stimulation did not re-induce it. Rather, octopuses contracted the injured area keeping it close to the body.

A subset of injured subjects ($n = 3$) used adjacent arms to guard their injury, wrapping their uninjured arms around the injured site. After 24 h the injured site was no longer contracted but light touch was enough to induce contraction that persisted throughout the behaviour test. Control subjects did not exhibit grooming or guarding behaviour. Arm injury also resulted in long-term sensitising effects in the injured and surrounding uninjured arms. After 24 hours, mechanical stimulation caused higher rates of spontaneous activity from intact arms in injured animals than sham-treated subjects. It is the directed wound attention that is particularly compelling evidence in relation to our criterion 6.

A separate study on a different species of octopus, the lesser octopus ($n = 12$), **Eledone cirrhosa**, also reports protective responses to injury (Polglase et al., 1983). In this study, all animals were anesthetised prior to wounding, two puncture wounds were then inflicted between the mantle apex and the siphon. The authors report that once the anaesthesia wore off the injured octopuses attended to the wound sites by stroking the tip of an arm across the injury. Note that this study does not report whether a subset of the subjects acted as control individuals that were sham treated. Nevertheless, similar wound-tending behaviour has been observed in octopuses following surgery to the optic capsule or cranium, although this observation is anecdotal (I. Gleadall, personal observation cited in Andrews et al., 2013). G. Fiorito also reports that octopus guard the mantle or cranium post-surgery (unpublished data and cited in Fiorito et al., 2015).

In a study discussed primarily under criterion 8, Crook (2021) found that octopods (**O. bocki**) injected with dilute acetic acid would groom the site with their beak, including stripping away some of the skin. As the grooming but not the skin-stripping behaviour is seen in response to other types of injury (arm crush, skin pinch, skin slice), Crook hypothesises that this could be a response that would work for noxious stings (to release the poison). If correct, this suggests that the octopus can represent the type of pain (mechanical or chemical) as well as its location.

Several studies on different species of octopod have shown that they withdraw, in a way that seems self-protective, from hermit crabs that bear stinging anemones on their shell (Polimanti, 1910; Boycott, 1954; Brooks, 1988; Ross, 1971; Hand, 1975; McClean, 1983; Brooks, 1988). This is not, by itself, compelling evidence in relation to criterion 6, because it is difficult to be sure whether such behaviours involve centralised representation of the bodily location of an injury or noxious stimulus. Common octopuses (**O. vulgaris**) are capable of reflex withdrawal in response to a noxious stimulus without reference to the brain (Hague et al., 2013).

There is limited peer-reviewed evidence of self-protective behaviour in response to noxious stimuli in cuttlefish. One study on learning in common cuttlefish demonstrates that they avoid the claws of crab prey after being pinched and learn to attack the crab from behind (Boal et al., 2000). Anecdotal evidence also suggests that cuttlefish can discriminate between different species of crabs and avoid attacking or hunting more aggressive crab species after being pinched (A.S. Darmaillacq, personal observation communicated in Andrews et al. 2013). Moreover, following surgery to the optic capsule, the cranium, the skin or the arms, common cuttlefish will exhibit directed wound attention and grooming, brushing their arms across the surgery site for several days to weeks (A.K. Schnell and C. Jozet-Alves, personal observation communicated to A.K. Schnell). Quantitative data on these observations were not recorded, but they can be regarded as credible anecdotal observations from cephalopod biologists with expertise in neuroethology.

Bazarini and Crook (2020) provide evidence of defensive behaviours in Hawaiian bobtail squid (*n* = 155), **E. scolopes**, following arm injury in response to noxious stimuli. Injured squid received a strong pinch to their left fin with grooved forceps. Injuries produced visible bruising of the tissue and some tearing along the crush margin. Control squid received the same procedure, but the forceps only lightly touched their fin. Following injury and sham treatments, the subjects were exposed to tactile and vibratory sensory tests at acute 6 h and chronic 14 days post-injury. Squid responded to tactile and vibratory sensory tests through defensive arm posture, which was sometimes accompanied by escape jetting or inking. This study shows that squid respond to noxious stimuli with defensive behaviours. Although wound grooming or guarding is not reported, it should be noted that the left fin would be difficult to reach with the squid's arms.

Another study by Crook et al. (2011) show that squid, **D. pealeii**, respond to minor arm injury with long-lasting enhancement of defensive responses to visual and tactile stimuli. In this study, squid (*n* =8) received an arm injury whereby one of the arms was removed using surgical scissors. Control squid (*n* = 10) were captured in the same way but rather than removing their arm, the arm was pressed with forceps for 1 s. Animals were tested 30 min prior to tissue injury and then following tissue injury at 10 min, 1 h, 6 h, 24 h and 48 h.

To investigate both visual and tactile responses subjects were divided into groups that had visual or no visual access (i.e. some subjects were blindfolded). All animals responded to the arm injury with escape jetting and ink release. Blindfolded injured subjects travelled slightly farther after injury than blindfolded, sham-treated squid. Time taken to settle and resume crypsis was significantly shorter among injured squid in the two sighted groups. Squid never displayed wound-directed attention (i.e. grooming or guarding). This absence is unlikely to be a result of the inability to reach or manipulate the injured area because the injured subjects were observed manipulating their blindfolds, which were close to the injured site.

These patterns suggest a strong effect of injury on visual responsiveness, but significant differences in response to touch between injured and sham-treated squid in the blindfolded group indicates that tactile sensitisation also occurs. In mammalian pain studies, long-term sensitisation of defensive responses has been used as an indicator of persisting pain. However, this criterion has been questioned because of the lack of evidence for centralised processing (e.g. Mogil, 2009) and we have decided not to regard sensitisation as evidence of sentience in this report. Overall, the results from Crook et al. (2011) show that arm injury in squid, **L. pealei**, did not lead to wound-directed behaviour but there was evidence of nociceptive sensitisation.

What explains the lack of site-specific wound-directed behaviour after injury in squid? The absence of pain, or something else? A different study by Crook et al. (2013) is relevant to this question. The researchers demonstrate that peripheral injury in squid (*n* = 42) resulted in pronounced, long-lasting spontaneous activity, as well as sensitisation to mechanical stimuli, in afferent neurons not only near the injury site but also on the other side of the body. Lack of localisation is consistent with the hypothesis that enhanced activity is part of a general behavioural state after injury in squid. This general behavioural state increases reactions to tactile stimulation *anywhere* on the body surface. This differs from mammalian nociceptors, which are assumed to be spatially associated with an injury, prompting pain-related self-protective behaviours directed at wound sites. Results from this study are important because they demonstrate that following injury, nociceptive sensitisation in squid appear to be widespread. The authors suggest that this phenomenon might function to initiate a generalised vigilance state. This explanation is consistent with other findings that show that minor injury in squid does increase risk of predation (Crook et al., 2014), thus a generalised vigilance state might help injured animals be more responsive to approaching predators.

2.7 Criterion 7: The animal shows associative learning in which noxious stimuli become associated with neutral stimuli, and/or in which novel ways of avoiding noxious stimuli are learned through reinforcement. ***Note: habituation and sensitisation are not sufficient to meet this criterion***

CONFIDENCE LEVEL	*We have very high confidence that octopods (Octopoda) and cuttlefish (Sepiida) satisfy criterion 7. We have high confidence that squid satisfy criterion 7 and medium confidence that nautiloids satisfy criterion 7.*
SUMMARY OF EVIDENCE	*Associative learning has been convincingly demonstrated in octopods and cuttlefish. Few studies have investigated associative learning in squid, but the overall evidential picture points towards associative learning being a shared capacity of the coleoid cephalopods. There are also few studied in nautiloids, but the evidence that does exist points towards a capacity for associative learning.*

Full review of evidence: What we are looking for here is robust evidence that the animal is able to form associations between noxious stimuli and neutral stimuli by, for example, learning to associate a particular place, or an otherwise neutral odour, with a noxious stimulus. We are also looking for evidence that an animal can learn a novel behaviour (distinct from any pre-existing reflex responses) that allows it to avoid a noxious stimulus.

We must distinguish associative learning from habituation, where an animal becomes less sensitive to a stimulus with repeated encounters, and from sensitisation, where an animal becomes more sensitive with repeated encounters. Habituation and sensitization are not enough. They are forms of learning, but they can be achieved without a brain, and without any integrative, centralised information processing at all (Ginsburg & Jablonka, 2019).

The link between associative learning and integrative processing is much stronger because representations of both stimuli have to come together in the same associative learning mechanism (Ginsburg & Jablonka, 2019; Birch et al., 2020). A recent study cast some doubt on this assumption by claiming to show associative learning in plants (Gagliano et al., 2016), but this study did not provide statistically significant evidence against a reasonable null hypothesis (Taiz et al., 2019) and the result has failed to replicate (Markel, 2020). As noted in Section 1.7, there is some evidence of unconscious associative learning (Greenwald and De Houwer, 2017), leading to on-going inquiry regarding which types of associative learning are most strongly linked to sentience and why. Instrumental learning (Skora et al. 2021), reversal learning (Travers et al. 2017), learning "incongruent" spatial relationships (Ben-Haim et al. 2021), and learning across temporal gaps between stimuli ("trace conditioning"; Clark et al. 2020) seem to have a particularly strong link to sentience. Our approach will be to take all evidence of associative learning as relevant to the overall evidential picture, without introducing any assumptions about which types of associative learning require sentience.

In general, it is a point of clear scientific consensus among cephalopod researchers that octopods and cuttlefish are readily capable of associative learning (Hanlon & Messenger, 2018; Hochner et al*.,* 2006; Marini et al., 2017; Mather, 1995, 2008; Schnell et al., 2020). The evidence is somewhat weaker in squid and nautiloids.

The brain of coleoid cephalopods is functionally specialised to facilitate learning. Based on electrophysiological studies, Hochner et al. (2006, p. 315) suggested that: "a convergent evolutionary process has led to the selection of similar networks and synaptic plasticity" involved in learning and memory in cephalopods and mammals. In particular, the vertical lobe-median superior frontal lobe complex has learning and memory functions analogous to the mammalian hippocampus (Hochner et al., 2006, Shomrat et al., 2015). Lesions inhibit performance in long-term learning tasks, such as visual discriminations, without affecting other survival behaviours (Boycott & Young, 1955; Maldonado, 1965; Young, 1960), and this structure develops concurrently with learning abilities in octopus (Fiorito & Chichery, 1995) and cuttlefish (Dickel et al., 2001).

Octopods. Octopods show a high capacity for associative learning and can be taught to associate reward or punishment with a variety of visual and tactile stimuli (reviewed in Schnell et al., 2020; briefly in Marini et al., 2017). For example, Papini and Bitterman (1991) trained the day octopus, **Octopus cyanea** (*n* = 37)*,* to associate a neutral stimulus with a food reward. Papini and Bittterman found that subjects that received larger rewards showed faster acquisition of the association than subjects that received smaller rewards. Moreover, when reinforcement was consistent, this induced better subsequent performance. Several other studies have shown that octopods can learn to associate between two different stimuli using rewarded or punishment training (i.e. electric shock) (Fiorito & Scotto, 1992; Kawashima et al., 2020; Mackintosh, 1964; Mackintosh & Mackintosh, 1963; 1964; Sutherland, 1962; Tokuda et al., 2015).

Recent work, rather than explicitly testing whether octopods can learn associatively at all, usually involves *training* octopods to learn some association as a first step towards testing some other cognitive ability. For example, studies have shown that octopods can perform spatial learning (Boal et al., 2000), social learning (Amodio & Fiorito, 2013; Tomita & Aoki, 2014), conditional learning (Hvorecny et al., 2007, Tokuda et al., 2015), and reversal learning (Mackintosh, 1962; Mackintosh & Mackintosh, 1963, 1964; but see Bublitz et al., 2017). However, we will not review these studies in detail here. The literature has been dominated by studies of **O. vulgaris**, but there are also some studies of **Octopus bimaculoides** (Boal et al., 2000), **Octopus ocellatus** (Tomita & Aoki, 2014), **Octopus aegina** (Kawamura et al., 2001) and **Abdopus aculeatus** (Kawashima et al., 2020).

Cuttlefish. In cuttlefish, learning has been extensively studied using the prawn-in-a-tube test (Agin et al., 1998, 2006; Boycott, 1961; Cartron et al., 2013; Chichery & Chichery, 1992; Dickel et al., 2000; Messenger 1971, 1973; Sanders & Young, 1940). The prawn-in-the-tube is a well-established setup for investigating learning and memory in cephalopods. It involves presenting the subject with a shrimp inside a glass beaker or test tube. Initially the subject attacks the prey item encased in the tube but quickly learns that the shrimp cannot be obtained. The ability of cuttlefish to succeed at the task is not in doubt, but a major challenge for researchers who use the prawn-in-a-tube task is to show that success involves associative learning (specifically, instrumental conditioning) and not just habituation (Agin et al., 2006).

Messenger (1973) showed that stronger punishments for attacking the prawn reduced the number of trials needed to reach criterion (i.e. the experimenter's standard for successful learning), whereas milder punishments increased the number of trials. However, these results did not rule out some combination of habituation to the prawn and a general reduction in responsiveness caused by punishment.

A key characteristic of habituation is dishabituation: the tendency for novel stimulus presentations to reverse the habituation process (Pinsker et al., 1970). Agin et al. (2006) tested dishabituation by giving the cuttlefish an alternative prey item (crab; exp. 1-2) or a novel stimulus (flashing light; exp. 3), before presenting the prawn-in-a-tube again. Despite the interpolated stimuli, there was no statistically significant tendency for animals to resume attacking the prawns. Nonetheless, the study suffered from a small sample size (exp. 1: $n = 8$; exp. 2: $n = 13, 9$; exp. 3: $n = 7$). Agin et al. do not report a power analysis, but null results in such a small sample are not compelling evidence of the absence of dishabituation.

Similar considerations apply to another study that attempted to disentangle associative learning from habituation (Purdy et al., 2006). The study found no evidence of a dishabituation effect in a study involving two groups of 7 cuttlefish (**S. officinalis**). This too is a small sample, but the two negative results taken together offer somewhat stronger evidence than either in isolation.

Darmaillacq et al. (2004) carried out the first study of taste aversion learning in cephalopods. They established whether cuttlefish (**S. officinalis**; $n = 66$) preferred crab or shrimp, before repeatedly presenting the preferred prey coated in distasteful quinine. Subjects rapidly learned to avoid these unpalatable prey items (mean ± SE: 8.1 ± 0.7 trials). This treatment group was compared to a control group, which was "trained" on preferred prey not coated in quinine. During choice tests either 24 or 72 hours later, 26 of 32 quinine-treated subjects avoided their originally preferred prey. Conversely, 26 of 34 control cuttlefish attacked their originally preferred prey. This is a high-quality study with a good sample size.

Cuttlefish research has focused on avoidance learning and mostly used **S. officinalis**, although other species also learn the prawn-in-a-tube task (e.g. **Sepia bandensis**: Bowers et al., 2020; **Sepia pharaonis**: Purdy et al., 2006). There is also evidence of classical conditioning (Agin et al., 1998, 2003; Cole & Adamo, 2005; Messenger, 1971), spatial learning (Alves et al., 2007, 2008; Scatà et al., 2016), and conditional learning (Hvorecny et al., 2007) in cuttlefish, but we will not review these studies in detail here.

Squid. We consider it unlikely that associative learning would be present in both octopods and cuttlefish but not squid. The evidence from octopods and cuttlefish, combined with evidence discussed elsewhere in the report regarding the phylogeny (Tanner et al. 2017), neuroanatomy (Andrews et al. 2013) and ecology (Mather and Kuba 2013) of the coleoid cephalopods, makes it much more likely that associative learning is a general trait of the coleoid cephalopods.

Nonetheless, compared to octopus and cuttlefish, there have been few learning studies on squid. Allen et al. (1985) investigated visual discrimination in Atlantic brief squid (**Lolliguncula brevis**). In the first experiment ($n = 3$), subjects were trained to attack a horizontal rectangle for a food reward and avoid a vertical rectangle or receive a 20 V electric shock. Squid subsequently attacked the horizontal rectangle in significantly more trials (39/39) than the vertical rectangle (7/35). There was some evidence for task retention after nine days, although no statistical analysis was reported. In the second experiment ($n = 1$), the positive stimulus was a white ball and the negative stimulus was a black ball. The white sphere was attacked in significantly more trials (58/58) than the black sphere (21/58). A limitation of this study is that the stimuli were not counterbalanced: horizontal/white stimuli were rewarded for all individuals, and vertical/black stimuli were punished for all individuals. This makes it difficult to disentangle learning from behaviour driven by properties of the stimuli, such as their visibility.

In a recent associative learning study, Zepeda et al. (2017) tested Hawaiian bobtail squid (**E. scolopes**) on the prawn-in-a-tube task. Subjects were trained in either massed (three 10-minute trials with 10-minute intervals) or spaced (three 10-minute trials with one-day intervals) sessions. The squid significantly reduced responding across the first trial. The data suggest that this reduced tendency to respond was retained for 8 days (in the massed treatment) and 10 days (in the spaced treatment) between tests. This retention is a form of long-term memory. However, this study also has limitations. Within trials, the authors compared the number of strikes in the first half with the number of strikes in the second half. A reduction in responding could be explained by depleted energy levels rather than learning. Even if learning were responsible, Zepeda et al. (2017) did not establish whether it was habituation or associative learning.

Nautiloids. Nautiloids have fewer neurons than coleoids and lack clearly differentiated lobes, including the vertical lobe-median superior frontal lobe complex linked to learning and long-term memory in coeloids (Young, 1965, 1991). Yet there is evidence for classical conditioning and potentially spatial learning in nautiloids.

Crook and Basil (2008) trained 12 chambered nautiluses (**Nautilus pompilius**) on a classical conditioning task. The unconditioned stimulus was food, the conditioned stimulus was a 0.5s blue light, and the responses were tentacle extension and rapid breathing. Although the authors had no criteria to establish that subjects had learnt the task, the conditioned stimulus induced significantly higher tentacle extension and breathing rates in the treatment group than an unreinforced control group three minutes and one hour after conditioning (i.e. short-term memory). There was no treatment difference for either measure at one hour, but significant differences reappeared at six and 12 hours (i.e. long-term memory). Crook and Basil equated this to the biphasic short- and long-term memory curve observed in coleoids (Agin et al., 2003, 2006). This functional analogy is surprising, given the structural differences between nautiloids and coleoids.

However, further research would be needed to allow high confidence that nautiloids satisfy criterion 7. The *p*-values for several time intervals, especially at three and 30 minutes, were only borderline significant (between 0.02 and 0.05). Moreover, we think it would have been appropriate to correct for multiple comparisons, such as by applying a Bonferroni correction. Had a correction been applied, the borderline significant findings may have been non-significant.

In another nautiloid study, Crook et al. (2009, exp. 1) found tentative evidence for spatial learning (learning the spatial configuration of a maze) in chambered nautilus (**N. pompilius**). Ten subjects were placed in a two-dimensional open-field maze with aversive bright light and shallow water. To escape these unconditioned stimuli, nautiluses needed to leave through an exit hole signalled by bubble wrap, a visual and tactile conditioned stimulus. Subjects underwent five 10-minute training trials, with a 15-minute inter-trial interval. Exit latency significantly decreased across the five training trials. Exit latency remained significantly below the naïve latency at 18 hours, 24 hours, 36 hours, 48 hours, 72 hours, 96 hours, 7 days, and 21 days. This retention time is substantially longer than the 12 hours observed in Crook and Basil's (2008) classical conditioning task.

However, it is unclear what the nautiloids were learning in this study. One interpretation is that they learnt to associate the bubble wrap with the exit hole. Alternatively, however, they began every trial opposite (180°) the exit hole, so may have learnt the orientation to escape, rather than the conditioned stimulus. It is also hard to rule out a general reduction in exploratory behaviour on repeated exposure to the same arena—a form of habituation.

2.8 Criterion 8: The animal shows that it values a putative analgesic or anaesthetic when injured in one or more of the following ways: (a) the animal learns to self-administer putative analgesics or anaesthetics when injured; or (b) the animal learns to prefer, when injured, a location at which analgesics or anaesthetics can be accessed; or (c) the animal prioritises obtaining these compounds over other needs (such as food) when injured

CONFIDENCE LEVEL	*There is recent evidence, in a just-published article, that octopods satisfy criterion 8. Although this is a single study, its high quality allows high confidence in the result.*
SUMMARY OF EVIDENCE	*The recent study noted above provides evidence that an octopus learns to prefer, when exposed to a noxious stimulus (acetic acid), a chamber in which a local anaesthetic can be accessed.*

Full review of evidence: One recent study bears on criterion 8. Crook (2021) asked: will an octopus (**O. bocki**), after being placed in their preferred chamber immediately after a potentially painful injection of acetic acid, learn to avoid that chamber in future? Moreover, will they learn to *prefer* a chamber in which they receive a local anaesthetic (lidocaine) when injured? Moreover, is this preference dependent on injury, so that the preference for the lidocaine-associated chamber is not formed when the animal is not injected with acetic acid? This is exactly the type of study that has the potential to provide high quality evidence for criterion 8 (via 8b) because it shows that the animal values an anaesthetic when injured.

Crook (2021) obtained clearly statistically significant evidence that the answer is “yes” to all three questions. Crook used a conditioned place preference (CPP) paradigm, a well-established paradigm for demonstrating the affective component of pain in mammals (Navratilova et al. 2013). Specifically, octopuses were introduced into a three-chamber apparatus and their preferred chamber was noted. Experimental subjects (n = 8) received a subcutaneous injection of dilute (0.5%) acetic acid in one arm and control subjects (n = 7) were injected with a saline solution. Results showed that experimental subjects avoided their initially preferred chamber, in which they were confined in after injection, and when presented with tonic pain relief (*i.e.* topical injection of lidocaine) the experimental subjects changed their chamber preference to the location in which they experienced pain relief. By contrast, control animals showed no change in chamber preference following injection of the saline solution and injection of lidocaine did not induce a change in chamber preference.

Moreover, Crook made electrophysiological recordings of activity in the brachial connectives, which connect the arm nerve cords to the brain (criterion 3). The recordings showed a prolonged period of activity that was then silenced by the injection of lidocaine. The overall structure of Crook’s experiment is shown in **Figure 3** (from Crook 2021).

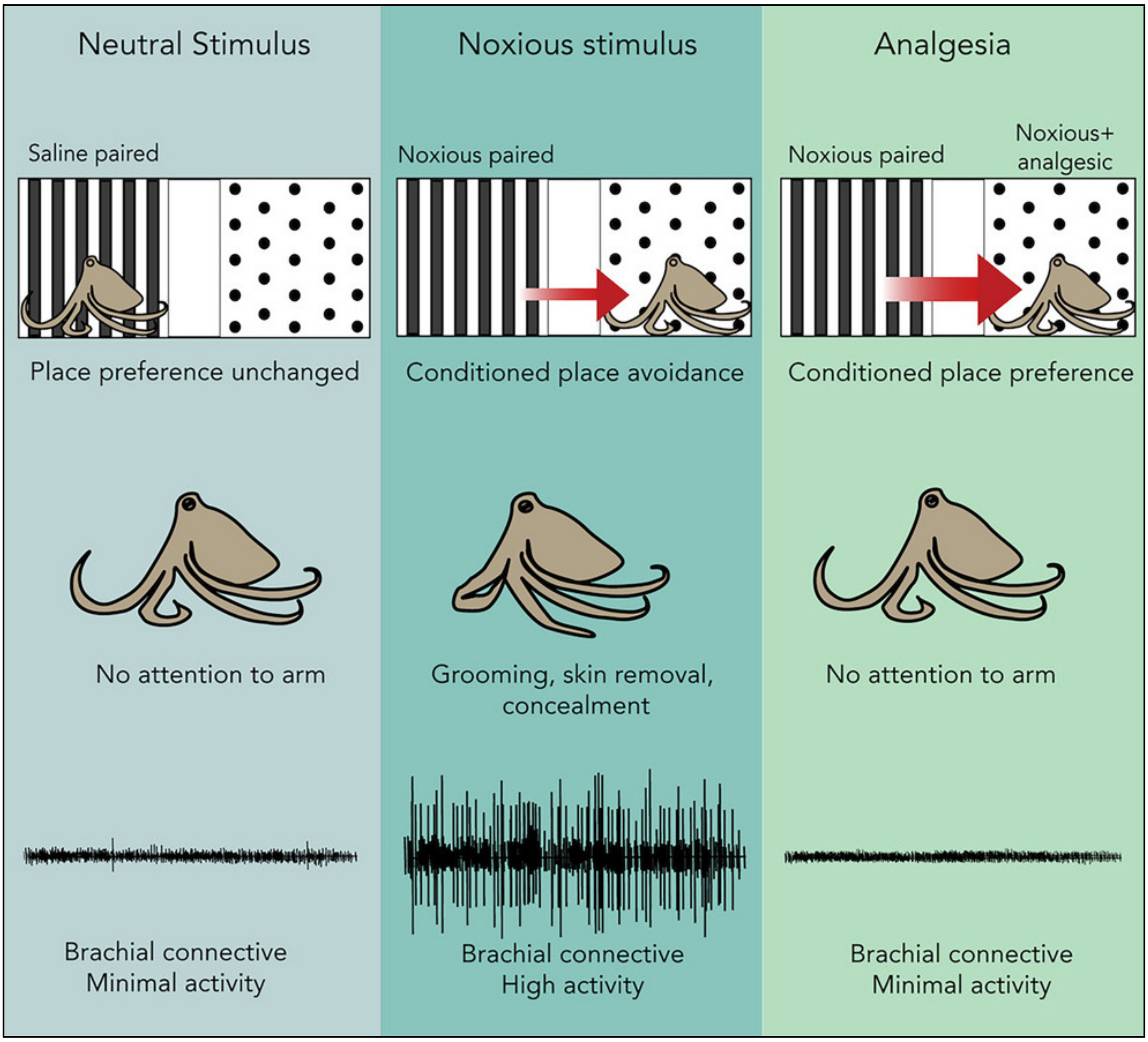

Figure 3: A key figure from Crook (2021). The experiment (which is relevant to our criteria 4, 5 and 8) involved four groups of animals (with either 7 or 8 in each group): a group injected with only saline solution; a second group injected with acetic acid; a third group injected with acetic acid and, later, lidocaine; and a fourth group (not shown) injected with saline and then lidocaine. After receiving acetic acid, the affected animals showed directed self-protective behaviour, increased neural activity, and avoidance of the chamber where they had received it. Lidocaine silenced the heightened neural activity, stopped the self-protective behaviour, and led to a conditioned preference for the chamber where the effects of the lidocaine were experienced. The figure is © Robyn Crook 2021, CC-BY-NC-ND 4.0 licensed. See the original source for further methodological details.

PART III. EVALUATING THE EVIDENCE OF SENTIENCE: DECAPODS

SUMMARY OF PART III

- There is strong evidence of sentience in true crabs (infraorder Brachyura). We have either high or very high confidence that true crabs satisfy criteria 1, 2, 4, 6 and 7.
- There is somewhat less evidence concerning other decapods. There is substantial evidence of sentience in anomuran crabs (infraorder Anomura). We have high confidence that they satisfy criteria 1, 2 and 6, and medium confidence that they satisfy criterion 5.
- There is also substantial evidence of sentience in astacid lobsters/crayfish (infraorder Astacidea). We have either high or very high confidence that these animals satisfy criteria 1, 2 and 4.
- In cases where we do not have high or very high confidence that a criterion is satisfied, this is invariably because of a lack of positive evidence, rather than because of clear evidence that the animals fail the criterion.

In this section, we review all evidence from decapods that bears on our eight criteria for sentience. Relevant past reviews on this topic since 2000 include Sherwin (2001), AHAW (2005), Elwood et al. (2009), Gherardi (2009), Broom (2014), Sneddon et al. (2014), Sneddon (2015), Burrell (2017), Walters (2018), and Elwood (2019a, b). Although these are all high-quality reviews, some new evidence has come to light since they were written, and they do not apply the framework we have set out in Part I. Rather than relying on past reviews, we have revisited all of the original evidence in order to produce a fresh review. Our conclusions, summarised above, are also summarised in **Table 3**.

Table 3. A summary of the evidence of sentience in decapods. *The colours and letters represent our confidence level that the criterion in question (column) is satisfied by the order (or orders) of animals in question (row). VH (dark green) indicates very high confidence, H (light green) indicates high confidence, M (dark yellow) indicates medium confidence, and L (light yellow) represents low confidence. Since we have not found evidence to support criterion 8 in any decapod, we have used the category of very low confidence (VL, light grey) in this case. Importantly, low/very low confidence implies only that the scientific evidence one way or the other is weak, not that the animal fails or is likely to fail the criterion.*

	Criterion 1	Criterion 2	Criterion 3	Criterion 4	Criterion 5	Criterion 6	Criterion 7	Criterion 8
True crabs (Brachyura)	H	VH	L	VH	L	VH	H	VL
Anomuran crabs (Anomura)	H	VH	L	L	M	H	L	VL
Astacid lobsters/crayfish (Astacidea)	H	VH	L	VH	L	L	M	VL
Spiny lobsters (Achelata)	H	VH	L	L	L	L	M	VL
Caridean shrimps (Caridea)	H	VH	L	M	L	M	L	VL
Penaeid shrimps (Penaeidae)	H	L	L	M	L	L	L	VL

3.1 Criterion 1: The animal possesses receptors sensitive to noxious stimuli (nociceptors)

CONFIDENCE LEVEL	*Genetic evidence and evidence from other arthropods leads us to have high confidence that nociceptors are widespread in the decapods. Direct neurophysiological evidence would be needed for us to have very high confidence.*
SUMMARY OF EVIDENCE	*Two main lines of evidence support the hypothesis that nociceptors are widespread in the decapods. First, nociceptors are present in other arthropods, such as insects. Second, the ion channel families involved in nociception are highly evolutionarily conserved, having been characterised in species ranging from flatworms to humans, and several homologous ion channel proteins have been found to be expressed in crabs and lobsters. Taken in conjunction with the behavioural evidence considered later, these lines of evidence together suggest that nociceptors are present in decapods.*

Full review of evidence: For the definition of a nociceptor, see Section 2.1 (we will not repeat this here). The most direct method for detecting nociceptors is by identifying peripheral sensory neurons that show altered electrical activity in response to potentially noxious stimulation. These methods have not so far revealed conclusive evidence for nociceptors in decapods (Sneddon et al., 2014; Walters, 2018). We have found only two electrophysiological studies explicitly exploring nociception in decapod crustaceans. To assess nociceptive responses to extreme pH, the second of two pairs of antennae of Louisiana red swamp crayfish (**Procambarus clarkii**) were severed, and extracellular recordings were taken from the nerve tip of each (Puri & Faulkes, 2010). Electrical activity of the nerves was compared when the antennae were washed or swabbed with sodium hydroxide (NaOH; alkaline), hydrochloric acid (HCl), benzocaine (a local anaesthetic in vertebrates) and the control solution (freshwater crayfish saline). The electrical responses differed greatly between individual antennae, showing no consistent increases or decreases in spike frequency between the different stimuli. Thus, the study yielded no evidence of nociceptors that respond to extreme pH in crayfish antennae (Puri & Faulkes, 2010). However, extreme pH is just one of various possible stimuli to which a nociceptor may respond.

In a follow-up experiment, the same procedure was used to investigate extracellular electrical responses of second antennal nerves from **P. clarkii**, but this time in response to extreme heat (washing with a small volume of water at 60 degrees celsius), control saline, capsaicin (the 'hot' chemical found in chilli peppers, dissolved in ethanol), isothiocyanate (the 'hot' chemical found in wasabi, dissolved in ethanol), or control ethanol solution (Puri & Faulkes, 2015). There was significantly more electrical activity in response to the hot water than to the control saline. There were no consistent differences in electrical activity between capsaicin, isothiocyanate, and ethanol, although statistical analysis of this was not reported. This provides tentative evidence for antennal nociceptors specialised for extreme heat. However, it is unclear if the receptors in fact also respond to moderate heat, because a range of temperatures was not tested, and because the small quantity of hot water will rapidly have been cooled by the much larger quantity of room temperature saline bathing the antennae. If the receptors respond to moderate heat, they would be thermoreceptors rather than nociceptors.

The distinction between 'extreme' and 'moderate' is not sharp. Even extreme stimulation should be within the bounds of what is evolutionarily relevant for the species, for a nociceptor to have any adaptive value to an animal in preventing it becoming physically damaged by stimuli. It may be necessary, therefore, to investigate nociception using stimuli ranging from mild up to increasing intensities/frequencies/durations. For example, heat sensitivity is often investigated in vertebrates using lasers, thermodes or hotplates, which can gradually become hotter until a nociceptive response is observed (see, e.g. Ashley et al., 2007 on rainbow trout). If nociceptors exist alongside other sensory receptors, we might expect electrical responsivity to be bi- or multi-modal, with some sensory receptors responding to moderate stimulation, and nociceptors responding only once the stimulation becomes more extreme. This is an evidence gap.

Another electrophysiological study was not intended to discover nociceptors but investigated the electrical responsivity to stimulation of sensory receptors on the inner edges of the pereiopod chelae (claws of a walking leg) in stone crayfish (**Austropotamobius torrentium**) (Altner et al., 1983). The authors did not claim to have found nociceptors but did note the existence of mechanoreceptors that were associated with external setae (bristles) and that only responded to “strong mechanical stimuli”. They did not quantify how strong, but it would be significant to know whether it was strong enough to cause avoidance behaviour, in which case they may be mechanical nociceptors.

Relatively recent molecular evidence provides indirect evidence for nociception in decapods. Transcriptomics can reveal which genes are being expressed as messenger RNA (mRNA) within an animal’s tissue, thereby causing specific proteins to be produced. Analysis of the transcriptomes of the Caribbean spiny lobster (**Panulirus argus**), clawed lobster (**Homarus americanus**), red swamp crayfish (**Procambarus clarkii**), and blue crab (**Callinectes sapidus**) showed that all four species express Transient Receptor Potential A (TRPA) channels (Kozma et al., 2020). The TRPA subfamily of ion channels is significant because some of its variants function as receptors for a variety of aversive stimuli across many bilateral multicellular animals. For example, the four decapods express homologues to TRPA1, which detects a very wide variety of potentially noxious stimuli across many animal species, ranging from flatworms (Arenas et al., 2017) to humans (Kádková et al., 2017). The decapods additionally expressed a homologue to the TRPA channel known as “painless”, so-called because when it is knocked out of the fruit fly (**Drosophila melanogaster**), the flies no longer avoid noxious thermal, mechanical or chemical stimuli (Tracey et al., 2003; Im & Galko, 2012).

Overall, the decapods expressed four to five TRPA homologues with other species, and two distinct TRPA channels with no insect homologues. They also expressed TRPV1 (Kozma et al., 2020), which is another highly conserved ion channel that is involved in polymodal nociception in mammals (Smith & Lewin, 2009). A related study showed that all of these ion channels were expressed in the antennae, limbs, and brains of **P. clarkii** to varying degrees (Kozma et al., 2018). These transcriptome data provide relevant evidence, but they are not enough for us to have very high confidence that nociceptors are present, because the same proteins can have different functions in different species and in different tissues within the same animal.

As noted for cephalopods (Section 2.1), it is relevant here that nociceptors are widespread across the animal kingdom. As well as existing in vertebrates, they have been found in annelid worms, nematode worms, gastropod molluscs and insects (Smith & Lewin, 2009; Walters, 2018). Crustaceans are a sister group to hexapoda (insects) within the arthropod phylum, and both derived from a common ancestor shared with nematoda (Halanych, 2004). It is unclear whether nociceptors evolved once in an ancient common ancestor and have been conserved in almost every species that followed, or whether they have evolved independently more than once via convergent evolution (Walters, 2018). If they evolved in any common ancestor shared between crustacea and hexapoda, then it seems unlikely that crustaceans would later have lost them, because nociceptors have clear survival value. On balance, this indirect evidence, when taken together with the behavioural evidence considered later, allows high (but not very high) confidence that nociceptors are present in decapods.

3.2 Criterion 2: The animal possesses integrative brain regions capable of integrating information from different sensory sources

CONFIDENCE LEVEL	*We have very high confidence that criterion 2 is satisfied by true crabs (infraorder Brachyura), anomuran crabs (Anomura), lobsters and crayfish (Astacidea, Achelata) and caridean shrimps (Caridea). It may be satisfied by other decapod infraorders, but many have not been studied in detail.*
SUMMARY OF EVIDENCE	*Decapods possess brains that integrate information from different sensory sources. The central complex, the hemiellipsoid bodies, and the accessory lobes perform integrative functions. True crabs (infraorder Brachyura) and anomuran crabs (Anomura) seem to have proportionally the largest and most developed hemiellipsoid bodies of the decapods studied so far, followed by caridean shrimps (Caridea). Lobsters and crayfish (Astacidea, Achelata) have relatively small hemiellipsoid bodies, but they integrate information using relatively enlarged accessory lobes.*

Full review of evidence: There is still much to discover about crustacean brains, with many parts not well understood. There is also enormous variation in brain structure across species, since different species are adapted to a wide range of different habitats and ecology (Sandeman et al., 2014; Strausfeld et al., 2020).

Nevertheless, across decapods, the brain is formed of three main collections of neuropils (densely interwoven neurons) (reviewed in Sandeman et al., 2014) that interlink with each other via an elaborate central complex (Utting et al., 2000). The first, dorsal-most, region of the brain is the protocerebrum, which primarily processes visual information. In fact, the lateral parts of the protocerebrum are physically located within the eyestalks of species that have eyestalks. The lateral protocerebra also contain the hemiellipsoid bodies, which will be discussed in more detail below.

The second region is the deutocerebrum, which receives information from the first set of antennae. This information comprises olfactory information, which is conveyed into the olfactory lobes of the deutocerebrum, as well as chemosensory and mechanosensory information. A large sweep of neurons connects the deutocerebrum with the protocerebrum and includes the olfactory globular tract. The deutocerebrum also includes the accessory lobes, which, in some decapods, help to integrate tactile and olfactory information with visual information from the protocerebrum (Sandeman et al., 1995).

The third, ventral-most, region is the tritocerebrum, which processes chemical and mechanical information from the second pair of antennae (antennules) and the mouthparts, and from the rest of the body. Mechanoreceptive information from the tritocerebrum is relayed up to the deutocerebrum, and some neurons with their cell bodies in the tritocerebrum extend all the way up into the lateral protocerebrum (reviewed in Sandeman et al., 2014).

It is clear that the crustacean brain structure has the potential to integrate information of different kinds, partly because of the extensive linkage of these three brain regions via the pathways and hubs of the central complex (Utting et al., 2000). Additionally, some specific structures are also known to integrate information, with the hemiellipsoid body being perhaps the most well understood integrative centre. Evidence has been gathering that hemiellipsoid bodies are homologous with insect mushroom bodies, which are also known to have integrative functions (Brown & Wolff, 2012; Sayre & Strausfeld, 2019). For example, a large study by Strausfeld et al. (2020) of nineteen decapods (and a stomatopod) found a protein in the hemiellipsoid bodies that plays a crucial role in learning and memory in fruit flies (Skoulakis et al., 1993). This is further evidence that the hemiellipsoid bodies are involved in integrating information.

Further evidence of the higher processing function of hemiellipsoid bodies has been found in the crab **Neohelice granulata** (Maza et al., 2016). The authors showed that it was possible to stain hemiellipsoid bodies with antibodies to proteins that are associated with memory processes (known as ‘p-CaMKII-α’), and with antibodies to a different protein (5-bromo-2′-deoxyuridine, or ‘BrdU’) that is produced by proliferating cells (production of new brain cells is a mechanism that underlies learning and memory). They also presented the crabs with a repeated ‘threatening’ visual stimulus, which initially caused them to try to escape and caused corresponding activity (a calcium cascade) in the hemiellipsoid bodies, with the crabs gradually stopping responding to the

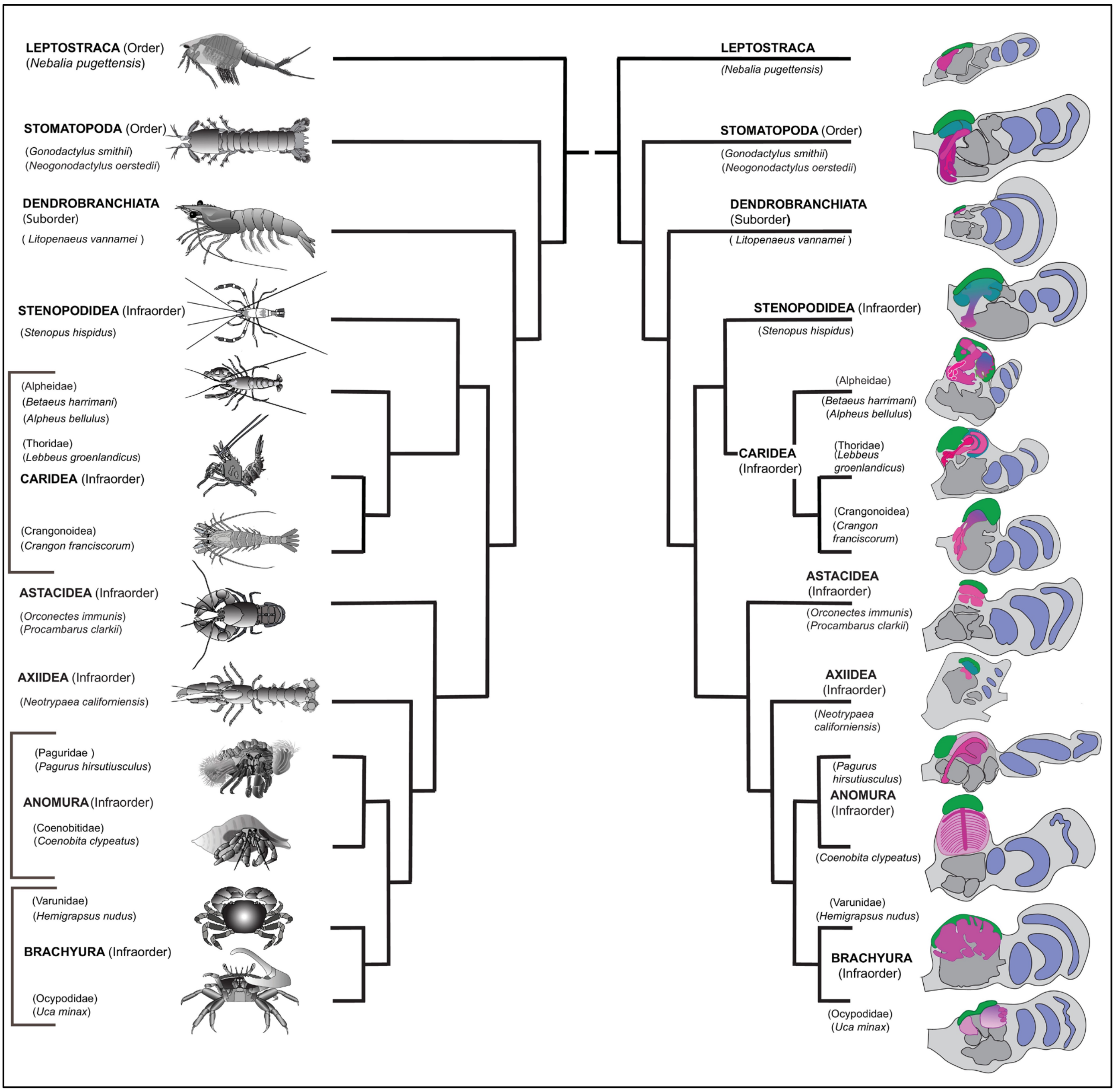

Figure 4: A key figure from Strausfeld et al. (2020). The pink regions indicate an integrative brain region associated with learning and memory (the hemiellipsoid body) in various species of crustacean, as identified using an immunostaining technique (N.B. Leptostraca and Stomatopoda are not decapods). This figure is © Strausfeld et al. 2020 / CC-BY-4.0 licensed. See the original source for full details of the technique used.

repeated stimulus. After a short interval, Maza et al. (2016) showed the crabs the stimulus again, either when the crabs were in the same environment as before, or when there was a different visual background. The crabs with the familiar background continued to ignore the cascade, but those with the new background reacted with renewed behavioural and hemiellipsoid activity. The authors therefore concluded that the hemiellipsoid bodies are involved in learning and memory (Maza et al., 2016), although it should be noted that the type learning here was probably habituation, not associative learning (see criterion 7).

One mechanism by which decapod hemiellipsoid bodies can integrate information involves interneurons known as ‘parasol cells’. These are located within the hemiellipsoid body and can integrate sensory information across modalities, with the ability to amplify signals (DeForest Mellon, 2003), and may play a role in decision-making and prioritisation. Without stimulation, the cells show consistent, synchronised pulses of activity. In crayfish, at least, individual parasol cells can receive either odour information from the olfactory lobe, visual information from the optical ganglion, or tactile, odour and visual information from the accessory lobe. When a strong stimulus activates any parasol cell, there is a burst of enhanced activity in that cell and in neighbouring cells, even if those neighbours did not receive the original stimulus input (DeForest Mellon, 2003). This cellular community-level activity may enable amplification of important signals, enabling important information to be prioritised. Neural mechanisms such as this could allow whole-organism perception, learning and decision-making about potential harms.

Some decapods have more developed hemiellipsoid bodies than others. Across 19 decapods studied, the groups with proportionately the largest hemiellipsoid bodies were true crabs (Brachyura), followed by anomuran crabs (Anomura), followed by various shrimps of the infraorder Caridea: reef dwelling shrimps (Alpheidae and Thoridae) (Strausfeld et al., 2020), and hydrothermal vent shrimps (exemplified by **Rimicaris exoculata**) (Machon et al., 2019). A key figure from this study is reproduced as **Figure 4**.

What about decapod species with relatively reduced hemiellipsoid bodies, such as crayfish and lobsters (Astacidea)? They may be integrating information using a different part of the brain. Specifically, the accessory lobe within the deutocerebrum is relatively large in astacids, and it can integrate multisensory information (Sandeman et al., 2014). In Australian freshwater crayfish (**Cherax destructor**), the relatively large accessory lobes receive input from deutocerebral interneurons that convey visual and tactile information from the hemiellipsoid bodies in the protocerebrum, as well as olfactory and other information from the deuto- and tritocerebra (Sandeman et al., 1995). The accessory lobe then projects information back upwards to the protocerebrum. Similarly, large and well-connected accessory lobes have been found in the spiny lobster (**P. argus**, infraorder Achelata) (Wachowiak et al., 1996), freshwater crayfish (**P. clarkii** and **Orconectes rusticus**), and the American clawed lobster (**Homarus americanus**).

The combination of relatively small hemiellipsoid bodies with large accessory lobes in astacids contrasts with the opposite found in other decapods, such as the coconut crab (**Birgus latro**, infraorder Anomura), which has an extremely small accessory lobe that is seemingly little connected with the rest of the brain, whilst its hemiellipsoid body is very large (Krieger et al., 2010). It is possible that integration of information can be done either primarily in the accessory lobe, as in crayfish and lobsters, or primarily in the hemiellipsoid bodies, as in crabs and some shrimp species (Sandeman et al., 2014).

3.3 Criterion 3: The animal possesses neural pathways connecting the nociceptors to the integrative brain regions

CONFIDENCE LEVEL	*We have low confidence that decapods satisfy criterion 3. This is solely because there is too little evidence for us to have medium or high confidence.*
SUMMARY OF EVIDENCE	*Neural pathways connect other sensory receptors to the integrative brain regions in decapods, and it is plausible that nociceptors would be connected to the same brain regions, but we have not found evidence that bears specifically on criterion 3.*

The nervous system is organised differently in elongated species, such as lobsters and prawns, versus compact species, such as crabs. In elongated species, the brain is connected to the ventral nerve cord, which runs along the length of the body. Each segment of the body contains a ganglion, and the nerve cord connects these together, conveying information to and from the brain. In compact bodied decapods, the segmental ganglia are not arranged linearly along a nerve cord, and instead form a single mass, the thoracic ganglion (Ruppert & Barnes, 1994; Smarandache-Wellmann, 2016). We note this here because it makes a difference to appropriate slaughter methods (see Part V).

As outlined within Criterion 2, decapod sensory receptors in general are connected to the integrative brain regions (Sandeman et al., 2014). This makes it plausible that nociceptors would also be connected. What is lacking for decapods is neurological evidence of the specific pathways involved in transmitting nociceptive information.

To be clear, the problem is the absence of high-quality evidence one way or the other—not that there is evidence *against* the nociceptors connecting to the integrative brain regions. It remains plausible that they do so.

3.4 Criterion 4: The animal's behavioural response to a noxious stimulus is modulated by chemical compounds affecting the nervous system in either or both of the following ways: (a) The animal possesses an endogenous neurotransmitter system that modulates (in a way consistent with the experience of pain, distress or harm) its responses to threatened or actual noxious stimuli; or (b) putative local anaesthetics, analgesics (such as opioids), anxiolytics or anti-depressants modify an animal's responses to threatened or actual noxious stimuli in a way consistent with the hypothesis that these compounds attenuate the experience of pain, distress or harm

CONFIDENCE LEVEL

We have very high confidence that criterion 4 is satisfied by true crabs (infraorder Brachyura) and astacid lobsters/crayfish (Astacidea). We have medium confidence that it is satisfied by caridean shrimp (Caridea) and penaeid shrimp (family Penaeidae). For other taxa, not enough evidence exists to allow us to have medium, high, or very high confidence.

SUMMARY OF EVIDENCE

Decapod crustaceans have endogenous neurotransmitter systems, including endogenous opioid, serotonergic, dopaminergic and octopaminergic systems. In true crabs (infraorder Brachyura), opioids mediate responsiveness to threatening stimuli and electric shocks. This effect is consistent with the hypothesis that opioids attenuate aversive experiences, while also being consistent with the hypothesis that opioids produce a general reduction in responsiveness. There is also evidence of a role for dopamine and octopamine in mediating learning from aversive and attractive stimuli (respectively). In both true crabs and astacids, there is evidence that serotonin mediates responses to stress, and evidence that antidepressant or anxiolytic drugs can be used to modulate the response. There is also evidence that the topical anaesthetic lidocaine modulates responses to injury in caridean and penaeid shrimps (Caridea, Penaeidae).

Full review of evidence: It is a clear point of scientific consensus that decapod crustaceans have endogenous neurotransmitter systems, including endogenous opioid, serotonergic, dopaminergic and octopaminergic systems (see Harlıoğlu et al., 2020 for a recent review).

Our interest here is specifically in the role of these compounds in modulating responses to threatened or actual noxious stimuli. We will consider criterion 4a and 4b together since many of the same studies investigate both endogenous neurotransmitters and drugs that reproduce the effects of these neurotransmitters. We will start with the true crabs (infraorder Brachyura), where there is the largest body of evidence, and then consider other decapods.

True crabs (infraorder Brachyura). In a series of experiments in the 1980s and 1990s, Hector Maldonado and colleagues at the University of Buenos Aires studied the effect of opioids on responses to danger and noxious stimuli in the crab **Neohelice granulatus** (formerly **Chasmagnathus granulatus**). Valeggia et al. (1989) and Romano et al. (1990) investigated escape responses to a "danger stimulus", a shadow passing overhead. They found that injecting crabs with naloxone, a drug that blocks the effects of opioids, blocked habituation to the danger stimulus, suggesting a role for endogenous opioids in producing habituation. Lozada et al. (1988) investigated defence responses (where the crab extends its claws and raises itself on its legs) to electric shock. They found that increasing the dose of morphine a crab received clearly inhibited its defensive responses to electric shocks. Maldonado et al.

(1989) investigated whether injecting morphine would reduce escape responses to the danger stimulus, and here too they found that found increasing the morphine dose inhibited responsiveness. In both studies, the effect was no longer found when naloxone was administered. A similar result was obtained using a synthetic analogue of the opioid met-enkephalin instead of morphine (Godoy & Maldonado, 1995).

These results raise the question of whether opioids work by attenuating an aversive state (a "pain-like" or "fear-like" state) or by generally inhibiting responsiveness to stimuli. Tomsic & Maldonado (1990) investigated whether morphine produced a general impairment of motor ability unrelated to responses to danger and could find no evidence of this in two test groups of 20 crabs each, relative to controls injected with saline. Tomsic et al. (1991) compared the effects of morphine with the effects of a neurotransmitter known to impair motor responses, gamma-Aminobutyric acid, or GABA. They found evidence that, although both GABA and morphine impair responsiveness, only morphine impairs long-term habituation to the danger stimulus.

These results can be contrasted with some rather different results from Barr & Elwood (2011). Barr and Elwood studied the effects of opioids on response to electric shock in the shore crab **Carcinus maenas**. The crabs were placed in a light area near to a dark shelter. Their aversion to light motivated them to enter the shelter, but sometimes they would receive an electric shock on entering. Barr and Elwood asked: would administering morphine make the crabs more likely to enter the shelter despite the risk of shock? What they found was that morphine made the crabs somewhat *less* likely to enter the shelter, seemingly due to a general reduction in responsiveness. The crabs injected with morphine "appeared limp and could not move their appendages in a normal manner" (2011, p. 342), a report at variance with Tomsic & Maldonado (1990). It may be that there are differences between crab species in responses to opioids. In this context, it is worth noting that Tomsic et al. (1993) tried to replicate their results concerning morphine and naloxone with another crab species, **Pachygrapsus marmoratus**, and were unable to do so.

The overall message is that opioids do mediate responses to noxious stimuli in both **N. granulatus** and **C. maenas**, and the way they do so is consistent with the hypothesis that opioids attenuate aversive experiences, but also (on current evidence) consistent with the hypothesis that opioids produce a general reduction in responsiveness. Clearly, evidence of this type is not conclusive evidence of valenced experiences—but no individual piece of evidence, considered in isolation, could settle this question. Variation among true crab species in their responses to opioids remain poorly understood.

There is a small amount of evidence concerning effects of drugs other than opioids on responses to threat in true crabs. When Maldonado's lab administered serotonin to **N. granulatus** instead of opioids, the crabs showed sensitisation to the danger stimulus, in line with the hypothesis that serotonin produces an anxiety-like state (Aggio et al., 1996). Hamilton et al. (2016) found evidence that administering fluoxetine, a selective serotonin reuptake inhibitor (anti-depressant), removed the preference for dark areas over light areas in the striped shore crab **Pachygrapsus crassipes**. Combined with the evidence from crayfish (see below), there is a strong case for the hypothesis that serotonin modulates responses to threatening and aversive stimuli in decapods.

Kaczer & Maldonado (2009) found evidence of a role for octopamine, often regarded as the invertebrate analogue of noradrenaline (norepinephrine), in improving appetitive (reward-based) learning and impairing aversive learning in **N. granulatus**, in a way consistent with the hypothesis that it facilitates experiences of reward but attenuates aversive experiences (see also Kaczer et al., 2011). Klappenbach et al. (2012) found evidence that dopamine plays approximately the opposite role to octopamine, improving aversive learning and impairing appetitive learning in **N. granulatus** in a way consistent with the hypothesis that it facilitates aversive experiences and attenuates attractive experiences. This role for dopamine differs from its role in the vertebrate brain but is in line with evidence from other invertebrate taxa, such as insects.

Other decapods. Eyestalk ablation is a controversial practice in shrimp aquaculture. The process involves cauterizing or cutting off one or both of the eyestalks of a mature broodstock female prawn in order to induce egg production. Two studies have examined the neurophysiological effects of eyestalk ablation in shrimp. Taylor et al. (2004) applied the topical anaesthetic lidocaine (branded Xylocaine) before eyestalk ablation of the whiteleg shrimp and observed that the swimming behaviour of shrimp (**Litopenaeus vannamei**) that had received the anaesthetic was much less erratic, with much less spiralling, than the swimming behaviour of shrimp that had received no anaesthetic. In a similar study, Diarte-Plata et al. (2012) applied lidocaine before eyestalk ablation of the caridean shrimp **Macrobrachium americanum**. They found a significant reduction in tail flicking. These studies, like those discussed above, raise the question of whether the anaesthetic is attenuating a valenced state or just inhibiting responsiveness. The current evidence, although compatible with the former possibility, leaves this question open.

Barr et al. (2008) studied grooming and rubbing behaviour in prawns (**Palaemon elegans**) in response to chemicals applied to the antennae. They found that applying hydrochloric acid (HCl) or sodium hydroxide (NaOH) to one antenna induced grooming and rubbing behaviour that was directed towards that antenna. This is relevant to criterion 6 and will be discussed again under that heading. They also found, unexpectedly, that the local anaesthetic benzocaine also triggered grooming. The anaesthetic also triggered tail flipping, a defensive behaviour, in 37/72 animals (compared with 0/72 when seawater was applied), suggesting it is strongly aversive. When animals to which benzocaine had been applied were then given HCl or NaOH, they were significantly less likely to display tail flipping or rubbing, compared with controls who had received seawater instead of benzocaine. However, this has to be interpreted in light of the aversive nature of the benzocaine. It may be due to an anaesthetic effect but may also be due to depleted energy levels following an aversive reaction to the benzocaine.

We also note that an attempted replication by Puri & Faulkes (2010) involving three other decapod species, white shrimp (**Litopenaeus setiferus**), grass shrimp (**Palaemonetes sp.**), and Louisiana red swamp crayfish (**P. clarkii**), failed to record any grooming or rubbing behaviour in response to extreme pH. On balance, this mixed evidential picture leads us to have medium confidence that caridean and penaeid shrimps satisfy criterion 4.

In a high-profile study published in the journal *Science*, Fossat et al. (2014) studied “anxiety-like” behaviour in crayfish (**P. clarkii**, infra-order Astacidea). Crayfish were placed in a maze in which they were free to explore both light and dark arms. When electrical fields were used to induce physiological stress in the animals, they became substantially less willing to enter the light arms. Crucially, there was evidence that the effect was mediated by endogenous serotonin. The brains of the stressed animals contained significantly higher levels of serotonin than the brains of the unstressed animals, as measured by a form of chromatography. Moreover, injecting unstressed animals with (exogenous) serotonin induced significantly more light avoidance behaviour than was found in controls injected with saline. Administering a common anxiolytic (anti-anxiety) drug, chlordiazepoxide, was found to restore a willingness to explore the light arms in the stressed crayfish, relative to a control group injected with saline. A follow-up study (Fossat et al., 2015) showed a positive correlation between the amount of serotonin in the brain and the degree of light avoidance, and again showed that administering chlordiazepoxide abolished the light avoidance behaviour. The large sample sizes used in these studies (267 crayfish in Fossat et al., 2014; 130 in Fossat et al., 2015) and low *p*-values inspire confidence in the reliability of the results.

Another study from the same lab (Bacqué-Cazenave et al., 2017), in which light avoidance behaviour was induced by aggression from another animal, led to similar findings: stressed animals had significantly higher levels of brain serotonin and displayed significantly greater light avoidance; the effect was again abolished by administering chlordiazepoxide. A study by Perrot-Minnot at al. (2017) found consilient results for an amphipod crustacean. Although amphipods are not decapods, this provides support for the hypothesis that the mechanisms involved are not distinctive to crayfish. In sum, this is high-quality evidence that serotonin regulates light avoidance behaviour induced by physiological stress in crayfish, in a way that can be modulated by anxiolytic drugs.

3.5 Criterion 5: The animal shows motivational trade-offs, in which the disvalue of a noxious or threatening stimulus is weighed (traded off) against the value of an opportunity for reward, leading to flexible decision-making. Enough flexibility must be shown to indicate centralized, integrative processing of information involving an evaluative common currency.

CONFIDENCE LEVEL	*We have medium confidence that motivational trade-offs are present in anomuran crabs (infraorder Anomura).*
SUMMARY OF EVIDENCE	*There is high-quality evidence that responses to electric shock in hermit crabs (infraorder Anomura) are modulated by odour. There is also evidence that hermit crabs' responses to electric shock are also modulated by shell quality, but this evidence is less reliable, because there are potential confounding factors, and the key results are only just statistically significant. There is high quality evidence that threat tolerance in crayfish depends on physiological stress, but this type of sensitivity is not evidence of a centralised decision-making system that weighs different needs against each other.*

Full review of evidence: As in Section 2.5, what we are looking for here is robust evidence that an animal is motivated to avoid a noxious stimulus, and that this motivation is *weighed* (*traded off*) against other motivations in a flexible decision-making system.

Work by Elwood's lab at Queen's University Belfast involving hermit crabs (**Pagurus bernhardus**) is especially relevant to this criterion. Hermit crabs live in shells produced by other animals. They prefer some types of shell to others and will swap a low-quality shell for a high-quality shell (Elwood et al., 1979; Elwood, 1995). This is itself a form of flexible decision-making (comparable, for example, to the ability of swarms of bees to choose a nest site; Seeley, 2010), but it does not by itself show that a motivation to avoid noxious stimuli is a factor in the animals' decisions.

To explore this possibility, Appel & Elwood (2009a) asked: if electric shocks are administered to the crabs when they are in the shells, will the crabs leave regardless of the quality of the shell, or will they be more reluctant to leave a high-quality shell than a low-quality shell? The latter would suggest that the disvalue of a noxious stimulus is weighed against other preferences. They compared the mean voltage required to induce a crab to leave a high-quality shell (Littorina) with the mean voltage required to induce a crab to leave a low-quality shell (Gibbula). They found that "hermit crabs in Littorina shells left the shells at significantly higher voltages than those in Gibbula shells" (Appel & Elwood, 2009a). The mean voltage required to induce a crab to leave a Littorina shell was 17.7V, compared with 15.0V for Gibbula.

However, some notes of caution are appropriate regarding the statistical significance of the result. The reported *p*-value was P = 0.0465 (Appel & Elwood, 2009a, p. 122). This level of significance was achieved with a *one-tailed* Mann-Whitney U test. What this means is that, assuming the null hypothesis of no effect of shell quality on mean evacuation voltage, there was a 4.65% probability of the results showing a difference of this magnitude *and direction*. It is more common to use two-tailed tests, and a two-tailed test in this case would have given a *p*-value of P = 0.093. This would not normally be considered a significant result.

In a separate study in which Elwood and Appel (2009) used a constant voltage of 8V, they found that crabs were more likely to evacuate the low-quality Gibbula shells (8/22 crabs evacuated) than the high-quality Littorina shells (4/33 evacuated). The *p*-value in this case was P = 0.047 (Fisher's exact test). Here too, a note of caution is appropriate, because the result is only just statistically significant.

Magee & Elwood (2016a) note a further limitation of the above approach: Gibbula and Littorina shells differ in shape, and it is impossible to be sure that the shape does not affect the transmission of electric shocks to the crab. They write: “it is possible that the wires fixed to the inner whorls of the shells to deliver the shock made closer contact with the crab’s abdomen when in the Gibbula shells than in the Littorina shells and thus the effect of the shock might have been physically greater in the Gibbula shells” (Magee & Elwood, 2016a, p. 32).

This led Magee and Elwood (2016a) to try a different approach. They asked: will hermit crabs trade-off shock avoidance with predator avoidance? In particular, will they be less likely to leave a shell when shocked, if the surrounding water contains the odour of a predator (a shore crab)? They did not find any difference in the mean voltage to evacuate between crabs exposed to this odour and crabs that were not. This can be considered an unsuccessful conceptual replication (but not a direct replication) of Appel and Elwood (2009a). What they did find, however, was that crabs exposed to an odour were substantially more likely to remain in their shells, even when given 25V shocks, than those exposed to no odour. Curiously, although an odour of a predator produced this effect, a strong, undiluted odour of a potential food source (a mussel) also produced it (Magee & Elwood, 2016a, Table 1). In this case, the *p*-values convincingly support an effect of odour on shell evacuation.

What does this mean in relation to criterion 5? The results show that decisions regarding shell evacuation are modulated by odour. They do not convincingly demonstrate a weighing of the relative value of shock avoidance against predator avoidance for two main reasons: the observation of an effect when the odour was not that of a predator, and the failure to find any trade-off between the voltage of the shock and the concentration of the odour. In sum, we can have only medium confidence that hermit crabs satisfy criterion 5 on the basis of these experiments.

Fossat and colleagues’ (2014) study of “anxiety-like” behaviour in crayfish (**P. clarkii**), mentioned in relation to criterion 4, is also relevant in this context. As explained above, crayfish were placed in a maze in which they were free to explore both light and dark arms. When electrical fields were used to induce physiological stress in the animals, they became substantially less willing to enter the light arms. This effect is clear, and we have no concerns about the statistical significance of the results. The study shows that decision-making in crayfish is stress-dependent, and that the dependency is mediated by serotonin. The effect of stress on behaviour has been replicated in follow-up studies (Fossat et al., 2015; Bacqué-Cazenave et al., 2017). A similar result was obtained in the amphipod **Gammarus fossarum** (not a decapod) by Perrot-Minnot et al. (2017).

This shows that the animal’s tolerance of a threat (exposure to light) is dependent on its internal state. A somewhat similar phenomenon has been observed in the nematode worm **Caenorhabditis elegans**, which shows greater tolerance of threats when hungry (Ghosh et al., 2016). We know from the case of C. elegans that this can be achieved by a simple mechanism in which hunger inhibits threat detection (Ghosh et al., 2016). A system that represents different needs, though not ruled out, is not required. The Fossat et al. (2014) results could be explained by a similar mechanism in which physiological stress increases sensitivity to threat, rather than by a decision-making system that weighs different needs against each other. So, while this is compelling evidence in relation to criterion 4, it is not compelling in relation to criterion 5.

3.6 Criterion 6: The animal shows flexible self-protective behaviour (e.g. wound-tending, guarding, grooming, rubbing) of a type likely to involve representing the bodily location of a noxious stimulus

CONFIDENCE LEVEL	*We have very high confidence that true crabs (infraorder Brachyura) satisfy criterion 6. We have high confidence that anomuran crabs (Anomura) satisfy the criterion. We have medium confidence that caridean shrimps (Caridea) satisfy the criterion.*
SUMMARY OF EVIDENCE	*Lines of evidence from five different studies support the hypothesis that species of true crab (infraorder Brachyura) are capable of targeting self-protective behaviours at the site of a noxious stimulus (e.g. claw, mouth, abdomen). While no single study would be fully convincing by itself, they provide good evidence when taken together. There are also credible reports of targeted grooming behaviour in hermit crabs (Anomura). Evidence of self-protective behaviour directed at the antennae in shrimps has been contested.*

Full review of evidence: As in Section 2.6, what we are looking for here is robust evidence of self-protective behaviours that go beyond reflexes: to meet this criterion, the animal should be able to vary its response in a targeted way, according to where on the body the noxious stimulus is administered.

Elwood et al. (2017) showed that applying acetic acid to the mouths of shore crabs (**Carcinus maenas**) caused the crabs to move their mouth parts, scratch at their mouth with their claws, and attempt escape significantly more than a control group. These effects were clear, with no concerns about the level of statistical significance. When the acid was applied to the eyes, the same responses were found, plus the withdrawal of the affected eye for longer than in the control group. This shows that shore crabs, when presented with a noxious stimulus at the mouth, can direct behaviours towards the mouth. What it does not show is the ability to target the response flexibly at different areas of the body.

McCambridge et al. (2016) compared edible crabs (**Cancer pagurus**) that had been manually declawed to crabs in which the autotomy (self-removal) of a cheliped had been induced. They found that manually declawed crabs were significantly more likely to touch the wound with the remaining claw or front walking legs (McCambridge et al., 2016, p. 1041). This is some evidence of wound-tending behaviour. The authors add that "although not part of the recording protocol, a number of manually declawed crabs showed a 'shudder' response when touching the wound" (McCambridge et al., 2016, p. 1042). They further add that "some manually declawed crabs shielded their wound by positioning the remaining claw in front of the wounded area" (McCambridge et al., 2016, p. 1042). No quantitative data on these observations was recorded, but they can be regarded as credible anecdotal observations from qualified experts.

Dyuizen et al. (2012) injected formalin into a cheliped (claw-bearing limb) of shore crabs (**Hemigrapsus sanguineus**) to study the effects on the nitric oxide system. They observed that active rubbing of the claw with the other claw was far more common than in crabs injected with saline solution (Mann–Whitney U-test, $P < 0.0001$). They also observed that, in the three minutes after injection, the crabs injected with formalin strongly preferred to use the uninjured cheliped when walking. The uninjured cheliped touched the ground approximately four times as often as the injured cheliped. The researchers also observed that the injured crabs "seemed to press their injured cheliped closer to the carapace compared with the intact cheliped until the end of the experiment" (Dyuizen et al., 2012, p. 2670) but no quantitative data was collected on this.

Another study of shore crabs (**H. sanguineus**) by Kotsyuba et al. (2010), also involving formalin injection, reports that "control and experimental crabs showed a sharp decrease in general activity within the first 3–5 s after injection: they came to a standstill, pressing the injured cheliped against the carapace. Later, crabs from experimental groups (formaldehyde injection) were hyperactive throughout the observation period; they made many movements of bending, unbending, and shaking the injured cheliped" (Kotsyuba et al., 2010, p. 203). No quantitative data on these behaviours were collected. The observed behaviours are not exactly the same as those reported by Dyuizen et al. (2012). Nonetheless, there is an important point of agreement: both report that shore crabs will target self-protective behaviours at the limb that is injured, rather than protecting all limbs equally.

Elwood & Appel's electric shock experiments with hermit crabs (**P. bernhardus**) have been discussed in relation to criterion 5. In two of the studies, Elwood and Appel noted one instance (in each study) of a crab grooming its abdomen after a shock. In a third (Appel & Elwood, 2009b), however, this behaviour was observed in 31/61 crabs which evacuated their shells. This is further credible observational evidence of targeted self-protective behaviour, this time in hermit crabs (Anomura). As the authors note, the dramatic difference between the studies may be explained by the use of a more effective shock procedure in the third study, but this is only a conjecture.

Diarte-Plata et al. (2012), in a study discussed above under criterion 4, investigated responses to eyestalk ablation in the caridean shrimp **Macrobrachium americanum**. Relevantly for criterion 6, they found a substantial majority of the shrimp rubbed the site of the wound, provided it was uncovered. Very few rubbed a wound that had been covered to prevent bleeding, and the difference between these groups was clearly statistically significant.

Barr et al. (2008), another study discussed above under criterion 4, applied hydrochloric acid and sodium hydroxide to one of the antennae of another species of caridean shrimp, **Palaemon elegans**, finding evidence of grooming and rubbing behaviour that was directed towards the affected antenna. They also found, unexpectedly, that the anaesthetic benzocaine also triggered grooming behaviour. However, an attempted replication by Puri & Faulkes (2010) involving three other decapod species, white shrimp (**Litopenaeus setiferus**), grass shrimp (**Palaemonetes sp.**), and Louisiana red swamp crayfish (**P. clarkii**), failed to record any grooming or rubbing behaviour in response to extreme pH.

3.7 Criterion 7: The animal shows associative learning in which noxious stimuli become associated with neutral stimuli, and/or in which novel ways of avoiding noxious stimuli are learned through reinforcement. *Note: habituation and sensitisation are not sufficient to meet this criterion.*

CONFIDENCE LEVEL	*We have high confidence that true crabs (infraorder Brachyura) satisfy criterion 7. We have medium confidence that lobsters/crayfish (infraorders Astacidea and Achelata) satisfy criterion 7.*
SUMMARY OF EVIDENCE	*Studies by Maldonado and colleagues provide convincing evidence of associative learning in the true crabs (infraorder Brachyura), although there are also some unconvincing studies and a notable null result. The study of associative learning in other decapod taxa (such as crayfish and lobsters) is at a comparatively early stage and has not yet produced compelling results. The literature highlights the challenges of developing experimental designs that rigorously distinguish associative learning from habituation and sensitisation.*

Full review of evidence: As in Section 2.6, what we are looking for here is robust evidence that the animal is able to form associations between noxious stimuli and neutral stimuli by, for example, learning to associate a particular place, or an otherwise neutral odour, with a noxious stimulus. We are also looking for evidence that an animal can learn a novel behaviour (distinct from any pre-existing reflex responses) that allows it to avoid a noxious stimulus. For discussion of why habituation and sensitization are not enough, and for discussion of which particular forms of associative learning may be most strongly indicative of sentience, see Section 2.6.

True crabs (infraorder Brachyura). First, some evidence that is not convincing. Dunn and Barnes (1981a) claimed to have shown that *decerebrate* shore crabs (**C. maenas**), in which the brain was separated from the thoracic nervous system, could still learn to hold the leg up to avoid electric shocks using the thoracic nervous system alone. If reliable, this would cast doubt on the wisdom of criterion 7, since it would show that avoidance learning is achievable without a brain. However, these results should not be considered reliable for two main reasons. First, Dunn & Barnes excluded 40% of their data from the analysis because "when data from all experiments were included, no significant differences between experimental (P) and control (R) animals emerged" (Dunn & Barnes, 1981a, p. 72). Second, Dunn and Barnes calculated twenty separate *p*-values for separate minutes of the experiment, in order to obtain three that were just statistically significant (0.04) and one that was under 0.01 (Dunn & Barnes, 1981a, pp. 73-4). In these respects, the work does not meet today's scientific standards for statistical analysis. A follow-up study (Dunn & Barnes, 1981b) used a problematic control procedure, and a similar investigation of decerebrate ghost crabs (**Ocypode ceratophthalm**) by Hoyle (1976) did not attempt statistical analysis ("the differences among individuals were enormous, ranging from one-trial learners to completely erratic ones, and so great as to make lumped data … of little meaning. ... Accordingly, in this paper individual results for selected animals are presented", Hoyle, 1976, p. 151). We consider it unlikely that these studies would pass peer review today.

In a study by Punzo (1983) of shock-avoidance learning in intact (i.e. non-decerebrate) mud crabs (**Eurypanopeus depressus**), the ten experimental animals appeared to learn swiftly and reliably to hold their leg out of the water and retained this behaviour after an hour. However, the control procedures used in this study are not clearly described, making it difficult to distinguish between effects due to learning and effects due to the shock itself.

In the late 1980s, Abramson, Feinman and collaborators investigated associative learning using the eye withdrawal reflex of the shore crab **C. maenas** (Abramson and Feinman 1987, 1988; Abramson et al., 1988; Feinman et al., 1990). In Abramson and Feinman (1988), a vibration to the carapace (presumed neutral) was paired with a puff of air aimed at the eye (presumed aversive). Experimental crabs were significantly more likely to retract the eye in response to a vibration alone compared to control crabs. The same conditioned response was found in an avoidance learning procedure, where retracting the eye prevented the air puff altogether (Abramson et al., 1988). One caveat is that it is surprising that the carapace vibration is described as a neutral stimulus, when it may be aversive. The results could be explained by the sensitisation effect of a doubly aversive stimulus (carapace vibration plus air puff).

In three notable studies, a team led by Hector Maldonado at the Universidad de Buenos Aires explored associative learning in the crab **N. granulatus**. In the first, Denti et al. (1988) showed that crabs which received an electric shock in a light chamber would subsequently take longer to enter that chamber from a dark chamber. The precise *p*-value is not stated (only that $P < 0.05$). It is hard to rule out the possibility that physiologically stressed crabs are less likely to explore a light chamber (see criterion 5, above), so this is not by itself a compelling demonstration of avoidance learning (a point made by Magee & Elwood, 2013). A second study by the same lab (Fernandez-Duque et al., 1992) sought to rule out the alternative explanation by using "yoked" control crabs who remained in the dark chamber but received exactly the same shocks as the crabs in the light chamber. The crabs shocked in the light chamber subsequently (after an interval between trials of 24 hrs) took significantly longer to enter it again than the yoked controls. In the third study, Dimant and Maldonado (1992) obtained similar results using food (a positive reinforcer) in place of electric shocks (a negative reinforcer): crabs which encountered food in the light chamber were significantly quicker to enter it after 24 hours, compared with controls which had received the same amount of food in the dark chamber. Magee and Elwood (2013, p. 354) remark, critically, that "shock increased a natural reluctance to enter the light area, but the results could be explained by an inhibition of walking rather than learning" (p. 354). However, this does not explain the difference between the experimental animals and yoked controls in Fernandez-Duque et al. (1992), or the difference between crabs fed in different chambers in Dimant and Maldonado (1992). Taken together, these studies provide good evidence of associative learning in **N. granulatus**.

Orlosk et al. (2011) attempted to train shore crabs (**C. maenas**) to associate light with food, and to search for food within a light beam shone on an arbitrary location. They report that 21/30 crabs were successfully trained. However, this study appears not to have used any control group or made any attempt to rule out the alternative explanation that habituation rather than associative learning was responsible for overriding the crabs' aversion to light.

Magee and Elwood (2013) asked whether shore crabs (**C. maenas**) could learn to avoid a shelter in which shocks were administered (a "shock-shelter"), in a setup where the crabs faced a choice between two shelters. They found no evidence of crabs avoiding the shock-shelter after one shock. However, they did find statistically significant increases in the number of shocked crabs switching shelter after 5 of the subsequent 10 trials. Curiously, crabs which received two shocks in the first two trials were no more likely to switch shelters in the third trial than crabs which had received one shock trial and one non-shock trial.

A limitation of this experimental design, highlighted by Magee and Elwood (2016b), is that crabs could move between shelters within a trial. This makes it hard to rule out an alternative explanation on which the crabs tended to return to the shelter they most recently encountered but would often move from the shock-shelter to the non-shock-shelter within a trial after a shock. Magee and Elwood (2013, p. 357) argue that this design is better than the latency-based design of Maldonado and colleagues, since a forced choice between shelters cannot be influenced by a general reduction in activity. Yet there is also a downside: because the crabs are free to move between shelters, it is impossible to compare a test group and a control group that have encountered exactly the same stimuli.

Magee and Elwood (2016b) sought to overcome this drawback of their (2013) design with a setup in which the test chamber (which still contained two shelters) was partitioned by an opaque screen. During training, the crabs (again **C. maenas**) were placed on either side of the partition in alternate trials, so that in each trial only one shelter was available to them. One shelter was randomly selected as the shock-shelter. Magee and Elwood asked: will this training, in which the shock- and non-shock-shelters were experienced sequentially, lead to the crabs avoiding the shock-shelter later on, when given a free choice of shelters (with the shocking mechanism now switched off)?

This setup had the potential to provide rigorous evidence of avoidance learning, but the key result was a null result: when given a free choice, 36/66 initially chose the former non-shock-shelter and 30/66 chose the former shock-shelter, which was not a statistically significant difference (Magee & Elwood, 2016b, p. 885). Offered the same choice again, 29/61 chose the former non-shock-shelter and 32/61 chose the former shock-shelter, which was again not a statistically significant difference (Magee & Elwood, 2016b, p. 885). There were also no significant differences between the test and control groups (Magee & Elwood, 2016b, p. 885).

A null result in this setup does not provide strong evidence *against* a basic capacity for avoidance learning, because the task was relatively difficult. Evidence from honey bees (**Apis mellifera**) suggests that learning from sequential stimuli is harder than learning from simultaneous stimuli (Dyer & Neumayer, 2005). Moreover, the crabs would have needed to form a memory of where they received a shock and apply that memory in a new context (with no partition in the chamber).

Taken together, the above experiments show the great challenges involved in rigorously demonstrating associative learning in any animal, but they also show ingenious ways of overcoming those challenges. The studies by the Maldonado group in the 1980s and 1990s provide good evidence of associative learning in **C. granulatus**, and those by the Elwood group in the 2010s provide some further positive evidence of associative learning in **C. maenas**, as well as a notable null result. This null result does not substantially undermine the earlier positive results due to the greater difficulty of the task. It is worth noting (in relation to the discussion in Section 1.6) that the type of associative learning being investigated in these studies is instrumental learning, and there is some evidence for a particularly close link between this form of learning and sentience (Skora et al. 2021).

Other decapods. Fine-Levy et al. (1988) investigated associative learning in spiny lobsters (**P. argus**). They asked: can the animal learn to associate an initially attractive odour (shrimp) with an aversive stimulus (a “pseudopredator” - a dark, fast-approaching object), so that the odour triggers avoidance behaviours? They found evidence of conditioning of grabbing, searching and active avoidance behaviours. One caveat about this study is that 14 different behaviours were separately analysed, with only 5/14 behaviours showing statistically significant evidence of conditioning. Another is that the analysis compares pre- and post-conditioning animals, rather than comparing conditioned animals with controls. It is not clear that the experimental animals displayed these behaviours to a significantly greater degree than controls.

Kawai et al. (2004) explored associative learning in crayfish (**P. clarkii**). They asked whether crayfish could learn to avoid mild (6.5V) electric shocks by walking from one compartment to another when a warning light was displayed. The crayfish showed significantly increased responsiveness to the warning light over many repeated trials (20 trials a day for 32 days). It is hard to rule out explanations here that appeal to sensitisation, especially given the very large number of shocks involved. Kawai et al. (2004) attempted to rule out this explanation with follow-up experiments but did not compare the test group to a yoked control group that experienced exactly the same number of shocks unpaired with a light.

Bhimani and Huber (2016) studied the crayfish **O. rusticus**. They improved on the Kawai et al. (2004) by using yoked controls, which received exactly the same mild (6V) electric shocks as the animals presented with the avoidance learning task (these animals are known in the literature as “masters”). The masters received a shock whenever they entered a specific area of an arena, marked out with a distinctive (hard or soft) substrate. The very clear result was that the masters soon started avoiding the shock-inducing substrate, whereas the yoked controls continued to explore the whole arena. Is this avoidance learning? An alternative explanation is that the shocks triggered an escape response followed by a period of slowed motion, a period which inevitably tended to occur on the non-shock substrate. To rule this out, it is crucial to test the trained crayfish on a new arena with the shocks switched off and the substrates differently positioned. As the authors note: “A possible alternate explanation may arise when master individuals simply slow their movements in safe quadrants as a case of negative electrostimulation taxis. Assessing the validity of this explanation will require further characterization of movement patterns for trained individuals utilising a rotated arena and the absence of shock” (Bhimani & Huber, 2016, p. 245). This follow-up study has not been carried out. Datta et al. (2018) used a similar experimental design with positive reinforcement (a dose of amphetamines) in place of electric shock, but this study is subject to broadly the same limitations.

Tomina and Takahata (2010) tested whether lobsters (**H. americanus**) could learn to grip a sensor to access food. The use of positive reinforcement (food) makes the work less directly relevant to questions of sentience (as defined in Part I) but not irrelevant. The master group of four lobsters showed a significant increase in gripping behaviour after training (relative to before training), and a group of four yoked controls did not. However, there appears to have been no direct statistical comparison of the master group with the control group.

In sum, there has been substantially less work on associative learning in other decapods, in comparison with the true crabs. The evidence that exists does not yet allow high confidence that lobsters and crayfish learn associatively, though it does allow medium confidence. Associative learning in other decapods should be regarded as a plausible, largely unexplored possibility.

Several other studies were also examined in relation to criterion 7 but were judged to offer insufficiently relevant and/or insufficiently significant evidence to merit detailed discussion: Stafstrom and Gerstein (1977), Wight et al. (1990), Abramson and Feinman (1990), Hermitte and Maldonado (1991), Panksepp and Huber (2004); Nathaniel et al. (2010); Tierney and Lee (2011).

3.8 Criterion 8: The animal shows that it values a putative analgesic or anaesthetic when injured in one or more of the following ways: (a) the animal learns to self-administer putative analgesics or anaesthetics when injured; or (b) the animal learns to prefer, when injured, a location at which analgesics or anaesthetics can be accessed; or (c) the animal prioritises obtaining these compounds over other needs (such as food) when injured

CONFIDENCE LEVEL	*Our confidence level is very low for all infraorders, because we have found no evidence either way.*
SUMMARY OF EVIDENCE	*We have found no evidence either for or against the claim that any decapod satisfies criterion 8.*

Full review of evidence: There is no evidence to review in this case. This criterion is an obvious evidence gap (see Part V) and an important direction for future research. We note that the self-administration procedure developed by Datta et al. (2018) for the self-administration of amphetamines in crayfish (O. rusticus) may provide a promising way to investigate criterion 8a in the future. The evidence reviewed in Sections 2.4, 2.8 and 3.4 suggests that lidocaine would be a particularly promising local anaesthetic to investigate in relation to 8a.

PART IV. WELFARE RISKS OF COMMERCIAL PRACTICES: CEPHALOPODS

Our aim in this section is not to provide a comprehensive guide to good practice for safeguarding the welfare of cephalopods. Our focus here will be on specific practices that potentially create a risk of poor welfare. We will consider what the existing literature can tell us about the welfare implications of these practices, and we will also highlight places in which there are evidence gaps.

4.1 Cephalopods in sea fisheries

SUMMARY OF EVIDENCE

Wild-caught cephalopods, if not dead already, usually die soon after being taken from the water, with significant welfare risks due to physical trauma and asphyxiation. The welfare issues are similar to those arising for wild-caught fish. There is no easy way to mitigate these risks, but codes of best practice should be developed for those cases in which cephalopods are caught alive.

There are several inshore cephalopod fisheries in the UK that target octopus, cuttlefish, and squid species (**Table 4**) (Pierce et al., 2010). Capture methods vary across fisheries and include netting, trapping, and dredging techniques. Unlike decapod fisheries, captured cephalopods are not transported alive and thus welfare risks for live maintenance and captivity are not considered here. This section will instead focus on the welfare risks that might arise from the point of capture to landing. Currently, there is limited scientific literature that explicitly identifies the welfare implications of commercial practices in cephalopod fisheries. Consequently, the welfare risks discussed in this section are largely based on capture, handling and transport data from studies that have captured cephalopods for scientific purposes.

Squid are caught using trawls, driftnets, and seine nets. Hand-jigging is also commonly used in squid fisheries (Pierce et al., 2010). We note that a substantial fraction of UK squid fishing occurs off the Falkland Islands. Squid caught in nets are generally dead when bought abroad, whereas squid caught through jigs are alive. Hand-jigs are considered the most humane live-capture method for squid but may not be appropriate for all species (Pierce et al., 2010). Jigging is also selective in the size range of animals captured (Rathjen, 1991), reducing the need to discard undersized animals.

Table 4: Landed cephalopod species and specific fishing gears used in inshore fisheries within the UK. *Sources: Pierce et al. (2010) and industry sources.*

Fishing method	Species
Gillnets, trammelnets, trawlnets	Sepia officinalis
Driftnets	Loligo vulgaris, Loligo forbesi
Inshore trawlnets	Loligo vulgaris, Loligo forbesi
Dredges	Octopus vulgaris, Eledone cirrhosa, Sepia officinalis, Loligo vulgaris, Loligo forbesi
Pots	Octopus vulgaris, Eledone cirrhosa, Sepia officinalis, Loligo vulgaris, Loligo forbesi
Devon spinners	Octopus vulgaris, Eledone cirrhosa
Hand-jigs	Loligo vulgaris, Loligo forbesi
Scottish fly-seine	Loligo vulgaris, Loligo forbes, Illex coindetii, Todaropsis eblanae, Todarodes sagittatus
Purse-seine	Illex coindetii, Todaropsis eblanae, Todarodes sagittatus

Octopus and cuttlefish are primarily caught using trawls, pots, and traps (Pierce et al., 2010). Cuttlefish can also be caught using nets (i.e. gillnets and trammelnets) and octopus can be caught as by-catch in pots and traps. Trawled or netted cephalopods are usually brought aboard the vessel dead or nearing death, whereas trapped animals are caught alive (industry sources). Dredging has also been used as a capture method for octopus, cuttlefish, and squid. Trawling and dredging are the most environmentally destructive methods and, in some instances, due to small size, undersized cephalopods are discarded and wasted (Pierce et al., 2010).

The following section will discuss the potential welfare risks associated with the different capture methods.

Physical trauma. Capture techniques can result in physical trauma to cephalopods. Specifically, physical trauma might arise from rough handling, causing the mantle to detach from the head of the animal (A. K. Schnell, personal observation). Raising benthic species too quickly can lead to buoyancy malfunction due to rapid changes in pressure (Forsythe et al., 1991; McDonald, 2011; Sherrill et al., 2000). However, unlike the swim bladder of fish, the buoyancy device in cuttlefish is unpressurized, so the volume is not markedly altered as the animal changes depth (Denton & Taylor, 1964; Sherrard, 2000). Nevertheless, rapid vertical movement may cause air to be trapped inside the mantle cavity (A. K. Schnell, personal observation) resulting in potential discomfort or pain.

During capture methods that involve nets, individuals might be pursued to exhaustion and then suffocate and become crushed under the weight of other animals. However, further research is required to determine the severity of this risk. Finally, collision with other animals or the side of the net routinely causes skin damage (Boyle, 2010). Cephalopods have soft skin and are particularly susceptible to skin ulcerations and fin injuries (i.e. specific to cuttlefishes and squids as octopuses do not have fins) that can result in permanent damage. These injuries encourage bacterial growth (Gestal et al., 2019) and can lead to disease or death (Hanlon et al., 1984; Boyle, 2010; Gestal et al., 2019).

Skin and fin injuries become a welfare concern if (i) individuals are left in nets for hours or days prior to landing and if (ii) undersized live animals are released back into the water with injuries. Skin plays a vital role in the survival of cephalopods as they use body patterns for both concealment and communication (Hanlon & Messenger, 2018). Moreover, research shows that minor injuries in squid increases risk of predation (Crook et al., 2014) and squid with skin and fin injuries do not respond favourably to changes in temperature and salinity compared to uninjured squid (Hanlon et al., 1983). The use of soft netting material or alternative capture methods (i.e. traps or jigging) might decrease some of the risks of physical trauma involved in netting capture methods (Iglesias et al., 2007), but this has not been systematically tested.

Aggression and cannibalism. Except for a few species, both octopods and cuttlefish are relatively solitary animals. Confinement within a small space, such as a pot or a trap, might not only cause stress but also result in fighting between individuals. Indeed, limb amputation is commonly observed in wild caught octopuses (Florini et al., 2011), which might be a result of autophagy/auto-mutilation (Budelmann, 1998; Reimschuessel & Stoskopf, 1990) or could be a product of fighting. Another risk is that all coleoid cephalopod groups have cannibalistic tendencies, particularly between individuals that are not size-matched and when insufficient food is provided (Aguado-Gimémenz & Garcia Garcia, 2002; Budelmann, 2010; Hayter, 2005; Ibáñez & Keyl, 2010; Jacquet et al., 2019; Moltschaniwskyj et al., 2007; Pierce et al., 2010).

Consequently, fisheries that include traps or pots to detain live individuals together should ensure that their devices are large enough for the species in question, baited with sufficient prey to sustain the total amount of captive individuals and frequently checked. Leaving the devices *in situ* for several days can cause discomfort, stress, and even death, as the confined space can provoke trapped animals to fight or eat each other.

Exposure to inappropriate salinity and temperatures. Cephalopods are highly stenohaline and stenotherm (Fiorito et al., 2015), meaning that they cannot tolerate a wide fluctuation in the salinity and temperature of the water (Moltschaniwskyj et al., 2007). Even in adults, changes in salinity, in particular, can result in visual indicators of stress or discomfort such as blanching of the skin and excessive inking and can lead to death (A. K. Schnell, personal observation). This underlines the point that commercial devices that trap live individuals (i.e. pots) should be frequently checked, especially during periods when sheltered inshore sites are susceptible to weather variations such as excessive rainfall.

Slaughter methods. Trawled or netted animals are usually brought aboard dead, whereas trapped or jigged animals are often alive (industry sources). If the animal is still alive, the animals die from asphyxiation prior to being iced. Asphyxiation is a welfare concern. Anecdotal evidence suggests that inhumane methods are sometimes used on European fishing vessels, such as clubbing, slicing the brain and reversing the mantle (Pereira & Lourenco, 2014).

However, there is currently an evidence gap about humane slaughter methods that are commercially practical and available. There are efforts to improve and standardise euthanasia in captive cephalopods used for scientific experiments (Andrews et al., 2013; Butler-Struben et al., 2018; Fiorito et al., 2015). These methods, however, are inappropriate for commercial practices because they often involve an overdose of anaesthetic (typically ethanol) that is not suitable for human consumption. Furthermore, mechanical methods that do not involve contamination, such as cutting or puncturing of the brain, require skilled handlers and are inefficient for large scale practices. Further research is needed to determine the most optimal slaughter methods for commercial cephalopod fisheries that expose the animal to the minimum amount of pain and distress.

The Association for Cephalopod Research (CephRes) is currently proposing to undertake such a project, which will evaluate different stunning methods in cephalopods for fisheries throughout the EU.

We have been unable to find any codes of best practice or voluntary guidelines that are specific to cephalopod fisheries. Even though cephalopods are often caught as by-catch, it would be sensible to develop codes of best practice for circumstances in which cephalopods are alive at the point of being caught.

4.2 Cephalopods in aquaculture

SUMMARY OF EVIDENCE

Although there is no cephalopod farming in the UK, there is some interest in it elsewhere in the world. However, cephalopods are typically solitary animals that are often aggressive towards each other in confined spaces, and there is no reliably humane slaughter method that could be performed commercially on a large scale. We have very high confidence that high-welfare commercial farming of cephalopods is currently impossible.

Although there is currently no cephalopod aquaculture taking place within the UK, we think it is worth discussing here. If large-scale cephalopod farming is developed elsewhere in the world, a question will arise as to whether the UK should allow imports of these products.

Globally, cephalopod aquaculture is currently small-scale and for few species (O'Brien et al., 2018). However, farms can be found in Europe, Australia, Latin America and Asia (Jacquet et al., 2019). Cephalopods are sometimes suggested as an attractive candidate for large-scale commercial aquaculture, due to increasing demand for cephalopod consumption, their high value, fast growth, high food conversion rate, high protein content and high fecundity (Pierce et al., 2010). **S. officinalis** and **O. vulgaris** have been described as promising candidates for commercial aquaculture in Europe, and some progress has been made in farming **O. vulgaris** in Spain.

Another commercial use of cephalopods is within the captive animal industry (zoos and aquaria). Cephalopods are usually housed in small numbers, with strict welfare requirements for accreditation in cases where the zoo or aquarium is accredited by BIAZA or another zoo association. We will focus here on the welfare issues raised by aquaculture of cephalopods for commercial purposes.

Hatchling mortality. One of the currently limiting issues in captive management of cephalopods is hatchling mortality. As well as limiting the viability of cephalopod farming, this can also be a welfare issue. For **O. vulgaris**, survival rates are at best around 30-40% at day 40 (Iglesias et al., 2007) and <10% by day 60 (Vaz-Pires et al., 2004). This is primarily due to problems with temperature, water quality, and nutrition (Boyle, 2010; Navarro et al., 2014; Vaz-Pires et al., 2004). Young require a large amount of live food (larval shrimp and other crustacea), which can be difficult to obtain (Iglesias et al., 2007; Pierce et al., 2010). Young animals dying of poor nutrition and inappropriate housing conditions are highly likely to suffer.

Capture and transport. As captive breeding efforts and rearing of young are often not successful, cephalopod aquaculture often takes the form of 'ranching' or 'rearing', in which young animals are captured and grown in captive tanks for eventual sale. Cephalopods in aquaria are also often wild-caught. Current guidelines appear to be based primarily on anecdotal evidence or on those developed for fish (e.g. Fiorito et al., 2015), and we have not found any studies explicitly assessing the different capture and transport methods for cephalopods, in terms of their welfare impact. We note that there is currently a working group through FELASA (Federation of European Laboratory Animal Science Associations) looking to provide a set of best-practice capture and transport guidelines appropriate to cephalopods.

As noted above (see Section 4.1), many capture techniques can be harmful to cephalopods. Transport can also be harmful. Cephalopods require highly oxygenated water, and prolonged transport can result in lowering oxygen and increasing nitrates. An air stone or aerator should be used when necessary (Fiorito et al., 2015; Iglesias et al., 2007; McDonald, 2011). Additionally, if the animals ink in the water and it is not subsequently cleaned (or the animal transferred), the ink can coat the gills and cause asphyxiation (Hayter, 2005; McDonald, 2011). Several species of octopus show stress-related biomarkers after trawl-catch such as immune system compromise, but typically show recovery within 24 hours (Barragán-Méndez et al., 2019). Some species appear more suited than others to these processes – for example, **O. vulgaris** and **S. officinalis** show some resistance to stress from handling and transport (Cooke et al., 2019, Vaz-Pires et al., 2004).

Poor nutrition. Poor nutrition is one of the primary problems in the establishment of large-scale aquaculture, as the animals are carnivorous and typically require live prey (Boyle, 2010; Navarro et al., 2014; Pierce et al., 2010). Although there is work on developing suitable alternatives, none has been successful enough for widespread use (Pierce et al., 2010). As it stands, there is insufficient understanding of the metabolism and nutritional needs to be able to formulate complete diets (O'Brien et al., 2018). Animals which fail to thrive on food sources provided will experience a range of welfare harms, such as hunger and nutritional and metabolic diseases.

Lack of cognitive stimulation. As well as concerns for physical health, there is also the potential for very poor psychological welfare for captive cephalopods, due to their behavioural and cognitive complexity (Cooke & Tonkins, 2015; Jacquet et al., 2019). Jacquet et al. (2019) are concerned about lack of cognitive stimulation for farmed octopus. They worry that the "tightly controlled and monotonous environments" typical of farming would not allow for the cognitive stimulation, exploration and environmental control necessary for psychological welfare. Cephalopods regularly show signs of stress in poor captive environments, such as irregular swimming patterns, depression, agitation and anorexia (McDonald, 2011) and stress can even result in autophagy (consumption of own limbs) (Hayter, 2005).

Lack of shelter. Cephalopods are soft-bodied and vulnerable to predators in the wild, typically using shelter and rapid retreat strategies when feeling threatened (Cooke & Tonkins, 2015), both of which could be restricted in captive settings. This can result in fear and stress, and animals without sufficient shelter can show depression and anorexia (Sherrill et al., 2000). It is thus important that animals are provided with ample hiding places, which will take the form of shelters/caves for octopods (Vaz-Pires et al., 2004), and for cuttlefish as either soft sand substrate in which to bury themselves, or environmental features that allow camouflage, such as artificial seaweed or even patterned wall coverings (Cooke et al., 2019; Tonkin et al., 2015). As squid are pelagic, they do not require shelter, but require more tank volume so as to prevent injury from jetting (Boyle, 2010).

Skin injury. A common startle or fear response for cephalopods, particularly cuttlefish and squid, is high speed 'jetting' away from the threat. In captivity, this frequently results in collision with tank sides and/or furniture and injury to the soft skin. These lesions often heal poorly, becoming infected, and can cause permanent damage, spread of infection to other tissues, and death (Cooke & Tonkins, 2015; Hanley et al., 1998; Sherrill et al., 2000). Cuttlefish can even fracture the cuttlebone (McDonald, 2011). Incidence of jetting can be reduced through provision of ample hiding places, visual barriers, and careful husbandry to ensure animals are not startled (McDonald, 2011). Injury can be minimised through use of rounded tanks, containing no rough surfaces or sharp objects (Fiorito et al., 2015, Slater & Buttling, 2011).

Inappropriate housing. The primary determinant of cephalopod health and welfare is the quality of the water they are housed in. Cephalopods are not very adaptable to changes in water conditions, and require strict monitoring of levels of oxygen, pH, CO_2, nitrates and salinity to ensure they stay within acceptable ranges, as well as rapid removal of ink when needed (Cooke et al., 2019; Fiorito et al., 2015; Hayter, 2005, McDonald, 2011; Sykes et al., 2012; Vaz-Pires et al., 2004). Poor water quality can result in poor health, infections, respiratory issues, agitation, increased incidence of inking and jetting, and death (Fiorito et al., 2015; Hanley et al., 1998; Hayter, 2005).

Other aspects of housing such as lighting, temperature and incidence of noise and vibrations, can impact welfare (Fiorito et al., 2015; Hayter, 2005). Cephalopods have different sensory abilities than our own, such as an ability to see polarised light, mechanoreception, and chemosensory, which will lead to corresponding unique environmental requirements we may not otherwise consider (Browning, 2019; Cooke et al., 2019). Temperature appears particularly important, as temperature will impact feeding, growth and lifespan (Aguado-Giménez & García García, 2002; Sherrill et al., 2000).

It is also important to house animals in appropriately sized social groups. Many species of cephalopod are solitary and should be housed individually, otherwise crowding can cause aggression and cannibalism (Aguado-Giménez & García García, 2002; Budelmann, 2010; Hayter, 2005; Jacquet et al., 2019; Pierce et al., 2010). Additionally, crowding can increase stress and decrease time spent resting and feeding (Cooke et al., 2019). The exceptions to this are some species of cuttlefish, which live in pairs (McDonald, 2011), and social squids which should be kept in groups (Fiorito et al., 2015).

Disease. Some of the factors already mentioned, such as stress, poor water quality and poor nutrition, can lead to disease. Stressed animals have compromised immune systems, which can lead to bacterial, viral, and fungal infections (McDonald, 2011, Sherrill et al., 2000). The cephalopod immune system is not well understood (O'Brien et al., 2018; Sykes & Gestal, 2014). Viruses are rare; bacterial infections are most common in skin lesions (as above), and gills (Fiorito et al., 2015; Sykes & Gestal 2014). Parasites are common in wild animals and can appear in captive stocks if live prey are used (Sykes & Gestal, 2014). UV sterilisation of water can help decrease presence of pathogens (Hanley et al., 1998). A lack of current knowledge of cephalopod analgesia and anaesthesia could also cause welfare concern when animals are injured or need to undergo medical procedures (Fiorito et al., 2015).

Slaughter methods. Currently, the only recommended method of humane slaughter for cephalopods is terminal overdose of anaesthetic, often followed by decerebration (Andrews et al., 2013; Boyle, 2010; Fiorito et al., 2015). However, this would be inappropriate for cephalopods slaughtered for human consumption. Mechanical slaughter involves cutting or puncturing the brain and requires careful and skilled operators to ensure it is performed correctly (Andrews et al., 2013; Boyle, 2010; Fiorito et al., 2015). This seems unlikely to be commercially viable on a large scale. There is, at present, no way for the commercial farming of cephalopods to use reliably humane slaughter methods. However, the same issue arises for cephalopods caught from the wild (see Section 4.1).

PART V. WELFARE RISKS OF COMMERCIAL PRACTICES: DECAPODS

As in Part IV, our aim is not to provide a comprehensive guide to good practice for safeguarding the welfare of decapods. Our focus here will be on specific practices that potentially create a risk of poor welfare. We will consider what the existing literature can tell us about the welfare implications of these practices, and we will also highlight places in which there are evidence gaps.

5.1 Handling during capture, transport, and sale

SUMMARY OF EVIDENCE

We have high confidence that declawing (removing one or both of the claws from a crab before returning it back to the water) causes suffering in crabs. We also have high confidence that the practice of nicking (cutting the tendon of a crab's claw) causes suffering and is a health risk. We have very high confidence that good welfare during transport and storage requires an appropriate stocking density, access to dark shelters and cool temperatures (for damp storage, no more than 8°C). Live, imported decapods can be ordered from online retailers, and we have very high confidence that this practice inherently creates a risk of poor handling and inappropriate slaughter methods.

Accidental injury. It is generally in the interests of the fishing industry to avoid damaging the decapods they catch, with intact animals fetching a much higher value than injured ones would, especially in larger species. Therefore, careful handling of decapods is already emphasised as good practice in industry guidance (e.g. Jacklin & Combes, 2005). Risk of physical damage is greater for catches that are intended for markets with less emphasis on the quality of individual animals, such as trawl caught species. Accidental physical injuries to decapods include cracked carapaces, damaged antennae, and loss of limbs. Haemolymph can rapidly leak from cracks, killing the animal. In species intended for relatively prolonged live storage or transport, industry guidance recommends that animals are carefully inspected, and those with damaged limbs should be prompted to cast off the limbs via autotomy (Jacklin & Combes, 2005). It is unclear what the relative welfare impact of external injury versus autotomy is to decapods, but risk of infection or rapid death is lessened with autotomy.

The risk of accidental injury can be reduced by refined capture methods. For example, brown crabs (**C. pagurus**) tend to cling to netting within the creels that are commonly used to capture them, so removing them from the creels can inadvertently tear the limbs. Smooth plastic inserts in the base of the creel may help reduce this (Jacklin & Combes, 2005). Lobsters (**H. americanus**) captured from deeper waters, and at commercial haulage speeds, were significantly more likely to show physiological stress and post-capture bacterial infection than those caught in shallower waters or at slower haulage speeds (Basti et al., 2010). The authors suggested that this could be due to rapid pressure decompression together with exhaustion from repeated tail-flipping during rapid haulage from deep waters. Onboard storage in recirculating seawater, rather than in damp storage, seemed to help lobsters partially recover from the effects of haulage. Assuming that commercial haulage speeds, and the depths at which lobsters are caught, cannot be reduced, recovery in recirculating seawater is therefore recommended (Basti et al., 2010). Langoustine (**Nephrops norvegicus**) are an example of a decapod species that can be caught via creels or trawling, and trawling has been shown to cause greater physiological stress, mortality and physical damage than creels (Ridgway et al., 2006; Albalat 2009). The same studies also showed that physical damage and mortality is more likely to occur during longer trawls, with season and time of day having additional effects. Similar results were found for shrimps (**Pandalus borealis**), with longer trawl times increasing mortality rates (Larssen et al., 2013). Trawling therefore poses a higher welfare risk to decapod species that are caught using this method, compared with creel catching, and there is already a wider discussion about the economic and environmental effects of trawling for decapods (e.g. Williams & Carpenter, 2016).

During transport and storage, the containers that the animals are held in can help reduce physical injury if well designed and species-appropriate. They should be resistant to crushing, should not allow limbs to become caught, should not contain so many animals that the animals below are crushed by the weight of the animals on top of them. Crabs (**C. pagurus**) transported from the UK to Portugal at the bottom of a vivier tank had more missing legs and claws than those transported at the top of the tank did, and they died sooner after arrival (Barrento et al., 2010). However, after 4 days in a recovery tank, similar overall mortality rates were seen in crabs from both transport positions. When lobsters are stored onboard in totes, they should be packed with their tails curled under them to protect their ventral surface from puncture, should face in the same direction, and be at a density that aids stability, but without pressing the animals too tightly together (Basti et al., 2010).

At all stages, handling of decapods should be careful and kept to a minium because it causes physiological stress (Jacklin & Coombes, 2005). If decapods are 'thrown' (e.g. Barrento et al., 2008) or 'tossed' (Lavallee et al., 2000) into containers, there is an increased risk of physical injury and loss of vigor compared with more careful placement. Careless and rough handling is a welfare risk and should be avoided.

Declawing. Declawing is the practice of removing one or both of the claws from a decapod. As discussed in Part III, McCambridge et al. (2016) found evidence that declawed crabs will tend their wound, shield it, and in some cases display a "shudder" response. They also found that declawed crabs are at a competitive disadvantage in contests with other crabs and are unlikely to mate. Duermit et al. (2015) found that declawed stone crabs (**Menippe** species) were less able to access one of their main food sources, bivalves. If the wound was greater than 7mm, the crabs died within days. A study by Patterson et al. (2009) showed that, even if a claw is removed through induced autotomy (self-removal) rather than through wounding, the ability of crabs (**C. pagurus**) to feed on bivalves was reduced. Another, by Patterson et al. (2007), showed that declawing produces a physiological stress response in **C. pagurus** (as indicated by glucose and lactate in the haemolymph) for at least 24 hours after the injury, and that the stress response is more severe for manual declawing than for induced autotomy.

Taken together with the evidence reviewed in Part III, it is reasonable to conclude (with high confidence) that the declawing of true crabs (infraorder Brachyura) causes suffering. Various shellfish industry representatives have told us that declawing is already frowned upon in the UK. The practice was banned in the UK from 1986 until 2000 (under S.I. 1986/496, The Crab Claws (Prohibition of Landing) Order 1986). In 2000, the relevant legislation was revoked (under S.I. 2000/1235, The Crab Claws (Prohibition of Landing) (Revocation) Order 2000), having been overridden by a European Union regulation (No 850/98), which allows 1% by weight of a catch of edible crabs (made by pots or creels) to consist of detached crab claws. Reinstating the ban on declawing in the UK would be an easy, low-cost intervention to improve the welfare of decapods.

Disabling of pincers (including nicking). Decapod pincers or large claws usually require disabling in some way, both to prevent injury to human handlers and to prevent injury to other animals sharing the same container.

For clawed lobsters, the usual method is to restrain the claws using elastic bands or cable ties (Jacklin & Combes, 2005). In American lobsters (**H. americanus**) with banded claws, recovery of haemolymph parameters after airfreighting on ice packs was compared between individuals with the bands versus individuals with their bands removed (Coppola et al., 2019). Those with the bands removed were placed into individual tanks to prevent fighting, so the effect of social condition differed as well as claw restraint. The recovery rate for almost all parameters, including glucose and lactate concentrations, was similar between the claw/social conditions. The one exception was that, although calcium initially decreased similarly in both groups, it increased again between 12 and 36h in the socially grouped lobsters with banded claws and remained significantly higher than in the isolated lobsters with freed claws for the remainder of the 4.5-day study. The welfare implications of calcium concentration is not well understood. The fact that the calcium levels in lobsters with banded claws increased to similar values seen immediately following air-freighting could indicate a deviation from homeostasis, but it is notable that the usual haemolymph indicators of physiological stress (glucose and lactate) were not similarly affected (Coppola et al., 2019). At present, there is little conclusive evidence about whether banding of claws or social grouping compromises welfare.

For brown crabs (**C. pagurus**), banding of claws is considered unsuitable within the shellfish industry, although we have not independently verified that it is unsuitable (Jacklin & Combes, 2005; industry sources). Instead, if live storage or transportation of the crabs is necessary, the tendon connecting the two parts of each claw are cut in a procedure known as ‘nicking’. Industry sources have told us that this only happens when crabs are intended for live export. In one study, nicking elevated glucose and lactate in the haemolymph compared with non-nicked controls, and it also increased the risk of muscle necrosis and pathology (Welsh et al., 2013). A further study showed that the effect of nicking is worsened at warmer temperatures, whilst colder temperatures helped reduce the risk of physiological stress and pathology (Johnson et al., 2016). Specifically, during 4h following nicking, increases in l-lactate and decreases in pH were only observed at 12°C, not at 8 or 4°C. Mortality was also greatly increased by nicking especially at higher temperatures, with 5/6 nicked crabs dying during 14 days at 12°C, 1/6 at 8°C and none at 4°C. Only one of the non-nicked crabs died (at 12°C). Haemolymph phenoloxidase activity, which is important in immunity and wound healing, showed a similar pattern, with both nicking and higher temperatures causing significant increases over 14 days (Johnson et al., 2016).

Nicking, especially under warm conditions, poses a risk to crab health and a welfare risk. For both reasons, alternatives to nicking should be developed and implemented. In Norway, the claws of brown crabs (**C. pagurus**) are at least sometimes immobilised using elastic bands (Woll et al., 2010). In blue crabs (**Callinectes sapidus**), elastic bands can be successfully used for binding claws if a small block or dowel is first gripped between the two dactyls of each claw and then left in place (Haefner, 1971). Another solution to prevent fighting could be to use individual compartments for storing crabs, equivalent to the ‘tubes’ used for **Nephrops.**

Social stress and aggression. The Seafish code of good practice for handling crustaceans recognises that aggression and stress sometimes occurs among decapods when many animals are trapped in the same creel (Jacklin & Combes 2005). Seafish recommends the use of creels with a second chamber and with escape gaps or a large mesh net (where practical) to allow by-catch to escape.

During storage, decapods that are usually solitary in the wild, such as lobsters, can be stored within the same tank. A study by Bacqué-Cazenave et al. (2017), discussed in Part III, found evidence that being on the receiving end of social aggression leads to an “anxiety-like” state in crayfish (**P. clarkii**), characterised by high levels of serotonin, and it is reasonable to assume that social aggression will produce similar states in other decapods. Social grouping of lobsters (**H. americanus**; n = 12) with bound pincers did not cause significant increases of haemolymph glucose or lactate compared to individual holdings (Coppola et al., 2019). This could suggest that social grouping without injurious aggression may not be especially stressful for lobsters, but statistically non-significant results, such as these, do not necessarily show the absence of an effect (e.g. a different measure of stress could reveal a previously unseen difference).

Low stocking density may be important in preventing social stress, but one survey conducted in Portugal showed that stocking densities can be very high (maximum reported: 300 kg m^{-3}) and sometimes exceeded recommendations (120 kg m^{-3}; Barrento et al., 2008). Carder (2017) investigated live lobster storage conditions at nine UK food retailers and found that lobsters were stocked at densities that caused some individuals to be on top of each other in 11 of the 26 display tanks observed; indeed, in four of the tanks, there were at least two full layers of lobsters. Similarly, Crustacean Compassion (2020) reported lobsters fighting in a wholesaler display tank, and up to 50 lobsters being displayed within a single tank. High stocking densities of socially stored decapods could be a welfare risk.

Exposure to inappropriate temperatures. The thermal preferences of decapods differ between species and depend to some extent on what temperature they are acclimatised to. For most species, the upper and lower temperatures that they choose to avoid are currently unknown (Lagerspetz & Vainio, 2006). Physiological stress, disease susceptibility, and mortality is increased in decapods transported or stored at excessively warm temperatures. This can occur whenever vessel- or shore-based storage containers cannot be cooled to an optimal temperature, such as during warm weather (Lavallee et al., 2000; Jacklin & Combes, 2005). As described above, in brown crabs (**C. pagurus**), haemolymph lactate and glucose increased, and the risk of pathology and mortality increased at 12°C compared with 8°C and 4°C, especially if the crab claws were nicked (Johnson et al., 2016). Simulated transport of brown crabs at 16°C resulted in 100% mortality, whereas most survived at 12°C if immersed in good quality seawater, or at 8°C if under damp conditions (Barrento et al., 2011). Similar results were found for the same species in another study of damp storage, where crabs showed reduced vitality at temperatures of 15°C and 20°C compared with 5°C and 10°C (Woll et al., 2006). Being immersed in warmer than optimal water caused farmed Asian tiger prawns (**Penaeus monodon**) to show stress responses including reduced feeding, red colouration and altered gene expression (de la Vega et al., 2007). Notably, the same study showed that very similar responses were observed under hypoxic conditions at cooler temperatures. Shrimps (**Pandalus borealis**) in Norway that were immersed in flowing water for 48h at 2 and 5^{o}C showed over 95% survival, reducing to 70% survival at 10^{o}C, and 50% at 15^{o}C (Larsson et al., 2013). It is therefore crucial that decapods in both immersed and damp storage are kept cool, below a maximum temperature threshold appropriate for their species (Jacklin & Combes, 2005).

Even during temporary storage, such as when onboard vessels and when awaiting transfer to vehicles or specialist storage, decapods should not be exposed to sunlight or warm ambient temperatures. For example, in one study, loss of vigor was significantly greater in lobsters landed on sunny days than on cloudy days, presumably because of exposure to sunlight (Lavallee et al., 2000). UK industry representatives have reported that onboard crab and lobster catches are often covered with fabric, such as carpet, and a cool, dark, damp environment is created using a constantly running seawater hose. Capture timings are often planned to avoid the hottest parts of the day, preventing spoilage of the catch, which would also help minimise the animal welfare risk of exposure to hot weather.

As well as risk of temperatures being too hot, it is also possible that temperatures may sometimes be too cold. Ice or ice-packs are sometimes used to cool decapod environments onboard vessels and during live transport, because it reduces the activity levels of the animals and decreases their oxygen requirements, helping prolong their lives (Jacklin & Combes, 2005). In scientific research, ice is assumed to anaesthetise or numb crustaceans, often being referred to as ‘cryoanesthesia’.

Ice should not be placed in direct contact with decapods. Fishing industry reports suggest that the sudden cold can stress and even kill many decapod species from UK waters (Jacklin & Combes, 2005). In some countries, including Italy and Switzerland, the displaying and transport of live crustaceans on ice or in icy water has been made illegal. Most decapods do not inhabit polar regions (the exception being certain caridean shrimp species), so they would rarely encounter ice in nature, and most become immobile at or below about 2°C (Frederich et al., 2002). They become inactive because, unlike other crustacean species that inhabit colder waters, decapods have relatively high concentrations of magnesium ions in their haemolymph, which immobilises the joints below this 2°C threshold (Frederich et al., 2000).

The reduced activity in decapods when cooled to close to freezing is sometimes termed ‘torpor’. It reduces the metabolic rate, which helps them survive short cold periods and regain activity once temperatures increase again. It is unlikely that decapods in UK waters enter torpor under natural conditions because UK coastal waters rarely reach temperatures below 4°C (Morris et al., 2018). Given this, we cannot assume that torpor is a ‘natural’ behaviour for decapods in UK waters.

The exact minimum temperature threshold that induces torpor seems to depend on how quickly the animals are cooled, and possibly differs between species. Temperatures of about 5°C already start to reduce activity levels in green shore crabs (**C. maenas**) (Young et al., 2006). In a study of warmer versus colder water dwelling brown crabs in Norway (Bakke et al., 2019), crabs of both populations showed preferences for water of about 12.5-14°C, and if cooled, 50% were unable to right themselves when inverted once temperatures declined to 1.3°C.

Whether near freezing temperatures cause nociception or pain in decapods is unknown. Research into this is urgently needed, especially because the assumption that extreme cooling has anaesthetic effects is in direct conflict with the possibility that it could cause avoidance, nociception, or pain. Even in humans, this paradox exists, because very cold temperatures can cause pain, but can otherwise numb certain other sources of pain (Yin et al., 2015), so the situation may also be complex in decapods. Cold nociception in general is not well understood across species, and it may have evolved later than heat nociception (Smith & Lewin, 2009). Interestingly, although the TRPA1 channel, which decapods possess, is activated by cold (among other noxious stimuli) in rodents and humans, it is instead activated by heat in *Drosophila* (Viswanath et al., 2003), so it cannot be presumed that it would respond to cold in decapods. That said, TRPA1 is not the only receptor involved in cold nociception.

This is an important evidence gap: there is a need for better knowledge of the lowest temperature that commercially important species of decapod can tolerate without harming health and welfare.

Storage and transport out of water. Some decapods, especially brown crabs, green crabs and lobsters, can typically survive for 2-3 days in ‘dry’ storage, as long as the conditions are sufficiently damp. This is sometimes known as damp storage or semi-dry storage. Containers used for damp storage include bongos, trays, nets and polystyrene boxes. Polystyrene boxes containing damp material and ice packs are commonly used for transporting live lobsters and crabs by air-freight. We have also encountered reports of decapods being stored alive at the bottom of fridges (Jacklin & Combes, 2005).

A study by Woll et al. (2010) investigated the effects of damp storage on brown crabs (**C. pagurus**) and reported that waste products, such as ammonia, started to accumulate in the haemolymph, since seawater is needed to remove them. This accumulation of waste products may or may not cause suffering—this is an evidence gap. Woll et al. found that “for crabs exposed at 10°C and 5°C, emersion (removal from water) for 36 h and 72 h, respectively, did not seem to have negative consequences for the animals” (Woll et al., 2010). Adverse health consequences were found in crabs that were already weak or moribund, and in crabs exposed to temperatures above 10°C (see also “Exposure to inappropriate temperatures”). This is in line with Seafish’s recommendation that temperatures should not exceed 8°C.

A key welfare risk to (non-amphibious) decapods is hypoxia (lack of oxygen), which causes lactate to build up in the tissues due to anaerobic respiration. In humans, this build-up of lactate is painful. Whether it is also painful in decapods is unknown—an evidence gap. Hypoxia can occur when an animal is removed from water because the gills can collapse. Decapods are exposed to air during damp storage, but also sometimes whilst awaiting transfer to vehicles or storage containers. For example, on a journey from the UK to Portugal, crabs were temporarily held in dry buckets for up to 2h while being loaded onto a vivier truck (Barrento et al., 2010). The crabs that were loaded into the top layer within the tanks, were held in the buckets for about 1h longer than those on the bottom layer, and had increased haemolymph L-lactate, acidity and haemocyanine before the journey. Moreover, their haemolymph pH remained lower than that of the crabs at the bottom even after the 58h journey (Barrento et al., 2010).

Hypoxia can also occur in seawater that is low in oxygen. Oxygen saturation can become low for many reasons including water being warm or overcrowding of animals in the water. In one study by Lorenzon et al. (2008) brown crabs had lower mortality and lower haemolymph lactate levels following 36 hours of commercial transport in damp containers than in seawater (both 10-13^{o}C), and the authors concluded that the seawater must have been poor quality (probably having low oxygen levels but perhaps also including contaminants).

Seawater oxygenation in the aforementioned vivier truck on the journey to Portugal started at only 3.5 mg l^{-1} and decreased during the journey to 2.8 mg l^{-1}, which is just below the minimum saturation suggested for crabs (Barrento et al., 2010). Keeping seawater clean and well-aerated can be challenging but is very important (Jacklin & Combes, 2005).

Other authors found that seawater transport at 12°C and damp transport at 4-8°C were equally viable in terms of relatively low brown crab mortality and haemolymph lactate and glucose levels (Barrento et al., 2011, 2012). The message is that one cannot simply say that storage in water is always preferable to storage out of water: a lot depends on the water quality and temperature. The maximum duration of damp storage should be investigated for key species to help prevent suffering.

Lack of food. Decapods in medium to long term storage, such as lobsters, are often not fed, partly to help prevent contamination and soiling of the water with uneaten food and waste products. They can survive without obvious weight loss or increased mortality risk for several weeks without food (e.g. Siikavuopio et al., 2018), although there are species differences (Sacristán et al., 2017). In the wild, decapods have periods of fasting as part of their moult cycle (Lipcius and Herrnkind, 1982). Recently moulted decapods are avoided in industry, because their flesh is very watery and their soft shells make them vulnerable to damage, so stored individuals will mostly comprise animals between moults that would be motivated to feed, and a smaller proportion that may have been preparing to moult and therefore would not feed. When intermolt European lobsters (**H. gammarus**) were held for 24 weeks at 4, 8, or 12°C, and either fed or unfed during that time, cooler water was shown to be necessary for the unfed lobsters to cope with lack of food (Albalat et al., 2019). Specifically, at 12°C the unfed lobsters showed significantly greater phenoloxidase activity in the haemolymph, greater water content in the muscle, and changes in the histology and lipid composition of the hepatopancreas, compared with all other groups. Unfed snow crabs (**Chionoecetes opilio**), kept for 100 days at 5°C, also showed a significantly greater reduction in relative hepatopancreas mass compared with those that were fed, but this did not impact on mortality rates (Siikavuopio et al., 2019). The resilience to starvation at cool temperatures in terms of body weight and mortality suggests that lack of food might pose little welfare concern, although this has not been tested directly, and fasting does have some gradual physiological effects.

Lack of access to dark shelters. Decapods in the wild will spend substantial amounts of time in dark shelters. Given a choice between a light area and a dark shelter, crabs will typically prefer the dark shelter (e.g. Barr & Elwood, 2011; Hamilton et al., 2016). Crayfish (**P. clarkii**) in an anxiety-like state will avoid bright areas (Fossat et al., 2014, 2015). Given this aversion to light, it is clear that good practice for handling decapods must involve providing them with access to dark environments. This is already recommended by Seafish as one of their “10 golden rules” for handling crustaceans (Jacklin & Combes, 2005). Yet there is evidence (obtained by the campaign group Crustacean Compassion) that supermarkets selling live lobsters in the UK commonly do not provide access to dark shelters (Carder, 2017) and display lobsters under bright lighting (Crustacean Compassion, 2020).

Online retail. Live decapod crustaceans can be ordered from Amazon and other online retailers. According to industry sources, only imported animals (from the USA and Canada) are sold in this way, although we have not independently verified this. There is no way to ensure welfare-sensitive handling when a live animal is delivered to a private home. This practice inherently creates a risk of poor handling and inappropriate slaughter methods (see also Section 5.2). Ending this practice would be a low-cost intervention to improve the welfare of decapods.

Wholesalers and supermarkets. A report by the campaign group Crustacean Compassion (2020) described highly inconsistent advice given to customers purchasing live lobsters in UK wholesalers on how to effectively transport, store or slaughter the animals. There is a need for enforceable codes of good practice regarding the advice and training that is provided in these settings. In our view, live animals should only be sold to customers who are trained in appropriate handling and slaughter methods.

5.2 Stunning

SUMMARY OF EVIDENCE

We have medium confidence that electrical stunning is effective at rendering decapods unconscious. There is evidence that it produces a seizure-like state in which the animal is unresponsive and plausibly unconscious. Pharmacological stunning is effective at immobilising animals, but its effectiveness rendering them unconscious is unclear. We have no confidence that chilling renders decapods unconscious.

To be effective, stunning must not only immobilise the animal but also render it unconscious. Electrical stunning has the potential to be an effective method. Electric shocks were the pain-inducing stimulus in many experiments reviewed in Part III. However, higher voltage and longer duration electric shocks, applied to neural tissue, can stun (and, at even higher voltages or longer durations, kill) crustaceans.

Roth and Øines (2010) concluded that electrical stunning was the most humane method to slaughter edible crabs (**C. pagurus**). As the only method effective within one second, it was considered preferable to chilling, boiling, and gassing with CO_2. Pre-slaughter stunning is a legal requirement in New Zealand and Switzerland. In the UK, two manufacturers produce most stunning equipment: Mitchell and Cooper Ltd (Crustastun) and Polar Systems Ltd. Crustastun units are designed to stun and kill lobster, crabs, and crayfish. The company manufactures both a single-animal unit for the hospitality sector and a large-scale stunner for processors. Polar Systems only manufactures a large-scale stunner, which is widely used in UK processing plants.

In non-peer-reviewed studies that are available online, Neil (2010, 2012) removed the carapace from six treatment (Crustastun: 110 V, 2-5 A, 10 s) and six control subjects of four species: lobster (**H. gammarus**), Norway lobster (**N. norvegicus**), shore crab (**C. maenas**), and brown crab (**C. pagurus**). This exposed the nerves of the central (circumoesophageal connective and, in lobsters, abdominal ventral nerve cord) and peripheral nervous system (legs). The Crustastun procedure usually ended all detectable neural activity. Electroshocked subjects did not autotomise; move their limbs, eyes or antennules, or recover (cf. Roth & Grimsbø, 2016; Roth & Øines 2010; Weineck et al., 2018). The only exceptions were two shore crabs, which each showed some neuronal recovery in one of the three legs tested (but not the central nervous system). A non-peer-reviewed study by Albalat et al. (2008) also found that Crustastun reliably kills langoustine (**N. norvegicus**). This suggests that the Crustastun was effective, but, since the results were not formally peer-reviewed, they allow only medium confidence.

In another non-peer-reviewed study on the physiological effects of Crustastun, Neil and Thompson (2012) subjected six lobster (**H. gammarus**) and six brown crab (**C. pagurus**) to electric shock. They compared haemolymph lactate concentrations in these animals to another six of each species, which were exposed to the same handling procedures but no electric shock (i.e. a control group). Every subject in the Crustastun treatment died, whereas every control subject was alive one week later. The handling procedure significantly increased haemolymph lactate concentrations in both lobsters and crabs. This increase was not significantly different between the Crustastun and control groups. The authors interpret this as indicating that electrical stunning does not increase stress levels over and above the stress of handling, emersion, and blood sampling, but the absence of a significant result is not a demonstration of the absence of an effect. Relatedly, a peer-reviewed study by Elwood and Adams (2015) found that, when controlling for activity level, shore crabs (*Carcinus maenas*) exposed to a weaker (10V) electric shock for a shorter time (200 ms) exhibited higher levels of haemolymph lactate than controls.

Previous studies inducing stress in crustaceans have recorded much higher haemolymph lactate concentrations than Neil and Thompson (2012) (e.g. Barrento et al., 2011; Lorenzon et al., 2007, 2008), indicating that a “ceiling effect” was not responsible for the lack of treatment differences.

While Neil and Thompson’s (2012) results suggest that Crustastun did not cause extreme physiological stress, we cannot conclude from this that it is painless (Stevens et al., 2016). Stress is one potential indicator of pain (Elwood, 2016), but this study should be considered in the context of how Crustastun affects other (neural) indicators. Haemolymph lactate alone, especially in an experiment with such a small sample size, is poor evidence that high-voltage shocks do not induce pain.

Fregin and Bickmeyer (2016), in a peer-reviewed study, found that the Crustastun induced a seizure-like pattern of increased neural activity in lobsters on either the "5 seconds" or "10 seconds" setting, combined with an absence of behavioural responsiveness to mechanical stimulation lasting between 10 and 60 minutes. In crayfish, the Crustastun induced "occasional" seizure-like states, whereas an alternative device (a "LAVES" device designed for stunning trout) regularly induced seizure-like states. They found that when crayfish were dropped into boiling water after induction of the seizure-like state, the neural response was much reduced, relative to controls, but not abolished. Fregin & Bickmeyer summarised their findings as follows: "electrical stunning induces epileptiform seizures but paralyses the animals and leads to a reversible decline of nerve system activity after seizure."

In truth, we do not know what the seizure-like neural activity induced by electrical stunning feels like from the animal's point of view. Diminished neural activity and behavioural unresponsiveness are consistent with total anaesthesia (which does not imply the total abolition of neural activity) but also consistent with some form of continuing experience (Alkire et al. 2008). So we are not in a position to conclude that electrical stunning produces total anaesthesia. More recent work found that electric shock immobilises and reduces heartrate in **P. clarkii** and **L. vannamei** (Weineck et al., 2018), but this still provides little insight into what the process feels like.

We can say with high confidence that the humaneness of electrical stunning is highly likely to depend on the electrical parameters used. Those parameters will need to be adjusted according to species, size, developmental stage and stage of moult of the animals.

Pharmacological anaesthesia is a possible alternative to electrical stunning. Two prime candidates are clove oil and AQUI-S, a clove oil-based product without the former's odour. In both, the active ingredient is eugenol (4-allyl-2-methoxyphenol). To our knowledge, pharmacological anaesthetics are rarely used on crustaceans in the UK. However, as a fish anaesthetic (Anderson et al., 1997; Keene et al., 1998; Soto, 1995), AQUI-S has been approved for human consumption in New Zealand, Australia, Chile, South Korea, Costa Rica, Honduras, and Norway, but not the EU or USA (Priborsky & Velisek, 2018).

Several studies indicate that clove oil and AQUI-S immobilise crustaceans. Eugenol immobilised blood-spotted crabs (**Portunus sanguinolentus**) in 14 minutes, with recovery taking 42 minutes (clove oil: 0.2 ml/l; Premarathna et al., 2016), and Australian giant crabs (**Pseudocarcinus gigas**) in 30 minutes, with recovery in 42 minutes (clove oil: 0.125 ml/l; AQUI-S: 0.5ml/l; Gardner, 1997). However, a study on three Pacific crab species reported much longer induction times – up to 188 minutes in hairy shore crabs (**Hemigrapsus oregonensis**; clove oil: 1-3 ml/l; Morgan et al., 2001). Recovery took 65 minutes for the shore crabs, but only 10 minutes for Dungeness crabs (**Cancer magister**; clove oil: 0.5-1.5 ml/l) and 14 minutes for kelp crabs (**Pugettia producta**; clove oil: 0.015-0.25 ml/l). Eugenol also immobilises other crustaceans, including lobsters (**H. americanus**: Waterstrat & Pinkham, 2005), langoustine (**N. norvegicus**; Cowing et al., 2015), crayfish (**Cherax quadricarinatus**; Ghanawi et al., 2019), prawns (**Macrobrachium rosenbergii**; Coyle et al., 2005) and shrimps (**Penaeus monodon**; Cai et al., 2012). However, these pharmacological studies typically use behavioural indicators of stunning, which do not distinguish anaesthesia from paralysis. Eugenol's mode of action is also poorly understood. Whilst pharmacological anaesthetics are potentially effective, more research is needed.

Chilling is another stunning technique. Crustaceans are "cold-blooded" (ectothermic): they rely on external heat to maintain their body temperature. When external temperatures drop below a certain threshold, crustaceans enter a state of torpor (see Section 5.1). This renders them immobile, preventing autotomy and aggression between individuals. Torpor also facilitates nerve centre destruction, allowing a faster and more humane dispatch.

However, it is unclear whether chilling-induced inactivity is associated with unconsciousness. Fregin and Bickmeyer (2016) kept lobsters (**H. gammarus** and **H. americanus**) and crayfish (**Astacus astacus** and **Astacus leptodactilus**) in 0°C tap water ice-slurry or −1.8°C seawater ice-slurry for one hour. After one hour, neural activity was still detectable in both conditions. This is inconclusive: neural activity alone does not imply consciousness, but the absence of neural activity, when reliably measured, does indicate unconsciousness. Weineck et al. (2018) immersed blue crab (**Callinectes sapidus**), red swamp crayfish (**P. clarkii**), and white-leg shrimp (**L. vannamei**) into ice-slurry between 0 and 4°C. Heartrate decreased in all three species, although most crabs still had a heartrate after five minutes. Crabs also exhibited central neural processing for muscle reflexes after two minutes. Cold shock did not influence haemolymph serotonin or octopamine levels in either the crabs or shrimp. Lobsters (**H. americanus**), spiny lobsters (**Panulirus japonicus**), and prawns (**Penaeus japonicus**) have cold-sensitive neurons in their ventral nerve cord, which increase their firing rate as temperature declines within a range of 0.5-5.5 °C (Tani & Kuramoto, 1998). Puri and Faulkes (2015) found no evidence for cold-sensitive nociceptors in crayfish (**P. clarkii**), but this study used a much colder stimulus (−78°C) than either conventional chilling methods or ecologically relevant conditions.

More research is needed to establish whether chilling itself is painful, and we need to remember that this may vary between decapod species. The existing literature leaves open the possibility that cold-induced immobilisation leaves crustaceans susceptible to pain from subsequent procedures.

UK fishers and processors rarely use chilling, but two methods predominate: chilling in air and chilling in slush-ice. At equivalent temperatures, torpor takes longer to reach in air, because air absorbs heat more slowly than water (AHAW 2005; Tseng et al., 2002; Morgan et al., 2004). Nonetheless, even slush-ice can take over 20 minutes to induce torpor (AHAW, 2005). Chilling is particularly ill-suited to temperate species, which are adapted to survive low temperatures. Slush-ice also presents another welfare concern. Salinity drops as the ice melts, which can lead to osmotic shock before torpor is induced, although maintaining salinity can resolve this issue (AHAW, 2005).

From a welfare perspective, crustaceans should be stunned before slaughter. Electrical and (potentially) pharmacological stunning are the most promising approaches. Future research could identify ways to stunning more practical and effective. The Humane Slaughter Association is currently funding research into effective methods of stunning and slaughtering crustaceans. The findings might improve the practicality and commercial viability of electrical stunning. Chilling may well paralyse crustaceans without anaesthetising them. We note that this method has been banned in Switzerland and in parts of Italy.

5.3 Mechanical slaughter (dispatch)

SUMMARY OF EVIDENCE

We regard the methods of double-spiking (for crabs) and whole-body splitting (for lobsters) as reasonable slaughter methods, given current evidence. There are greater welfare risks associated with single-spiking, head-only splitting, tailing, and high-pressure processing.

The shellfish industry uses the term “dispatch” to refer to the slaughter of decapods. We use the two terms interchangeably in this report.

Unlike vertebrates, crustaceans have a decentralised nervous system. Crabs have two main nerve clusters (ganglia), and lobsters have 13 interconnected ganglia down the ventral nerve cord. The result is that methods that target only the brain will not necessarily kill the animal quickly (Roth & Øines, 2010).

Spiking involves piercing the underside with a spike, destroying the ganglia. This method is recommended for crabs, because the brain (or cerebral ganglion) and ventral nerve mass (or thoracic ganglion) can both be spiked in rapid succession in a procedure known as “double spiking”. An early study for the Universities Federation for Animal Welfare (UFAW) recommended double spiking as the most humane method for slaughtering crabs (Baker, 1955). Although double spiking is relatively quick, it is not instantaneous. At present, most UK crab processors only destroy one ganglion (“single spiking”). Single spiking creates a welfare risk because it is less likely to kill the animal quickly and reliably (Roth & Øines, 2010). Regulations requiring double spiking (coupled with education about why this matters) would improve UK welfare standards.

Spiking is unsuitable for lobsters, because their chain of ganglia cannot be individually pierced quickly and accurately. To destroy all 13 ganglia, lobsters’ under-surface must be severed down the longitudinal midline using a knife. This process, known as **splitting**, is common in restaurants (industry sources). Due to the demand for whole lobsters, chefs typically only split the head (head splitting), rather than the whole body (complete splitting). However, head splitting leaves the posterior ganglia intact, raising the chance of continued survival. We cannot be confident that head splitting reliably renders the animal unconscious immediately. From a welfare perspective, lobsters should be split from head to tail, destroying all 13 ganglia and killing the animal. Whole-body splitting should take less than 10 seconds when performed by a skilled practitioner. We note, however, that there is a risk of the procedure failing to kill the animal quickly if it is performed incorrectly by an untrained person.

Tailing involves separating the thorax from the abdomen. On Nephrops (langoustine) vessels, for instance, the abdomen is usually twisted away from the thorax (industry sources). Large vessels may chill the Nephrops beforehand, inducing immobility but without necessarily achieving anaesthesia. As well as Nephrops, crayfish and occasionally crab are slaughtered using tailing in the UK (industry sources). Whereas spiking and splitting (properly performed) destroy all the animal’s ganglia, tailing does not.

High-pressure processing involves exposing batches of crustaceans to very high water pressure. It is claimed that high-pressure processing kills crustaceans in <6 seconds, equivalent to spiking and splitting (industry sources). We have not been able to find robust scientific evidence confirming this. High-pressure processing without effective prior stunning has the potential to cause pain, even if it is over quickly. Although it is the most common form of dispatch in the USA, this practice is rare in the UK and any legislation to prohibit their use would primarily be pre-emptive (industry sources).

Correctly practised, spiking and splitting are relatively quick dispatch methods. Quickly destroying every ganglion before further processing (e.g. boiling, freezing, or chopping up) ensures that the animal is dead and may not feel further pain. However, both tailing and routine spiking/splitting practices (especially single spiking and head splitting) do not destroy all ganglia. Double spiking crabs and completely splitting lobsters would align the UK with international best practice. Nevertheless, all manual mechanical dispatch methods take several seconds and may sometimes leave ganglia intact. Ideally, crustaceans should be effectively stunned beforehand.

5.4 Slaughter (dispatch) using extreme temperatures

SUMMARY OF EVIDENCE

We have high confidence that chilling in a home freezer is an inhumane slaughter method, since it takes more than one hour for animals to die. We have high confidence that live boiling (without prior stunning) is an inhumane slaughter method for relatively large decapods, which may take more than 2 minutes to die. We have low confidence that gradually raising the water temperature is more humane than live boiling.

Chilling. Decapods are sometimes dispatched using extremely low temperatures. The welfare issues outlined in the section on stunning also apply here: nervous system activity continues after chilling, melting slush-ice can cause osmotic shock, and death is slow. Gardner (2004) argued that this method of dispatch is slow, inconsistent, and aversive. As noted in Section 5.3, the evidence is inconclusive on this issue, with some evidence of cold-sensitive neurons (Tani & Kuramoto, 1998) but also a failed attempt to identify cold-sensitive *nociceptors* in crustaceans (Puri & Faulkes, 2015). If future research confirms their absence at more realistic temperatures in more species, low temperatures could conceivably be a humane slaughter method.

Chilling is a rare slaughter method in UK industry, because it reduces meat quality (industry sources), but is common in domestic kitchens. This is concerning as, unlike commercial blast freezers, home freezers do not reduce temperature rapidly. Crustaceans in home freezers must, therefore, be left to die over a period of more than one hour (Roth & Øines, 2010). Edible crabs autotomise during freezing (Roth & Øines, 2010), and this may be considered a credible indicator of distress against a background of considerable evidence of sentience (see Part III). This prolonged suffering may be worse than fast methods considered inhumane (e.g. boiling).

Boiling. Boiling is perhaps the most controversial dispatch method, having been banned in several jurisdictions (Switzerland, New Zealand, and parts of Italy). Immersion in boiling water is nonetheless common in UK restaurants and domestic kitchens for lobster, Nephrops (langoustine), small crabs, crayfish, shrimps, and prawns, as well as on-vessel for brown shrimp.

Boiling elicits various behavioural and physiological symptoms of distress. Baker (1955) reported that edible crabs (**C. pagurus**) immersed in boiling water rapidly autotomised and displayed behavioural signs of distress, such as uncoordinated movements and escape attempts. More recent work on lobsters and cuttlefish did not observe such behaviours but did find that intense neural activity continued for up to 30-150 seconds after immersion (Fregin & Bickmeyer, 2016). This suggests a period of up to 2.5 minutes of continued sentience, potentially involving extreme suffering. Smaller individuals died much faster than larger ones, suggesting that boiling involves less prolonged suffering for smaller crustaceans (e.g. shrimps). The estimate of 2.5 minutes aligns with Roth and Øines (2010) estimate, obtained by a different method.

To address welfare concerns about live boiling, some authors have recommended immersing crustaceans in cold water and slowly raising the temperature (e.g. 1°C per minute). Using this method, a few studies have found that crabs, lobsters, and crayfish do not elicit behavioural responses indicating pain and distress (e.g. tail-flipping or escape behaviour; Fregin & Bickmeyer, 2016; Gunter, 1961). Fregin and Bickmeyer (2016) also observed that CNS electrical activity decreased to zero above 32°C in lobsters (**H. gammarus and H. americanus**) and crayfish (**A. astacus** and **A. leptodactilus**).

However, in other studies, slowly heated edible crabs (**C. pagurus**; Baker, 1955) and red swamp crayfish (**P. clarkii**; Adams et al., 2019) displayed behaviours indicating distress, including escape attempts, uncoordinated movements, and autotomy. Adams et al. (2019) also found that, despite looking dead, immobile crayfish still had a heartbeat at 40°C. Heartbeat alterations in response to touch and sensory neuron recovery were recorded up to 44°C, indicating a functional nervous system in apparently unresponsive crustaceans. Hence, a lack of behavioural responses to boiling may not indicate total anaesthesia. We cannot have even medium confidence that gradually raising water temperature (without prior stunning) is more humane than dropping an animal into boiling water. There is still a serious risk that it causes suffering over a period of minutes.

5.5 Slaughter (dispatch) using freshwater immersion

SUMMARY OF EVIDENCE

We have high confidence that freshwater immersion is an inhumane slaughter method. It may lead to more prolonged suffering than faster methods considered inhumane, such as boiling.

Crustaceans immersed ("drowned") in freshwater must usually be left overnight. This practice is rare in the UK, as it reduces meat quality, but sometimes practised on lobster and brown crab (industry sources). From a welfare perspective, it cannot be recommended. Baker (1995) reported that an edible crab (**C. pagurus**) immersed in freshwater exhibited signs of distress, such as uncoordinated movement and increased respiration. After 10 minutes, Australian giant crabs (**Pseudocarcinus gigas**) autotomised and tore at their legs and abdomen (Gardner, 1997). Freshwater immersion potentially leads to more prolonged suffering than faster methods considered inhumane, such as boiling.

5.6 Decapods in aquaculture

SUMMARY OF EVIDENCE

Eyestalk ablation is a common practice (internationally) in shrimp aquaculture, but one that poses a serious welfare risk if the animals are sentient.

There are several lobster hatcheries in the UK, mostly specialising in the clawed lobster **H. gammarus**. We know of one company (RAS Aquaculture Research) that has developed techniques for farming the spiny lobster **P. elephas**. The above considerations regarding the handling of lobsters also apply, of course, to lobsters in hatcheries. We also know of two operations (Great British Prawns and FloGro Systems) that specialise in hatching the whiteleg shrimp or king prawn, **L. vannamei**. However, the vast majority of prawns are imported.

As noted in Part **III**, eyestalk ablation is a controversial practice in shrimp aquaculture that involves removing one or both of the eyestalks of a mature broodstock female prawn in order to induce egg production. A study by Taylor et al. (2004) on **L. vannamei** found that eyestalk ablation provoked a recoil reaction and recommended the use of an anaesthetic (lidocaine) to dampen this reaction.

Another study by Diarte-Plata et al. (2012) found that ablated shrimp (**M. americanum**) were much more likely to flick their tails and rub the site of the wound than non-ablated controls. They found that covering the wound significantly reduced these responses, and that lidocaine also significantly reduced them.

There is little evidence one way or the other regarding sentience in penaeid shrimps (see Part **III**), but we do have high confidence that, if they are sentient, eyestalk ablation poses a severe welfare risk.

In recent years, experiments with ablation-free approaches by Zacarias et al. (2019, 2021) have suggested that eyestalk ablation may not be necessary for economically viable shrimp aquaculture, and that avoiding it leads to better reproductive performance from the breeding females and more resilient offspring with lower mortality rates.

As far as we know, the two UK-based shrimp aquaculture companies source their fry (hatchlings) from overseas rather than breeding them in-house. One of them, FloGro Systems, confirmed to us that it does not use eyestalk ablation. The other, Great British Prawns, did not reply to our emails. Assuming eyestalk ablation is not practised in the UK, there would be no major downside to banning eyestalk ablation within the UK, but any immediate welfare benefit would be limited.

PART VI. EVIDENCE GAPS

Our aim in this part of the report is to draw attention to evidence gaps that have come to light in the course of our inquiry. These evidence gaps are important directions for future research.

Analgesia and anaesthesia. For both cephalopods and decapods, there is a lack of knowledge regarding analgesia, including which drugs are useful in preventing pain. Research into analgesia should be a high priority. Research into anaesthesia is also limited, although some promising local and general anaesthetics have been identified (Butler-Struben et al., 2018).

Slaughter methods. Currently, the only approved method of humane slaughter for cephalopods is through terminal overdose of anaesthetic, which cannot be used in animals destined for human consumption. Mechanical methods (brain cut/puncture) are time-consuming and require expertise to be performed correctly (and we are not confident that they are humane even when performed correctly). We recommend research into development of humane slaughter methods for cephalopods that can be performed immediately post-catch. The Association for Cephalopod Research (CephRes) has told us that it plans to evaluate different stunning methods in cephalopod fisheries.

For decapods, the slaughter methods that are most likely to be humane are double-spiking (for crabs), whole-body splitting (for lobsters), or electrocution until dead using a specialist device designed and validated for that purpose. Yet even these methods may take 10-15 seconds, and the first two require specialist skills. Research into methods of killing a decapod reliably and humanely in less than 10s is an obvious priority for future research, as the Humane Slaughter Association has recognised.

Nicking. We are concerned about the practice of nicking, in which the tendons in the claw of a brown crab (**C. pagurus**) are cut. There is a view in the industry that this is necessary because no effective banding is possible for brown crabs, but we can neither confirm nor deny this. We think further research into alternatives to nicking in brown crabs would be worthwhile.

Chilling and contact with ice. It is well known that decapods will enter a state known as torpor at temperatures close to freezing, though the precise thresholds for different species are not well known. It is not known whether torpor renders animals unconscious or merely immobile. It is also not known whether decapods have nociceptors for cold temperatures, which could be activated by direct contact with ice or ice-packs. Decapods clearly require cool temperatures, but the dangers of cooling them too much are poorly understood. There is a need for better knowledge of the lowest temperature that commercially important species of decapod can tolerate without harming health and welfare.

Stunning. Current evidence suggests that electrical stunning can induce a seizure-like state in astacid lobsters and crayfish, and that this state diminishes, without wholly abolishing, the nervous system's response to boiling water (Fregin & Bickmeyer, 2016). However, there is a striking lack of solid evidence in this area. More evidence is needed about how electrical stunning affects other commercially important decapod species, how smaller species (such as shrimps) can be effectively stunned, and how stunning technology might be made to work on boats.

When sentience begins. Little is known about the development and maturation of neural networks involved in pain in cephalopods or decapods. The evidence we have reviewed concerns adult animals.

Best-practice guidelines. We have not found standardised best-practice guidelines for the capture, transport, breeding, housing and husbandry of cephalopods outside scientific contexts (on scientific contexts, see Fiorito, et al., 2015). The development and implementation of such guidelines is important for ensuring the welfare of cephalopods outside scientific settings. Although there are some guidelines for decapods, drawn up by Seafish (Jacklin & Combes, 2005), we recommend the development and implementation of guidelines that focus more heavily on welfare than on product quality. Although welfare and product quality are related, they are not the same thing.

Stocking density and packing. It will be important to establish an evidence-based maximum stocking density for storing decapods to prevent social stress. A maximum bulk weight for packing decapods for live transport would also help to reduce crushing of those at the bottom of the container and hypoxia for those awaiting loading into the top of large containers.

Nautiloids. There is currently no research into the presence of nociceptors or nociceptive responses in nautiloids. Further neurophysiological and behavioural research to establish these capacities (if present) would provide more insight. However, nautiloids are not a commercially important taxon in Europe. All nautilus species are threatened due to overfishing for their shells (especially in Indonesia and the Philippines) but conservation is not the topic of this report.

Relevant nociception and aversion thresholds in decapods. Research into nociception and aversion in decapods should focus on industry-relevant stimuli such as ice, ambient temperature, oxygen saturation, concentrated ammonia or urea (as accumulates around the gills during damp storage), and lactic acid (as accumulates in tissues during hypoxia). Experiments should incorporate methods to minimise bias, such as randomisation and blinding. For both ethical and scientific reasons, nociceptive studies should consider use of stimuli that gradually increase in intensity until either a nocifensive response is made or a humane endpoint is reached (whichever is reached first).

PART VII. OVERALL FINDINGS AND RECOMMENDATIONS

7.1 The question of sentience

Our review presents a complicated evidential picture. The evidence of sentience is very strong for octopods (order Octopoda) and strong for true crabs (infraorder Brachyura). There is substantial evidence for other coleoid cephalopods (squid and cuttlefish) and for some other decapod taxa (anomuran crabs, astacids, and caridean shrimps). The picture is summarised in **Table 5**.

Three general observations are worth emphasising. First, the amount of evidence for a given biological taxon is largely dependent on how much scientific attention that taxon has received in relation to sentience. Octopods and true crabs have received sustained scientific attention, whereas (for example) nautiloids and penaeid shrimps have barely been studied. Various other taxa (e.g. squid, cuttlefish, and anomurans) have received an intermediate level of sentience-focused attention, resulting in an intermediate amount of evidence.

Second, there are no cases in which we have very high/high confidence that a taxon *fails* a criterion. While this may seem surprising, it should be noted that cephalopods and decapods were selected for scrutiny precisely because they seem like plausible candidates for sentience. If we had reviewed evidence for other invertebrate animals (e.g. jellyfish), we might well have ended up with very high confidence that the criteria are failed.

Third, there is no dramatic difference in the quality or volume of evidence regarding cephalopods as opposed to decapods. We thought we might find a dramatic difference between cephalopods and decapods, or between octopods and everything else, but this is not reflected in the current scientific literature. There is more evidence for sentience in octopods than in true crabs, but the difference is not vast, and the evidence for sentience in true crabs is actually slightly more substantial than the evidence for sentience in other, less-studied cephalopods. This leads us to recommend that, if cephalopods are to be included in the scope of animal welfare laws, decapods should also be included.

How should policymakers respond to this complicated evidential picture?

Our central recommendation

We recommend that **all cephalopod molluscs and decapod crustaceans** be regarded as sentient animals for the purposes of UK animal welfare law. They should be counted as “animals” for the purposes of the Animal Welfare Act 2006 and included in the scope of any future legislation relating to animal sentience.

The Animal Welfare Act 2006 (**AWA**) states that the power to extend the scope of the Act “may only be exercised if the appropriate national authority is satisfied, on the basis of scientific evidence, that animals of the kind concerned are capable of experiencing pain or suffering.” We recommend that Defra considers this threshold to have been satisfied by both cephalopods and decapods.

Table 5. A summary of confidence levels regarding the evidence of sentience in cephalopods and decapods (a duplicate of Table 1). *The colours and letters represent our confidence level that the criterion in question (column) is satisfied by the taxon in question (row). VH (dark green) indicates very high confidence, H (light green) indicates high confidence, M (dark yellow) indicates medium confidence, L (light yellow) represents low confidence, and VL (light grey) represents very low confidence. For descriptions of the criteria, see the main text. Importantly, low/very low confidence implies only that the scientific evidence one way or the other is weak, not that the animal fails or is likely to fail the criterion.*

	Criterion 1	Criterion 2	Criterion 3	Criterion 4	Criterion 5	Criterion 6	Criterion 7	Criterion 8
Octopods (Octopoda)	VH	VH	H	H	M	VH	VH	H
Cuttlefish (Sepiida)	H	VH	H	L	M	M	VH	L
Other coleoids (squid, all orders)	H	VH	H	L	M	L	H	L
Nautiloids	H	L	L	L	L	L	M	VL
True crabs (Brachyura)	H	VH	L	VH	L	VH	H	VL
Anomuran crabs (Anomura)	H	VH	L	L	M	H	L	VL
Astacid lobsters/crayfish (Astacidea)	H	VH	L	VH	L	L	M	VL
Spiny lobsters (Achelata)	H	VH	L	L	L	L	M	VL
Caridean shrimps (Caridea)	H	VH	L	M	L	M	L	VL
Penaeid shrimps (Penaeidae)	H	L	L	M	L	L	L	VL

Why are we making this recommendation? There is very strong evidence of sentience in octopods (order Octopoda) and strong evidence in true crabs (infraorder Brachyura). In other cases, we found evidence that was (by the lights of our framework) substantial but not strong.

This is likely to reflect disparities in the amount of scientific attention different taxa have received. Scientific attention has gravitated towards some taxa rather than others for reasons of practical convenience (e.g. which animals can be kept well in labs) and geography (e.g. which species are available where a lab is located). Because of this situation, we think it would be inappropriate to limit protection to specific orders of cephalopod, or to specific infraorders of decapod. Such an approach has never been taken with vertebrates. For example, we do not protect lab rats (**R. norvegicus**) while excluding other, less studied mammalian species, even though much of the evidence regarding sentience in mammals comes from lab rats. In the case of vertebrates, legislators in the UK have been willing to generalize from well-studied lab animals to other relevantly similar species. It would be consistent to take the same approach regarding invertebrate taxa.

One alternative option would be to count only octopods as sentient, on the grounds that the evidence is stronger for octopods than for any of the other taxa we have considered. However, because the evidence is also strong in true crabs (see **Table 5**), such a move would exclude decapods on the basis of a fairly small difference in the amount of evidence of sentience.

A second alternative option would be to count only octopods and true crabs as sentient animals. However, this approach would also face serious problems. Although the evidence of sentience is stronger for true crabs than for anomuran crabs (infraorder Anomura) and astacid lobsters/crayfish (infraorder Astacidea), the difference is not vast and plausibly results from disparities in how scientists have allocated their attention. A law that protected true crabs but not anomuran crabs and lobsters would be highly confusing (because the various infraorders of decapod are not widely known) and would, in effect, write into law a contingent fact about which species scientists have chosen to study most intensively.

If astacids were included, the exclusion of other decapod taxa (such as spiny lobsters and caridean shrimps) would be subject to the same criticisms: it would be both confusing and a reflection of disparities in scientific attention.

We have noted that there is very little evidence of sentience at present in penaeid shrimps. However, if caridean shrimps were included, but penaeid shrimps excluded, the potential for confusion would be high. Therefore, on balance, we reject the suggestion that protection should only be extended to specific infraorders of decapod. We note that this is a point of agreement with the earlier AHAW (2005) report.

The AWA refers to “animals of the kind concerned”, leaving open the question of the most appropriate grain of analysis for animal welfare law. Our recommendation is that “decapod crustacean” is a good category for legislative purposes. Protecting specific infraorders (such as Brachyura) but not others in a general animal welfare law would lead to the problems explained above. A better approach, in our opinion, would be to protect all decapods in general legislation, while also developing enforceable best-practice guidance and regulations that are specific to the welfare needs of commercially important species.

We note here that Swiss animal welfare legislation (the Tierseuchenverordnung, or Animal Protection Order) uses the category of “Reptantia”, a category encompassing all those decapods that move primarily by walking rather than swimming. We highlight this as a possible alternative option. However, any restriction of animal welfare law to walking decapods would questionably exclude caridean shrimps, which move primarily by swimming, but for which the evidence of sentience is as strong as it is for (e.g.) spiny lobsters.

Similar considerations apply regarding the cephalopods. The evidence is strongest for octopods. But while it is possible in principle to protect only octopods (indeed, ASPA protected only one species of octopus, **O. vulgaris**, between 1993 and 2012), the exclusion of squid and cuttlefish, especially if combined with the inclusion of some or all decapods, would give undue significance to contingent facts about how scientists have allocated their attention.

Similarly, the exclusion of nautiloids, if combined with the inclusion of all decapods, would lead to the same problems. So, we recommend including all cephalopods in the scope of general animal welfare law, while also developing enforceable best-practice guidance and regulations that are specific to the welfare needs of commercially important species.

In making this recommendation, we have considered the fact that legislation relating to animal sentience also includes the Animals (Scientific Procedures) Act 1986 (**ASPA**).

We recommend that all decapod crustaceans are brought within the scope of ASPA.

All cephalopod molluscs are already included the scope of ASPA, so we are, in effect, recommending that, in addition, decapods in science are brought within this regulatory framework.

In practice, this would mean that scientific work on decapods would require an ethical review by an Animal Welfare and Ethical Review Body (AWERB), which would weigh the harms and benefits of the work and ensure that researchers are following the imperative to “reduce, refine, and replace” (the 3Rs). We also recommend the development of best practice guidelines for decapod research, and we note that the Universities Federation for Animal Welfare (UFAW) is already working on this.

We do not think this is likely to obstruct scientific progress, for two main reasons. First, some institutions already require ethical approval for crustacean research under internal, non-ASPA (or “NASPA”) procedures. Second, we think ethical review can improve the quality of scientific research, because justifying a study forces researchers to clarify its rationale, hypotheses, and potential impact (Prescott & Lidster, 2017). The question of decapods in science is considered in greater detail in recent reviews by Rowe (2018) and Passantino et al. (2021).

7.2 Commercial practices, including slaughter (dispatch)

Our review of the welfare implications of commercial practices leads to some further recommendations:

We recommend reinstating the ban on declawing, the practice of removing one or both of the claws from a decapod before returning it back to the water. We have high confidence that this practice causes suffering.

We recommend a ban on the sale of live decapod crustaceans to untrained, non-expert handlers. We have very high confidence that this practice inherently creates a risk of poor handling and inappropriate slaughter methods.

Ending these practices would be low-cost interventions to improve the welfare of decapods.

The Welfare at Time of Killing (England) Regulations 2015 (**WATOK**) already mandate humane slaughter, but more detail about appropriate slaughter methods is needed. Our recommendation is that effective electrical stunning should be implemented wherever possible (but it is not clear that it is practically possible at present on small boats, or for small species). A relatively quick and effective slaughter method should then be used, such as double-spiking (for crabs), whole-body splitting (for lobsters), or electrocution until dead using a specialist device designed and validated for this purpose.

These methods are still often slower than one would ideally like, since they may take 10-15 seconds to be effective. The development of reliably fast slaughter methods for decapods should be a priority for further research in this area, as the Humane Slaughter Association has recognised. Policy in this area needs to recognise that more humane stunning and slaughter methods may become available in the future, and that the evidence in favour of any particular method or device is limited at present.

We cannot be confident that a single head wound that immobilises an animal without reliably and immediately killing it will render it unconscious, so we cannot recommend single-spiking or head-only splitting. For the same reason, we cannot recommend tailing (removing the abdomen from the thorax, or the head from the thorax) as a humane method. We also cannot recommend slaughter by freshwater immersion, by rapid boiling, by slowly raising the temperature of water, or (given our current limited knowledge) by chilling. These methods simply cannot be relied upon to kill the animal as quickly as possible.

We recommend that clearer regulations are drawn up for decapod slaughter and that they ban the least humane slaughter methods in cases in which a more humane slaughter method is clearly available. These regulations should include all cases in which a relatively large decapod (crab, lobster, or crayfish) is slaughtered on land. In short:

We recommend that the following slaughter methods are banned in all cases in which a more humane slaughter method is available, unless preceded by effective electrical stunning: boiling alive, slowly raising the temperature of the water, tailing (separation of the abdomen from the thorax, or separation of the head from the thorax), any other form of live dismemberment, and freshwater immersion (osmotic shock).

We note that an earlier review (AHAW 2005) arrived at concordant recommendations, as did another recent review, conducted independently of this one (Conte et al., 2021).

Stunned decapods should not be mechanically slaughtered if they show signs of recovery from stunning, such as resistance to handling, controlled limb movement or reactions of the eyes and mouthparts to touch. More research is recommended on the question of how to achieve effective electrical stunning, and on the question of how electrical stunning may be implemented when decapods are slaughtered at sea.

Globally, in shrimp aquaculture, it is a common practice to sever the eyestalks of breeding females to accelerate breeding (“eyestalk ablation”). To our knowledge, this does not currently occur at the UK’s two penaeid shrimp hatcheries, because they source their hatchlings from abroad. A ban on eyestalk ablation in the UK would be a reasonable precautionary measure but might not generate an immediate welfare benefit.

Our aim has not been to draw up a code of best practice for the treatment or humane slaughter of cephalopods and decapods. That would be a separate project. Defra may wish to consider adding legal force to Seafish’s existing code of best practice for handling and storing live decapod crustaceans. It is particularly important to require access to suitable dark shelters and appropriate temperatures for the species in question (e.g. 8°C or less for damp storage). We recommend more research on the welfare needs of decapods, especially research that can provide more precise insight into the appropriate temperature ranges for different species. For brown crabs (**C. pagurus**), we also recommend the development and implementation of alternatives to nicking.

The welfare issues concerning cephalopod molluscs are somewhat different. There is an important evidence gap relating to humane slaughter methods for cephalopods in cases where they are trapped alive. Our evidence suggests that various different methods are currently used in European waters, including clubbing, slicing the brain, reversing the mantle and asphyxiation in a suspended net bag. We are not able to recommend any of these methods as humane. We encourage Defra to consider introducing legislation in the future which would protect the welfare of wild-caught animals, to the extent that this is possible.

We are concerned about the growing interest elsewhere in the world (e.g. Spain, Australia, Japan, Mexico) in octopus farming. Octopuses are solitary animals that are often aggressive towards each other in confined spaces. In our opinion, high-welfare octopus farming is impossible. To be clear, this is not a comparative remark about the welfare of farmed animals compared to their wild counterparts: welfare problems arise in both cases. The government may wish to consider a ban on imported farmed octopus. A pre-emptive ban on octopus aquaculture in the UK could be considered but might have no immediate welfare benefit.

In sum, our view is that the time has come to include cephalopod molluscs and decapod crustaceans in UK animal welfare law in an explicit way, and to take proportionate steps to regulate practices that are a source of reasonable and widespread animal welfare concerns.

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Figure 5: European Lobster. *Image by Cefaclor, Wikimedia Commons, CC-BY 2.5 licensed.*

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