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Food for Thought

Review of some scientific issues related to crustacean welfare

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The scientific literature on the subject of welfare and pain in crustaceans is immature. It is based largely on a few dubious and disputed studies done on a small number of decapod species in instances where nociception was not confirmed, laboratory artefacts occurred, all variables that potentially influence the results were not fully controlled, and interpretations of results were questionable or contradictory. The proposed criteria for pain being applied to crustaceans since 2014 has set the "evidential bar" for pain so low it is impossible to have confidence that the behaviours observed in many experiments are even due to nociception, extinguishing scientific confidence that these behaviours are in any way analogous to how the word pain is defined, used, and understood by humans. Given the critical flaws in design and interpretation of several crustacean "pain" studies, acceptance of claims of pain for these animals, even as a precautionary measure, represents acceptance of a much lower evidential bar than is usually dictated by normal scientific standards. This may lead to circumstances whereby the precautionary principle, underpinned by weak science, is used by decision makers to justify unnecessary constraints on scientific research or other uses of crustaceans, imparting significant costs to scientific programs (and potentially food production industries), which are likely to exceed any benefits from changes in welfare status that may (or may not) accrue to these animals.

Keywords: anthropomorphism, crab, crustacea, decapoda, lobster, nociception, pain, prawn, shrimp.

Introduction

An opinion by the European Food Safety Authority's Scientific Panel on Animal Health and Welfare (EFSA, 2005) concluded "The largest of decapod crustaceans are complex in behaviour and appear to have some degree of awareness. They have a pain system and considerable learning ability", and that "all decapods should receive protection". However, decapod crustaceans were subsequently not included for protection under EU animal welfare legislation, due to debate and "fierce resistance" from sections of the scientific community in Europe at the time (Birch, 2017). In the decade since, there have been repeated calls by animal welfare lobby groups in several countries to include crustaceans under welfare legislation as a precautionary measure (Birch, 2017), resulting in the recent inclusion of lobsters and crayfish in welfare legislation in Switzerland on 1 March 2018 (https://www.blv.admin.ch/blv/de/home/tiere/tierschutz/revision-verordnungen-veterinaerbereich.html). The initial scientific resistance back in 2005 was, at least in part, based upon doubt regarding the scientific validity of some of the "pain criteria" being used for crustaceans. Several studies found inconsistencies such as a lack of morphine analgesia to mild electric shocks (Barr and Elwood, 2011), lack of evidence of the presence of nociceptors [the first report of nociceptors in crustaceans was published a decade later by Puri and Faulkes (2015)], and other scientifically critical issues, some of which remain unresolved today (Rose *et al.*, 2014; Puri and Faulkes, 2015; Stevens *et al.*, 2016). The confusion regarding the state of knowledge in this field is exemplified by a recent review by Sneddon (2018), who does not cite Puri and Faulkes (2015) and claims "no studies as yet have identified nociceptors or receptive fields in decapods."

Since 2005 the volume of literature relating to crustacean welfare has increased significantly, as has the number of peer reviewed papers and rebuttal letters highlighting scientific flaws in some of the key "crustacean pain" research papers (e.g. Rose *et al.*, 2014; Puri and Faulkes, 2015; Stevens *et al.*, 2016). In any developing area of scientific research, it is important that points raised by critical reviews and rebuttal papers are also considered by decision makers and legislators whenever the merits of the original literature are assessed. Given that interest in crustacean welfare continues to increase, a critical review of the available

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scientific literature on the subject of nociception and pain in crustaceans is needed. However, it should be noted that in conducting this review, the author is examining the science and is in no way advocating for careless or indiscriminate use of crustaceans by researchers or industry, out of fundamental respect for life itself (Adamo 2016a).

Definitions of pain

The scientific problems that have been identified in the field of crustacean pain arise in part from the difficulties with dealing scientifically with subjects that are often subjective. The word "pain" was first developed to describe a human emotional experience often (but not always) associated with trauma or injury (https://www.iasp-pain.org/terminology?navItemNumber=576), so the word "pain" may be accurately used when discussing the relative experiences of humans and closely related primates, other mammals, or even birds. However, as taxa further and further away in evolutionary and morphological terms from humans are considered, it is reasonable to ask how analogous their experiences to noxious stimuli are to the human experience, and therefore how relevant phylogenetically retrospective use of the word "pain" becomes (Derbyshire, 2016; Diggles, 2016). For this reason, some scientists consider it inappropriate (or even mischievous) to use the word "pain" to describe behaviours and experiences of fishes (and crustaceans), as this is essentially a form of anthropomorphism (Rose, 2007; Rose *et al.*, 2014) that invites false equivalence between the experience of those animals and that of human pain (Derbyshire, 2016).

For example, the low-voltage electric shocks used by some research groups to generate behavioural changes in crabs have not been shown to specifically induce nociception (Puri and Faulkes, 2015), and therefore could represent an "irritation", "stimulus", "unpleasant sensation", "buzz", "itch", or "tingle" if applied to human skin under similar circumstances. In such contexts, the word "pain" is being used instead of other arguably more appropriate terms, possibly because other words do not carry the same legal meaning or headline potential (Stevens *et al.*, 2016; Boutron and Ravaud, 2018). For these reasons, a valid working definition of pain is vital when studying its underlying mechanisms.

The key features of the definition of pain by the International Association for the Study of Pain (IASP) are that pain is *(i) an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage; (ii) pain is always subjective; and (iii) pain is sometimes reported in the absence of tissue damage and the definition of pain should avoid tying pain to an external eliciting stimulus* (https://www.iasp-pain.org/terminology?navItemNumber=576, last accessed 8 May 2018). The IASP also define nociception as "*the neural process of encoding noxious stimuli*, though *pain sensation is not necessarily implied*", nociceptors as "a high-threshold sensory receptor of the peripheral somatosensory nervous system that is capable of transducing and encoding noxious stimuli", and noxious stimuli as "stimulus that is damaging or threatens damage to normal tissues".

Because nociception occurs widely in the animal kingdom (Tobin and Bargmann, 2004; Smith and Lewin, 2009; Sneddon, 2018), it is very important to understand that "activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state" (https://www.iasp-pain.org/terminology?navItemNumber=576, last accessed 8 May 2018). With this in mind, it is clear that nociceptors are not "pain receptors", as pain (as humans know and understand it) is experienced emotionally in the brain and therefore is likely to only be experienced by animals with brains that are sufficiently developed to generate phenomenal consciousness and sentience (Broom, 2013; Rose *et al.*, 2014; Key, 2015). Hence, conclusions by the EFSA (2005) that decapod crustaceans "have a pain system" were premature, especially considering their statement predated the first scientific confirmation of the presence of nociceptors in a decapod crustacean by a decade (Puri and Faulkes, 2015).

Operational definitions of pain have been inconsistent or absent

Other problems exist in the area of definitions. Scientists operating in the field of fish and crustacean welfare cannot meet the criteria for pain established for humans, as some of these criteria are untestable in animals. As a response to criticism about their assumptions and conclusions (e.g. Rose, 2002, 2003, 2007; Puri and Faulkes, 2010; Browman and Skiftesvik, 2011; Rose *et al.*, 2014), alternative criteria for defining animal pain were developed (Sneddon *et al.*, 2014). These criteria extended the earlier much criticized "more than a mere reflex" definition and the authors proposed that "if animals fulfill (the) criteria… they should be considered capable, beyond a reasonable doubt, of experiencing pain with implications for their health and welfare" (Sneddon *et al.*, 2014).

The criteria included a list of minimal anatomical prerequisites including presence of nociceptors, central processing in the brain, efficacy of analgesia (but see Barr and Elwood, 2011) as well as behaviours including avoidance responses, behavioural changes, motivational states, and other characteristics, some (but not all) of which needed to be fulfilled in order to "characterize pain beyond a reasonable doubt" (Sneddon *et al.*, 2014). While more detailed than the "more than a mere reflex" definition, the new definitions remain "loose". For example, Sneddon *et al.* (2014) do not specify if a minimum level of brain development is required. In the case of crustaceans, this is important as there are over 67 000 described species (Zhang, 2011) exhibiting a huge range in size and anatomical complexity (Regier *et al.*, 2010). Furthermore, as pointed out by Browman and Skiftesvik (2011), the situation leaves room for researchers to pick and choose the criteria that they want to use, and ignore the ones that don't work for them. The end result has been publication of several more studies since Sneddon *et al.* (2014) with the same methodological and interpretational problems as the earlier studies. However, these more recent papers not only ignore the earlier criticisms of their methodological and interpretational flaws, but use Sneddon *et al.* (2014) to support the interpretation that they have found behavioural evidence that is "consistent with criteria for pain" or "consistent with the idea of pain" (e.g. Elwood and Adams, 2015; Rey *et al.*, 2015; Magee and Elwood, 2016; Elwood *et al.*, 2017). In effect, the criteria published by Sneddon *et al.* (2014) have simply "moved the goalposts", without addressing the earlier scientific criticisms and have thereby effectively lowered the "evidential bar" needed to claim that the criteria for pain have been fulfilled in fish and invertebrates. The risk of this approach is evidenced by the fact that robots also fulfil many of these criteria (Adamo, 2016a, b).

In summary, the criteria for pain proposed by Sneddon *et al.* (2014) set "the bar" for pain too low to establish any real

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Table 1. Summary of studies using electric shocks on decapod crustaceans.

Study	Species	Voltage (V)	Current (mA)	Frequency (Hz)	Duration (s)	Outcome
Maldonado and Miralto (1982)	Mantis shrimp *S. mantis*	n/a	6.4–74.4	n/a	0.1	Most animals respond with tail flick between 8 and 38.1 mA current. "dose response" to morphine analgesia[a].
Lozada *et al.* (1988)	Ghost crab *C. granulatus*	1–10	n/a	50	1	A total of 22.6% of crabs respond at 6 V, 77.4% respond at 10 V, rest unresponsive. Morphine reduces crabs defensive response to shock in a dose-dependent manner[a].
Kawai *et al.* (2004)	Crayfish *P. clarkii*	6.5	0.68 V/cm	n/a	0.4 s every 3 s	Learning to avoid electric shock after 20 trials/day for 32 days was context dependent. Around half of crayfish facing an escape door learned to avoid shock by walking through the door, vs. 0% for those facing away from the door.
Appel and Elwood (2009a)	Hermit crab *P. bernhardus*	0.2–19	n/a	200	1 s every 2 s	Crabs respond to electric shock at 9.0–9.1 V regardless of shell type, but more voltage required to evacuate a more preferred type of shell[b]. Autotomy in 10% of crabs.
Appel and Elwood (2009b)	Hermit crab *P. bernhardus*	10	n/a	200	0.2 s every 20 s	Shocked crabs more likely to abandon shell and move into new shell faster than unshocked crabs[b]. Female crabs have lower shock response threshold than males.
Elwood and Appel (2009)	Hermit crab *P. bernhardus*	8	n/a	200	1 s every 20 s	Shocked crabs more likely to abandon shell and move into new/less preferred shell than unshocked crabs[b].
Barr and Elwood (2011)	European shore crab *C. maenas*	8	n/a	180	0.2	Morphine inhibits movements with or without shocks[b].
Magee and Elwood (2013)	European shore crab *C. maenas*	10	n/a	180	0.2 s every 5 s	Crabs learned to avoid multiple shocks by avoiding specific shelters[b]. Autotomy in 18.9% of crabs, 10 crabs that autotomized 1 leg did not exhibit different behaviour.
Elwood and Adams (2015)	European shore crab *C. maenas*	10	n/a	180	0.2 s every 10 s	Shocked crabs had higher haemolymph lactate than controls[c].
Magee and Elwood (2016)	Hermit crab *P. bernhardus*	1–25	n/a	180	0.2	Initial response 4–6 V (range 1–10 V) in all crabs, but 35.3% of crabs did not evacuate shell up to 25 V[b]. Crabs less likely to evacuate shells when exposed to odours from predators or potential food items[d].

n/a = data not shown, therefore difficult to determine shock intensity.
[a]Result may be due to "generalized non-responsiveness" due to morphine rather than analgesia of nociception.
[b]Nociception unknown/doubtful at voltages used in absence of current data.
[c]Result may be due to uncontrolled factors such as muscular contraction or respiratory/cardiac apnoea and/or cardiac bradycardia.
[d]Result may be due to uncontrolled/unrelated factors such as olfaction/gustation.

confidence that the alleged "pain behaviours" in crustaceans (or fish for that matter) are truly related to anything in any way analogous to the definition and use of the word pain that we are familiar with as humans (see Derbyshire, 2016).

Problems with experimental administration of putatively "painful" stimuli

Application of mild, low-voltage (1–10 V, unknown current) electric shocks to crabs (e.g. Elwood and Adams, 2015), though likely to be sufficient to initiate muscle activity (Stevens *et al.*, 2016), may not represent a truly noxious stimulus to crustaceans as they are unlikely to cause tissue damage (Puri and Faulkes, 2010, 2015). This is an important point that was suggested experimentally by the lack of morphine analgesia to such shocks in shore crabs (*Carcinus maenas*) (Barr and Elwood, 2011, though see section on Misinterpretation of "morphine analgesia").

The underlying problem is the absence of validated, reliable methods for initiating and measuring nociception in crustaceans using electric shocks. Indeed, electrical stimulation of peripheral nerves has been used to treat pain in humans (Mobbs *et al.*, 2007), demonstrating that all electrical shocks are not necessarily "painful". Because of the absence of published electrophysiological data on nociceptor activation in crustaceans exposed to mild electric shocks and undisclosed currents, more recent research by Magee and Elwood (2013, 2016) and Elwood and Adams (2015) is critically flawed as activation of nociceptors has been assumed by the authors, but not demonstrated (Table 1).

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Puri and Faulkes (2015) noted that the behavioural data produced in the various papers by Elwood *et al.* can be interpreted in ways that do not require nociception to explain the results, and several researchers have pointed out that electric shocks are likely to activate any electrically excitable cell, including non-neural ones (Puri and Faulkes, 2015; Stevens *et al.*, 2016). If nociceptors are not being specifically stimulated by the undisclosed electrical currents used in these experiments (it is electrical current, not voltage, that activates tissues), the behaviours of the crustaceans studied could therefore simply be habituation or associative learning in response to an irritating stimulus, rather than a specific nociceptive response to tissue damage (Puri and Faulkes, 2015; Stevens *et al.*, 2016), and learning in invertebrates is not evidence of awareness or pain (Tobin and Bargmann, 2004; Broom, 2013; Rose *et al.*, 2014). Because of these reasons, studies that utilize electric shock on crustaceans without disclosing electrical current data or demonstrating nociceptor activation should be discouraged.

Negative results and alternative interpretations are often ignored

It has been previously pointed out that many studies about aquatic animal welfare "ignore negative results" and "inflate the science boundary" (Browman and Skiftesvik, 2011; Rose *et al.*, 2014). When reviewed objectively, scientific claims that fish or crustaceans "may feel pain" have been largely based on a few dubious and disputed studies done on a small number of animals and species in instances where laboratory artefacts are known to have occurred, all variables that potentially influence the results are not fully controlled, and interpretations of results have been questionable and sometimes contradictory (e.g. Newby and Stevens, 2008, 2009; Newby *et al.*, 2009; Puri and Faulkes, 2010; Rose *et al.*, 2014; Stevens *et al.*, 2016; Diggles *et al.*, 2017; Key *et al.*, 2017).

For crustacean research, these problems are explored in more detail in the section on Specific problems with the scientific literature on putative pain in crustaceans, but in many cases the flaws in these studies have arisen in part due to failure to control other variables known to affect animal behaviour (e.g. chemosensation, olfaction, gustation), and failure to consider alternative, more parsimonious interpretations (Rose *et al.*, 2014; Stevens *et al.*, 2016; Diggles *et al.*, 2017; Key *et al.*, 2017; Boutron and Ravaud, 2018). Notably, when some of these studies (e.g. Sneddon, 2003; Barr *et al.*, 2008) have been repeated by other research groups (Newby and Stevens, 2008; Puri and Faulkes, 2010), some of the "key findings" were not replicated (Newby and Stevens, 2009; Puri and Faulkes, 2010). These problems are all signs of research in an emerging scientific field that is immature.

Specific problems with the scientific literature on putative pain in crustaceans

A more detailed review of key crustacean welfare papers highlights several specific scientific problems that are evident in the literature on putative pain in crustaceans.

Misinterpretation of "morphine analgesia"

Earlier studies (Maldonado and Miralto, 1982), reported apparent dose-dependent "morphine analgesia" after electric shocking (range 8−38.1 mA current) in mantis shrimp (*Squilla mantis*). A few years later Lozada *et al.* (1988) reported similar results for a varunid ghost crab (*Chasmagnathus granulatus*), using low voltages (1–10 V) but unreported amperages (Table 1). However, Bergamo *et al.* (1992) observed that threat responses in *Carcinus mediterraneus* tapped on the carapace with a 10 g weight were also halted when the crabs were dosed with morphine, a result consistent with later research showing that morphine has various non-specific effects on crustacean behaviour (Nathaniel *et al.*, 2010, Imeh-Nathaniel *et al.*, 2017). Subsequent studies on shore crabs noted that morphine may result in "general non-responsiveness" (Barr and Elwood, 2011) that could explain the claimed "analgesic effect" of morphine reported in the earlier studies, leaving open the question of whether nociception was ever achieved by Maldonado and Miralto (1982) and Lozada *et al.* (1988) using electric shocks, especially given nociception was not demonstrated in either study.

Experiments employing electric shocks: technical shortcomings and misinterpreted results

Other literature relating to application of electric shocks to crustaceans are summarized in Table 1. Kawai *et al.* (2004) examined if crayfish (*Procambarus clarkii*) could learn to avoid electric shocks (6.5 V for 0.4 s, every 3 s) by exiting a compartment via an escape door. Electric shocks initiated tail flip escape responses. However, over time around 50% of crayfish could learn to avoid the electric shock by walking through the escape door, but only if they were facing toward the escape door. Crayfish oriented away from the escape door never learned to avoid the shock, showing that avoidance learning was context dependent. Appel and Elwood (2009a) reported motivational trade-offs in hermit crabs (*Pagurus bernhardus*) exposed to electric shocks as evidence of a "potential pain experience". They found crabs first reacted to electric shock at the same voltage (9.0–9.1 V) regardless of shell type, but it took more voltage on average to evacuate a more preferred shell type (*Littorina*, 17.7 V) compared with a less preferred shell type (*Gibbula*, 14.9 V). However, no measurement of the amperage used was reported in that study (Table 1), and no electrophysiological evidence of presumed nociceptor activity was provided, hence it is difficult to ascertain if sufficient electrical current was used to activate tissues, and what receptors (if any) were activated. Elwood and Appel (2009) exposed the same species to mild electric shocks (8 V) intended, in the authors words "to be below the level that would cause the crab to leave the shell and thus judged not to be severe". Hence given the absence of current (amperage) data or electrophysiological evidence of nociceptor activity, and given that electric shocks would interact with a wide range of cells and receptors (Derby and Steullet, 2001; Puri and Faulkes, 2010, 2015; Stevens *et al.*, 2016), it is again impossible to determine whether nociception occurred at all, or if so, whether it occurred in the absence of other confounding factors in the Elwood and Appel (2009) study, or for that matter in any of their subsequent studies in which electric shocks were used and electrical current and electrophysiological data were not reported (Appel and Elwood, 2009b; Barr and Elwood, 2011; Magee and Elwood, 2013, 2016; Elwood and Adams, 2015; Elwood *et al.*, 2017; Table 1). The main problem with electric shock, especially for aquatic animals, is that it non-specifically activates any electrically excitable cell, including non-neural ones (e.g. muscle tissue), meaning that assumed "nociceptive behaviours" triggered by such stimuli may represent abnormal responses of the nervous system (or other systems, see Derby and Steullet, 2001), rather

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than reveal the workings of a nociceptive sensory system tuned to tissue damage by evolution (Puri and Faulkes, 2015).

These issues with research using electrical shocks on crabs were highlighted upon publication of a paper by Elwood and Adams (2015) who exposed shore crabs (*C. maenas*) to electric shocks (10 V, but again unknown current), and measured increased haemolymph lactate in shocked crabs, concluding that their study "fulfils the criteria expected of a pain experience". A comment on the article (Stevens *et al.*, 2016) highlighted some of the scientific flaws in Elwood and Adams (2015) and advised policy-makers not to make inappropriate decisions about crab welfare based on such studies. First, Stevens *et al.* (2016) pointed out that Elwood and Adams conflicted the terms "stress" and "pain", which involve two separate pathways in all animals. This is because Elwood and Adams (2015) used a known stress marker (haemolymph lactate) to suggest that the shocks used induced pain in the experimental crabs without inducing muscle activity, thus correlating stress with pain. Stevens *et al.* (2016) pointed out that it has been known for many decades that elevated lactate in crabs occurs in association with many other conditions, for example, during any muscular activity or with an increase in water temperature, and that some earlier studies showed the lactate levels reported by Elwood and Adams (2015) were within the normal range for *C. maenas*. Furthermore, they also pointed out that other studies have reported lactate levels in exercised crabs that were ten times higher than those reported by Elwood and Adams (2015). It is also known that exposure to a "wide range of chemical and environmental stimuli" such as flashes of light or touching the eyes or carapace (Wilkens *et al.*, 1974), and thus also presumably electric shocks, can stop the heartbeat and breathing of crustaceans for short periods (cardiac bradycardia and/or respiratory apnoea), increasing lactate via temporary anaerobic metabolism as part of a normal anti-predator "startle response" that could hide the crustacean from predators that can detect weak electric fields (such as sharks and rays) (Wilkens *et al.*, 1974; Stevens *et al.*, 2016).

In summary, Stevens *et al.* (2016) found that the methods used by Elwood and Adams (2015) could not distinguish between normal stress or startle responses and pain, and that there were reasonable alternative explanations for the elevated lactate reported (i.e. alternative and more parsimonious explanations for their results apparently were not considered and certainly were not tested experimentally).

Interactions between electric shocks and learning behaviours in various decapods continue to be investigated, and the observations continue to be interpreted as being "consistent with the idea of pain" (Magee and Elwood, 2016). However, this more recent work has the same problems as the previous studies. For example, in a study by Magee and Elwood (2016), 35.3% of hermit crabs (*P. bernhardus*) did not evacuate their shells despite having been exposed to voltages of up to 25 V. In the absence of electrical current and electrophysiological data, it is impossible to determine whether non-evacuation of shells was due to insufficient current, as well as the odour interactions that were also being studied. For the latter, hermit crabs were less likely to evacuate their shells when exposed to "predator odours" (water from tanks containing *C. maenas*), but unexpectedly, hermit crabs were also less likely to evacuate shells when an undiluted "non-predator odour" (=mussel odour) was added. Magee and Elwood (2016) speculated that "This could be due to the concentration of the odour being unusually high and hence novel when compared to a rocky shore, where there would be regular flushing. Alternatively, it could be due to an association of mussel beds with predators of hermit crabs, such as larger shell breaking crabs". The authors did not mention that mussels are a potent food source for crustaceans, containing large quantities of attractants, which are known olfactory and gustatory stimulants (Kasumyan and Doving, 2003; Derby *et al.*, 2016), which can influence crustacean behaviour (e.g. Stocker and Huber, 2001). Hence, failure of a hermit crab to evacuate its shell in Magee and Elwood (2016) could also be associated with olfactory, gustatory, or other chemosensory stimuli that may have initiated other unforeseen behavioural interactions (e.g. preparation for exploratory food searches).

Effects of chemical applications are often not replicable and their interpretation is controversial

Several studies have examined the effects of chemicals, temperature, and other potentially noxious non-electrical stimuli on crustacean behaviour (Table 2). Barr *et al.* (2008) studied the effects of 10% acetic acid (vinegar), bases (sodium hydroxide), and anaesthetic (benzocaine) on glass prawns (*Palaemon elegans*), concluding that by grooming and rubbing antennae exposed to these compounds (including benzocaine), treated prawns were "attempting to ameliorate a painful effect of the stimulus.....consistent with the idea of pain". However, other researchers repeated the study using three other crustacean species [crayfish *P. clarkii*, white shrimp (*Litopenaeus setiferus*), and grass shrimp (*Palaemonetes* sp.)] and failed to replicate these results (Puri and Faulkes, 2010). Instead, they found no responses to extreme pH (hydrochloric acid and sodium hydroxide) in any of these species. They also failed to find any behavioural or physiological evidence (including electrophysiological evidence) that antennae contained nociceptors, suggesting either that the ability to respond to pH was not universal in crustaceans or (more likely), that Barr *et al.* (2008) had mischaracterized normal grooming or other behaviours as evidence of nociception (Puri and Faulkes, 2010).

Kotsyuba *et al.* (2010) injected the claws of shore crabs (*Hemigrapsus sanguineus*) with formalin to investigate how stressors affected nitric oxide production. Formalin-injected crabs exhibited hyperactive movement of that cheliped followed by autotomy of the appendage in 80% of crabs from a polluted area, and 60% mortality within 4 h. In contrast, rates of both autotomy and mortality in crabs taken from a relatively unpolluted area were only 10%, showing how environmental stressors could significantly affect results. Dyuizen *et al.* (2012) also injected formalin into the claws of *H. sanguineus*, at a dose rate of 50% of that used by Kotsyuba *et al.* (2010). Formalin-injected crabs exhibited increased flexion, movement and rubbing of the injected claw in first 3 min post injection, with autotomy of the injected claw occurring in 20% of crabs within 32 s. The remaining crabs reduced use of the injected cheliped over the next 10 min compared to saline-injected controls (none of which autotomized any claws) before reverting to normal behaviour. The autotomy behaviour in these two studies is notable as its prevalence was dependent on the formalin dose used. However, given that autotomy of claws in edible crabs (*Cancer pagurus*) was no more stressful than handling alone, based on haemolymph glucose and lactate measurements (Patterson *et al.*, 2007), the relevance of autotomy to crab welfare in this context remains unclear.

Aggio *et al.* (2012) investigated exposure of blue crabs (*Callinectes sapidus*) to chemical deterrents and found that they

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Table 2. Summary of studies using chemicals and temperature variations on decapod crustaceans.

Study	Species	Chemicals/temperature	Concentrations	Outcome
Barr *et al.* (2008)	Glass prawn *P. elegans*	Acetic acid, sodium hydroxide, benzocaine	10% (acetic acid, sodium hydroxide), or 2% benzocaine	Increased grooming and rubbing of treated antennae (including antennae treated with benzocaine)[a].
Puri and Faulkes (2010)	Crayfish *P. clarkii* white shrimp *L. setiferus* grass shrimp *Palaemonetes* sp.	Hydrochloric acid, sodium hydroxide, benzocaine	6 mol/l HCL and NaOH for *P. clarkii* and *L. setiferus*, 1 mol/l HCL and NaOH for *Palaemonetes* sp., 2% benzocaine for *P. clarkii*	Responses to extreme pH were not observed in any of the species examined. No behavioural or electrophysiological evidence was found that antennae contained nociceptors for extreme pH or benzocaine/ethanol.
Kotsyuba *et al.* (2010)	Shore crab *H. sanguineus*	Formaldehyde	1% formaldehyde in physiological solution, Dose 0.001 ml/g body weight	Crabs from a polluted area had higher haemolymph nitric oxide levels than crabs from a clean area. Formalin-injected crabs (into the right cheliped) exhibited hyperactive movement of that cheliped. Autotomy of injected cheliped occurred in 80% of crabs from the polluted area (60% mortality), but only 10% of crabs from the clean area exhibited autotomy (10% mortality).
Aggio *et al.* (2012)	Blue crab *C. sapidus*	*Aplysia* ink, denatonium, quinine, caffeine, cinnamaldehyde	Undiluted (ink) or 5 mmol/l (all others)	Crabs always searched for deterrent laced food and place it in their mouth parts. The deterrent effect manifests via oesophageal taste receptors as either rejection or extensive manipulation, but in both cases crabs bit the adulterated food.
Dyuizen *et al.* (2012)	Shore crab *H. sanguineus*	Formaldehyde	1% formalin in artificial seawater. Dose 0.0005 ml/g body weight	Formalin-injected crabs (into the right cheliped) exhibited increased flexion, movement, and rubbing of that cheliped in first 3 min post-injection. Autotomy of injected cheliped occurred in 20% of crabs within 32 s. Reduced use of injected cheliped in remaining crabs over next 10 min post-injection.
Puri and Faulkes (2015)	Crayfish *P. clarkii*	Low and high temperatures, capsaicin, isothiocyanate	Soldering iron at 54°C, dry ice at −78.5°C fed peppers (capsaicin) or wasabi (isothiocyanate) *per os ad libitum*	No aversion or grooming responses to capsaicin or isothiocyanate, no response to low temperatures, however vigorous escape responses when touched with a hot soldering iron but no long-term trauma noted. Nociceptors sensitive to heat confirmed by electrophysiology[b].
Elwood *et al.* (2017)	European shore crab *C. maenas*	Acetic acid, capsaicin, mineral oil	10% acetic acid (vinegar), 0.018 g capsaicin per 10 ml mineral oil, undiluted mineral oil	Application of acetic acid to the mouth and eyes resulted in vigorous movement of mouth parts[c] and in the case of application to the mouth rapid movements interpreted as attempts to escape the enclosure[d].

[a]Result probably due to mistaking normal grooming or chemosensory behaviour as nociception.
[b]First demonstration of nociceptors in crustaceans.
[c]Nociception not demonstrated by electrophysiology.
[d]Movements may be due to uncontrolled/unrelated factors such as preparatory food finding exploration following olfaction/gustation.

always searched for deterrent-adulterated food and placed it in their mouth parts, with rejection of adulterated feed only occurring after tasting and extensive manipulation. This suggests that chemicals that are deterrents for other animal species are not necessarily deterrents for crabs. In a landmark study Puri and Faulkes (2015) were the first scientists to report the presence of nociceptors in decapod crustaceans when they found behavioural and electrophysiological evidence of activation of nociceptors sensitive to heat by touching crayfish (*P. clarkii*) with a soldering iron set at 54°C. However, they failed to find any

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evidence of nociception when crayfish were exposed to dry ice at −78.5°C, or to nocigenic chemicals in foods including capsaicin (in peppers) or isothiocyanate (wasabi) (Puri and Faulkes, 2015). The recent animal welfare legislation in Switzerland states that lobsters and other crayfish cannot be transported on ice or in ice water (https://www.blv.admin.ch/blv/de/home/tiere/tierschutz/revision-verordnungen-veterinaerbereich.html). The evidence used for this decision is unclear, given the observations of Puri and Faulkes (2015) that suggests the absence of nociception in crayfish exposed to cold temperatures. Perhaps this is because, in contrast with crustacean welfare literature published by some other research groups, the study of Puri and Faulkes (2015) did not gain international headlines, but instead they prudently cautioned readers against over interpreting their results.

A study by Elwood *et al.* (2017) tested behavioural responses of the European shore crab (*C. maenas*) to 10% acetic acid (vinegar) and capsaicin solutions applied to the mouth and eyes. Like Puri and Faulkes (2015), Elwood *et al.* (2017) did not find evidence of any nocigenic effects of capsaicin. However, application of acetic acid to the mouth and eyes of *C. maenas* resulted in vigorous movement of mouth parts and, when applied to the mouth rapid movements that were interpreted as "attempts to escape the enclosure" (Elwood *et al.*, 2017). When acetic acid was applied to an eye, the mouth parts moved and the claws scratched at the mouth in a similar manner to when acetic acid was applied to the mouth, but the eyes tended to be held down for longer. The authors suggested that one possible reason for the mouth part movements in response to the eye treatment was that a small groove near to the base of each antenna might allow some of the acetic acid to trickle down to the mouth area. Elwood *et al.* (2017) claimed (again without electrophysiological evidence) that the increased activity in crabs exposed to acetic acid was evidence of nociception and subsequent behaviours were "consistent with the idea of pain". They also noted that while Puri and Faulkes (2010) found no evidence of nociception in response to hydrochloric acid, Barr *et al.* (2008) and Elwood *et al.* (2017) found that "acetic acid has considerable effect when applied to antennae, eyes and mouth. . ..(and) it seems unlikely that this is a species effect and it might be that different acids act differently on nociceptors".

A more plausible interpretation of the results of Elwood *et al.* (2017) is that some acidic compounds act on chemoreceptors that are not nociceptors (see Diggles *et al.*, 2017). The fact that different acids affect behaviour of aquatic animals in different ways was demonstrated by Lopez-Luna *et al.* (2017), who found zebrafish (*Danio rerio*) larvae exposed via the water to acetic acid exhibited different behaviour compared to fish exposed to citric acid in water of the same pH. Fish exposed to citric acid remained active even at concentrations of 5% when pH dropped as low as 2.6, while fish exposed to the acetic acid showed an initial increase in activity at low concentrations (0.1%), followed by a decrease in activity at higher acetic acid concentrations. The decreased activity was assumed by Lopez-Luna *et al.* (2017) to be due to "pain", however earlier researchers had assumed that increased (not decreased) activity was evidence of nociception in zebrafish larvae exposed to acetic acid via the water (Steenbergen and Bardine, 2014), calling the construct validity of the assay into question.

Administration of acetic acid via the water (in fish) or via topical application (crabs) has not been shown to trigger nociceptors in any electrophysiological studies to date in either fish (Diggles *et al.*, 2017) or crustaceans, despite the fact that nociception has been assumed to occur by several authors (Steenbergen and Bardine, 2014; Elwood *et al.*, 2017; Lopez-Luna *et al.*, 2017). A critical point that has been overlooked in these studies is that some acids trigger chemoreceptors that initiate non-nociceptive behaviours (Derby and Steullet, 2001; Derby *et al.*, 2016). For example, citric acid is a potent feeding stimulant for some fish species invoking strong exploratory behaviour (Kasumyan and Doving, 2003; Diggles *et al.*, 2017), while dilute (10%) acetic acid (vinegar), has a distinctive taste and smell which is a potent olfactory/gustatory stimulant for humans and arthropods (e.g. *Drosophila*, see Landolt *et al.*, 2012; Joseph and Carlson, 2015). It is well-known that crustaceans will attempt to eat a wide variety of foods, even foods laced with compounds that are considered by humans to be deterrents (Aggio *et al.*, 2012; Puri and Faulkes, 2015) including unpalatable acids (Derby *et al.*, 1984), although it appears that no research has been published to date in the scientific literature on the chemosensory attractiveness of acetic acid for fish or crustaceans.

Hence the "escape" behaviours observed by Elwood *et al.* (2017) (and for that matter, the "pain"/grooming behaviours reported by Barr *et al.*, 2008) may actually be food seeking exploratory behaviours in animals where chemoreceptors (particularly olfactory and gustatory receptors) have been triggered by vinegar (Table 2). Given the unsubstantiated assumption that exposure to dilute acetic acid/vinegar via the water "only stimulates nociceptors", research is needed to determine whether acetic acid/vinegar is also a chemosensory (particularly olfactory and gustatory) stimulant for fish and crustaceans, as it is for insects and humans.

Only a narrow range of decapod species have been studied to date

The literature in Tables 1 and 2 summarizes studies that have been undertaken on ten species of decapod crustaceans. Even within such a small range of species, a wide range of responses to putatively noxious stimuli have been reported, with few consistent scientifically valid outcomes except for the apparent lack of response to the supposedly nocigenic compound capsaicin (Puri and Faulkes, 2015; Elwood *et al.*, 2017). With many different classes and orders of crustaceans (e.g. Martin and Davis, 2001) being utilized by humans, and no exploration of nociception or welfare outside of a miniscule proportion of the Order Decapoda, it is clear that the scientific literature on this subject is limited and immature.

How high should the scientific bar be set?

In the context of the above, there is a conspicuous lack of scientifically valid evidence of pain in crustaceans at this time, including several examples of nonreplicable results and/or overinterpretation of behavioural responses to assumed aversive stimuli (Boutron and Ravaud, 2018) in situations where it is questionable whether nociception has even occurred (Rose *et al.*, 2014; Puri and Faulkes, 2015; Stevens *et al.*, 2016). Hence, in a strict sense there is currently no scientifically valid reason to change the welfare status of any crustaceans. The scientific problems surrounding methodologies for confirming the presence and activation of nociceptors and initiating nociception, physiological, and behavioural definitions, the problem of inconsistent results that are difficult to interpret, as well as of other perplexing issues such as the

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significance of autotomy and regrowth of limbs must all be resolved, before this field of research can move forward.

The question of "when is the right time?" to protect crustaceans under welfare legislation is a loaded one, and will depend on the height at which the "evidential scientific bar" is set (Birch, 2017). Consequently, there may never be a "right time" based on scientifically valid criteria, if these criteria cannot be met. In the field of aquatic animal welfare this difficult problem usually leads to discussions relating to invoking "the benefit of the doubt", and the precautionary principle.

Birch (2017) discussed the application of the precautionary principle to the problem of animal consciousness/sentience (one of the pre-requisites for pain). He defined the precautionary principle in the context of animal sentience as follows:

> Where there are threats of serious, negative animal welfare outcomes, lack of full scientific certainty as to the sentience of the animals in question shall not be used as a reason for postponing cost-effective measures to prevent those outcomes.

Birch (2017) then outlined how decisions to enact the precautionary principle in this context come down to two main decision criteria, first a burden of scientific proof:

> When there is a live scientific hypothesis that posits a causal relationship between human action and a seriously bad outcome, we should set an intentionally low evidential bar for the acceptance of that hypothesis in the context of formulating policy.

Second, there is a decision rule:

> Once we have sufficient evidence of a threat of a seriously bad outcome, we should act, in a timely and cost-effective manner, to prevent that outcome. The implication is that the goal of preventing the seriously bad outcome deserves sufficient priority that, once the evidential bar is cleared, it is inappropriate to delay action further.

Based on these decision criteria, Birch (2017) pointed out that the key to precautionary reasoning is to delimit carefully what constitutes "seriously bad outcomes". This is because if there is no imminent threat of seriously bad outcomes, there is no urgency to lower the "evidential scientific bar". In the context of holding live crustaceans captive at a research facility (for example) the imminent threat of "seriously bad outcomes" must be compared with a valid benchmark—which if a nature-based definition of welfare is used (Diggles *et al.*, 2011), could well be the living conditions of those animals in the wild.

Wild caught prawns (*Penaeus* spp.) held at an aquaculture research facility as broodstock will be used here as a case study to examine their welfare needs (see Specific manipulations used in a prawn research facility for a more detailed case study). Prawns are important components of the natural food chain in the wild and are subject to high predation pressure (Salini *et al.*, 1990). Removing prawns from their natural environment and rearing them in captivity for use as broodstock thus releases them from external predation pressure (though they do cannibalize if given insufficient food), and therefore reduces the majority of the imminent threat of "seriously bad outcomes" for those individuals. Given the high economic value of broodstock and expense of research, much effort is then expended to ensure any experimental manipulations are not compromised by artefacts from captive holding, which is why husbandry procedures aim to maximize survival of captive prawns by optimizing water quality, food supply and biosecurity to control and/or eliminate naturally occurring diseases. In effect, the chances of "seriously bad outcomes" for these experimental animals are extremely low, far less than for prawns in their natural environment, being restricted to only the specific experimental manipulations they are given. For a more detailed assessment of the welfare issues related to such experimental manipulations on prawns, see the section on Specific manipulations used in a prawn research facility.

The problem with the "benefit of the doubt" or "precautionary principle" is that by inviting a lowering of scientific standards, it can be misused or lead to undesirable, unintended consequences. It has been suggested that the precautionary principle is basically a socio-political manoeuvre that effectively excludes valid science from policy, and that allowing the "benefit of the doubt" is not benign, nor may it be the best way to protect aquatic animal welfare (Rose *et al.*, 2014). Perhaps this is why Birch (2017) insists that there should *not* be any lowering of scientific standards, at least initially when considering new taxa. The aim of his appeal to uphold "normal scientific standards" revolves around a premise that "a low evidential bar should not be applied when inferring the presence of credible indicators of sentience. It should instead be applied at a later stage: it should be applied when making a precautionary attribution of sentience on the basis of a single credible indicator, and when extrapolating across a whole order from a single species. There should not be any lowering of standards with regard to the methodology of experiments, or with regard to the analysis of experimental data" (Birch, 2017).

Birch (2017) highlighted the problems that surface if scientific standards are lowered with his example of reversal of the burden of proof to assume an animal is sentient unless there is conclusive evidence otherwise. The major problems with this were identified by him as "being unscientific or anti-scientific", and that such a position would make "the science of animal sentience. . . more or less irrelevant to the scope of animal protection law: all animals would be assumed sentient unless proven otherwise, and it is hard to see how research could prove otherwise", leading to "inclusion of nematodes and insects within the scope of animal protection legislation, creating significant practical obstacles to biomedical research" (Birch, 2017).

On initial assessment, to the scientist, decision makers or interested layperson the prospects of this reversal of burden of proof actually occurring might appear remote (despite the wishes and activities of animal rights organizations), however in the field of aquatic animal welfare it is already happening given the nascent uncritical acceptance of the "animal pain" criteria summarized by Sneddon *et al.* (2014) (https://www.hakaimagazine.com/features/fish-feel-pain-now-what/). These criteria essentially lower the "evidential bar" needed to claim pain in fish and invertebrates to a point where insects measured against the criteria are already being considered as "probably sentient" (Barron and Klein, 2016, Klein and Barron, 2016), and robots also fulfil many of the criteria (Adamo, 2016a, b). It is known, however, that insects are descended from crustaceans (Regier *et al.*, 2010). Therefore, even speculation about the possible existence of consciousness and "pain" in insects (Fischer, 2016) does not require that it also occurs in crustaceans (Puri and Faulkes, 2015), as it is possible

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that nociception evolved in insects after their split from crustaceans, although some authors group all arthropods together in this regard (Mallatt and Feinburg, 2016).

Given that crustaceans and other arthropods also commonly and naturally exhibit behaviours such as autotomy [loss and regrowth of entire claws and limbs (Maruzzo *et al.*, 2005; Patterson *et al.*, 2007, Appel and Elwood, 2009a; Kotsyuba *et al.*, 2010; Dyuizen *et al.*, 2012; Magee and Elwood, 2013], this brings the validity of many of the criteria presented by Sneddon *et al.* (2014) into serious question if the word pain (as used and understood by humans) is to be applied to crustaceans. Certainly, it is difficult to understand what adaptive advantage an ability to "feel pain" would confer to a crustacean that spontaneously sacrifices appendages by autotomy during normal development or when under stress (Patterson *et al.*, 2007; Kotsyuba *et al.*, 2010), only for those appendages to be grown back in later moults (Maruzzo *et al.*, 2005). In fact, Patterson *et al.* (2007) found that induced autotomy of a claw in edible crabs (*C. pagurus*) was no more stressful than handling alone, based on measurements of haemolymph glucose and lactate. Indeed, autotomy in crustaceans (which can occur in the absence of mechanical loading), appears fundamentally different to autotomy which follows mechanical damage in lizards or mammals such as African spiny mice (*Acomys* spp., see Seifert *et al.*, 2012). The same can be said for insects that exhibit behaviours such as eating their own innards and continuing feeding while being eaten (Adamo, 2016a). Accepting pain (in any human understanding of the word and its meaning) for animals that can naturally undergo such behaviours seems bizarre.

Another problem with the definitions for pain presented by Sneddon *et al.* (2014), is that the requirement for "central processing in the brain" does not specify the minimal level of brain complexity needed to achieve the processing required for a "pain" emotional response to nociception. In discussions of insect and crustacean awareness, it has been suggested that while animals with decentralized nervous systems (e.g. jellyfish, annelids) do not meet the criteria for phenomenal consciousness, the number of neurons in those invertebrates with a central ganglia that could be considered as a primitive, crude brain is unimportant (Klein and Barron, 2016). Others, however, consider this position extremely liberal and that it is unscientific to assume that small neuronal numbers are unimportant for phenomenal consciousness, an important pre-requisite for pain (Adamo, 2016b). Indeed, sceptics note if neuron number is not considered important (a honey bee has < 1 million neurons, a mouse 68 million and a human 86 billion, see Klein and Barron, 2016) and "subjective experience" is defined solely as an ability to react to the environment in any purposeful way (e.g. learning and motivated behaviours, see Sneddon *et al.*, 2014), then speculation that nematodes (Tobin and Bargmann, 2004), insects (Adamo, 2016b), and crustaceans may be conscious is likely to be upheld based on existing claims, suggesting that by this definition it would be entirely plausible that they "have feelings" and therefore potentially "feel pain" (Fischer, 2016; Elwood, 2017).

It should be made clear that the word "feeling" is consistently misused or unqualified in discussions of animal pain or consciousness. There is abundant evidence that conscious feelings are distinct from unconscious emotions, just as conscious pain is distinct from unconscious nociception (Le Doux, 2012; Rose *et al.*, 2014). In fact, unconscious emotions are better designated as actions of unconscious neural survival circuits, which are doubtless present in all forms of animals (Le Doux, 2012).

On the other hand, misuse and loosely defining of the word "feeling" would also mean that the same definitions could be used to argue (despite the protests of some, see Elwood, 2017), that robots can have subjective experiences, which could include pain (see Adamo, 2016b). This possibility puts the argument about the validity of the pain criteria of Sneddon *et al.* (2014) into perspective and brings a whole range of new problems into welfare science, including such issues as the thousands (or millions) of allegedly "sentient beings" you would kill while driving your car across the countryside (Fischer, 2016).

In other words, in embracing such liberal definitions, taking them to their inevitable conclusion, and then suggesting that regulatory changes are required to protect these "sentient beings" from possible harm, there is a risk of making welfare science effectively meaningless (Fischer, 2016). This is exactly why some scientists advocate for more pragmatic, evidence-based approaches to fish and crustacean welfare that are based on objective assessments of measureable, well-established physiological stress and health-related indices, rather than on speculation as to what fish, crustaceans or other invertebrates may (or may not) be feeling (Diggles *et al.*, 2011; Rose *et al.*, 2014; Stevens *et al.*, 2016). Because of the many problems and uncertainties regarding crustaceans (and fish, nematodes and insects) summarized above, and in the absence of imminent threats of "seriously bad outcomes" (see the section on Specific manipulations used in a prawn research facility), there is no urgency to lower the "evidential scientific bar" for these groups of animals at this time, and in the case of decisions affecting regulation of research and food production industries, strong arguments can be made to maintain the highest scientific standards possible when considering such questions, particularly regarding the methodology of experiments and analysis and interpretation of data (Birch, 2017). Otherwise if the precautionary principle is enacted too early, a paradoxical ("Catch 22") situation may arise and restrictive animal ethics requirements may hinder or even prevent studies that could provide the data needed to solve the unresolved scientific questions (Rose *et al.*, 2014).

Anthropomorphic interpretations of stress in crustaceans

Unlike the issue with sentience and pain, stress is well established and well defined and can be assessed in fish and crustaceans by measuring hormonal, biochemical, or other changes to the normal physiological state of an animal as it tries to adapt to changing environmental conditions (Stoner, 2012). Stress modulates the immune response and is one of the key factors influencing health and disease states (Adamo, 2012). Therefore, there has been a large amount of research defining useful stress indicators for measuring crustacean welfare (e.g. Paterson *et al.*, 2001, 2007). Stress in crustaceans can be assessed by measuring changes in immune function such as total haemocyte counts and prophenoloxidiase markers (Soderhall and Cerenius, 1992). Crustaceans subject to stressors also release biogenic amines such as epinephrine and serotonin (Adamo, 2012) or hormones such as crustacean hyperglycaemic hormone (CHH), which elevates haemolymph glucose concentrations in response to stressors such as moulting, emersion, and eyestalk ablation (Chang *et al.*, 1999). Measurements of haemolymph lactate have also been used to

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measure responses to exercise stress (Booth and McMahon, 1985) as well as natural (Wang *et al.*, 2018) or anthropogenic stressors (Patterson *et al.*, 2007; Bakke and Woll, 2014), while measurements of other haemolymph parameters such as metabolites (urea, ammonia, nitric oxide) or even total protein concentration are also useful (Kotsyuba *et al.*, 2010; Dyuizen *et al.*, 2012; Bernardi *et al.*, 2015), as are assessments of impaired reflexes (Stoner, 2012).

While the fact that crustaceans become stressed in some situations is not controversial, several authors have attempted to attribute emotional states to stressful situations, including Elwood and Adams (2015) who exposed shore crabs (*C. maenas*) to electric shocks and attempted to relate a stress marker (increased haemolymph lactate in shocked crabs) to "a pain experience". Recent studies have also reported that responses of crayfish (*P. clarkii*) to prolonged (30 min) exposure to stressful stimuli (electric fields) raised serotonin levels in the brain and resulted in avoidance of light for 30–90 min (Fossat *et al.*, 2014). Inhibition of the behaviour by injection of serotonin antagonists, and the lack of involvement of dopamine (Fossat *et al.*, 2015) led to claims that the behaviour was similar to anxiety in humans as defined by "a behavioural response to stress, consisting in lasting apprehension of future events", and speculation that crayfish have emotions (i.e. conscious feelings) (Fossat *et al.*, 2014, 2015). Similarly, Bacque-Cazenave *et al.* (2017) studied aggression and fighting and dominance hierarchies in *P. clarkii* and found that serotonin levels increased in losers, which often continued to be attacked by dominant crayfish leading to them to speculate that the "hostile behaviour resembled psychological harassment in humans". However, this seems extremely speculative, especially considering that the ongoing "harassment" observed may simply be an experimental artefact of confinement of crayfish in small aquaria (leading to an inability of the loser to leave the home range of the dominant crayfish), while previous studies in other species of crayfish (*Astacus astacus*) and squat lobsters (*Muninda quadrispina*) linked increased serotonin to aggression, not anxiety (Antonsen and Paul, 1997; Huber and Delago, 1998). The claims of Bacque-Cazenave *et al.* (2017) and Fossat *et al.* (2014, 2015) appear to be classic cases of anthropomorphism, as not only are some of their results inconsistent with previously published work, there is absolutely no empirical justification for casual, inappropriate, and misleading use of language from the field of human psychology to explain crayfish behaviour.

The recent increase in use of fish and invertebrates as model organisms by researchers who usually study human health-related topics using more traditional mammalian laboratory animals (such as mice), seems to have led to increased attribution of human-like traits to the subject animals. This is anthropomorphism which, when applied uncritically in the context of animals that are a large distance in evolutionary and morphological terms from humans, reduces the credibility of aquatic animal welfare science (Rose, 2007; Browman and Skiftesvik, 2011; Boutron and Ravaud, 2018). It seems in the field of aquatic animal welfare science, more than most others, there are "many examples of selective preference of scholars and audiences for exciting research results over metaphorical buckets of ice water" (Allen-Hermanson, 2017). The reality is that, using the above instances as an example, dominance hierarchies in crayfish are a behaviour evolved by natural selection to assist in partitioning of sometimes scarce natural resources (e.g. food, shelter) and determine gene progression through mate selection (Stocker and Huber, 2001). Size, past experience, visual cues, olfactory cues, previous social history, moult stage, crayfish density, and a range of other factors influence the outcomes of fighting behaviours in crayfish (Rubenstein and Hazlett, 1974; Schneider *et al.*, 2001; Stocker and Huber, 2001; Cook and Moore, 2009). Even though some of the hormonal mechanisms associated with such behaviour may be conserved between crayfish and humans, crayfish did not evolve from humans so attribution of the human feeling state to the crayfish is highly speculative and must be viewed with extreme caution. The nature of the extent of such speculation could also be illustrated by way of asking whether adult crayfish might experience feelings like sadness, remorse, or guilt while cannibalizing their offspring.

Specific manipulations used in a prawn research facility

A case study examining welfare of broodstock prawns held in a research facility is of direct relevance to researchers and the prawn (shrimp) farming industry in the Americas and Asia-Pacific regions. This study is performed to clarify whether these captive experimental animals may be exposed to "seriously bad outcomes" requiring intervention from welfare legislation during typical experimental manipulations, including electro ejaculation, pleopod clipping, haemolymph sampling, eye stalk ablation, tagging, stunning, and euthanasia.

Electro-ejaculation of male prawns as part of reproductive management

For research into methods of improving production of larval and juvenile prawns, manual and electro-ejaculation methods are used to obtain sperm from male broodstock prawns for fertilization and/or sperm assessments (e.g. Sellars *et al.*, 2013). Manual methods involve applying slight pressure to the prawn body to examine whether sperm are released. Electro-ejaculation involves placing two electrodes on each side of the thelycum and applying controlled low (<10 mV) currents from 3 to 9 V to initiate muscle contractions that release spermatophores (Soundarapandian *et al.*, 2013). Single application of the procedure has no notable side effects, but repeated forced ejaculation may be associated with increased frequency of melanization of the reproductive tract (Braga *et al.*, 2018). If results from Appel and Elwood (2009a, b), Elwood and Appel (2009), or Elwood and Adams (2015) were accepted uncritically, the electro-ejaculation process could possibly be considered "painful" due to the use of electric shocks strong enough to trigger muscle contractions. However, as pointed out in the section on Experiments employing electric shocks: technical shortcomings and misinterpreted results, in the absence of evidence of nociceptor activation triggered by such stimuli, there is no scientifically valid evidence that the process of electro-ejaculation in prawns causes nociception, hence it cannot be considered a "potentially painful process". Instead, the process is likely to result in stress equivalent to exercise (from muscle contraction) and handling, hence suitable precautions to reduce handling and exercise stress should be used to minimize any adverse effects from electro-ejaculation procedures. It is notable that in other areas of veterinary medicine and animal science, it is common to collect semen from domestic ruminants using electro-ejaculation without sedation or anaesthesia.

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Pleopod clipping for sample acquisition

Clipping of between 10 and 75% of the distal part of the pleopods of prawns or lobsters is usually done using sterilized scissors to obtain samples of tissue for genetic analysis, or to test for moult stage, or disease agents as part of routine disease screening (Sahul Hameed *et al.*, 2005) or development of specific pathogen-resistant (SPR) or specific pathogen-free (SPF) stocks (Wyban, 1992; Lightner, 2011). Pleopods are poorly vascularized appendages, and due to the nature of the appendage the majority of the tissue sampled is of ectodermal origin (i.e. cuticle or carapace) that is naturally lost and regenerated each moult, meaning that the clipped pleopod is quickly regenerated. While clipping causes mechanical damage, it is not known whether the pleopods of crustaceans have nociceptors. However, there are many studies of stress markers and immune parameters in crustaceans that show that pleopod clipping is no more stressful than handling alone (e.g. Paterson *et al.*, 2001). Because of the minimal invasiveness and natural redundancy (due to moulting) inherent with this procedure, pleopod clipping should be considered a minor manipulation provided suitable precautions to reduce stress from handling and exercise are employed.

Injections and haemolymph sampling

Sampling of haemolymph from juvenile and adult prawns is usually done using suitable gauge needles (e.g. 25–27 G hypodermic needles) for a variety of tests (e.g. measurement of haemolymph immune or stress parameters). Sterile needles are inserted through an arthrodial membrane previously swabbed with ethanol, haemolymph drawn into the syringe (which is often pre-primed with anticoagulant), then the needle is removed and the sample is processed. The haemolymph of healthy crustaceans exhibits rapid clotting so the small needle puncture wound closes and heals rapidly. As for pleopods, it is not known whether the arthrodial membranes of crustaceans have nociceptors, however there are many studies of stress markers and immune parameters in crustaceans that show haemolymph sampling is another minor manipulation that is likely to represent little more than the stress usually encountered by handling alone (e.g. Paterson *et al.*, 2001). Again, this example is analogous to human and veterinary medicine; analgesics are not employed when taking a blood sample.

Eye stalk ablation of female prawns to stimulate maturation and spawning

The eyes of prawns contain a gland called the X organ, which secretes hormones including gonad inhibiting hormone (GIH) that can inhibit maturation of the eggs and sperm of broodstock prawns. Eyestalk ablation is a process involving cauterizing or cutting of one of the eyestalks of a mature (>10-month old) broodstock female prawn using flame-sterilized hot forceps or ligation in order to alter the hormonal balance by reducing production of the inhibition hormones, allowing the prawn to undergo the final stages of maturity in captivity (Uawisetwathana *et al.*, 2011). Use of flame-sterilized forceps ensures a rapid (< 2 s) ablation process as well as cauterization and sterilization of the wound. This process was developed in the 1970s to enable larval production to occur over a narrower time frame, and has allowed the prawn farming industry to develop worldwide as the procedure ensures reliable and timely hatchery production schedules (http://www.fao.org/fishery/culturedspecies/Penaeus_monodon/en). Without eyestalk ablation, spawning is not guaranteed in some prawn species (e.g. *Penaeus monodon*), making it very difficult to structure research or breeding programs. It is also notable that, like autotomy and regrowth of limbs, some broodstock prawns may grow back ablated eyes over time (<6 months) if permitted to do so (Desai and Achuthankutty, 2000), although this may not be a common occurrence.

A study by Taylor *et al.* (2004) suggesting eyestalk ablation in *Penaeus vannamei* was stressful (based on observations of erratic or spiral swimming) was not controversial at the time as it is well-known that crustaceans can experience physiological stress. Later, Diarte-Plata *et al.* (2012) repeated the same study on a different host species but used the word "painful" instead of stressful, based on an unsubstantiated assumption that tail flicking, rubbing, and non-sheltering were evidence of "pain". However, tail flicking escape responses and rubbing are not specific to nociception and have not been validated as evidence of "pain" in crustaceans (Puri and Faulkes, 2010; Rose *et al.*, 2014). Furthermore, rubbing or tail flicking do not always occur when prawns have their eyestalks ablated (B. K. Diggles, personal observations). While it would be expected that heat from flame-sterilized forceps would activate nociceptors (Puri and Faulkes, 2015), responses to heat occurred in around 2 s in their study (Puri and Faulkes, 2015), hence rapid action (< 2 s for hot forceps) would reduce the duration of the noxious stimulus during ablation. Puri and Faulkes (2015) also stated in their discussion that their results "suggests that crayfish have nociceptors specialized to detect noxious high temperature stimuli. Nevertheless, whether a species has nociceptors or not is not conclusive evidence that the species feels pain".

So while eyestalk ablation procedures lasting >2 s may induce nociception (Puri and Faulkes, 2015) and be stressful, nociception does not necessarily lead to pain, and stress does not equal pain (Stevens *et al.*, 2016). However, if the stress involved with the handling and ablation process is to be minimized, the option to use a topical anaesthetic such as xylocaine (Taylor *et al.*, 2004) may be considered appropriate if its efficacy has been demonstrated and its use does not interfere with other variables to be measured in the experiment. The option to replace ablation with alternative procedures (such as natural spawning) may not be appropriate for those prawn species that do not respond well to environmental manipulations aimed at inducing spawning (e.g. *P. monodon*). Furthermore, given that the number of eggs obtained from non-ablated *P. monodon* is significantly lower than that obtained by ablated prawns (Uddin and Rahman, 2015), alternatives to ablation for such species would require use of many more broodstock prawns, which contravenes a fundamental welfare principal of reduction of the numbers of animals impacted by human manipulations.

Tagging by injecting elastomer implants, PIT tags, ring tags, or glued tags

To facilitate identification of individual prawns, various tagging procedures can be used, including injecting elastomer implants, injecting Passive Integrated Transponder (PIT) tags, gluing tags onto the carapace, or placing circular "bird-band" tags around the eye stalk. The issues relating to the injection of elastomer implants or PIT tags into the muscle of the abdomen are virtually identical to those of injection and haemolymph sampling (see section on Injections and haemolymph sampling) with sterilization of equipment reducing chances of bacterial infection while

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the small puncture wound from the needle closes and heals rapidly around the tag such that there are no significant health complications with the tagging process when done correctly. The advantage of the injection of tags is that they can persist in growing animals between moults, reducing handling requirements compared to simply gluing tags onto the carapace or use of bird-band tags around the eyestalk. Neither of the latter procedures are invasive, but both require repeated handling of prawns over time and potential loss of data as the tags are shed with the exoskeleton during each moult. Given that repeated handling is likely to be as stressful (or more so) than the process of injection of the tags, there would seem to be no valid reason to require replacement of invasive tagging procedures with non-invasive ones if invasive tagging (elastomer tags, PIT tags) provides advantages for a given experimental design. Moreover, in veterinary medicine, analgesics are not employed when inserting PIT tags in cats or dogs.

Stunning and euthanizing prawns (ice slurry, anaesthetics)

For purposes of producing healthy prawn stocks free of pathogens, best practice biosecurity arrangements are commonly employed, which involves destructive disease testing of broodstock prawns after spawning to ensure that the progeny stocks will be free from diseases that impact their future health (Wyban, 1992). While this may appear wasteful, euthanizing the relatively small numbers of short-lived broodstock (the lifespan of penaeid prawns seldom exceeds 2 years) can ensure better health outcomes for the millions of progeny animals produced. As such, this approach is considered best practice because control of disease is considered the highest priority for overall welfare of the captive prawn population (Lightner, 2011).

Euthanizing prawns is usually done using an ice slurry (a 0°C mixture of >2 parts crushed ice to 1 part salt water) or, very occasionally, anaesthetic (Aqui-S, isoeugenol active). Submerging adult broodstock *P. monodon* in an ice slurry for >1 min is an effective way to euthanize these large prawns that are reared at tropical water temperatures (29°C) (B. K. Diggles, unpublished data). Ice slurry is also effective for sedating or euthanizing large mud crabs (*Scylla serrata*), see https://www.youtube.com/watch?v=i-wkdRmdrrE. Broodstock prawns placed in an ice slurry appeared to be initially stunned by the temperature difference, almost ceasing activity and exhibiting no more than two or three tail flips in the first 30 s, before ceasing movement altogether. However, if prawns were removed from the ice slurry before 1 min and placed back in 29°C seawater, it was possible for them to recover to normal activity over the next 30 min or so (B. K. Diggles, unpublished data).

In contrast, use of the recommended commercial concentration of Aqui-S anaesthetic (17 ml per 1000 l or 17 mg/l) in 29°C seawater did not induce noticeable anaesthesia after 60 min (B. K. Diggles, unpublished data), resulting in numerous tail flip escape attempts whenever the prawns were touched. This was not surprising, as Coyle *et al.* (2005) found that MS222 and 2-phenoxyethanol were ineffective while isoeugenol was effective for anaesthetizing freshwater prawns (*Macrobrachium rosenbergii*), but only at concentrations 5–10 times higher (100–200 mg/l) than those used on finfish. It is notable that exposure to such high concentrations of anaesthetics poses a health and safety risk to researchers, particularly if they are exposed for long periods. Given the fact that low temperatures do not activate nociceptors in some crustaceans (Puri and Faulkes, 2015), ice slurry appears a highly effective, non-chemical method for humanely stunning and euthanizing prawns and also other tropical crustaceans that should be considered superior to the use of anaesthetics, which besides having questionable efficacy for crustaceans (and occupational health and safety risks for users when used at high concentrations), require disposal of the chemicals into the waste water stream (usually down the sink or drain). The latter contributes to unintended downstream effects on the welfare of wild fishes and shellfish as the chemicals enter the waste water stream and, ultimately, the environment as organic contaminants (Diggles *et al.*, 2011, 2017).

Conclusions

(1) Given the critical flaws in design and interpretation of several crustacean "pain" studies, and the inconsistent results obtained from the extremely narrow range of crustacean species studied to date, acceptance of claims of pain for these animals, even as a precautionary measure, would represent acceptance of a much lower evidential bar than is usually dictated by normal scientific standards. The proposed criteria for animal pain (Sneddon *et al.*, 2014) effectively set "the bar" for pain and sentience so low that it is impossible to have confidence that the behaviours observed in many experiments are even due to nociception, let alone in any way analogous to how the word pain is used and understood by humans.

(2) The present situation may be leading to circumstances whereby weak scientific literature on crustacean welfare is being used by decision makers to justify additional, possibly unnecessary constraints on scientific research that uses crustaceans, imparting significant costs (in time and experimental flexibility) to scientific programs (and potentially also food production industries), which would likely exceed any minor beneficial changes in welfare status that may (or may not) accrue to the experimental animals.

(3) There are already several examples of non-replicable results in the literature on alleged "pain" in crustaceans (Puri and Faulkes, 2010) and/or overinterpretation of behavioural responses to presumed aversive stimuli (Puri and Faulkes, 2015; Stevens *et al.*, 2016). In the crustacean welfare literature to date, more biologically plausible and parsimonious alternative explanations to conclusions of crustacean "pain" are often being ignored (e.g. Rose *et al.*, 2014; Puri and Faulkes, 2015; Stevens *et al.*, 2016; Allen-Hermanson, 2017; Diggles *et al.*, 2017; Key *et al.*, 2017). This leads to the conclusion that the scientific literature on this subject is immature, and does not include scientifically valid evidence of pain in crustaceans.

(4) Appeals for application of the precautionary principal extend the "benefit of the doubt", which requires that the "scientific evidential bar" for formulating policy be lowered when there is a scientific hypothesis that posits a causal relationship between human action and "seriously bad outcomes" (Birch, 2017). However, in the absence of imminent threats of "seriously bad outcomes" (such as in the case study herein related to prawn aquaculture research), there is no urgency to lower the "evidential scientific bar" for including crustaceans under animal welfare legislation at this time.

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(5) If the precautionary principle is enacted too early, a paradoxical "Catch 22" situation may arise in that restrictive animal ethics requirements may hinder or even prevent the high quality studies needed to provide the data to solve unresolved scientific questions. Furthermore, animal ethics regulations for use of animals for scientific purposes in some countries appear to have no mechanism for reviewing or withdrawing existing protections once enacted (e.g. NHMRC, 2013). This means that inclusion of new taxa into welfare legislation is often a one way street that should, therefore, require considerable weight of evidence that should require no lowering of scientific standards with regard to the design, methodology, and replication of experiments, or the analysis and interpretation of data (Birch, 2017). It remains the task of the broader scientific community to ensure that the highest scientific standards are upheld in this regard. Alternatively, if the precautionary principle is used to justify inclusion of certain animal groups under welfare regulation, policy-makers should be obliged to regularly review the scientific criteria used to justify such decisions, and/or include provision for "sunset clauses" for the withdrawal of such taxa from protection if more robust scientific data becomes available at a later date which invalidates the preliminary results used to trigger the precautionary decision.

(6) High quality science is expected from national research institutions studying, for example, problems in crustacean food production industries that may underpin a nation's food security. This does not mean that crustaceans should be used (or abused) for such activities carelessly or indiscriminately, but it is important that the quality of any research that may influence regulatory decisions that constrain such national research institutions (or food production industries) should be equally high. In the case of decisions affecting regulation of crustacean research, strong arguments can be made to maintain the highest scientific standards possible when considering such questions.

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Declaration of interest

The author is an independent scientist who works on topics related to aquatic animal health and welfare for a wide range of government, aquaculture, and fisheries institutions in the Asia-Pacific region. He acts as a reviewer for several aquatic animal health journals and has developed, and administers publically available aquatic animal welfare tools such as the ikijime.com website and ikijime tool phone applications in his own time, at his own expense. The author has previously published articles identifying weaknesses in the science underlying aquatic animal welfare.

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