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Strong inferences about pain in invertebrates require stronger evidence

Commentary on [Crump et al.](#) on *Decapod Sentience*

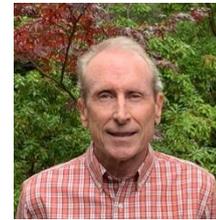
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Abstract: Evidence for sentience in animals distantly related to humans is often sought in observations of behavioral and neural responses to noxious stimuli that would be painful in humans. Most proposed criteria for painful sentience in “lower” animals such as decapod crustaceans have no necessary links to the affective (“suffering”) component of pain. The best evidence for painful affect in animals is learned aversion to stimuli associated with noxious experience, and conditioned preference for contexts associated with relief from aversive consequences of noxious experience, as expressed in voluntary behavior. Such evidence is currently lacking for any invertebrate except octopus.

[Edgar T. Walters](#), Professor of Integrative Biology and Pharmacology, investigates plasticity of neural and behavioral responses to bodily injury in molluscs (*Aplysia* and squid), cellular mechanisms of persistent pain induced by central or peripheral injury in rats, mice, and humans, and the evolution of pain-related mechanisms.

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1. Pain-related research using invertebrates is uncommon but has potentially important implications. The thought-provoking target article by Crump et al. (2022) proposes eight criteria for inferring the existence of painful sentience in decapod crustaceans or any other animals incapable of verbal communication. While the authors’ title suggests an inquiry into sentience in general, the criteria listed focus on the experience of pain, in part because of the authors’ interest in animal welfare.

Ethical considerations in pain-related research are significant both for the welfare of animal subjects and for human needs (e.g., informing rational but costly welfare policies for economically important species). The promise of eventual human health benefits is standard when justifying stressful experimental procedures in biomedical research with animals. Whereas animal studies primarily utilize a few mammalian species, several invertebrates have contributed to basic knowledge about mechanisms of nociception and adaptive responses to injury; notably, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Aplysia californica*, and, to a lesser extent squid, leeches, crayfish, and a handful of other invertebrates (Burrell, 2017; Walters, 2018; 2020).

Most of the information from these studies is only indirectly relevant to the ethically important question of whether any invertebrates, including decapod crustaceans, have a capacity for pain. Remarkably little is known about the critical aversive (potentially affective) aspects of responses to noxious stimulation for any invertebrate except octopus, as I detail here briefly while addressing Crump et al.’s proposed criteria for painful sentience.

1. **Nociception**
2. **Sensory integration**
3. **Integrated nociception**
4. **Analgesia:** (a) endogenous (b) exogenous
5. **Motivational trade-offs**
6. **Flexible self-protection**
7. **Associative Learning**
8. **Analgesia preference:** (a) self-administer (b) location (c) prioritised

Crump et al.'s 8 criteria

2. Less informative criteria for painful sentience. The target article's pain criteria 1 to 4 and 6 represent features that are either expressed too ubiquitously in the Animal Kingdom to be of much use in inferring pain or can be mediated straightforwardly by automatic mechanisms without a need for emotional contributions. Virtually all species, even placozoans and poriferans lacking neurons, show adaptive reactions to noxious stimulation. This stimulation is detected by specialized receptors in subsets of cells (often nociceptive sensory neurons—nociceptors—but also other cell types including immune cells and epithelial cells that can communicate humorally). All nervous systems can integrate local information with global information (e.g., about injury) communicated by specialized neural pathways, by less specialized nerve nets, and/or by far-reaching humoral signals (e.g., hormones, cytokines, growth factors, damage-associated molecular patterns). These may, in various combinations, carry complex information about noxious events, permitting some flexibility in nociceptive responses in even the simplest animals (Walters, 2019; Romanova et al., 2020; Moroz et al., 2021).

The existence of alternative pathways that could convey information about injury and inflammation means that neither conventional nociceptors nor obvious neural pathways to integrative centers are logically necessary for painful sentience. Nevertheless, nociceptors are clearly useful neurons that enable rapid communication of nociceptive information to central integrative networks, and they have been identified in nematodes, annelids, molluscs, chordates, and in one class of arthropods—Insecta (Walters, 2020). Whereas I agree that nociceptors (perhaps related to the class III and IV multidendritic nociceptors in *Drosophila*) are likely to be found eventually in crustaceans, the evidence cited in the target article is still very weak and sometimes incorrect (e.g., TRPV1 has not been documented in arthropods or any other invertebrate).

Integration of disparate sources of information by neurons in nociceptive networks will not be unique to animals with painful sentience. Every animal species examined appropriately has revealed inhibitory as well as excitatory and facilitatory chemical signals that can modulate defensive responses, as indicated by effects on neuronal membrane potentials and/or neuronal G protein-coupled receptor signaling in those species' nociceptive pathways. Furthermore, suppression of nocifensive responses by anesthetics says nothing about the quality and often little about the function of the affected neuronal activity (depending upon how precisely targeted the anesthetic delivery is). The suggestion that suppression of defensive responses in shrimp by the anesthetic lidocaine implies some homology between human and shrimp nociceptive modulation mechanisms is almost meaningless because

lidocaine-sensitive, voltage-gated sodium channels are evolutionarily ancient and expressed in most if not all animal phyla, including Cnidaria (which the authors and most of us consider unlikely to have painful sentience).

Inferring pain-related or even nociception-specific functions from the application of human analgesics to animals is also problematic. Meaningful conclusions require extensive molecular and pharmacological controls that are almost never adequate in studies of invertebrates; and drug companies have little incentive to develop or refine analgesics for non-mammalian species. As one prominent example, Crump et al. cite several of the numerous studies claiming that opioid signaling has pain-related suppressive functions in decapod crustaceans and other invertebrates. Most of these studies have used pharmacological and immunohistochemical methods that are notoriously nonspecific. Modern genomic analyses have failed to find genes convincingly related to vertebrate opioid receptor genes or opioid precursor genes in any invertebrate, indicating that opioid signaling first evolved in chordates and is absent in invertebrates (Huang et al., 2022).

Finally, long-lasting, site-specific responses to noxious stimulation can be both complex and mediated by relatively simple circuits, such as found in *Aplysia* (Walters and Erickson, 1986; Walters, 1987). If an animal lives long enough to benefit from wound-tending and has the motor apparatus to do so, there is no logical need to assume that such tending is not largely reflexive and unconscious. For example, at least one grooming behavior, directed scratching, can occur in decorticate mammals (Frigon, 2012).

3. More informative criteria for painful sentience. The most important question raised by the target article is whether invertebrates such as decapod crustaceans suffer in response to noxious stimulation. The best available evidence for negative affect in any animal is a demonstration through voluntary behavior that a nociceptive state is aversive – that, given the opportunity, an animal will choose to avoid that internal state. Such aversion doesn't prove the state involves conscious pain, but it is arguably the closest we can get to inferring a state of injury-associated negative affect that motivates flexible behavioral decisions and resembles the unpleasantness and cognitive correlates of human pain. In addition, painful aversion should be greater in animals showing nociceptive sensitization (functional equivalents of human hyperalgesia and allodynia) (Walters, 1994).

Voluntary behavior showing motivational trade-offs (target article criterion 5) can reveal the potency of an aversive state induced by noxious stimulation. The cited studies on hermit crabs by Elwood and colleagues provided evidence that aversion caused by presumably noxious electric shock in a shell could be traded off against the benefits of occupying a high-quality shell or of avoiding predator odor around a low-quality shell. These observations are interesting from a cognitive perspective, but they suggest that the motivational state induced by the applied shock was not very strong; its potency appears to have been lower than the conflicting motivation to occupy a desirable shell or to refrain from changing shells in the presence of predator odor.

What would be observed with a stronger shock or more noxious stimulus that might evoke more pain? In rats a different choice was found when substantial pain was in conflict with another strong rodent motivation: to explore. Neuropathic and postsurgical pain decreased voluntary crossings of a surface of sharp probes in an unfamiliar chamber. Given the experimental conflict between strong exploratory drive and pain stimulated by exploration,

exploratory drive dominated in normal rats, but pain dominated in injured, hyperalgesic rats (Odem et al., 2019).

Two additional operant paradigms (also dependent on voluntary behavior) for testing affective pain in mammals correspond roughly to target article criteria 7 and 8. Although not yet tested with decapod crustaceans, an elegant recent study with octopus combined these operant paradigms to provide the most convincing evidence to date that any invertebrate has painful sentience (Crook, 2021). Acetic acid was injected into an octopus arm in one of three experimental chambers. The octopuses remembered the injection chamber and subsequently avoided it (conditioned place aversion, CPA). In some octopuses the acetic acid injection was followed later by lidocaine injection into the same site immediately prior to placement in a different chamber. Subsequent testing long after recovery from the lidocaine revealed conditioned place preference (CPP) for the chamber associated with lidocaine's block of the ongoing nociceptive activity induced by the acetic acid. Thus, after only a single experience with each contingency, this invertebrate remembered both the context (place) where ongoing nociceptive activity had been initiated and the place where relief from the continuing aversive state driven by the nociceptive activity was obtained. This informative experimental design illustrates how an invertebrate's voluntary behavior can be used to reveal a link between flexible cognitive processing (including complex associative learning, memory, and decision making) and a potent aversive state induced by noxious stimulation.

4. Conclusions. Determining whether animals in various lineages, such as decapod crustaceans, experience painful sentience is a goal with profound evolutionary interest and important ethical implications. Mammalian pain research has made substantial advances in identifying the affective component of pain and optimizing methods to detect it (Kuner and Kuner, 2021; Tappe-Theodor et al., 2019). However, the more sophisticated methods have rarely been applied to invertebrates. Indeed, strong evidence for noxious stimulation driving a relatively long-lasting aversive state linked to flexible cognitive processing has only been documented rigorously in a single invertebrate species, *Octopus bocki* (Crook, 2021). Similar strong evidence from CPA and CPP tests for the aversiveness of nociceptive states is also lacking for non-mammalian chordates, including fish (Sneddon et al., 2018; Walters, 2018).

Carefully controlled operant tests analogous to those used recently to infer affective pain in rodents and octopus are feasible for many species, including decapod crustaceans. CPA, CPP, and motivational conflict tests with and without prior nociceptive sensitization are powerful, well-described indicators of nociceptive aversive states. Without such experiments, the evidence for painful sentience in these and other invertebrates will remain weak. Human impacts on decapod crustaceans and various other invertebrates with relatively complex nervous systems are frequent and often violent. More compelling experimental evidence for painful sentience in these animals could help in efforts to reduce their mistreatment.

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