"I'll have the fish and shrimps": pain and analgesia in invertebrates and fish

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Introduction

Less than 4 decades ago, it was relatively routine for human physicians to forego administering analgesic drugs to human infants, even after significant invasive surgical procedures. At the time, some human physicians and neuroscientists believed that human infants had an immature central and peripheral nervous system, which was neither structurally, nor functionally, capable of receiving and processing noxious stimuli. The thinking was that analgesic drugs would not only be ineffective, but they may contribute to deleterious physiological side-effects, which might further jeopardise the recovery and, ultimately, the health of the infant. In veterinary medicine, our understanding of pain and analgesia in domestic mammals has grown exponentially during the past 20 years, yet we still have a long way to go. Many veterinary clinicians still argue that the administration of analgesics is risky to the patient and may mask behavioral signs of pain, which are considered evolutionarily adaptive for survival. However, veterinarians have an ethical obligation to treat painful conditions in all animals, as effective pain management reduces stress-induced disruption to homeostatic mechanisms, and also decreases morbidity and mortality associated with trauma or surgery. The objective is to describe and highlight what is known with respect to our understanding of pain (nociception) and analgesia (antinociception) in invertebrates and fish. The evolution of nociception and antinociception is also salient within this discussion.

The primary question is whether fish and invertebrates "experience" pain or are they merely capable of demonstrating a "reflexive" response to a noxious stimulus (nociception)? According to the International Association for the Study of Pain (IASP), nociception is defined as "the neural processes of encoding and processing noxious stimuli", while pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (Merskey et al. 1994). More recently, a group of veterinarians, human physicians, and scientists attempted to clarify and expand upon the definition of pain as written by the IASP, and defined animal pain thus: "… animals feel pain and that although it is unclear at this time at what taxonomic level nociception is associated with pain and whether all species, including humans, feel pain with the same qualities and intensities, operationally vertebrates and some invertebrates experience pain" (Paul-Murphy et al. 2004).

Perhaps, more importantly, can we recognise pain in fish and invertebrates? Is the perception of pain by a fish or an invertebrate equivalent to that of a mammal? We will never be able to fully and objectively answer these questions, because the animals simply cannot tell us. Many would argue that fish and invertebrates do not have the same anatomical and/or physiological capabilities to "process" pain. In other words, fish and invertebrates are merely responding and passively reacting to stimuli to which they are exposed, with little or no ability for cognition or self-awareness. However, recent research in fish, amphibians, reptiles, and birds has demonstrated that the transmission of peripheral sensory signals, via the spinal cord, to

midbrain and forebrain regions are homologous to mammalian cortical and limbic structures. Additionally, the endogenous opioid system, which is activated in response to nociception and contributes to analgesia, is also well conserved throughout vertebrate phylogeny. Thus, the physiological and anatomical requirements for pain and analgesia appear to be remarkably similar among all vertebrate species. While much less is known about invertebrates, many species (especially the cephalopods) have well-developed nervous systems, and some species respond to exogenous opioids in a similar manner to that of mammals.

Measuring pain in fish and invertebrates is the most difficult hurdle in the study of pain and analgesic efficacy. Like all nonhuman species, fish and invertebrates are unable to verbally communicate pain. Discriminating normal versus abnormal behaviour can be difficult to define operationally and to measure, and typically requires a clearly defined ethogram. Species-specific behaviour must also be taken into consideration. In addition, painful behaviour is context-specific, such that behaviour in an unfamiliar hospital setting may be very different to behaviour exhibited in a home enclosure. Rather than behaviour, many investigators choose to measure physiologic parameters, such as heart or respiratory rate, body temperature, blood pressure, corticosteroids, catecholamines, amongst others.

Methods for measuring pain in animal species include the adaptation of pain rating scales developed for evaluating pain in human infants. However, pain scales, while objective, are not easily adapted to different animal species. Animal pain, or lack thereof, is also commonly assessed before and after surgical procedures. This method requires the development of a behavioural ethogram, which, in turn, requires the observer to become well versed in subtle behavioural differences through many hours of observation and analysis (videotaped or live observation). Physiological parameters, such as heart rate, respiratory rate, blood pressure, plasma cortisol/corticosterone and/or catecholamine concentrations, and serum inflammatory markers have also been used to assess an animal's response to pain. An alternative to studying post-surgical pain is to measure pain under strictly controlled laboratory conditions using established behavioural models during which noxious stimuli (e.g., mechanical, thermal, chemical) are applied to an anatomical location on the fish or invertebrate subject. Analgesic drugs can be administered and the response compared to baseline responses.

The quandary with respect to our interpretation of whether fish and invertebrates experience pain is in developing and attempting to answer scientific questions regarding pain, versus the clinical and animal welfare obligations toward treating pain. While the anatomical and physiological mechanisms underlying pain and analgesia in fish and invertebrates (or amphibians, reptiles and birds for that matter) are scientifically interesting, especially from an evolutionary perspective, are we obligated to base our clinical judgment solely on our interpretation of peer-reviewed published data? Our limited understanding of pain and analgesia in fish and invertebrates should not obscure our clinical decisions, and we should err on the side of fish and invertebrate wellbeing by making the assumption that conditions considered painful in humans and other mammals should be assumed to be potentially painful across all other vertebrate and invertebrate species. Could it be that recognition of pain in fish and invertebrates is impeded by our inability to empathise with species that do not convey distress through facial expressions, do not vocalise in response to distress, and are not warm and fuzzy?

Do fish feel pain and why do we care?

From an animal welfare perspective, there are significant sociopolitical concerns regarding the minimisation of pain and distress in animals maintained for food, sport, and research purposes. In the United States, fish represent nearly 25% of all animals used for research and education, and pet fish out-number all other pet species. Millions of fish are also maintained in zoos and aquaria throughout the world. Regulation of fish welfare could have an enormous economic impact as future global fish consumption will continue to increase and, currently, global aquaculture produces billions of dollars in revenue. In addition, there would be a dramatic impact on worldwide commercial and sport fishing industries. In Germany, fish welfare is already covered under the German Animal Welfare Act (Tierschutzgesetz; http://www.animallaw.info/nonus/statutes/stdeawa1998.htm), which covers all vertebrates, although it distinguishes between "warm-blooded" and "cold-blooded". The methods for killing sport fish are strictly regulated and anyone wishing to

fish in Germany must take lessons in fish welfare and pass an exam before being allowed to obtain a fishing license. There are also fines imposed and possible incarceration for causing harm and/or suffering.

From a clinical perspective, it is imperative that veterinarians provide pain relief to all animals, and they are ethically obligated to humane treatment of all animals, including fish. Therefore, a lack of understanding of pain in fish should not negate use of analgesics in situations considered painful in mammals. However, the choice and dosage of analgesics must be based on sound research, and sometimes the best we can do is extrapolate information from related species. Therefore, pursuit of clinically relevant pain and analgesia research is critical for expanding our understanding of these issues in fish.

There is substantial evidence that fish have the appropriate neuro-anatomical structures and pathways to experience pain (Sneddon et al. 2004; Braithwaite & Boulcott 2007; Sneddon 2009; Weber 2011). However, there are two recent, conflicting scientific perspectives, which have been at the forefront of the issue regarding fish pain. One is that fish lack a neocortex, and therefore, are unable to experience "pain" as we recognise it (Rose 2002). Rose argued that it was anthropomorphic of investigators to suggest that fish experience pain; rather, without a neocortex, a fish moving away from an electric shock or the teeth of a predator is analogous to a reflex or an unconscious behaviour. Alternatively, Sneddon and her colleagues argued that fish have peripheral nociceptors, functional neuroanatomical structures (the telencephalon is likely responsible for processing pain in fish, as in birds and mammals), and appropriate behaviour to substantiate that fish, in fact, can experience pain (Sneddon 2003; 2004; 2009; Sneddon et al. 2003a; 2003b).

While Rose continues to argue from the perspective that fish can't possibly experience pain because they lack a neocortex, others continue to provide experimental evidence in support of fish feeling pain. One argument against the hypothesis that a neocortex is necessary for an animal to experience pain is that birds, while lacking a neocortex, experience pain, which can be ameliorated using opioid drugs (Sladky et al. 2006; Paul-Murphy et al. 2009a; Paul-Murphy et al. 2009b; Cole et al. 2009). Avian brain-imaging studies support the hypothesis that portions of the telencephalon and hippocampus are associated with pain perception, and the strongest evidence across phyla is that the telencephalon, not the neocortex, is the essential brain structure required for consciousness in animals (Merker 2007).

In human brain imaging studies, only the anterior cingulate gyrus (a 'limbic' system brain component) has demonstrated a consistent response during the conscious experience of pain (Shackman et al. 2011). Current evidence suggests that the fish telencephalon is considered the centre for pain processing as in other animals (Dunlop & Lamming 2005). Anatomical and brain lesion data support that the lateral and medial pallial regions (telencephalon) of the forebrain in bony fish are homologous to the mammalian hippocampus (learning and memory) and amygdala (emotion) (Sneddon 2009). As an example, goldfish and trout exposed to noxious needle pricks and heat, as well as gentle stroking with a paint brush, demonstrated neural responses recorded in the dorsal horn of the spinal cord and ascending pathways through cerebellum and tectum and up to the telencephalon (Dunlop & Lamming 2005).

Peripheral nociceptors in fish are thought to be analogous to those in mammals. Single unit recordings were made from receptive fields on the head of rainbow trout innervated by the trigeminal nerve, and five different nociceptor subtypes were determined in trout (Sneddon 2003; Sneddon et al. 2003b). In addition, endogenous opioids and opioid receptors were found in spinal cords and brains of fish (Rodriguez et al. 2000; de Velasco et al. 2009; Gonzalez-Nunez & Rodriguez 2009; Sanchez-Simon et al. 2010; Sundstrom et al. 2010). Expression of mu-, kappa-, and delta-opioid receptors in zebra fish has been localised throughout the brain and spinal cord (Gonzalez-Nunez & Rodriguez 2009). In zebra fish, the distribution and type of opioid receptors in the central nervous system supports a sensory or analgesic role for those receptors (Gonzalez-Nunez & Rodriguez 2009).

Behavioural patterns in fish under experimental conditions also support that fish experience pain. For example, elevated respiratory rate was associated with pain or distress in trout and koi (Sneddon 2009). Fish showed tail flick responses to electric shock, fin pinching, and needle pricks, and morphine attenuated

these responses, while naloxone reversed the effects of morphine (Sladky et al. 2001; Dunlop & Lamming 2005; Braithwaite et al. 2007; Sneddon 2009). In a study in which trout lips were injected with bee venom or acetic acid, fish took longer to return to feeding, had increased respiratory rate, rocked side to side, and rubbed their lips in the bottom of their home aquarium (Sneddon 2003; Sneddon et al. 2003b). Morphine lessened these behavioural reactions to lip injection of bee venom and acetic acid (Sneddon et al. 2003b). Acetic acid injected into trout lips also caused impairment of novel object avoidance compared with fish having saline administered into their lips (Sneddon et al. 2003a).

The interpretation of these results suggested an impairment of avoidance behavior due to distraction caused by pain. A comparable phenomenon was described in humans with pain causing concentration and short-term memory deficits (Oosterman et al. 2011). With respect to efficacy of opioids in ameliorating post-surgical pain, koi undergoing a surgical procedure were administered butorphanol (kappa-agonist, mu-antagonist) or saline pre-surgically. Those receiving saline showed decreased activity, swam lower in water column, and had decreased feeding, while fish receiving butorphanol showed no significant change from pre-surgical behavior (Harms et al. 2005).

In my own recent experiments, koi undergoing a unilateral gonadectomy were more likely to return to normal behavior (food intake, increased activity, lack of hiding behavior, increased responsiveness to novel stimuli, rate of respiration post-surgically if they received morphine (5 mg/kg) compared with both saline and butorphanol (10 mg/kg) (Sladky, unpublished data). Untoward side-effects of opioids included hyperactivity in a few fish receiving morphine and buoyancy abnormalities in some of the fish receiving butorphanol. In pharmocokinetic studies of morphine in two fish species, trout and flounder, there was a rapid distribution after intracoelomic administration (within 30 minutes), but slow elimination (approximately 2 days) compared with mammals. Perhaps most importantly, the environmental (water) temperature in which the fish were maintained had a significant effect on the pharmacokinetics of morphine, with slower distribution and elimination at colder water temperatures (Newby et al. 2006).

Fish experience chemical and physiological stress responses similar to mammals. Fish produce "stress hormones" (cortisol and adrenaline) and release them in a similar way as mammals (Weber 2011). Like mammals, fish release dopamine and serotonin under stressful conditions, and substance P receptors have been found in some fish species (Moons et al. 1992). Substance P is produced in small-diameter sensory pain fibres and released into the dorsal horn of the spinal cord following noxious peripheral stimulation, promoting an increased sensitivity to pain. Substance P has been found in the central nervous system (CNS) of some fish species, with highest concentrations in the hypothalamus and forebrain (Batten et al. 1999). An evolutionarily ancient peptide neurotransmitter found in mammals, FMRFamide, was identified in trout brains (Castro et al. 2001). FMRFamide functions in analgesic and aversive responses in mammals.

What do we know about invertebrate pain?

If our understanding of pain and its measurement is limited in fish, and we potentially have little to no empathy for fish pain and distress, how do we begin to understand and measure pain and distress in invertebrates? From a scientific perspective, research evaluating pain and analgesia in invertebrates provides an additional key component to understanding the evolution of nociception. From a clinical perspective, as in fish, when we are uncertain about a painful procedure, we should err on the side of the individual animal's welfare if at all possible, and this should include invertebrates. With increasing animal care and use regulations for all research animals, scientists are moving down the phylogenetic tree, using animals, such as invertebrates, that are not yet regulated.

From an invertebrate welfare perspective, cephalopod (e.g., octopus, cuttlefish, squid) welfare is considered salient in many countries (Mather 2001; Moltschaniwsky 2007). In New Zealand (Animal Welfare Act, 1999), Australia (Queensland Government Animal Care and Protection Act, 2001), and Norway (Animal Welfare Act, 2009), cephalpods and crustaceans (e.g., crabs, lobsters, crayfish, prawns) are included in animal welfare legislation. Currently, while there is no universal legislation concerning cephalopod welfare, cephalopod welfare is legislated in Canada, the United Kingdom, and legislation is

in place in Australia and the United States in some institutions. *Octopus vulgaris* has been protected from invasive experimental research in the United Kingdom under The Animals (Scientific Procedures) Act of 1986 because of their large brains and highly developed learning skills. This legislation occurred years before invasive research on chimpanzees was restricted or banned in the United Kingdom (1997), New Zealand (1999), and The Netherlands (2002). In 2010, the European Union adopted a new set of requirements for the protection of animals in scientific procedures including those for research, education, and training, which will go into effect in 2013. This Directive will cover the welfare of all cephalopods. However, there is concern about the welfare of farmed aquatic invertebrates (e.g., shrimp, prawns, crabs, crayfish, mussels, oysters, lobsters, squid, octopus, cuttlefish, sea urchins, sea cucumbers, scallops, snails, clams, etc.). Many aquatic and terrestrial invertebrates are maintained as pets, in zoological institutions, in aquaria, and in research facilities.

What scientific evidence exists for invertebrates experiencing pain? Invertebrate nervous systems are highly diverse, ranging from the "nerve net" of the hydra, jellyfish, or sea anemone, to the relatively complex brain of the cephalopods. Except for cephalopods, "higher" invertebrates (arthropods, crustaceans, insects, arachnids) possess nervous systems consisting of several to many ganglia associated with body segments, which culminate in a primitive brain (Tobin & Bargmann 2004; Zullo & Hochner 2011). Although an octopus brain differs from a typical vertebrate's brain, it shares key features such as folded lobes, a hallmark of complexity, and distinct visual and tactile memory centers (Mather 2001; Grimaldia et al. 2007). When an electroencephalogram (EEG) is applied to the octopus brain, similar electrical patterns to vertebrates are generated, which is different from all other invertebrates studied (Zullo & Hochner 2011).

Although peripheral nociceptors have not been identified in cephalopods, there are no published reports that anyone has investigated peripheral nociception in cephalopods. On the other hand, nociceptors have been identified in anemones, sea cucumbers, leeches, nematodes, *Drosophila*, and many other insects (Kavaliers 1988; Tobin & Bargmann 2004; Xu, et al. 2006; Smith & Lewin 2009; Puri & Faulkes 2010). The leech has a segmented body; each segment possesses a ganglion containing T (touch), P (pressure) and N (noxious) cells. N cells respond to acid, capsaicin and heat (Tobin & Bargmann 2004). Other invertebrate species with peripheral nociceptors include sea slugs, fruit flies, and nematodes (Smith & Lewin 2009).

Many invertebrate species (earthworms, round-worms, molluscs, *Drosophila*) possess endogenous opioid receptors (Dalton & Widdowson 1989; Tobin & Bargmann 2004). Immunohistochemical staining indicated the presence of endogenous opioid receptors in nematodes (Prior et al. 2007). Mussels possess benzodiazepine and opioid receptors in their nervous systems (Gagne et al. 2010). In addition, there is genetic and physiologic evidence that invertebrates and vertebrates may have similar capacities with respect to pain and analgesia. The "Painless gene" in *Drosophila* is necessary for the flies to detect noxious heat (Xu et al. 2006). "Painless" encodes for the Transient Receptor Potential (TRP) ion channel, which is the evolutionary homolog of mammalian TRPA1, the ion channel that is responsible for sensing environmental irritants, pain, cold and mechanical stretch. As mentioned with fish, the FMRFamide-related family of peptides has been identified in molluscs and was implicated in modulation of nociception (Manev & Dimitrijevic 2004).

Pain-associated behaviour of invertebrates has been described in multiple species. In sea anemones, crabs, crayfish, sea slugs, snails, flatworms, crickets, praying mantis and *Drosophila*, withdrawal responses are observed with thermal and mechanical noxious stimuli (Zabala et al. 1984; Kavaliers 1988; Valeggia et al. 1989; Kavaliers et al. 1997; Wittenberg & Baumeister 1999; Kavaliers et al. 2000; Tobin & Bargmann 2004; Drew & Wood 2005; Xu et al. 2006; Pryor et al. 2007; Miller-Perez 2008; Smith & Lewin 2009; Nathaniela et al. 2010). Application of an irritant to one side of a cockroach evoked an accurately directed scratch reflex from the ipsilateral leg (Gritzay et al. 1998). Electric shock causes limb withdrawal in praying mantis (Zabala et al. 1984) and defensive responses in shrimp (Elwood & Appel 2009). It has also been demonstrated that morphine attenuated these nociceptive responses, and naloxone reversed the antinociceptive morphine effects, which implied that appropriate peripheral or central opioid receptors

are present in these invertebrate species. Land snails and cockroaches withdrew foot/limb in response to a noxious thermal stimulus, and mu-opioid agonists increased the withdrawal latency (Prato et al. 1995; Kavaliers et al. 2000; Gritzay et al. 1998). Naloxone was shown to block this increased withdrawal latency. Using an aquatic invertebrate species, applying acid to the antennae of prawns caused them to immediately begin rubbing their antennae (Elwood & Appel 2009). However, a separate study failed to replicate this in three species of shrimp, and extracellular recordings of antennal nerves showed no changes after application of acids (Puri & Faulkes 2010).

In an interesting behavioural learning study, electric shocks administered to hermit crabs caused them to vacate their shells. If novel vacant shells were provided, shocked crabs entered the new shells and never re-entered old shells, implying that the crabs associated their original shells with a negative experience. The crabs also showed post-electric shock related behaviours, such as rubbing affected body parts. These rubbing behaviours disappeared after morphine administration (Barr & Elwood 2011). In my own research, tarantulas consistently withdraw a limb after application of a noxious thermal stimulus using the Hargreaves apparatus (Sladky, unpublished data). When morphine (100 mg/kg, intracoelomic) was administered, limb withdrawal increased compared to butorphanol (20 mg/kg, intracoelomic) or saline. However, at the high morphine dosage, some of the tarantulas exhibited ataxia, although all appeared to return back to normal within 4-6 hours.

Cephalopods present a unique invertebrate phylogenetic dilemma with respect to our understanding of pain and analgesia. While no specific pain research has been conducted in cephalopods, several investigators believe that octopuses exhibit conscious behaviour (Mather 2001; Hochner et al. 2006; Moltschaniwsky et al. 2007; Mather 2008). Octopuses solve mazes, learn cues, play with objects, and remember solutions (Hochner et al. 2006; Mather 2008). Individual octopuses appear to have distinct personality traits, the first ever measured in an invertebrate species (Mather 2008). For example, octopuses confronted with the same threat alerts and food stimuli react in different ways. One might flee, but another might fight or show curiosity.

Conclusion

The clear distinction that once existed between the terms "pain" and "nociception" has become blurred recently, to the point that many neuroscientists and clinicians no longer make a distinction; that is, most accept that nociception is equivalent to pain. There is substantive and compelling evidence from the neuroanatomical, neurophysiological and behavioural literature to suggest that, at some level, a variety of fish species experience pain under certain contexts. In my clinical experience, mu-opioid agonists appear to be most effective in providing pain relief, particularly post-surgically. In my research with koi, morphine sulfate (5 mg/kg, IM) is the most effective analgesic drug with few side-effects, and I tend to use morphine or hydromorphone (2-3 mg/kg, IM) in clinical practice. The evidence in support of invertebrates experiencing pain under a variety of conditions remains inconclusive, but is compelling, nonetheless. Subjectively, in my research using tarantulas, there is little question that they react to noxious thermal stimuli in a similar manner to mammals, birds, and reptiles. In addition, hypodermic needle insertion into the exoskeleton incites an immediate withdrawal reaction, followed by limb rubbing at the site of needle insertion. The cephalopods remain the enigmas of the invertebrate world and may soon become the poster animals for increased regulation and oversight for invertebrate care and welfare, as they are believed to learn, remember, play, and possibly exhibit consciousness.

Many veterinary clinicians argue that the administration of analgesics is risky to the patient and may mask behavioural signs of pain, which are considered evolutionarily adaptive for survival. However, veterinarians have an ethical obligation to treat painful conditions in all animals, including fish and invertebrates, as effective pain management reduces stress-induced disruption to homeostatic mechanisms, and also decreases morbidity and mortality associated with trauma or surgery. However, several obstacles limit successful analgesic use, including subjectivity in pain assessment, inadequate knowledge of analgesic efficacy across species, pharmacokinetics of analgesic drugs, and the unknown relationship between risks and benefits for specific drugs. It is my hope that future research will

help us to determine if, and at what phylogenetic level, fish and invertebrates feel pain. Until then, we must use all available evidence, especially in those species most closely related to the species being studied, to err on the side of animal in subjectively assessing that a procedure considered painful in a mammal, should also be considered potentially painful in an invertebrate or fish species.

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