Ethical Issues on using Invertebrates in Environmental and Biomedical Practices – A Case Study on Living Fossil Horseshoe Crab

Akbar John B[1] , Hassan I. Sheikh[2], Ibrahim Shogar[3], Abdurrezak A.H[4], Kamaruzzaman, B.Y[2], Jalal, K.C.A[2], Ailin Razali[5],
[1]Institute of Oceanography and Maritime Studies (INOCEM),International Islamic University Malaysia,Kuantan.
[2]Department of marine science,International Islamic University Malaysia , Kuantan.
[3]Department of Computational and Theoretical Sciences, Kulliyyah of Science,International Islamic University Malaysia ,Kuantan,
[4]Department of Biotechnology,International Islamic University Malaysia , Kuantan.
[5]Kulliyyah of Medicine, International Islamic University Malaysia , Kuantan.

ABSTRACT

Increasing utilization of living animals in ecological and biomedical research has drawn serious concerns in terms of animal welfare and ethical practices in animal handling. Significant attention has been given to animals of higher taxonomical hierarchy especially vertebrates such as fishes, rodents, reptiles and mammals, while ethical framework on invertebrate handling and welfare is less addressed (except for cephalopods). The definition of 'Animal' itself by any international consortia or Animal Research Act (ARA) does not include invertebrates as an animal entity. This is due to the lack of standard ethical framework to understand the pain and other physiological stress experienced by the invertebrate test animal. One such example would be the living fossil 'horseshoe crab' which is extensively bled to obtain its blue blood that is used for endotoxin quantification in biological samples. The biomedical bleeding itself leads to 15-30% post bleeding mortality of crabs, while pain and stress caused by the bleeding practice is not studied. Hence, this paper discusses the technicality of establishing standard framework for invertebrate handling. The paper also highlights the *shari'ah* (Islamic law) principles on scientific experimentations on animal subjects, particularly the norms related to the adoption of invertebrates in environmental and biomedical practice. Comprehensive review of ethical regulations in animal experiments, especially invertebrates, would be beneficial for revising and improving existing animal ethical practices.

KEYWORDS: Invertebrates, animal welfare, horseshoe crab, Animal Research Act, *shari'ah* principle

INTRODUCTION

Among the bewildering array of animal taxa (excluding microbes), invertebrate constitutes more than 90% of the estimated more than 10 million animal species, mainly arthropods.[1] Their utilization as a major source of animal protein (especially shrimps, lobsters, crabs, clams, squids etc.) and their aquaculture potential as candidate species contributes considerable GDP to any country.[2] Invertebrates are also valued for their ability to make luxurious products such as silk, pearls, and shells; which are used for ornamental and decorative purposes.[1] Besides their direct tangible benefits to human, their complex interaction with ambient environment plays a key role in maintaining balance in biodiversity and species-species interaction.[3] A number of invertebrate especially benthic forms have been used as an indicator species to understand the health status of the environment.[4] Despite their importance, majority of invertebrates are excluded from legislation regulating scientific research on animals due to the existing criteria that only consider species/group of species as 'animal' if they experience pain through their nociceptors. Though, invertebrates experiences pain, discomfort or stress due to environmental stimuli, the knowledge on the structures and pathways involved in perceiving pain in invertebrates are limited compared to vertebrates.[5,6] Mollusks exposed to noxious stimuli that mimics the induction of pain in vertebrates exhibited similar physical response as vertebrates.[7] However, their experience of pain is different due to the absence of central nervous system (CNS) or myelin nerves that are the main structures that process pain in vertebrates including human.

Corresponding author:
Akbar John,
Institute of Oceanography and Maritime Studies (INOCEM),
International Islamic University Malaysia,
Jalan Sultan Ahmad Shah
25200 Kuantan
Pahang, Malaysia.
Email: akbarjohn50@gmail.com

Animal Utilization in Research

Current recommendations and legislation for ensuring appropriate animal care and use in biomedical research are based on the three guiding

principles of Reduction, Refinement and Replacement (3Rs) (8-11). '*Reduction*' is achieved by adopting methods which will '*minimize animal use*' by urging researchers to optimize the levels of information from fewer animals or to obtain as much information from the same number of animals, thereby reducing future use of animals. '*Refinement*' is to improve existing scientific procedures and animal husbandry which will '*minimize actual or potential pain*', suffering, distress or lasting harm and/or improve animal welfare in situations where the use of protected animals is unavoidable. '*Replacement*' refers to methods that avoid or '*replace the use of animals*' defined in an area where they would otherwise have been used.

Invertebrates as an Animal Model

Many invertebrates are used as model animal in biomedical research especially mollusk and few other arthropods as they exhibit fundamental insights into a range of biological processes involved in action potential generation, synaptic transmission, learning, memory, and more recently, nociceptive biology.[7] For instance, *Drosophila* expresses biological response similar to the rats when their $GABA_B$ receptor system is enzymatically blocked leading to compromised nociceptive response and showing a higher threshold to thermal shock. This has led to the use of *Drosophila* as animal model in various pharmacological studies, especially neurological and analgesic drug discovery.[12]

However, the definition of animal (for experimental use) by any of the international consortia or Animal Research Act (ARA) does not include invertebrates (excluding cephalopods) as test animals and hence does not require ethical committee approval to use invertebrates (except cephalopods) as test organism. Currently, most countries do not have ethical guidelines or framework on the use and handling of invertebrates in research.[13,14] The US Animal Welfare Act excludes fish and invertebrates from ethical justification in their use in research even though the Public Health Service requires it regardless of legal definition of an animal under this policy. In the United Kingdom, the Cruelty to Animals Act 1876 specifically excludes invertebrates. Recent directive from the European Parliament (2010/63/EU) have also included cephalopods (squids, cuttlefish, octopus and Nautilus etc.) in the animal use protection legislation.[15] Similarly, cephalopods are protected in Canada, but legislative protection in Australia and the United States are not at the national level and thereby are limited to institutional guidelines.[15] The Council of Europe issues a "Charter on Invertebrates" that recognizes the "compelling positive values of invertebrates including their use in science and medicine". Many institutions have developed a policy of reviewing all protocols involving invertebrate use, whereas other IACUCs may refuse to review invertebrate protocols in jurisdictions where invertebrates do not meet the legal definition of "animal." Thus, the use of Cephalopods such as cuttlefish, nautilus, octopus and squid requires IACUC review, while the use of decapods such as crabs, crayfish and lobster does not require it. The inclusion of cephalopods in these guidelines is due to an intense neurological study conducted on this animal group, while similar study on other invertebrate is still limited.[16]

Challenges in Establishing Ethical framework for Invertebrates

In comparison to the vertebrates, complexity of species diversity in invertebrates makes it difficult to come up with a standard ethical framework to address how they respond to external stimulus. Even within one group of animals, there is diverse response to the same stimulus over time. The response to the external stimulus is also influenced by age, sex and physiological condition of the test animal.[17] Therefore, many researchers argued that central nervous system provides limited clues about the potential to experience pain, while physiological changes in response to noxious stimuli or the threat of a noxious stimulus and behaviour of the organism might prove useful in formulating new ethical guidelines. However, to date, such applications on invertebrates is limited.[6,18,19]

Pain in human is experienced in the cerebral cortex, whereas fish, for example, lack this structure.[20] Therefore, if we consider only animals with pain response mechanisms and structures that are similar to those of the human brain, fish and numerous other organisms will be left out. Some invertebrates have structures analogous to the cortex. For example, specific brain areas in the octopus are specialized for sensory analysis, memory, learning, and decision making and thus may be considered analogous to the human cerebral cortex.[21] Due to this fact, the cephalopods are the only group of invertebrates coming under the current ethical framework.

Invertebrates could also respond to a noxious stimulus in an adaptive fashion via a nociceptive reflex. Previous studies proposed many criteria that better measure or demonstrate pain in various animals including amphibians [22,23], fish [24], and other invertebrates.[6,25] These criteria and animal response differ from species to species. For instance, Sea anemones respond to mechanical stimuli and to the stings of other anemones but not to thermal stimuli.[26] Annelids have nociceptors that respond to acid, capsaicin, and heat.[27] The snail (*Cepaea nemoralis*) responds to a hot plate at >40°C by lifting the anterior portion of its foot.[28] Overall, it can be argued that pain experience is associated with tissue damage typically depends on nociception, therefore lack of nociceptors would misleadingly suggest that the animal is insensitive to noxious stimuli and could not experience pain.[29] Hence, a more comprehensive investigation of the scope of the word "pain" is needed to establish a more inclusive ethical framework that covers invertebrates.

'Pain or Pain like'?

All organisms are susceptible to a variety of naturally occurring hazards that can cause tissue damage. However, animals have mechanisms that enhance their ability to maintain the integrity of their tissues through the detection of noxious stimuli and reflexes to get away from them and/or minimize their deleterious effects. The main challenge in including invertebrates in animal welfare legislation is the debate on whether invertebrates experience pain and suffering or if they simply exhibit nociception.[30]

Nociception is the ability to detect and respond to a noxious stimulus that causes an emotional perception of pain.[19,31] Nociception is defined as "the neural processes of encoding and processing noxious stimuli" or "the detection and reaction to stimuli that may compromise their integrity". The sensory systems that respond to noxious stimuli and immediate protective reflexes are termed nociceptors.[31] In contrast, the International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage".[31] The difference between nociception and pain can become clearer in Drosophila larvae. For instance, when a Drosophila larvae was attacked by a parasitoid wasp, a potential danger, it responded by rolling toward the stimulus, which caused the wasp's ovipositor to pull out and the wasp to leave.[32] This shows nociceptive behaviour of the larvae when an external stimulus is given perceptual mechanism coupled with the organization of responses in respond to an external stimulus. On the other hand, when the same larvae were exposed to thermal or mechanical stimuli, a potential tissue damage, they failed to roll away from those stimuli. This might be due to a lack of a particular functional gene that can process heat as an "unpleasant sensory" or as a "potential tissue damage". Therefore, a new definition on nociception that address the physical damage or emotional distress experienced by invertebrates is needed.

Horseshoe Crab – Case study

Horseshoe crabs are unique in their genetic makeup and are commonly known as "Living Fossil". An economic analysis indicates that the annual social welfare benefit of the fishery from horseshoe crab along the entire Atlantic coast is about $150 million for the biomedical industry and $21 million for the commercial eel and whelk fisheries.[33] However, the largest industry that utilize horseshoe crabs nowadays is biomedical industry. Horseshoe crab is the sole source of LAL/TAL (Limulus Amebocyte Lysate/Tachypleus Amebocyte Lysate) in biomedical industries.

LAL is used to detect bacterial contamination in parenteral drugs, devices, infusion and transfusion solutions. The advent of LAL has made this step easy and the reliability of LAL test is recognized by the Food and Drug Administration as an "End product" test. This triggers the biomedical companies to concentrate on LAL production using horseshoe crabs as the sole source. In the United States alone, the number of horseshoe crabs (*L. polyphemus*) harvested for the biomedical industry has nearly doubled from 1.3×10^5 to 2.5×10^5 since 1989 (34). In China alone, the total number of crabs harvested in recent years for biomedical bleeding (*n=600,000*) ended up with hundred percent mortality, as the crabs were bled to death and then sold to restaurants for human consumption.[35] The biomedical bleeding process together with handling, transportation and other external environmental stresses directly increased the mortality rate of the horseshoe crabs and thus threatens their existence. Post bleeding mortality due to stress was recorded to be between 15-30%.[34] At present, no study has been carried out to address the pain caused by bleeding horseshoe crabs as well as post bleeding stresses experienced by the animals in terms of physiological and immunological responses to the ambient conditions.[36] Hence, horseshoe crab is a typical case of a heavily utilized and unregulated invertebrate that contributes a lot to humans.

Sharia (Islamic Law) Perspective

According to the Islamic principles, it is not permitted for humanity to do everything to other living things and lives must only be taken if necessary. Furthermore, there are Islamic restrictions on manipulating animals, such as limited hours of work and maximum burden that they should bear. Hunting of young birds for pleasure is forbidden by Islam. Several Islamic manuscripts state that animals have their own position in the creation hierarchy and humans are responsible for the facilities and animals at their disposal, including animals' health and feed. Al-Quran emphasized that *"We have not created the earth and sky and whatever among them for fun, but because of justice [wisdom and necessity]; however most of them are not aware of it"* (44:38-39). *"Certainly, there is no living thing on earth or on the wing unless it belongs to its own group the same way that you humans belong to your own race; We have not ignored anything in the book [of creation] and eventually all [the living things] will be resurrected and return to their Lord"* (6:38). These two verses clarify the point that all parts of the universe have their own positions and nothing is created in vain. Al Quran also stated that humans are allowed to make use of animal for their livelihood *"And He created the cattle for you; you have in them warm clothing and (many) advantages, and of them do you eat. And there is beauty in them for you when you drive them back (to home), and when you send them forth (to pasture). And they carry your heavy loads to regions which you could not reach but with distress of the souls; most surely your Lord is Compassionate, Merciful. And (He made) horses and mules and asses that you might ride upon them and as an ornament; and He creates what you do not know" (An-Nahl 16: 5-8)"*. A question arises on 'how much we human is allowed to make use of animals'. Though, all animals are created

subservient to human being, in the Islamic perspective, human manipulation of animal life is not without restrictions and humans are only allowed to take animals' lives if and only when necessary. Numerous hadith (sayings of Prophet Mohammed [pbuh]) suggested the importance of animal care in Islam to the extent that Islam prohibits from slaughtering animals in front of one another and forbids hunting baby birds before they have left the nest. Shaddad ibn Aws reported: The Messenger of Allah, peace and blessings be upon him, said: 'Verily, Allah has prescribed excellence in everything. So if you have to kill, then kill in the best manner. If you slaughter, then slaughter in the best manner. Let one of you sharpen his knife so his animal feels no pain' (Sahih Muslim Hadith Number 1955). 'Ali Ibn Abu Talib (pbuh) says, 'Be obedient to Allah regarding His subjects and the lands at your disposal, for you are responsible even for the survival of the animals'. Hence, according to Islam, animal utilization for human benefit is allowed as long as their rights are fulfilled and un-abused.[37]

Public Perspective

The general public tends to express feelings of aversion or fear towards most invertebrates due to concerns of disease and stings from some species. Others are known as being pests/invasive species that eat people's food or even regarded as highly unattractive animals such as spiders.[1] Public concern is also economically motivated and hence empathy of people on different invertebrates varies differently based on their economic potential. The scientific community even values minimal ethical concerns for invertebrates as they are currently used as models for many experiments instead of vertebrate animals, which receive greater ethical considerations.[24] The negative feelings people have towards invertebrates makes conservation and welfare efforts challenging to be introduced.[1] While it is unlikely that humans will develop affinities for many invertebrate species, however, public awareness on invertebrate's science and their contributions to humans will help reduce negative perceptions of invertebrates.

CONCLUSION

In conclusion, unlike vertebrates, little concern is shown for the welfare of invertebrates. The challenges in establishing a standard ethical framework for handling invertebrates in biomedical experiments is mainly due to the diverse physiological and behavioural response shown by different species and ages. The new ethical framework must have a broader definition of "animal" to include invertebrates, and an extended definition of "nociception" that covers a wider range of emotional stress and physical damage. More studies are also needed to identify structures and pathways involved in invertebrate's nociception. Finally, negative perception of people towards invertebrates makes conservation and welfare efforts challenging to be introduced. Public awareness campaigns that highlight the contributions of invertebrates to science, education and humans will make conservation efforts easier to be implemented.

ACKNOWLEDGEMENT

This research work was financially supported by FRGS 15-210-0451, Ministry of Education Malaysia and RPDF 18-004-0004, IIUM.

REFERENCES

1. Kellert SR. Values and Perceptions of Invertebrates. Conserv Biol. 1993; 7(4): 845-855.
2. FAO. The State of World Fisheries and Aquaculture 2014. http://fao.org/2/sofia14e; http://www.fao.org/news/story/en/item/231522/icode/;.
3. Covich AP, Palmer MA, Crowl TA. The Role of Benthic Invertebrate Species in Freshwater Ecosystems: Zoobenthic species influence energy flows and nutrient cycling. BioScience. 1999; 49(2): 119-127. doi: 10.2307/1313537
4. Ellis DV, Agan Pattisina L. Widespread neogastropod imposex: A biological indicator of global TBT contamination? Mar Pollut Bull. 1990; 21(5): 248-253. doi: http://dx.doi.org/10.1016/0025-326X(90)90344-8
5. Gherardi F. Behavioural indicators of pain in crustacean decapoda. Annali Istituto Superiore Di Sanità 2009; 45.
6. Sherwin CM. Can Invertebrates Suffer? Or, How Robust is Argument-by-Analogy? Anim Welfare. 2001; 10(1): 103-118.
7. Crook RJ, Walters ET. Nociceptive Behavior and Physiology of Molluscs: Animal Welfare Implications. ILAR Journal. 2011; 52(2):185-195. doi: 10.1093/ilar.52.2.185
8. Buchanan-Smith HM, Rennie AE, Vitale A, Pollo S, Prescott MJ, Morton DB.. Harmonising the definition of refinement. Anim Welfare. 2005; 14(4): 379-384.
9. Kilkenny C, Parsons N, Kadyszewski E, Festing MFW, Cuthill IC, Fry D, Altman DG. Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals. PLoS ONE. 2009; 4(11): e7824. doi: 10.1371/journal.pone.0007824
10. Manciocco A, Chiarotti F, Vitale A, Calamandrei G, Laviola G, Alleva E. The application of Russell and Burch 3R principle in rodent models of neurodegenerative disease: The case of Parkinson's disease. Neuroscience & Biobehav Rev. 2009; 33(1): 18-32. doi: http://dx.doi.org/10.1016/j.neubiorev.2008.08.002
11. Richmond J. The Three Rs *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals* (pp. 3-22): Wiley-Blackwell. 2010.
12. Manev H, Dimitrijevic N. Drosophila model for in vivo pharmacological analgesia research. Eur J Pharmacol. 2004; 491(2–3): 207-208.

doi: http://dx.doi.org/10.1016/j.ejphar.2004.03.030
13. Animal Ethics Policy. IIUM (Ed.), IIUM press, Malaysia, 2012: p15.
14. Moltschaniwskyj NA, Hall K, Lipinski MR, Marian JEAR, Nishiguchi M, Sakai M, Warnke K. Ethical and welfare considerations when using cephalopods as experimental animals. Rev Fis Biol Fish. 2007; 17(2): 455-476. doi: 10.1007/s11160-007-9056-8
15. EUPCEU. The European Parliament and the Council of the European. Union. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Off J Euro Uni. 2010; 53: 33-79.
16. Huffard CL. Cephalopod neurobiology: an introduction for biologists working in other model systems. Invert Neurosci. 2013; 13(1): 11-18.
17. Lewbart GA. Invertebrate Medicine (G. A. Lewbart Ed. 2nd ed.): Wiley Online. 2011.
18. Elwood RW. Pain and Suffering in Invertebrates? ILAR Journal 2011; 52(2), 175-184. doi: 10.1093/ilar.52.2.175
19. Elwood RW, Barr S, Patterson L. Pain and stress in crustaceans? Appl Anim Behav Sci 2009; 118(3–4): 128-136. doi: http://dx.doi.org/10.1016/j.applanim.2009.02.018
20. Rose JD. The neurobehavioral nature of fishes and the question of awareness and pain. Revie Fisher Sci 2002; 10: 1-38.
21. Budelmann BU. Octopus. physiology and behaviour of an advanced invertebrate. Behav Process. 1978; 3(4):356-357. doi: http://dx.doi.org/10.1016/0376-6357(78)90009-8
22. Machin KL. Amphibian Pain and Analgesia. f Zoo and Wildlife Med. 1999; 30(1): 2-10.
23. Stevens CW. Opioid research in amphibians: an alternative pain model yielding insights on the evolution of opioid receptors. Brain research. Brain rese rev. 2004; 46(2): 204-215. doi: 10.1016/j.brainresrev.2004.07.003
24. Sneddon LU, Braithwaite VA, Gentle MJ. Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. Proc Roy Soc B: Bioll Sci 2003; 270(1520):1115-1121. doi: 10.1098/rspb.2003.2349
25. Broom DM. Cognitive ability and sentience: Which aquatic animals should be protected? Dis Aquat Organ 2007; 75(2):99-108.
26. Mather JA. Philosophical Background of Attitudes toward and Treatment of Invertebrates. ILAR Journal. 2011; 52(2): 205-212. doi: 10.1093/ilar.52.2.205
27. Smith ESJ, Lewin GR. Nociceptors: a phylogenetic view. Journal of Compar Physiol A. 2009; 195(12): 1089-1106. doi: 10.1007/s00359-009-0482-z
28. Kavaliers M, Hirst M, Teskey GC. A functional role for an opiate system in snail thermal behavior. Science. 1983; 220(4592): 99.
29. Key B. Fish do not feel pain and its implications for understanding phenomenal consciousness. Biol Philos. 2015; 30(2): 149-165. doi: 10.1007/s10539-014-9469-4
30. Harvey-Clark C. IACUC Challenges in Invertebrate Research. ILAR Journal. 2011; 52(2): 213-220. doi: 10.1093/ilar.52.2.213
31. IASP. International Association for the Study of Pain. IASP Taxonomy. 2012. http://www.iasppain.org/Content/NavigationMenu/GeneralResourceLinks/PainDefinitions/default.htm.
32. Hwang, RY, Zhong L, Xu Y, Johnson T, Zhang, F, Deisseroth K, Tracey WD. Nociceptive Neurons Protect Drosophila Larvae from Parasitoid Wasps. Curr Biol. 2007; 17(24): 2105-2116. doi: http://dx.doi.org/10.1016/j.cub.2007.11.029
33. Manion MM, West RA, Unsworth RE. Economic assessment of the Atlantic coast horseshoe crab fishery. Arlington. Virginia. 2000.
34. John BA, Jalal KCA, Zaleha K, Armstrong P, & Kmaruzzaman BY. Effects of blood extraction on the mortality of Malaysian horseshoe crabs (*Tachypleus gigas*). Mar Freshwater Behav Physiol. 2011; 44(5): 321-327. doi: 10.1080/10236244.2011.642505
35. Carmichael RH, Botton ML, Shin PKS, Cheung SG. Changing Global Perspectives on Horseshoe Crab Biology, Conservation and Management. 2015; Springer International Publishing.Netherland.
36. Anderson RL, Watson WH, Chabot CC. Sub-lethal behavioral and physiological effects of the biomedical bleeding process on the American horseshoe crab, *Limulus polyphemus*. Biol Bull. 2013; 225(3):137-151.
37. Reza Gharebaghi MRVM, Hasan Ghasemi, Heidary ADAF. Animal rights in Islam. Alter Anim Test Exper. 2007; 14: 61-63.