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HOW ONE HEALTH AND ONE WELFARE CAN STRENGTHEN THE EVIDENCE OF A MANAGEMENT PROCEDURE - A CASE STUDY OF EYESTALK ABLATION IN FARMED SHRIMP

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Abstract

One Health is connected with the One Welfare through links between animal and human welfare, and with sustainable animal-keeping systems. This connection fosters interdisciplinarity, and helps ensure human and animal wellbeing, while addressing more effectively current societal challenges in a more sustainable way. There is a knowledge gap regarding some species-specific operational welfare indicators in some aquaculture species such as crustaceans with potential to impact human wellbeing, and justifying a One Welfare approach. A review of the scientific literature based on PRISMA protocols has been carried out within this study. The review focuses on pain indicators and nociception, and the potential impact on the welfare of shellfish, as well as on the physiological and molecular mechanisms associated with the use of eyestalk ablation (EA) in adult female shrimp in aquaculture facilities. Through the One Health and One Welfare approaches, an assessment of whether the EA procedure is valid for use in shrimp management protocols was also carried out. The case study concluded that alternatives to EA should be sought to ensure compliance of this practice with the One Health and One Welfare concepts.

Key words: *aquaculture shrimp, eyestalk ablation, one health*, *Penaeus spp.*

INTRODUCTION

The concept of One Health has been practiced since ancient times, yet we have come a long way to the One Health concept as we understand it today. One Health has been recently defined by the One Health High Level Expert Panel (OHHLEP) as the concept which "recognizes that the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and interdependent" (Adisasmito et al., 2022). One Health is contributing to sustainability, addressing collective needs, such as the requirement for safe and nutritious food, as well as other needs (WOAH/OIE, 2019). One Health, used for the human, animal and environment components of health, partially overlaps with One Welfare, thus extending One Health's approach to a much broader view (Pinillos et al., 2016). Consumer protection, food safety and public health are intrinsic components of One Health, wherein traceability of animals and animal products in animal farming and the food production industry are of paramount importance, aligning One Health with One Welfare. As food provenance and farm assurance gain traction, consumers are increasingly seeking information on conditions under which their food is produced (Nicolae et al., 2017). Among known good practices, wellbeing of the producers of animal-derived food, and welfare of the farmed animals (including humane handling, and the prevention of animal disease), are required (WOAH/OIE, 2019). The importance of welfare of farmed animals is now well known, although there are still many conflicting aspects arising from its translation into practice. Identifying all (farmed) sentient non-human animals to whom strict welfare criteria should be applied is a daunting task. Ideally, a gold standard should be available to benchmark sentience amongst different species. Such a

standard is currently lacking (Regan, 1985; Diggles et al., 2024). This paper discusses the practice of eyestalk ablation (EA) in penaeid shrimp, currently applied in some shrimp (and other shellfish) maturation facilities to help overcome captivity induced to resolve the inhibition of maturation in females (Meng et al., 2020). Using the example of EA, the authors explore existing links between One Health and One Welfare, while assessing whether EA is appropriate for use in shrimp management protocols. Based on the assumptions of Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA protocols) (Moher et al., 2015), in order to be more sensitive and capture as many relevant references as possible, this review used the term shrimp interchangeable with prawn, and shellfish with crustacean. Pain was first used to describe a human emotional negative experience, and is defined by the International Association for the Study of Pain as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage" (Yue, 2008; Raja et al., 2020). Invertebrates demonstrate behavioural and neural plasticity that is similar to that of vertebrates. There is an increasing number of studies providing evidence of sentience in cephalopods due to their cognitive ability and response to noxious stimuli and pain killers (Powell, 2022). Although pain may not be directly measurable to observers of invertebrates, pain-like states can be inferred in these animals based on animal behaviour, underlying nervous activity in the nociceptive systems that process information related to an injury, sound and "vocalization", which approximately 100 invertebrate species are reported capable of producing to communicate among themselves (Walters, 2018; Miles et al., 2022). Pain has survival and adaptive values, helping to increase the chance of passing on genetic makeup to future generations, and leading to species preservation. Diggles (2019), argues that the scientific literature of welfare and pain in crustaceans is immature, based largely on a few dubious and disputed studies conducted in a small number of decapod species. Despite recognition that there is a growing focus on invertebrates such as cephalopods and crustaceans, further research is needed to provide more insight into the sentience of these animals, and in this case, particularly in penaeids (Proctor et al., 2013). Nociception research has been conducted mostly on the search for nociceptors, or pain receptors, the sensory neurons responsible for signalling potential damaging stimuli (Zimmerman, 1986; Taylor et al., 2004; Tobin & Bargmann, 2004; Kristiansen & Bracke, 2020; Olsson et al., 2021;). In this paper, the authors chose the criterium of pain because it has a direct impact on the welfare of commercially farmed animals. This is the reason that eyestalk ablation (EA) is discussed here. The specific objectives of this study are to review the scientific literature about pain indicators and nociception, and the potential impact on the welfare of crustacea, review the physiological and molecular mechanisms associated with the use of EA in adult female shrimp in aquaculture facilities, try to determine through the One Health and One Welfare concepts whether this procedure is valid for use in shrimp management protocols, and provide suggestions for alternatives to EA.

MATERIALS AND METHODS

A review of the scientific literature on pain indicators and nociception, and the potential impact on the health and welfare of shellfish following EA was performed based on the assumptions of PRISMA-P (Moher et al., 2015). PRISMA uses a set of items for reporting in systematic reviews and meta-analyses, focusing primarily on the reporting of reviews evaluating the effects of interventions and critical appraisal of published systematic reviews. The information sources were identified through the National Library of Medicine databases [PubMed, PubMed Central (PMC)] and Web of Science. Google Scholar, which is a much broader, yet less focused search, was not included with this study. The search strategy for this review consisted in use of the term 'shrimp' interchangeable with 'prawn', and 'shellfish' with 'crustacean' to be more sensitive, and to capture as many relevant references as possible. These terms are used interchangeably across the globe with unclear distinction between them. Shellfish and shrimp

were replaced with crustacea* and prawn respectively, in some searches. The eligibility criteria for the main search were the findings based on the following keywords: 'eyestalk', 'ablation method', 'eyestalk ablation', 'shrimp', 'shellfish', 'pain indicators', 'welfare', 'nociception', 'consciousness' and 'emotions'. Figure 1 shows the examples of the search methodology used for this research.

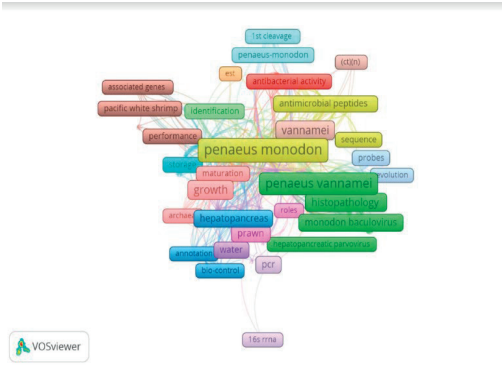

Figure 1. Search methodology by key words - adapted from PRISMA-P 2020 (Moher et al., 2015)

This search was indicative of potential issues with EA but not sufficient for it to provide conclusive evidence of pain in penaeids. Therefore, we initiated an additional generic search to include 'one health', 'one welfare', 'Specific Pathogen Free AND shrimp / prawn', 'Genomic selection AND White Spot Syndrome Virus AND Pacific white shrimp / crustacea*', 'ablation method AND shellfish / crustacea AND prawn / shrimp'.

RESULTS AND DISCUSSIONS

When the words 'ablation method AND shellfish AND shrimp' were used, one paper was obtained revealing that EA-induced responses of the neuroendocrine-immune system in *Penaeus vannamei*, unique differentially expressed genes are observed in the eyestalk, brain and thoracic ganglia (Liu et al., 2020). When the words 'pain indicators AND shellfish AND shrimp' were used, only one paper was found, and it was not related to the topic of interest (Rapala et al., 2005). When the words 'pain indicators AND welfare AND shellfish AND shrimp' were used in PubMed, 3 of the 10 papers retrieved were related to shrimp and one to crayfish (Sakaew et al., 2013; Adams et al., 2019; Chandararathna et al., 2021; Passantino et al., 2021). When the same keywords were used in PMC, 3 of 10 papers were related to shrimp and antimicrobial resistance (Sakaew et al., 2013; EMA, 2017; Chandararathna et al., 2021). When using the words 'pain indicators AND welfare AND shellfish AND shrimp' to search the PMC database, five papers were listed. These were physiological and behavioural indicators to measure crustacean welfare, the need to enact legislation to protect crustaceans, potential pain in fish and decapods, animal welfare issues in capture-based aquaculture and Entomophagy (the practice of eating insects and invertebrates (Adams et al., 2019; Elwood, 2021; Pali-Schöll et al., 2019; Chandararathna et al., 2021; Passantino et al, 2021;). A search using the words 'shrimp AND sentient' provided 1 and 30 papers in Pubmed and PMC, respectively, but only 1 paper referred to 'sentient' (Browning & Birch, 2022). The original keyword search, although using appropriate terms (in the opinion of the authors), returned few relevant papers on the subject, although these papers indicated that the EA procedure might be problematic from a management and welfare perspective and there was some evidence that shrimp might be considered sentient (Wyban & Sweeney, 1991; Bray & Lawrence, 1992; Hoang et al., 2002; Almeida et al., 2004; Taylor et al., 2004; Sainz-Hernández et al., 2008; Chung et al., 2011; Uawisetwathana et al., 2011; Asusena et al., 2012; Diarte-Plata et al., 2012; Pamuru et al., 2012; Shen et al., 2013; Burrell, 2017; Rowe, 2018; Aguiñaga-Cruz et al., 2019; Sathapondecha & Chotigeat, 2019; Liu et al., 2020; Zhang et al., 2020; Jin et al, 2021; Laphyai et al., 2021; Albalat et al., 2022; Ortiz-Gullién et al., 2022; Walters, 2022; Zhang et al., 2022; Mood et al., 2023). However, this information was far from conclusive to form a robust opinion, or as a guide for policymakers or the public. It was decided to expand the literature search to a more generic one to include wider areas such as welfare, veterinary, regulatory, public health, genetics, and to assess eyestalk ablation (EA) under One Health and One Welfare concepts. One Health had to be searched for as one word (One-Health rather

than One Health, with WOS for example, yielding 143 for the former but 3,394 for the latter). Including One Welfare allows for additional consideration of, and reflection on human and animal wellbeing, consumer opinion and food production policy making, but there were no relevant returns for including One Welfare with aquaculture and shrimp / crustacea*. These latter searches returned several additional results. The search using the key words 'ablation method AND crustacea AND prawn' yielded two publications of relevance, one paper on the role of methyl farnesoate in growth and maturation of the ovary, and another paper about the mechanisms of eyestalk ablation-induced ovarian maturation in the swimming crab (Ayanath & Raghavan, 2020; Xianliang et al., 2020). The search using the key words 'impacts of EA on molting' returned seven papers of relevance (Almeida et al., 2004; Sainz-Hernández et al., 2008; Chung et al., 2011; Diarte-Plata et al., 2012; Pamuru et al., 2012; Shen et al., 2013; Aguiñaga-Cruz et al., 2019). The search 'pain in invertebrates' was far too generic but returned nine papers of relevance (Buckingham et al., 2005; Marder, 2007; Blitz & Nusbaum, 2011; Crook et al., 2013; Mason et al., 2014; McMackin et al., 2016; Burrell, 2017; Crook, 2021; Walters, 2022;). The search for 'Genomic selection AND White Spot Syndrome Virus AND Pacific white shrimp' returned seven papers of relevance describing genetic evaluation of shellfish and genetic tests in relation with White Spot Syndrome Virus in shrimp (Zwart et al., 2010; Lillehammer et al., 2020; Hernández-Montiel et al., 2021; Onihary et al., 2021; Trang, 2021; Parrilla-Taylor et al., 2022; Medrano-Mendoza et al., 2023) (Table 1).

Eyestalk ablation (EA) refers to the removal or cutting of one (unilateral) or both (bilateral) eyestalks from an adult female shrimp. Another practice which induces the same effect is eye ligature by tying a thread around the base of the eye stalk then burning through the base of the eye stalk with a surgical forceps (Browdy & Samocha, 1985). EA is the most expedient method used for the induction of ovarian maturation and spawning in penaeid and non-penaeid shrimp (Primavera, 1983; Browdy, 1992; Vaca, 1999). This procedure was a major breakthrough in shrimp farming and commercialization, as it increased maturation and fertility of the eyestalk ablated shrimp brood stock females, making these hatcheries profitable (Primavera, 1985). Eyestalk ablation was routinely practiced on adult female shrimp in almost every research and commercial marine shrimp maturation or reproduction facility in the world, in both research and commercial settings. Commercial maturation of female penaeids used to rely almost exclusively on the technique of unilateral EA (Fingerman, 1997). It gave predictable peaks of maturation and spawning, but problems were reported with its use in penaeids and non-penaeids, like reduced reproductive performance, and deterioration in spawn quality and quantity over time and conflicting results on spawn size, hatch success and other variables (Emmerson, 1980; Tsukimura & Kamemoto, 1991; Kannan et al., 2015; Anand et al., 2019; Rodrigues et al., 2022). Today, several large-scale shrimp maturation facilities no longer use this procedure because non-ablated females live longer and produce eggs and nauplii of higher quality. The most economically important species are currently the giant tiger prawn *Penaeus monodon* and *P. vannamei*. Availability of fast-growing, specific pathogen-free (SPF) *P. vannamei* has contributed to make this species the most important aquaculture species worldwide. In most cases, female shrimps raised in captivity suffer from inhibition of ovarian maturation. Following uni- or bilateral EA, female shrimps develop ovaries and spawn in captivity, as complete ovarian development often ensues within 3 to 10 days. The presumed mechanism of the EA-induced fertility in shrimp female brood stock is that, following EA, the gonad inhibitory hormone (GIH) is not released from the eyestalk neurosecretory complexes, thereby lessening the inhibitory effect on the ovaries. GIH releases naturally in the non-breeding season. The fact that ovaries do not reach maturity in captivity is correlated with elevated levels of GIH in these females. EA lowers the haemolymph titter of GIH. The exact mechanism of EA on ovarian maturation is not known. This practice is of welfare concern as the EA technique is often applied without anaesthesia, while impaired vision and/or blindness is debilitating and secondary

infection can ensue (Albalat et al., 2022). Through a participatory approach and trans-disciplinarity, One Health aims to achieve outcomes not achievable by silo mentality (Zinsstag et al., 2022). From a holistic perspective, and taking into account all inputs into the whole-of-chain approach, consumer protection is an intrinsic component of One Health. Traceability is a priority in the whole-of-chain approach to food safety. Competent authorities assure consumers of transparency and security at all stages of the food production continuum, as they are entitled to wholesome, and nutritious food.

Table 1. Relevant papers describing genetic evaluation of shellfish and genetic tests in relation with White Spot Syndrome Virus in shrimp

Key words used	**PubMed**	**PMC**	**WOS**
	0 (0)	17 (2)	0
eyestalk AND pain AND shrimp AND shellfish			
eyestalk ablation AND shrimp AND shellfish	4	19	10
eyestalk AND ablation AND shrimp AND shellfish / crustacea	4 / 55	19 / 175	51.817[a]/ 42
eyestalk AND ablation AND shrimp OR crustacea	50.157	14.176	31.993
Eyestalk ablation AND crustacea* OR prawn	2.271	4.567	9.921
ablation method AND shellfish / crustacea AND shrimp / prawn	1 / 4	57 / 172	1 / 1[g]
pain AND shrimp AND shellfish / crustacea*	1 / 4	370 / 453	0[e]/ 9
eye peduncle[b] AND shrimp AND ablation	2	130	0
eyestalk AND shrimp AND ablation	61	202	224[d]
nociception AND sentience AND shrimp AND shellfish	4	77	0[d]
pain indicators AND sentience AND shrimp, shellfish	6	160	0[d]
pain indicators AND nociception AND shrimp	0	28	0[d]
pain indicators AND welfare AND shrimp / crustacea*	0 / 3	70 / 62	0 / 3
Shrimp / crustacea* welfare	35 / 77	1566 / 2064	246 / 396
welfare AND shellfish AND shrimp	6	316	42
welfare AND crustacea AND prawn	4	84	11
welfare AND prawn OR crustacea*	22.221	34.326	64.397
nociception AND welfare AND aquatic	2	102	4
nociception AND emotions AND shellfish / crustacea*	0 / 1	3 / 24	0 / 0
nociception AND consciousness AND shellfish	227	10	1[e]
Ablation AND Welfare	184	9516	968
Genomic selection AND White Spot Syndrome Virus AND Pacific White Shrimp / crustacea*	2 / 5	239 / 293	7 / 5
One-Health AND aquaculture	1743	20.717	3394
Pain in invertebrates	3423	9712	148
Impacts of Eyestalk ablation on molting	1	16	1
One-Health AND aquaculture AND shrimp / crustacea*	128 / 56	4524 / 3025	17 / 6
One-Welfare AND aquaculture AND shrimp / crustacea*	0 / 0	1 / 3[f]	0 / 0
Shrimp aquaculture practices	171	1,571	454
Specific Pathogen Free AND shrimp / prawn	79 / 5	4349 / 712	139 / 15

[a]Results were too vague and returned same number with eyestalk ablation AND shrimp OR shellfish, or replacing shellfish with Crustacea*
[b]Searches performed by using the words "*eye peduncle*" instead of "*eyestalk ablation*", used by the shrimp industry in Ecuador
[c]Replacing AND with OR shellfish yielded over 32,000 articles, many on seafood safety
[d]Same result substituting prawn for shrimp, or crustacea* for shellfish
[e]Valente, 2022 but with crustacea* instead of shellfish
[f]No relevant articles

Food traceability is also important during primary production, as it ensures the traceability of the raw material inputs into the food chain, based on quality, certification and accreditation of their products. Despite facing global challenges, such as the requirement to develop sustainability in food production for future generations, recognizing and acting upon existing and/or emerging animal welfare issues should not be neglected. Eyestalk ablation is an example of a technique used to support intensive production of some aquacultured crustacea, originally meeting increasing consumer demand for these products. It could be argued that food security was being addressed by applying techniques that are potentially stressful and harmful to these farmed species. This would seem contrary to any One Welfare, One Health paradigm discussed earlier. There are indications that, through better management, non-EA female brood stock can reach a comparable level of productivity, providing they are adequately fed The problem the authors perceive with EA is threefold: as consumers are more aware of food provenance, including the welfare applied to animal food producing systems, and similar to the drive for free range hen eggs in some sectors, those consumers investigating their food supply chains may be unwilling to purchase and consume shrimp obtained via EA protocols (Sampson et al., 2021). Secondly, most consumers are not aware of EA that is applied to some seafood, which may be translated into a lack of transparency in the food traceability process. This raises the question of whether industry should pre-empt any perceived lack of communication with public discourse. Consumers indicate a higher willingness-to-pay for improved animal welfare (Van Loo et al., 2014). Calls to include details about the way farmed seafood is obtained in the existing traceability requirements would not only meet what we perceive as the need for more transparency of this production chain to inform the consumer, and to empower their decision making, but it would also motivate possibly higher costs of production of welfare-based seafood, for which consumers would be willing to pay (Żakowska-Biemans & Tekień, 2017; Balzani & Hanlon, 2020). Thirdly, EA may not just relate to the wellbeing of these animals and the social and mental wellbeing of consumers, but it may also impact the mental health of staff working in the industry, such as shrimp farmers/producers and veterinarians/health care assistants of these farms. The attitude of those working on the EA shrimp farms towards animal welfare is based on many factors, including the ability of those workers to bond with their stock (Balzani & Hanlon, 2020). Involving farmers in their view and value of farm animal welfare can lead to better management and animal health outcomes. The shrimp EA protocol is for most, if not all welfare advocators, a non-necessary, stressful procedure, with a high potential to cause distress in the animal subjected to EA. As observed by animal welfare regulators, cases where animal welfare conditions are not met may cause the development of mental health issues of individuals working on such farms, and potentially affect their families. The Canadian Farmed Animal Health and Welfare Council recognizes the existence of a wide range of “unique occupational stressors” in the animal food producing industry, calling for “an effective mental health support system to recognize these unique and specialized stressors”, suggesting the need for appropriate intervention to ensure the welfare of the affected animals, and to facilitate the recovery of those experiencing distress from animal welfare incidents. The fact that it is impossible to actually know which of invertebrate species are sentient makes invertebrate sentience an important issue (Mood et al., 2023). To prove sentience in these species, a clear understanding of the way consciousness occurs, and identification of the structures allowing for consciousness to take place would be necessary. While such knowledge is currently lacking science-based proofs, different indicators that help estimate which invertebrates are sentient are being used. There are different attitudes towards different animal uses. Some are driven by the fact that the use of animals by humans is often associated with animal welfare costs. Sometimes, these are due to deliberate harm, but most often, they are due to inadequate husbandry, breeding and management protocols. Animal welfare is an interdisciplinary field where many have a role to play. One way to ensure animals are

provided good welfare is to use the Five Freedoms as benchmark for meeting animals' needs (Van Loo et al., 2014). It is recognized that little is currently known about some of the five dimensions as they relate to penaeid shrimp, and despite being regarded as outdated by some, the Five Freedoms may be a good place to start (Webster, 2008; McCulloch, 2012; Mellor, 2016). They include, among other freedoms, the freedom from discomfort (by providing adequate environment), freedom from physical problems (pain, injury and disease), and freedom from fear and distress (treatment and conditions which avoid animal suffering). Building consensus over the utilization of a shared working definition for welfare, pain and sentience is of paramount importance, especially in those animals raised to enter the food chain. This is a strong aspect of One Welfare. Pain is an emotion which evolved by natural selection and it may be shared by many animal species (Wadiwel, 2016). It is unlikely that pain arose *de novo* in humans, but rather it is a product of the evolutionary process, developed under selection pressures. Chordates, of course, share a common ancestor with many invertebrate phyla, such as molluscs and arthropods. While most research studies addressing pain have focused on humans and other mammals, current findings in invertebrate species are shedding light on nociception and pain-related functions, indicating that these may be ancient adaptive mechanisms present across phylogenetically related taxa, and that their welfare should be considered (Walters, 2018). Operation Welfare Indicators for capture-based aquaculture (CBA) protocols and provisions of the Animals (Scientific Procedures) Act 1986 should also be taken into consideration. Research on the ability of invertebrates to feel pain is both controversial and relatively rare, and response to noxious stimuli might be one way of investigating further. Invertebrates are underutilized in nociception studies, although there is perceived potential that these studies could promote development of novel therapies of pain in humans and other species. It is also expected that nociception studies conducted in invertebrates will reveal new insights into the mechanisms of those stimuli causing behavioural changes (Burrell, 2017). Insights into the fundamental mechanisms of nociception are provided by comparative approaches which use a wider range of animal species as model systems. Despite the increasing number of studies on pain, it is still difficult to be defined, as it cannot be soundly, scientifically proven, and it seems in most cases impossible to reproduce in other individuals. This indicates the "broad" feeling of pain, and may explain why experimental results are not relevant in these cases. Perhaps a preliminary definition of pain should be accepted in shellfish and other non-human species, to have the welfare concept applying to all animal species. From our literature research conducted on pain definitions and characterization, we suggest the definition proposed by Walters (2018): "Pain is a complex phenomenon, which is different in every individual and involves both a sensory (nociception) and affective (emotional) component." Behaviour can be interpreted as associated with pain but since we accept "our" interpretation of behaviour as indicating pain in more familiar mammals e.g., dogs and cats, why do we dispute behaviour in fish and invertebrates?

CONCLUSIONS

Eyestalk ablation, used widely in decapod crustaceans to expedite ovarian maturation and spawning, has a negative impact on the immunocompetence of the eyestalk-ablated females; state-of-the-art genetic findings may lead to new ways to induce ovarian maturation without having to perform EA.

Adopting more welfare-friendly handling techniques such as the LSDOT (Low Salinity and Diet, Optimal Temperature), or using topical anaesthetics, such as XylocaineR, prior to EA may reduce stress. Application of a coagulating agent (FibraseR) and antibiotics, diminish the time required for haemolymph clotting at the ablation site, minimizing haemolymph loss, and helping to prevent potential infections after the procedure.

From the animal welfare perspective, it is necessary to amend welfare assessment platforms, such as the Animal Welfare Assessment Grid (AWAG), currently used in mammals and birds, to achieve objective outcomes for welfare of decapods. In light of

new developments in pain and sentience, novel approaches to scientifically explore these concepts in animals should be proposed in the future.

Species-specific operational welfare indicators for all farmed Crustacea should be developed, and appropriate intervention to facilitate the recognition and recovery of those potentially experiencing distress due to welfare incidents in EA shrimp farms must be considered.

To assess the validity of the study (i.e., bias, confidence in estimates etc.), further systematic reviews on the topic are required.

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