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Review Article

Welfare of Decapod Crustaceans with Special Emphasis on Stress Physiology

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Despite the growing concern on animal welfare in crustacean farming, both from legislative bodies as well as the common public, studies on welfare are limited and transfer to routine farming is missing. While biocertification schemes such as the Aquaculture Stewardship Council (ASC) involve a welfare dimension, these dimensions cannot be communicated to the consumer in a scientifically sound manner. Animal welfare is recognized as integral part of sustainability due to the losses associated with bad animal welfare standards and is considered highly relevant by consumers around the world. On the other hand, increasing animal welfare is also required for the optimisation of aquaculture technology. Behaviour of the animals suggests that decapod crustaceans experience nociception and there are several indications of pain perception as well. Also, distress has rarely been evaluated under routine aquaculture conditions and markers for chronic stress detection need to be identified. Indeed, most work on welfare of crustaceans focuses on cellular, oxidative stress only. Here, a comprehensive assessment of chronic stress should be carried out to optimize rearing technology in nurseries, during ongrowing, harvesting, anesthesia, transportation, and humane slaughter in terms of a good aquaculture practise.

1. Introduction

Over the past decade, among decapod crustaceans, shrimp farming doubled its global production (2008: 5 million t, 2018: 9.4 million t) [1, 2]. Among the species farmed, the Pacific Whiteleg shrimp *Penaeus vannamei* is by far the most important species assigned to approximately 53% of total crustacean production [2]. Next to salmon, tuna, hake and herring, shrimps are among the most consumed aquaculture species in Germany [3]. To cover this demand, approximately 47 000 t have been imported [4]. Most of these imports are produced in open pond systems, reaching a global yield of 5 mio t annually [5]. In contrast, there is a small, emerging recirculating aquaculture system (RAS) based production of approximately 447 t in Europe [6]. RAS-based production is often sold by direct marketing as fresh products, advertising a sustainable image. Although the RAS production is so small, it has been a nucleus for the integration of welfare dimensions in crustacean farming (mainly shrimp but also lobster), but systematic scientific evaluation of animal welfare in shrimp is scarce.

Indeed, fundamental principles of animal welfare in husbandry such as the "Five Freedom Concept" (Five Provision Concept) [7] have not been evaluated nor adapted to shrimp farming as a prerequisite of a knowledge-based evaluation of animal welfare in farming. In contrast to other livestock including fish, rearing technology has hardly been evaluated in the context of welfare criteria in routine farming and can therefore rarely be communicated to the consumer. The safety of the food chain is directly connected to the welfare of those animals farmed for food production [8]. As part of the Animal Welfare Strategy, the European Commission called for measurable animal welfare indicators to reinforce the scientific-based good husbandry [9], but invertebrates are so far excluded as recently announced for the revision of the EU animal welfare laws [10]. However, the EU parliament adopted the Farm to Fork resolution, which calls on the Commission to support and encourage the development of higher welfare standards for marine invertebrates such as decapod crustaceans [11]. In the UK, decapod crustaceans are now recognised as "sentient" in the sentience bill and changes in legislation are foreseeable, too.

Animals face noxious hazards that may cause distress and tissue damage. To protect them from such adverse effects, crustaceans have nociceptive reflex responses. Still, there is a fundamental difference between nociception and pain, the first has a physiological dimension while the second has an emotional/mental one [12–15]. The specific receptors that are sensitive to injury are called nociceptors and have been conserved throughout the animal kingdom [14, 16]. In principle, they are sensory organs that process noxious stimuli, either chemical, mechanical, thermal or combinations of these in a reflex to prevent serious injuries [14, 15]. If the experience is processed in the brain and linked to an aversive experience, it has a highly adaptive value including avoidance learning to terminate the noxious stimulus. These attempts to escape the stimulus goes beyond the reflexive response seen with nociception and might thus be more successful than with the mere response. Such experience is consequently termed pain. Due to its salience, it is likely to be remembered as well as the situation that resulted in the nociception. The discussion on pain and suffering in decapod crustaceans is rather controversial and far from being solved. Some authors demonstrated that responses of decapods cannot be explained as pure reflexes whereas others state that the literature is inconclusive and that there are no welfare concerns [17, 18].

In contrast, stress is a physiological response of an organism that helps reconstitute homeostasis after interference of an adverse external stimulus (stressor). Experiencing intense chronic stress, the stress response may lose its adaptive function and becomes dysfunctional (distress). When stress is prolonged, it can elicit behavioural adaptations often referred to as state of higher alertness. In vertebrates a tripartite stress concept has been formulated [19, 20]. Although crustaceans lack the endocrine system of vertebrates, a similar concept can be applied to crustaceans (Figure 1) [21–23, 26]. In a primary reaction, neuroendocrine factors are released which trigger the secondary stress response. In the secondary stress response, metabolic changes shifting energy to restore the homeostasis occur (alteration of glucose, lactate, and glycogen). In the tertiary response, after prolonged exposure to the stressor, changes in the performance of the animal are observed, for example, reduced growth, decreasing disease resistance, or behavioural changes [21]. Prolonged stress with serious impact on the performance is generally called distress. It is generally accepted that prolonged stressful farming conditions translate into reduced health and, subsequently, to outbreaks of diseases, often involving total loss of stocks.

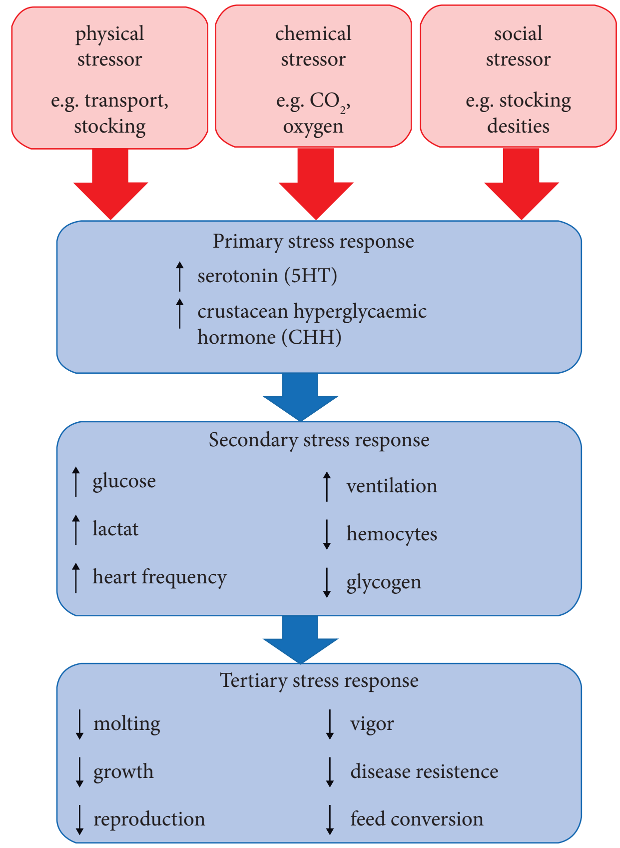

Figure 1: Overview of some physiological responses in decapod crustaceans exposed to physical, chemical, or social stressors. Stressors trigger a neuroendocrine response (primary response) that evokes physiological and behavioural effects (secondary response) to restore homeostasis. If persistent, stressor may induce systemic changes affecting the performance of the animal [21–25].

In this review, we will focus on the physiological aspects of welfare and welfare monitoring. The impact of diseases on welfare has been presented in detail recently [27]. We will review the existing literature on pain and distress in crustaceans—particularly in shrimp—to support a precautionary principle on animal welfare as established in fish and terrestrial livestock [13]. Furthermore, we will highlight those welfare indicators established supporting future attempts to optimise existing practice in the farming of crustaceans. We will furthermore identify risks related to welfare in practice, in particular nurseries, ongrowing, harvesting, anesthesia, transportation, and humane slaughter, and pinpoint those issues relevant for the sustainable optimisation of technology, preferably in Pacific Whiteleg shrimp.

2. Animal Welfare Concept

In the farming of aquatic animals, the pure number of species farmed (>400 species) by far exceeds those of terrestrial animals. Species-specific welfare information is often limited. For example, approximately 70% of the farmed aquatic species have no publications on welfare [28].

Currently, the rapid growth in species such as shrimp is outpacing welfare information available [28]. Indeed, Shrimp production is intensifying whereas shrimp sentience is still not commonly accepted [29, 30].

Defining welfare is difficult due to the complexity of the subject and the missing welfare criteria. The Five Freedom concept was established in the 1960s in response to a governmental report in the UK on livestock husbandry. It states that any aquatic farmed animal should at least have freedom of hunger and malnutrition by adequate access to a diet to maintain full health and vigour, freedom from discomfort and exposure by providing an appropriate environment including shelter and resting area, freedom from injury and disease by prevention or rapid diagnosis and therapy, freedom to express normal behaviour by providing sufficient space, proper facilities, and company of conspecifics, and freedom from fear, pain, and distress by ensuring conditions and treatment which avoid distress and suffering. Indeed, complete freedom from these negative states is not possible and the aim should focus on minimising them as part of a good aquaculture practice strategy. The European Welfare Quality assessment system for farm livestock specially excludes the utilisation of the Five Freedoms concept and enunciates four "welfare principles" of "good feeding," "good housing," "good health," and "appropriate behaviour" [31–33]. Similarly, Mellor et al. [34–36] advocated a concept of Five Provisions, including "good nutrition," "good environment," "good health," "appropriate behaviour," and "positive mental experiences," focusing attention on the practical measures needed to achieve desirable welfare outcomes. Likewise, the Five Domains Model for animal welfare identifies "nutrition," "environment," "health," "behaviour," and "mental state" as physical/functional domains drawing attention on the practical management of animals [27, 36–39], minimising negative experiences and promoting positive achievements. Recently, noninvasive indicators for shrimp farming have been presented, introducing a comprehensive scoring system based on the domains nutrition, environment health, and behaviour [29]. In this system, reference values such as temperature or pH and indicators assigned to the respective domain are scored.

Broom pointed out that there is no single parameter to assess welfare in husbandry [40]. The need to incorporate an interdisciplinary approach based on behavioural, physiologic, and health parameters is widely accepted today [41, 42]. Ashley [43] highlighted the importance of physiological parameters based on sensitivity and diagnostic value supporting a gradual evaluation of welfare aspects.

3. Nociception

Nociception is the sensory mechanism that allows animals to rapidly sense and avoid potentially tissue-damaging stimuli, either heat, mechanical, or chemical [14]. Downstream of the nociceptive input, the neural signals trigger protective (nocifensive) processes that reach the nervous system. Subsequently, they are processed and may be perceived as "painful" [16]. Nociception is rather plastic and by no means returns to normal function after damage has occurred [44, 45]. Consequently, an increase in sensitivity is often observed after injury referred to as sensitization. Clearly, decapod crustaceans show nociceptive behaviour [46–48], but primary nociceptors have only recently been described in one species [49]. It should be noted that, in contrast to *Drosophila*, crayfish do not respond to nocigenic chemicals such as capsaicin [49], isothiocyanate [49] or extreme pH [50].

There are many molecules and corresponding receptors linked to nociception such as transient receptor potential (TRP) channels and opioid receptors that have been reported in decapod crustaceans [24, 51–54]. TRP channels control temperature and mechanical nociception, transducing noxious stimuli into currents [54]. Opioids and opioid receptors are intrinsically linked to nociception and pain, modulating the actual stimulus on the level of the brain. There are three G-protein coupled opioid receptors, namely, μ-, δ-, and κ- and the respective ligands enkephalin, *ß*-endorphin, and dynorphin [53, 55].

Several studies demonstrated that acute and chronic noxious stimulation induces the expression of the nitric oxide synthetase (NOS) which synthesizes the neuronal messenger nitric oxide (NO) in mammals and invertebrates [56–58]. In Shore crabs, it has been suggested that NO is not just involved in sensory processing but also in motor programme modulation such as tail flipping [58]. Increased NO therefore may affect sensory signalling, central neural processing, and generation of efferent motor signals after exposure to a noxious stimulus, similar with the mechanisms observed in mammals.

4. Pain

Although most animals have nociceptive reflex responses that protect them from noxious influences, some taxa have evolved a capacity to experience pain, presumably to enhance long-term protection based on remembrance of the noxious nature of pain. Zimmerman stated that pain can be identified as an aversive sensory experience caused by injury that elicits protective motor and vegetative reactions and results in learned avoidance in the future [59]. An animal in pain should therefore quickly learn to avoid the noxious stimulus. Bateson [12] suggested eight criteria indicative with regard to the sensation of pain. The first three define a set of functional prerequisites—the possession of nociceptors, a brain-like central structure similar to the cerebral cortex of humans as well as endogenous modulators such as opioids and opioid receptors. Furthermore, analgesics should modify the response to the noxious stimuli. The remaining are purely behavioural stating that the animal should avoid noxious stimuli experienced and that this experience should be relatively persistent and that the animal should associate the neutral events with the noxious stimuli. Indeed, if many of these criteria are fulfilled, one may conclude that it is probable that the respective species experiences pain. Evidence from behavioural studies suggests that decapod crustaceans have a capacity to "experience pain/suffer" because they show responses consistent with pain and have a relatively complex cognitive capacity. Sneddon [14] extended these criteria (Table 1), but the criteria have been questioned to "set the bar for pain too low." One has to keep in mind that introspection is probable and that animal pain is not necessarily the same like our own feelings, particularly in phylogenetically distant species such as crustaceans [60, 61]. Indeed, pain describes a human emotional experience and analogy with any animal cannot be ultimately proven. The further the phylogenetic distance, it is reasonable to ask how analogous the experience to noxious stimuli is to the humane experience. Pragmatically, one could define pain as a nonreflexive response to a noxious, potentially tissue-damaging stimulus that alters future behaviour [17]. This definition gets away from the idea of feelings or consciousness, which are impossible to access or prove.

Wounded crustaceans show activities such as rubbing, limping or grooming that indicate awareness of the site of the wound and some attempt to reduce further damage [15, 24]. Indeed, such behaviour has been interpreted as being consistent with pain [62]. In extreme, injured appendages can be cast off (autotomy). It has been suggested that autotomy is mediated by pain-like experience [61, 63]. Also, autotomy increases mortality in crustaceans [64]. In addition, autotomy can reduce the harvest biomass because individuals utilize energy resources to regrow limbs at the expense of body growth [65]. Crayfish *Procambarus clarkii* tend to prefer dark to light environments. However, if they are first exposed to the electric shock that induces escape responses, they show a much stronger avoidance of the light environments and appear to become risk averse, which has been described as "showing anxiety." This "anxiety" is accompanied with higher levels of serotonin (5HT) in the brain [66, 67]. At the same time, noxious stimulation induces the release of the crustacean hyperglycaemic hormone (CHH), which is analogous to the stress hormone cortisol in that it mobilizes energy reserves to restore homeostasis (Figure 1). Also, as a consequence, lactate was elevated in those shocked crabs as well, documenting the interlinkage of pain and stress.

It has been argued that decapods do not have a sufficiently large complex nervous system. Still, some decapods possess a brain as large as in some fish and they have a high degree of functional compartmentalization allowing for surprisingly advanced cognitive processes [68, 69]. Nevertheless the criteria described by Sneddon et al. [15] (Table 1), have been questioned [18] and further research is needed. Some of the questions to be addressed are even related to functional aspects such as the possession of an opioid system modulating noxious stimulation, a morphological/functional description of nociceptors or the interlinkage between pain perception and distress. Also, the use of a truly relevant—for example, in ecological terms—noxious stimuli has been raised [18].

5. Distress

We define distress as prolonged exposure to a stressor or repeated stressors with a serious impact on the homeostasis with adverse, long-lasting effects on the overall performance of the animal (tertiary stress response). Indeed, the highly evolved stress system of vertebrates had its origin in simpler in the invertebrate nervous system [26], which will be outlined for shrimp in the following sections.

5.1. Primary and Secondary Stress Response in Crustaceans. In invertebrates, numerous studies identified a neuroendocrine system analogous to the hypothalamic-hypophysial system of vertebrates with substances closely resembling vertebrate neuropeptides and hormones, for example oxytocin, vasopressin, adrenocorticotropin (ACTH), α melanocyte stimulating hormone (MSH), somatostatin and others [26, 70–72]. More than 600 mio years ago, specialized neuroendocrine cells were already present in cnidarians and neuroendocrine signalling persists in both, the protostome and deuterostome lineages [72–75]. Yet, the endocrine systems of arthropods and vertebrates evolved independently and differently since more than 540 million years ago [72]. Since arthropods lost the ability to synthesize cholesterol [76], steroids, presumably, were limited in opportunity to evolve within this lineage as cholesterol is the precursor of steroid hormones [72].

A keyplayer of the decapod endocrine system is the eyestalk-derived crustacean hyperglycaemic hormones (CHH). This neurohormone is primarily involved in glucose homeostasis [77] (Figure 2). Lorenzon et al. reported that CHH is involved in a stress response after environmental stress such as elevated temperatures [80], lipopolysaccharide injection [81], infection with white spot syndrome virus [82], transport [83], changing salinity [80], or heavy metal exposure [84]. CHH primarily originates from the eyestalk which is commonly regarded as integrative neuroendocrine centre [78, 85]. Here, CHH is synthesized by the X-organ of the eyestalk and subsequently stored in the sinus gland next to the blood sinus [78]. Two splice variants, a long (CHH-L) and a short (CHH), which share the same sequence for the first 40 residues but thereafter differ considerably, have been identified [78, 86]. Sub- or neofunctionalisation of these paralogues remains to be resolved before CHH can be used as a stress marker.

During ecdysis, CHH peaks in the hemolymph [78, 87]. Still, this peak originates from the gut not the eyestalk [88]. Upon CHH secretion, mobilization of glycogen in the target tissue increases glucose and stimulates glycolysis and the citric acid cycle (CAC) (Fig.). In parallel, lipolysis is increased furthermore stimulating CAC. As in vertebrates, glucose and lactate in the hemolymphe can hence be used as stress makers [89]. Indeed, lactate is regarded as main end product under stress conditions [90]. At the same time, CHH restores Na^+ and K^+ concentrations in the hemolymphe to prestress levels [80]. In the Pacic Whiteleg shrimp *Penaeus vannamei* CHH increased pathogen clearance and survival of pathogen-infected shrimps [91], elevated hemocyte count and phagocytic activity of hemocytes [92] and upregulated the expression of several immune genes including antimicrobial peptides and superoxide dismutase [92, 93]. Also, CHH inhibits ecdysteroid synthesis and may thus slow down growth [87, 94, 95].

Table 1: Criteria for pain according to Sneddon et al. [15].

	Criteria
1	Nociception by nociceptors
2	Central processing of nociception involving a brain-like organ that regulates behaviour
3	Nociceptive processing sensitive to endogenous modulators (opioids and opioid receptors)
4	Nociception activates physiological responses (change in in respiration, heart rate, or hormonal levels)
5	Evidence that responses are not just a nociceptive reflex (physiological and behavioural modulation)
6	Alterations in behaviour that reduce encounters with the stimulus (avoidance learning)
7	Protective behaviour, such as wound guarding, limping, rubbing, or licking
8	All of the above reduced by analgesia or local anesthetics
9	Self-administration of analgesia
10	Pay a cost to access analgesia
11	Selective attention whereby the response to the noxious stimulus has high priority over other stimuli (e.g., reduced performance in learning tasks)
12	Altered behaviour after noxious stimulation where changes can be observed in conditioned place avoidance and avoidance learning paradigms
13	Relief learning
14	Long-lasting change in a suite of responses, especially those relating to avoidance of repeat noxious stimulation
15	Avoidance of the noxious stimulus modified by other motivational requirements as in trade-offs
16	Evidence of paying a cost to avoid the noxious stimulus

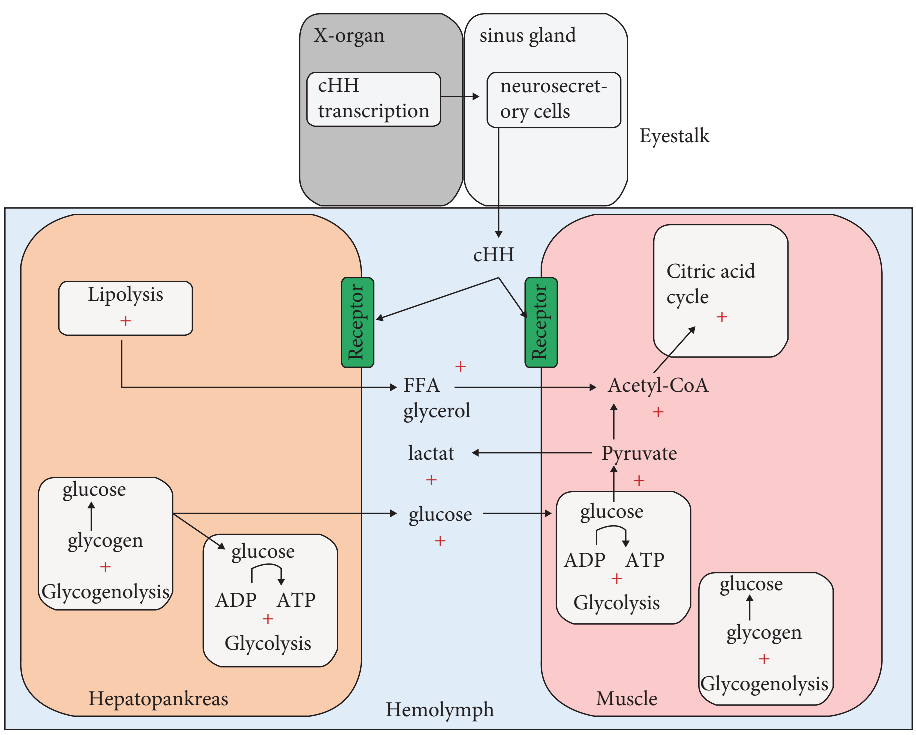

Figure 2: Metabolic role of cHH in the hepatopancreas and the muscle in crustacea. FFA: free fatty acids (modified from Chen et al. [78] and Li et al. [79]).

Lorenzon et al. have demonstrated that serotonin (5HT) increases glucose levels whereas dopamine (DA) decreases glucose levels in most species [85]. This effect is mediated by CHH secretion, which is inhibited by DA [96] and stimulated by 5HT [97, 98]. Similarly, methionine-enkephalin (M-enk) induced hyperglycemia [99, 100] but also hypoglycaemia [101, 102] in a species-specific manner. After eyestalk ablation, no effects are observed, strongly confirming the involvement of CHH from the eyestalk [85]. CHH peptides exhibit much greater variety with commonly four or more different isoforms [103, 104]. Consequently, due to sub- or neofunctionalization, the use as welfare indicator needs careful evaluation in the respective species. Changes in 5HT in the brain seem to induce anxiety-like behaviours in crayfish in an intensity-dependent manner [66]. This may suggest an interlinkage between a putative stressful event and a pain-like sensation here. Also, injection of 5HT induced such anxiety-like behaviour and could be prevented by the injection of 5HT antagonists [66, 67]. Therefore, as in mammals, crustaceans seem to exhibit a behavioural and a metabolic stress response [66].

It is known that confrontation with a range of chemical and environmental stimuli such as light flashes or touching, can arrest the heartbeat and breathing of crustaceans for short periods (cardiac bradycardia and/or respiratory apnoea), increasing lactate as part of a normal antipredator response that could hide the crustacean from predators that use weak electric elds to detect the prey (such as sharks and rays) [105, 106].

5.2. Tertiary Response and Distress. As outlined, CHH secretion may interfere with the immune response on a large scale, including reduced total hemocyte counts or prolonged clotting time [107–109]. Also, altered CHH can affect moulting and thereby inhibit growth [110]. The effects on osmoregulation have been observed in several species and may increase energy expenditure on a large scale [111]. Still, specific mechanisms are rarely described. In this context, a modulation of Na^+-K^+-ATPase mRNA has been reported [112]. Chronically, energy metabolism is affected by this osmoregulatory stress [113]. Low salinity and high densities also reduce the ability of Pacific Whiteleg shrimp to cope with acute stress [114]. Although Whiteleg shrimp tolerate a wide range of salinities, salinity below the optimal range affects growth and survival. Furthermore, low salinity reduces the ability of the shrimps to cope with an acute stressor, for example hypoxia or escape reaction [114].

High stocking densities in Pacific Whiteleg shrimp farming cultivation influence the activity of e.g., superoxide dismutase, catalase, glutathione peroxidase, and heat shock protein to face this chronic stress [115]. Also, suboptimal rearing temperatures cause stress in shrimp: Low temperatures are particularly problematic for Pacific Whiteleg shrimp [116] and lead to an increase of dopamine and CHH level in the haemolymph, higher hemocyte counts, autophagy and a disturbance of the fat and protein metabolism [117]. In shrimp aquaculture suboptimal oxygen supply may occur and recurrent hypoxia has a severe impact on shrimps, negatively affecting survival, growth and disease susceptibility [118].

Although autotomy is often regarded as a welfare indicator, it has been shown that autotomy is not more stressful than normal handling, exhibiting similar lactate and glucose levels in edible crabs *Cancer pagurus* [119].

6. The Missing Aspect: Welfare Measures Based on Behavioural Expressions

As mentioned above, the assessment of stress, suffering, and pain as a basis of ensuring animal welfare and health and species-appropriate, sustainable breeding, needs a combination of behavioural, morphological as well as physiological parameters. In practice, however, behavioural indicators of stress, suffering, and pain can be considered to promote preventive health care, and thus early detection of stress, injuries, and diseases but their scientific foundation through a coupling with physiological or morphological indicators are hardly done yet in crustaceans.

Next to the detection of stress and suffering, knowledge of the behavioural patterns expressed by crustaceans during all life stages helps to promote crucial aquaculture measures like feeding efficiency, growth rates, and breeding success [120]. For example, knowledge on a species' feeding patterns helps refine feeding practices [121]. Verifying the time of the day when shrimps are most likely to perform searching behaviours allows feed provision at a time when shrimps are most likely to eat it, thus reducing feed waste and ensuring better water qualities [122]. For successful breeding, knowledge of the activity patterns during the moulting cycle help selecting appropriate handling practices that reduce stress and enhances offspring production. Therefore, behaviour observations are already an utmost important observation for the farmer.

Besides behavioural indicators of stress, suffering, and pain or those that improve culturing, the "freedom to express natural behaviour" is considered as fundamental for good animal welfare [123]. These considerations arrive from the assumption that animals need to express their innate or "natural" behavioural repertoire in order to live in a positive state ("be happy"). Natural behaviour, however, can only be expressed in nature because the encountered environments of captive held animals substantially deviate from the environment in which the wild ancestors live (captivity: lack of predators, sufficient high quality food, restricted space, often unnatural individual densities, etc.). Thus, several natural behaviours are either not expressed (predator avoidance reactions) or when expressed, they become maladaptive in captivity and then are even detrimental for the animal's health [123]. As an example, aggressive interactions among conspecifics are adaptive in the wild when individuals' defend certain scare resources like mating partners or food sources [124]. In captivity, where there is no need to defend food or mating partners and space limitations prevent from escape, aggression induces stress and injuries and is thus detrimental to animal welfare. The human care takers are then required to take measures reducing or suppressing the motivation to express these natural but now maladaptive behaviours. In finfish aquaculture, aggression is, therefore, often suppressed through increased rearing densities that prevent animals to show motivation to establish territories they want to defend or by providing structural enhancements (shelter and hiding spaces) that allow for better avoidance of conspecific encounters.

In order to be able to identify negative states, to promote beneficial cultural practices, and to allow animals to express behaviours necessary for their well-being, we advocate that a first step must be to establish which behaviours are normal under certain environmental conditions [122]. Normal here means that these behaviours are predictably expressed under similar environmental conditions. In a second step, physiological as well as morphological indicators should be combined to evaluate the importance of these behaviours for the animal's well-being, e.g., to categorize certain behaviours as either promoting or decreasing animal welfare.

Methodologically, advances in camera systems and automated animal tracking software enable us to survey farmed animals in great detail [110]. For example, measures of movement activity that inform about moulting phase or food search as well as establishment of aggressive encounter prevalence as well as interindividual distances have great potential to compliment physiological and morphological indicators of animal well-being. In fish farming, real-time video monitoring systems have been successfully established [125–130], but they are mostly lacking in shrimps farming [121, 131]. This is in part due to high turbidity in at least open systems, while the biofloc system completely depends on monitoring of physiological welfare indicators [132].

7. Implications for Good Aquaculture Practice

The goal of good working practice in aquaculture is a sustainable and safe production. In addition to a responsible utilization of resources, animal welfare is essential. Farmed organisms in aquaculture can experience stress in a variety of ways. The understanding of interrelationships is necessary for the recognition of problems and, in turn, to compensate them.

From an applied perspective, the production cycle is divided into periods, and the respective periods will be discussed with regard to the major concerns for animal welfare. It has been claimed that studies on pain in crustaceans have been "largely based on a few, dubious, and disputed studies done on a small number of animals" [18]. Unfortunately, welfare with regard to relevance to aquaculture has hardly been assessed systematically in crustaceans. Indeed, most work focuses on oxidative stress (Table 2). Here, we present selected studies that have been carried out with regard to the most common stressors. Establishing reliable parameters for the evaluation of welfare in farming may support future development of the existing technology. In crustaceans, reducing stress has been carried out in order to reduce susceptibility to diseases [144–146], improve growth and feed conversion after improved energy allocation [110, 144, 147, 148], and reduce cannibalism and aggression within the farming system [149–152]. Indeed, a stress evaluation may be used to comprehensively optimize and monitor rearing conditions (water parameters, stocking densities, feeding regime, and so on) in terms of good aquaculture practice. Therefore, evaluations under realistic, up-scaled conditions are needed in the future.

To enforce welfare and aquaculture best practice, Birch et al. [153] recommended a ban on the live sale of decapods to untrained, nonexpert handlers. Undoubtedly, ending such practice would improve animal welfare of decapods.

7.1. Broodstock and Reproduction. The broodstocks are reared under optimal conditions in terms of density, water parameters, and feeding to support the best possible reproductive output. For optimal reproduction, maturation is still induced by eyestalk ablation. This procedure reduces the secretion of moult-inhibiting hormone (MIH) from the *X*-organ of the eyestalk and initiates the resumption of gonad maturation. Using flame-sterilized hot forceps ensures rapid (<2 s) ablation and sterilization of the wound [18]. In some species (e.g., *Penaeus monodon*, *Penaeus vannamei*), efficient reproductive management requires such ablation. Negative consequences include physiological imbalances [154, 155], reproductive exhaustion [154, 155], physical trauma [156], regulation of immune-related genes [157, 158], stress [156, 157], and mortalities. In a long-term view, predictable maturation and efficient spawning have been envisioned as golden goals [159, 160]. Recently, comparable reproductive performance has been achieved with nonablated females in *Penaeus vannamei*, paving the road for improved reproductive management [160]. Indeed, there are a couple shrimp hatcheries that produce their settlings without eyestalk ablation [27].

7.2. Nursery. Commonly, to increase the quality, growth, and survival of the shrimps, a nursery phase is carried out before the actual growth-out phase. A common method to assess and ensure the quality of postlarvae is to perform "stress tests" in small batches of postlarvae [154, 161–163]. In fact, these tests are rather lethal tests and the diagnostic outcome for the subsequent performance of the postlarvae can be questioned. The larvae are exposed to very high stress levels (e.g., salinity, temperature, ammonia, and formalin), and the number of survivors is determined. This procedure profoundly disrupts metabolic mechanisms, causing weaker individuals to die. In the future, molecular markers and behavioural tests may provide more sensitive and meaningful assessment tools. Indeed, the outcome of the mortality tests is often not tightly correlated to the subsequent performance during farming, and more reliable assays are desirable.

7.3. Growth-Out. From the farmer's point of view, the ongrowing phase is certainly the most important period in terms of commercial success as it involves the highest expenditure and determines revenue. The interaction between fluctuating, and in some cases suboptimal, conditions and animal health plays a crucial role for the economic success.

Water turbidity in intensive shrimp farming is a major stressor, particularly in earthen ponds with aerators (Table 2). Increasing clay particles induced a rise in lactate and glucose levels and resulted in higher mortality rates [89]. Also, particle concentration induced an elevation of oxidative stress biomarkers such as superoxide dismutase (SOD) activity and resulted in an elevated total hemocyte

Table 2: Stress evaluation and respective parameters in decapod crustaceans.

Stressor	Marker	Effect	Species	Ref
Air exposure	SOD	Increased	Pacific whiteleg shrimp *Penaeus vannamei*	[108]
	Mortality	Increased		
	TAC	Increased		
	Glucose	No change	Harbour crab	[133]
	Lactate	Increased	*Liocarcinus depurator*	
	pH	Decreased		
	SOD	Increased	False king crab	[134]
	CAT	Increased	*Paralomis granulosa*	
	GST	Increased		
Air exposure at higher *T*	Hemocyanin	Decreased	Swimming crab	[135]
	Glucose	Increased	*Portunus trituberculatus*	
	Lactate	Increased		
	Glycogen	Decreased		
Ammonia	THC	No change	Pacific whiteleg shrimp *Penaeus vannamei*	[136]
	SOD	Decreased		
	PO	Decreased		
	Na-K-ATPase mRNA	No change	Amazon river shrimp	[137]
	THC	Decreased	*Macrobrachium*	
	SOD	Increased	*Amazonicum*	
	HSP70 mRNA	Increased	Asian paddle crab	[138]
			Charybdis japonica	
Nociception	iNOS	NO signalling may modulate noticeptive behaviour	Shore crap *Hemigrapsus sanguineus*	[58]
pH (acid, alkaline)	SOD mRNA	Increased	Pacific whiteleg shrimp *Penaeus vannamei*	[139]
	CAT mRNA	Increased		
	GPx mRNA	Increased		
	ROS	Increased		
Salinity	THC	Slightly decreased	Pacific whiteleg shrimp *Penaeus vannamei*	[107]
	Glucose	Slightly increased		
	HSP70 mRNA	Increased	Mitten crab	[111]
	HSP90 mRNA	Increased	*Eriocheir sinensis*	
	Na^+-K^+-AtPase mRNA	Increased		
Stock density	GSH	Increased	Narrow clawed crayfish	[140]
	GPx	Increased	*Astacus leptodactylus*	
	CAT	Decreased		
	HSP70	No change		

Table 2: Continued.

Stressor	Marker	Effect	Species	Ref
Temperature	THC	Decreased	Pacific whiteleg shrimp *Penaeus vannamei*	[141]
	PO	First increased then decreased		
	NOS	First increased then decreased		
	SOD	First increased then decreased		
	Transcriptomics	Differentially regulated	Shrimp	[142]
	HSPs	Increased	*Palaemon* spp	
	THC	Decreased	Mediterranean green crab	[143]
	SOD	Not significant	*Carcinus aestuarii*	
	PO	Increased		
	CAT	Not significant		
	Hematocrit	Decreased		
	Glucose	Not significant		
Turbidity	Lactate, glucose	Increased	Pacific whiteleg shrimp *Penaeus vannamei*	[89]
	Gill histology	Lamellar fusion, hyperplasia, hypertrophy		
	THC	Increased		
	SOD	Increased		
	PO	Increased		
	Gill flaring	Increased		

CAT: catalase; FCR: food conversion ratio; GSH: glutathione; GST: glutathione-S-transferase; GPx: glutathione peroxidase; HSP: heat shock protein; NOS: nitric oxide synthase; PO: phenoloxidase; SOD: superoxide dismutase; TAC: total antioxidant capacity; THC: total hemocyte count; WG: weight gain.

count (THC). Carrying out such a marker-based stress evaluation enabled the recommendation of safe levels of turbidity (<30 NTU). In another study, after exposure to ammonia, SOD decreased, whereas THC was not affected [136]. Under acid and alkaline pH, oxidative stress markers increased substantially but returned to normal after a day, suggesting that adaptive restoration of homeostasis occurred relatively fast [139]. In *Astacus leptodactylus*, glutathione (GSH) and SOD, as well as heat shock protein, increased with higher stocking densities [140], suggesting a strong oxidative stress response upon crowding. In juvenile *Penaeus vannamei*, substantial changes in behaviour were observed with increasing stocking densities [122]. Here, an ethogram-based evaluation can be used to characterise social stress and recommend optimized stocking densities [122]. Although shrimps are considered less cannibalistic [152], cannibalism can be exacerbated when disease or environmental conditions (e.g., high ozone, low dissolved oxygen) induce soft shells [149, 164, 165]. Longer periods of sub-optimal feeding seriously affect shrimp, causing potential economic losses to farmers that need to be avoided [166]. It has also been reported that starvation induces cellular immunity in *Penaeus vannamei* and impacts disease resistance. Mostly, with the exception of RAS, on-growth takes place in open systems, that are exposed to fluctuating environmental influences. Especially, temperature and salinity changes were identified as stressors under these conditions. Low salinity stress directly influences growth and osmolality- and metabolism-related genes [112]. Pacific Whiteleg shrimp respond to rapid temperature changes by activating antioxidant enzymes and stress-related biomarkers [167].

As a conclusion, with regard to the stress biomarkers assessed, stressors need to be evaluated carefully, and there is a need for distress-specific, chronic markers in order to optimise existing technology and define ranges to safeguard the animals. So far, very few parameters have been assessed (mainly biomarkers for oxidative stress), and there is an urgent need for universal distress-related markers.

7.4. Harvesting. Harvesting is one of the most stressful steps during the production cycle and inevitably involves physical handling. The animals are assembled and removed from the water using nets or pumps. This procedure triggers flight behaviour and represents stress for the animals, which can cause physical injuries and may reduce meat quality. Under these circumstances, heart rates are substantially increased [168].Also, ammonia, glucose, and lactate rise [169, 170]. Upon arrival at a processing factory, crustaceans are often (rarely shrimp) resubmerged, allowing some recovery [171, 172]. After new removal from the water, individuals are immediately sorted and transferred to ice water for stunning and killing.

Crustaceans, such as lobsters and crabs, are often kept alive for long periods before being submerged in boiling water. Therefore, it is reasonable to ask if the animals have some degree of consciousness and will experience the treatment as painful.

7.5. Anesthesia. Decapods can be anesthetized with a wide variety of anesthetics, which are either administered by injection or as anesthetic bath for experimental purposes [173, 174]. Since crustaceans from aquaculture are produced for human consumption, the use of chemical narcotics is forbidden due to human safety concerns. For the stunning of tropical shrimp, two main standardized methods are currently recommended cooling and electroshock [175]. A viable method to anesthetize lobsters (*Homarus americanus*) is to slowly increase the temperature, as neuronal activity in the animals comes to a halt at higher temperatures without causing behavioural abnormalities. Cooling is not effective in this species, as the animals still react to external stimuli [176]. In contrast, standardized cooling is used successfully in tropical shrimps and is usually carried out on ice slurry. Submersion in ice slurry for >1 min is an effective way to euthanize most shrimp species reared at tropical water temperatures. Given the fact that low temperatures do not activate nociceptors in some crustaceans [49], ice slurry appears to be a highly effective, easy-to-perform, non-chemical method for humane stunning. However, this method is not recognized by law in some countries, e.g., Switzerland or Italy. In fact, there have been suspicions that chilling may only paralyze the animals without anesthetizing them. Electrostunning has been controversial since it induces epileptic seizures in lobster and crayfish [176]. Electrostunning seems to paralyze species such as crabs and lobster [176–178], but evidence of its effectiveness is scarce [175]. In shrimp, electrostunning can be recommended [178].

7.6. Transport. Several crustacean species are marketed as live products (review, see [179]). Adequate shelter has been recognized to improve animal welfare during transport [153]. Temperature variation and air exposure are recognized stressors for those species transported alive [135]. Also, handling, increased densities, and deteriorating water parameters, such as carbon dioxide and ammonium concentration, are stressful, especially for *L. vannamei* [110, 180]. Several guidelines for transportation have been established (seee.g., [181, 182]), partly based on the evaluation of stress-related markers. Generally, in most countries, transportation is allowed on ice, but in Switzerland, it has to be carried out in seawater.

Although some crustaceans are facultative air breathers, such as *Carcinus maenas*, transportation on ice is a potential stressor for most species that may disturb oxygen consumption, leading to an accumulation of anaerobic metabolites such as lactate. Air exposure also induces CHH, triggering a stress response [22, 183]. To promote lethargy and reduce stress, alive crustaceans are cooled before transport. During cooling, autotomy frequently occurs in some species, although the interpretation of autotomy with regard to its relevance to its welfare is unclear. At slower cooling rates, autotomy is reduced [179]. As a recommendation, a relatively low temperature (5–10°C), a humid atmosphere, and avoiding disturbance improve animal welfare during transport [179]. As a whole, transport is particularly challenging and causes substantial stress for the transported animals. Therefore, husbandry and handling parameters during transport should be kept within an optimal range for the respective species.

7.7. Humane Slaughter. Recent studies demonstrated that some responses of decapods cannot be explained as reflexes [17, 61, 184], opening the possibility that they can experience pain. Recently, there is a substantial consumer concern about the slaughter of certain decapod crustaceans—e.g., lobster and humane killing has been subject of recent studies [185]. In Swiss and New Zealand, boiling lobster whilst alive has been banned, and lobster needs to be stunned before boiling, but in most countries, decapods are still excluded from animal welfare legislation.

The most humane killing method is probably an electrical stunning device called CrustastunTM [175] for larger animals such as lobster. Still, in smaller animals, such as shrimp, this method has not been certified so far, requiring further research [153]. Electrical stunning kills the animal within a second, and no stress has been observed in animals transferred to the device (monitored as lactate concentration). Immersion in ice slurry equally causes sedation in shrimp and crayfish within minutes, but crabs remain sensible for some time and retain a functional neural circuit. Splitting is a humane slaughter technique in lobster but requires experience. Here, the nervous system is destroyed by cutting along the longitudinal midline [176].

8. Conclusions

Particularly for decapod crustaceans, aquaculture growth is outpacing welfare knowledge. With regard to the ongoing debate on welfare in decapods, a better understanding of the mechanisms underlaying nociception, pain, and distress is required to safeguard good animal husbandry according to the Five Freedom concept. Scientific evidence on pain perception is mainly based on behaviour studies and pain-related mediators, such as opioid receptors or nociceptor-related TRPs, have not been identified so far. Studies measuring physiological parameters in routine aquaculture monitoring are largely lacking. There is a need for easy to access physiological biomarkers and comprehensive behavioural studies to monitor welfare in practice. Undoubtedly, scientific-based welfare considerations should be used to further improve existing technology, communicate welfare to customers, and better safeguard welfare in crustacean farming.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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