



Welfare in Farmed Decapod Crustaceans, With Particular Reference to *Penaeus vannamei*

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The farming of decapod crustaceans is a key economic driver in many countries, with production reaching around 9.4 million tonnes (USD 69.3 billion) in 2018. These efforts are currently dominated by the farming of Pacific whiteleg shrimp, *Penaeus vannamei*, which translates into approximately 167 billion farmed *P. vannamei* being harvested annually. Further production growth is expected in the future and hence the need for more research into its health and welfare is required. Herein, from an extensive survey of the available literature, we scrutinise farming practices and the challenges associated with the production of *P. vannamei* from an animal-centric welfare perspective (1), we propose potential welfare indicators (2) and we critically review current scientific evidence of sentience in penaeid shrimp among other commercially important decapods (3), since it is plausible that in the near future not only the largest, but in fact all decapod crustaceans will receive welfare protection. This review highlights that despite the wide knowledge on crustacean stress physiology and immunology as well as disease control, still little is known about some key parameters related to the five welfare dimensions. We recommend that further research should focus on developing a systematic integrated welfare assessment encompassing all the different aspects of the crustaceans farming and life cycle up to slaughter. Furthermore, direct and indirect species-specific operational welfare indicators should be developed for all decapod crustaceans currently farmed, similar to the ones suggested in this review for *P. vannamei*.

Keywords: sentience, penaeids, farming, welfare, disease, aquaculture, humane slaughter

1 INTRODUCTION

True prawns belonging to the family Penaeidae comprise more than 400 described species and include commercially extremely valuable species such as the giant tiger prawn (*Penaeus monodon*) and the Pacific whiteleg shrimp (*P. vannamei*, synonym *Litopenaeus vannamei*). As a typical example of their biology, *P. vannamei* is native to the Eastern Pacific coast from Sonora, Mexico in the North, through Central and South America as far South as Tumbes in Peru, in areas where water temperatures are normally >20°C throughout the year. They occupy tropical marine habitats, where

adults mature at 6–7 months and live and spawn in the open ocean. The larval stages are carried towards the shore by tidal currents, and postlarvae move further inshore to spend their remaining pre-adult stages in coastal estuaries, lagoons or mangrove areas (FAO, 2009).

The artificial rearing of a penaeid species for aquaculture can be traced back to more than 70 years ago with the rearing of Kuruma prawn *P. japonicus* larvae (Hudinaga, 1935; Hudinaga, 1942). However, while initial work was performed in this species, rearing and developmental work especially during the 1960's and 1970's expanded quickly in other geographical areas for various penaeid species (Cummings, 1961; Elwald, 1965; Muthu et al., 1974; Thomas et al., 1974). From a commercial production perspective, the work by Hudinaga (Hudinaga, 1966; Hudinaga, 1967) is considered the basis from which the penaeid shrimp industry developed.

The first captive spawning of *P. vannamei* was achieved in Florida in 1973 from nauplii shipped from a wild-caught mated female from Panama. Following the development of intensive breeding and rearing techniques, the discovery that unilateral eyestalk ablation and adequate nutrition promote maturation, commercial culture of *P. vannamei* began from the mid-1970s in South and Central America and subsequently in mainland United States of America, with strict quarantine laws or bans to prevent importation of exotic pathogens with new stocks. Although a non-indigenous species in Asia, from the beginning of the millennium *P. vannamei* has become the dominant species in penaeid shrimp aquaculture in many Asian countries, notably China, Vietnam, Thailand and India, replacing local species such as *P. monodon*.

In 2018, the total aquaculture production of crustaceans was estimated to be 9.4 million tonnes (USD 69.3 billion) (FAO, 2020). Of this production, more than 50% is accounted for by the farming of *P. vannamei* (4.96 million tonnes) while *P. monodon* production is around 8% (750K tonnes). The main producing countries are now based in the Asia-Pacific region (80% production) led by China, while in Latin America the main producing country is Ecuador with 600K tonnes farmed in 2019. While the production is dominated by these two species, knowledge on rearing methods is available for many penaeid species including *P. aztecus*, *P. chinensis*, *P. merguensis*, *P. duorarum*, *P. japonicus*, *P. orientalis*, *P. setiferus*, *P. stylirostris* and *P. indicus* among others (see for example Cook and Murphy, 1969; Heng and Rui-yu, 1994). At the time of writing, the only complete genomes available for the family Penaeidae are *P. vannamei* and *P. monodon* (Zhang et al., 2019; Uengwetwanit et al., 2021). However, given the importance of biodiversity from a food security context (Metian et al., 2020) it is possible that this situation will change in the future and necessary tools and SPF-free seed will be available for other species.

While growth in this sector has been very significant, the sector has suffered from a number of production challenges, mostly driven by disease (see *Evaluation of Key Challenges Associated With the Production P. vannamei From a Welfare Perspective*). In fact, the main driver for the introduction of *P. vannamei* to Asia was the perceived differences in its disease

susceptibility to white spot syndrome virus (WSSV) compared to *P. monodon* (Funge-Smith and Briggs, 2003). However, incidences of disease in *P. vannamei* have flourished with the development of the industry and major crises have been experienced all over the world (Linda et al., 2005; Kumar et al., 2014). In this context, diseases such as acute hepatopancreatic necrosis disease (AHPND), hepatopancreatic microsporidiosis (HPM), hepatopancreatic haplosporidiosis (HPH), aggregated transformed microvilli (ATM) and covert mortality disease (CMD) have been added to the list of previous known threats to the commercial production of *P. vannamei*, such as white spot disease (WSD), yellow head disease (YHD) and infectious myonecrosis (IMN) (see review Thitamadee et al., 2016). Disease of course plays a significant role in animal welfare. In fact, the word 'dis-ease' refers to a state that lacks 'ease' or well-being. Thus, one of the outcomes of poor welfare is expressed in the form of disease (Butterworth and Weeks, 2009).

1.1 The 'Five Domain Model' as a Framework for Animal Welfare Assessment

While it is clear the importance that disease place within the context of animal welfare, health is only one of the animal welfare pillars within the so-called 'Five Domains Model' (Mellor and Reid, 1994). In this model, good welfare or well-being is described as the state that is manifest in an animal when its (1) nutritional, (2) environmental (physical environment), (3) health, (4) behavioural and (5) mental needs are met. The first four domains focus on the animal's physiological and behavioural needs and encompass pathophysiological disturbances caused by nutritional, environmental and health-related issues. According to Mellor and Beausoleil (2015) once these factors are assessed their anticipated consequences are assigned to the (5) 'mental' domain and it is these affective experiences that determine the animal's welfare state (Mellor et al., 2009). These five domains are essentially equal to the 'five freedoms' formulated by the Farm Animal Welfare Council in a number of countries, including the UK (Mellor and Reid, 1994).

From a more fundamental perspective, the term welfare is intrinsically linked with the absence of suffering, which include anxiety, fear, pain and distress and more recently increasingly linked to the promotion of positive states (Mellor and Beausoleil, 2015). However, these experiences, according to most national and international policy frameworks, only apply to animals that are sentient as non-sentient animals are not capable of feeling.

Therefore, in this review we will evaluate the extent to which the five domains of welfare are being met by current commercial practices in the key penaeid commercial species, *P. vannamei*, and propose future developments that are needed to identify relevant indicators for the evaluation of the welfare of this species when cultured. We will also assess the current evidence for sentience in penaeid and, by extension, other decapod crustacean species, when relevant. We believe these are important considerations *per se* and especially if increased legislative protection on these animals is to be implemented.

2 EVALUATION OF KEY CHALLENGES ASSOCIATED WITH THE PRODUCTION OF *P. VANNAMEI* FROM A WELFARE PERSPECTIVE

2.1 Maturation of Female Broodstock and Current Production Practices: Eyestalk Ablation

In natural environments, *P. vannamei* spawn under specific environmental cues (a combination of optimal water temperature, salinity and dissolved oxygen mainly) stimulating ovary development and spawning by neurosecretory centres (Calderon-Perez et al., 2007). In production systems, parameters such as water temperature, salinity, and dissolved oxygen, nitrogen compounds, photoperiod, light intensity, maturation room and tank characteristics, and diet may be manipulated and adjusted (Browdy, 1998; Whetstone et al., 2002; Jorry, 2012). In general, *P. vannamei* should be held for maturation under specific conditions that include low light (10–30% of natural or artificial light), ideally with a system to control photoperiod (maintained at about 10–12 dark and 12–14 hours light) (Treece and Fox, 1993; FAO, 2003). Noise (particularly loud or intermittent noise), movement and other disturbances should be kept to a minimum level. Maturation tanks should be round (but in Asia square or rectangular tanks are also used), dark-coloured, smooth sided, and of approximately 3–5 m diameter (FAO, 2003). Stocking densities of around 6–15 shrimp m⁻², with a male to female ratio of 1:1, 1:1.5 or 1:2 are optimal. Water temperature, salinity, dissolved oxygen, pH, alkalinity and ammonia are usually maintained at the range of 28–29 °C, 30–35 ppt, ≥4 mg L⁻¹, 8–8.2, ≥100 mg L⁻¹ CaCO₃ and < 1 mg L⁻¹ respectively (FAO, 2003). It is also important to make sure that the broodstock have good nutritional status and appropriate size (at least 30 g and >40 g for male and female, respectively), age and origin (i.e., grow-out conditions and genetic background) (Racotta et al., 2003).

The external factors mentioned above are considered to improve success in inducing sufficient egg production to meet commercial schedules, but insufficient to ensure consistent and predictable production scheduling, leading the *P. vannamei* sector to continue reliance on manipulating the endocrine system of penaeids using eyestalk ablation to improve reproductive performance.

Ablation of eyestalks is broadly used in commercial hatcheries as a crude method of hormonal manipulation to induce maturation and spawning in *P. vannamei*. It involves the removal or constriction (through cutting, cauterizing or tying) of one (unilateral) or two (bilateral) eyestalks to reduce the level of gonad inhibiting hormone (GIH/MO-IH) which is produced by the X-organ and sinus gland complex situated in the optic ganglia of the eyestalk (Kang et al., 2014; Wang et al., 2019; Magaña-Gallegos et al., 2021). Eyestalk ablation is important as ovarian maturation of *P. vannamei*, is controlled by GIH hormone and is presumed to inhibit vitellogenesis (Kang et al., 2014). Thus, the eyestalk ablation accelerates ovarian maturation in the female, resulting in spawning (Palacios et al., 1999a). However, from a welfare perspective, both unilateral and bilateral ablations have detrimental effects on females (Table 1).

Due to the multiple negative effects associated with ablation (Table 1) and concerning the welfare of *P. vannamei* broodstock, predictable maturation and spawning of captive specimens without the use of eyestalk ablation has been considered as a long-term goal for the industry (Quackenbush, 2015). Recent reports have suggested that similar productivity in broodstock can be obtained without eyestalk ablation through the application of husbandry interventions, including pre-maturation conditioning, increased stocking density and/or altered sex ratios (Zacarias et al., 2019). Trials conducted using these practices have demonstrated that rapid maturation and re-maturation of non-ablated *P. vannamei* females can be obtained while maintaining similar production levels of eggs/nauplii compared to ablated females (Zacarias et al., 2019; Zacarias et al., 2021). In addition, genetic background has been

TABLE 1 | Summary of eyestalk ablation effect in Penaeids (includes data from *P. vannamei*, *P. monodon* and *P. stylirostris*).

Eyestalk ablation impact in penaid species

| Reported Effects | References |
|--|--|
| Positive | |
| Reduce gonad inhibiting hormone | (Treeratrakool et al., 2014; Das et al., 2015) |
| Induce rapid maturation and spawn | (Chamberlain and Lawrence, 1981; Palacios et al., 1999b) |
| Negative | |
| Physiological imbalance | (Palacios et al., 1999b; Das et al., 2015) |
| Reproductive exhaustion | (Palacios et al., 1999b; Das et al., 2015) |
| Physical trauma | (Taylor et al., 2004; Bae et al., 2013) |
| Stress | (Taylor et al., 2004; Bae et al., 2013; Treeratrakool et al., 2014) |
| Reduction of shrimp hyperglycaemic and moulting inhibiting hormones | (Sainz-Hernández et al., 2008) |
| High energy demand | (Racotta et al., 2003) |
| Activation/reduction of immune related genes | (Sainz-Hernández et al., 2008; Bae et al., 2013; Treeratrakool et al., 2014) |
| Influence on metabolism of macronutrients | (Racotta et al., 2003; Sainz-Hernández et al., 2008) |
| Alteration of biochemical pathways | (Racotta et al., 2003; Sainz-Hernández et al., 2008) |
| Weight loss | (Palacios et al., 1999b) |
| Drop of haemocyanin and glucose in hepatopancreas | (Palacios et al., 1999b) |
| High broodstock mortality | (Zacarias et al., 2019) |
| Compromise offspring quality (e.g., decrease robustness to diseases) | (Palacios et al., 1999a; Zacarias et al., 2021) |

suggested to play a role in the production without eyestalk ablation. Therefore, ablation of eyestalks may no longer be required, which would represent an important step towards improved animal welfare. As an example, there are a few regular maturation facilities in Central and Latin America (Brazil, Colombia, Ecuador, and México), and Asia (Thailand) that are no longer using eyestalk ablation (Patrick Sorgeloos, 2020. Pers. Comm; Robsons McIntosh, 2020. Pers. Comm.). To support the phase-out of such practice, further studies to quantify the trade-offs from both production and economic perspectives would be very useful for the industry.

2.2 Disease and Stress-Immunity Mechanisms in Farmed Tropical Penaeids

Disease remains the key limiting factor for sustainable food production from crustacean aquaculture and fishing efforts. At the time of writing, 10 out of 11 diseases listed for crustaceans by the World Organisation for Animal Health¹ blight penaeid shrimp aquaculture (listed in **Table 2**). Viral (IHHNV, TSV, WSSV, YHV) pathogens dominated a series of debilitating disease outbreaks across Asia in the 1980s and 1990s, whereas bacterial (*Vibrio parahaemolyticus*) and fungal (*Enterocytozoon hepatopenaei*) pathogens emerged in the 2000s and 2010s (Thitamadee et al., 2016; Shinn et al., 2018; de Souza Valente and Wan, 2021). Substantive financial losses are incurred due to high mortality rates, up to 100% within days-weeks in many cases, and shrimp that survive are usually stunted and deformed, leading to high levels of waste (some features are listed in **Table 2**).

2.2.1 Biological Defences

To prevent, and manage, the spread of disease in decapod aquaculture, extensive efforts have been made to characterise their biological defences (Bachère, 2000; Tassanakajon et al., 2018; Gong and Zhang, 2021). Simply, innate immunity is split across three categories of defence (**Figure 1**): physical barriers (1), cell-directed responses carried out by haemocytes (2), and humoral factors produced by a variety of tissues that recognise, immobilise, and kill pathogens (3). What follows is a synopsis of the major features of decapod innate immunity – the articles cited provide more detail of these topics.

Physical (1). Disease-causing agents need to enter a host for survival, to replicate, and occasionally, complete their lifecycle. The penaeid carapace represents an important biological barrier – cuticle layers formed with chitinous polymers are hardened through the addition of calcium salts (external surface > epicuticle > exocuticle > endocuticle > epidermis > haemocoel > organs). The exoskeleton restricts most pathogens – some fungi and oomycetes are exceptions – to a few sites of entry (Coates et al., 2022); the gastrointestinal, reproductive, and respiratory systems as well as the nephrocomplex (i.e., antennal gland) (De Gryse et al., 2020; Liu et al., 2021). For example, Qi et al. (2017) highlighted the importance of the intestinal barrier in *shrimp-vibrio* antibiosis. The authors interrogated the intestinal

transcriptome of *P. vannamei* infected with the causative agent of acute hepatopancreatic necrosis syndrome (AHPNS), namely *V. parahaemolyticus*, to reveal ~2,500 upregulated genes with functions in immunity and cellular restructuring, e.g., pathogen recognition, phenoloxidase activity, antimicrobial peptides.

Cellular (2). Should the physiochemical barriers fail, a heterogeneous population of circulating immune cells termed haemocytes (granular, semi-granular and hyaline cells), can intercept the pathogenic intruders, reviewed by Johansson et al. (2000). Crustacean haemocytes perform tissue surveillance and recognise ‘non-self’ carbohydrate-lipid complexes that coat the surfaces of microbes; β -glucans and mannans for fungi (Vargas-Albores and Yepiz-Plascencia, 2000; Yepiz-Plascencia et al., 2000), lipopolysaccharides (endotoxins) and lipoteichoic acids for Gram-negative and Gram-positive bacteria (Phupet et al., 2018), respectively. Depending on the size of the pathogen (micro vs macro), the numbers of intruders present and the target tissue, haemocytes respond in several well-defined ways (**Figure 1**).

- Phagocytosis – direct ingestion of the microbial target into the cytoplasm and subsequent destruction *via* lysis facilitated mostly by enzymes, reviewed by Liu et al. (2020).
- Degranulation – the release (extra-cellularisation) of cytoplasm-derived granules containing a concoction of anti-infective factors, including antimicrobial peptides, adhesives, and the components of the phenoloxidase cascade (Smith and Söderhäll, 1983). Degranulation precedes nodule/capsule formation.
- Nodule formation – cellular aggregates consisting of numerous microbes (usually bacteria or yeast-like cells), host haemocytes, and melanotic debris. Such nodules are usually sequestered to specific tissues like the hepatopancreas and gills (Smith and Ratcliffe, 1980; Davies et al., 2020).
- Encapsulation – the ‘walling off’ or ‘ensheathment’ of microbes, microeukaryotes, parasitoids (too large to phagocytose), and damaged host tissue in recurrent layers of haemocytes. After multiple layers of haemocytes are in place, melanin is used to coat the capsule (van de Braak et al., 2002; Costa et al., 2009).
- Extracellular trap release (ETosis) – discharge of decondensed chromatin embedded with proteinaceous factors. Microbes are caught in the ‘chromatin net’ and are vulnerable to multiple biocidal agents, including histone-derived peptides and peroxidase (by)products (Ng et al., 2013; Robb et al., 2014).

Humoral (3). There is much coordination and crosstalk between the so called cellular and humoral immune reactions in decapods – wound repair, clot formation (haemostasis) and pro-phenoloxidase activation being key examples (Fontaine and Lightner, 1973; Söderhäll and Smith, 1983; Maningas et al., 2013). A myriad of enzymatic and non-enzymatic factors, including agglutinins, lysins, cytokine-like molecules and antimicrobial peptides (e.g., crustins; Bartlett et al., 2002), are found in the haemolymph of decapods. Many are derived from

¹World Organisation for Animal Health (OIE) 2022. Aquatics, Crustaceans. Available at: https://www.oie.int/en/what-we-do/animal-health-and-welfare/animal-diseases/?_tax_animal=aquatics%2CCrustaceans

TABLE 2 | Marked infectious diseases and syndromes of cultured penaeid shrimp.

| Disease | Causative agent | OiE listed* | Gross features (and notes) | Reference (not exhaustive) |
|--|--|---------------------|--|--|
| Abdominal segment deformity disease Acute hepatopancreatic necrosis disease [Early mortality syndrome] | Retrovirus-like element (abdominal segment deformity element or ASDE). NLRs, non-long terminal repeat retrotransposons. <i>Vibrio parahaemolyticus</i> isolates | - YES | Twisted and enlarged abdominal segments, with some observations of opaque muscle (necrosis and degeneration). The midgut, hepatopancreas and stomach of shrimp visible externally turned brown in infected shrimp. Sloughing of epithelial cells in the tubules of the hepatopancreas. Extensive haemocyte infiltration of tissue, and secondary bacterial septicaemia prior to <i>in exitus</i> . Vermiform structures appear in hepatopancreatic tubules and midguts. | (Sakaew et al., 2008; Sakaew et al., 2013) (Lightner et al., 2012; Tran et al., 2013; Kumar et al., 2020) |
| Aggregated transformed microvilli [White faeces syndrome] | Prokaryotic and microsporidian pathobiome. <i>Enterocytozoon hepatopenaei</i> are found in the white faeces of some affected shrimp. | - | Extensive sloughing and aggregation of microvilli coincide with the production of white faecal strings. | (Sriurairatana et al., 2014; Tang et al., 2016; Prachumwat et al., 2021) |
| Covert mortality disease | Covert mortality nodavirus | - | So named due to shrimp dying at the bottom of ponds. Nuclear enlargement in the hepatopancreas, muscle whitening and necrosis | (Zhang et al., 2014; Zhang et al., 2017) |
| Hepatopancreatic microsporidiosis | <i>Enterocytozoon hepatopenaei</i> (<i>Agmasoma penaei</i>) | YES | Substantial growth retardation in <i>P. vannamei</i> due to disrupted gut epithelium and nutrient absorption. Intracytoplasmic - connective tissue (<i>A. penaei</i>) and tubules (<i>E. hepatopenaei</i>) of the hepatopancreas. | (Tourtip et al., 2009; Chaijarasphong et al., 2021; Geetha et al. (2022) |
| Hepatopancreatic haplosporidiosis | <i>Haplosporidium</i> sp. | - | Melanisation of the cuticle, hepatopancreas shrinkage (pale and atrophied), reduced growth, and flaccid bodies. Histology revealed tubule epithelia cells contained cytoplasmic multinucleate plasmodia and trophonts. | (Utari et al., 2012; Thitamadee et al., 2016) |
| Infectious hypodermal and haematopoietic necrosis | Infectious hypodermal and haematopoietic necrosis virus | YES | Interferes with developmental stages from eggs to post-larval. The virus infects epidermal and mesodermal tissues. | (Lightner et al., 1983) |
| Infectious myonecrosis | Infectious myonecrosis virus | YES | Cuticular deformity, notably a bent rostrum (not always) Focal to extensive muscle whitening along the abdomen and tail regions. In some shrimp, regions become necrotic and reddened Lymphoid organs tend to. Be atrophied and enlarged. Rapid onset has been linked to feeding prior to exposure to stress. | Lightner et al., 2004; Poulos et al., 2006; Prasad et al., 2017) |
| Male reproductive tract degenerative syndrome | Auto-immunity? [some links to temperature, diet, and microbes] | - | Deformed and melanised sperm held in the spermatophores alongside the accumulation of amorphous debris. Dark discolouration is visible externally upon inspection of the shrimp ventrum. | (Talbot et al., 1989; Diamond et al., 2008) |
| Necrotising hepatopancreatitis | <i>Hepatobacter penaei</i> | YES | Shell softening, flaccid limbs, discoloured (black) gills, and white, atrophied hepatopancreas. Some shrimp showed reductions in feeding and changes in behaviour. | (Frellet et al., 1993; Nunan et al., 2013) |
| Taura syndrome | Taura syndrome virus | YES | Acute: anorexia, lethargy and erratic swimming. Opaque tail muscle, soft cuticle, and sometimes, reddening of the tail. Transition: melanotic lesions of the cephalothorax and tail cuticle. Chronic: lymphoid organ spheroids (sometimes they appear ectopically). | (Hasson et al., 1995; Bonami et al., 1997; Tumburu et al., 2012) |
| White head disease | Decapod iridescent virus [1] (Haemocyte iridescent virus) | YES | Can target haemocytes, haematopoietic and lymphoid tissues. Whitening of the carapace at the base of the rostrum. Red body discolouration. Atrophy of the hepatopancreas. | (Liao et al., 2020; Qiu et al., 2020) |
| White spot disease | White spot syndrome virus | YES | Shrimp are usually lethargic with reduced levels of feeding and preening, and sometimes, a loose cuticle. Characteristic white, calcified spots can be visible on the dorsum of the carapace. Some have discoloured hepatopancreatic tissue. | (van Hulten et al., 2001; Verbruggen, 2016) |

(Continued)

TABLE 2 | Continued

| Disease | Causative agent | OiE listed* | Gross features (and notes) | Reference (not exhaustive) |
|----------------------------|---|-------------|--|--|
| White tail disease | <i>Macrobrachium rosenbergii</i> nodavirus Extra small virus | YES | Detected in <i>P. vannamei</i> , <i>P. indicus</i> , and <i>P. monodon</i> . Opacity and milky/white appearance of the abdomen in larval to juvenile shrimp. Reduced feeding and swimming behaviour is often associated with uropod and telson degeneration. | (Bonami et al., 2005; Ravi et al., 2009; Senapin et al., 2012; Senapin et al., 2013) |
| Yellow head disease | Yellow head (nido)virus (complex) Gill associated virus | YES | Distinct yellowing/bleaching of the cephalothorax/carapace, and enhanced feeding. Abnormally yellow (and soft) hepatopancreas. The virus targets multiple tissues including the gut, antennal gland, nervous tissue, and gonads. | (Wijegoonawardane et al., 2008; Mohr et al., 2015); |

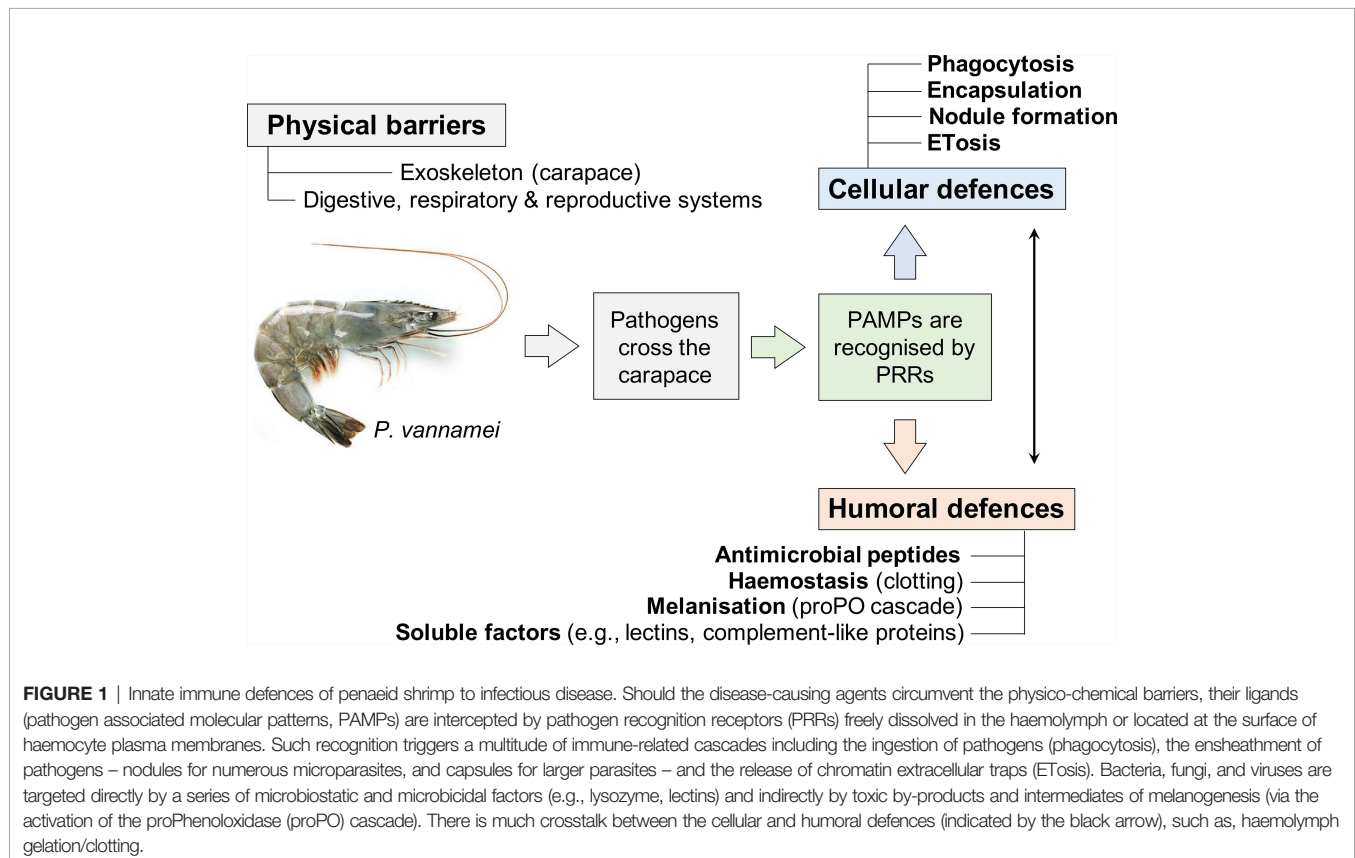
*World Organisation for Animal Health (OIE 2022; https://www.oie.int/en/what-we-do/animal-health-and-welfare/animal-diseases/?_tax_animal=aquatics%2Ccrustaceans)

the haemocytes (e.g., clotting pathway components), while others are released constitutively by diverse tissue types including the hepatopancreas, with the remainder being acute phase (Smith and Chisholm, 1992; Suleiman et al., 2017; Tassanakajon et al., 2018). Arguably, the most identifiable response is the generation of the black/brown pigment melanin from phenoloxidase activities. Melanin itself is microbiostatic and virustatic, whereas the by-products generated from its biogenesis (e.g., oxygenic radicals), are highly toxic (Cerenius et al., 2010; Coates and Talbot, 2018). Multiple enzymes – laccase, tyrosinase, catecholoxidase and haemocyanin – are capable of catalysing simple phenols into quinones, which go on to form melanin (Coates and Costa-Paiva, 2020). These

enzymes are usually referred to collectively as phenoloxidases and display multiple immune properties (Cerenius and Söderhäll, 2021).

2.3 Environmental and Biological Conditions During Grow-Out: Health-Stress-Disease Axis in Ponds

The health of farmed fish is inextricably linked to their welfare (Segner et al., 2012), and unsurprisingly, this is also the case for decapod crustaceans such as penaeid shrimps, as illustrated by the Disease triangle in **Figure 2**. There remains a deficit with respect to welfare indicators or standardised reference intervals for monitoring penaeids in captivity, partly because it is



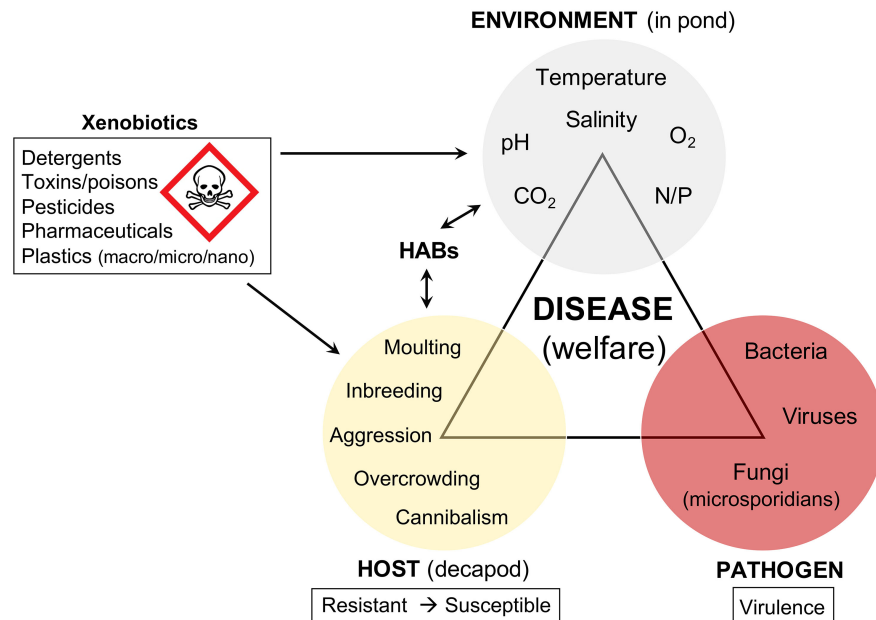


FIGURE 2 | Disease triangle in the context of penaeid welfare (in pond). Growth and maintenance of shrimp under culture conditions poses several challenges for shrimp health and welfare. Susceptible hosts, combined with virulent pathogens and unfavourable environmental conditions encourage disease outbreaks. The presence of immune-modulating xenobiotics, fluctuating abiotic factors leading to hypoxia, hypercapnia, or acidosis can tip the balance in favour of the pathogen(s). HABs, harmful algal blooms; N, nitrogen; P, phosphate.

challenging to define what constitutes a healthy shrimp (Coates and Söderhäll, 2021). Recent efforts have looked to the gut microbiome as a means of measuring 'healthy' penaeids, including immune vigour (Li et al., 2018; Holt et al., 2021). Unsurprisingly, the resident microbes of penaeid tissues are linked to disease and health outcomes – the composition, diversity, and balance between pathobionts and symbionts.

Acute, subacute, prolonged, and chronic perturbations in pond abiotic factors – temperature (Cheng et al., 2005; Rahman et al., 2006), pH (Gunalan et al., 2010), salinity (Li et al., 2008), nitrites (Tseng and Chen, 2004), and hypoxia/hypercapnia (Burgents et al., 2005) – influence decapod susceptibility to disease. Acute stress can stimulate the immune system and tends to have transient impacts on the host, sometimes promoting stress tolerance to subsequent exposure. Prolonged stress, from one or more source (chemical versus physical) can lead to shifts in metabolism, immune dysfunction, and health declines. Imbalances of dissolved oxygen and carbon dioxide in the water (hypoxia, and hypercapnia, respectively) can lead to tissue dysoxia and lactate accumulation – the switch to suboptimal glycolytic metabolism over oxidative phosphorylation. Temperature, pH and dissolved gas content are inextricably linked. As penaeids are ectotherms, temperature extremes outside of their tolerance range can have profound effects on general physiology. Indirectly, higher water temperatures retain less O₂ and increased pCO₂ in the water leads to acidosis. CO₂ can also bind with H₂O in the

haemolymph to form H₂CO₃ (carbonic acid) (reviewed by Coates and Söderhäll, 2021; Millard et al., 2021).

From a biological perspective, one of the key questions for both welfare and production is the effects of stocking density. For this reason, there are numerous studies that have assessed the effects of stocking density on *P. vannamei* at different life stages (Froes et al., 2013; Wasielesky et al., 2013; Esparza-Leal et al., 2015) and under different production systems (Cuvín-Aralar et al., 2009; Wasielesky et al., 2013; Durairaj et al., 2018). While most of these studies have concentrated on the impacts from a production perspective there are some studies available on the effects of stocking density on the behaviour of *P. vannamei* (da Costa et al., 2016; Bardera et al., 2021). Results from (da Costa et al., 2016) showed that under aquaria conditions stocking density affects the behaviour of *P. vannamei* juveniles and that there is an optimum animal density that promotes behaviours such as 'searching' and 'feeding'. Dominance hierarchies have been reported and results revealed that at least at the juvenile stage, higher densities (around 25 juveniles m⁻² compared to 6 and 12 juveniles m⁻²) maximise feeding behaviour while minimising the effects of dominance. However, the translation of such observations to real productions systems is challenging and therefore studies on real production systems are mainly based on production outputs. In this sense, the effect of stocking density in relation to temperature has been modelled and data indicate the importance of tailoring stocking density based on the production system and temperature as even minor

differences in temperature can produce significant differences in growth and therein profits (Villanueva et al., 2013; Araneda et al., 2020).

In summary, it is difficult to recommend unique values for the main biological factors as they are extremely dependent on the life stage, the actual production system (sometimes associated with different regions) and the environmental conditions the animals experience. However, standardised farming management practices (including animal handling), stable pond/tank conditions (e.g. water quality parameters remaining in ideal range, enough space for shrimp to grow, presence of natural diet and beneficial microbial communities), and routine health checks are necessary to avoid stress, fatalities and financial loss.

3 WELFARE INDICATORS IN DECAPOD CRUSTACEANS

3.1 Current State on Physiological Biomarkers

In decapod crustaceans, as in vertebrates, disturbances in the nutritional, environmental and health welfare domains can lead to allostatic overload (Freire et al., 2020). Allostasis, defined as the adaptive process for actively maintaining stability through change (Sterling and Eyer, 1988) is relevant when trying to establish reference physiological intervals. In this sense, physiological responses that can be measured and that are associated with homeostasis and allostasis could be relevant as direct welfare indicators. The concept of using physiological measures as welfare indicators is particularly relevant for aquatic species where assessing behaviour, pain and suffering is not easy or not possible due to lack of knowledge (Jerez-Cepa and Ruiz-Jarabo, 2021). For these species, the physiological framework is based on the fact that a number of physiological parameters will move outside their allostatic range (overload). These changes must be associated with the different types and degree of stress that the animals experience. Decapod species, like any other organism, respond to stressors by mounting a stress response that involves behavioural and physiological changes at multiple biological levels. The concept of finding suitable biomarkers that can be applied in the context of decapod welfare has been mentioned in few studies (Rosas et al., 2004; Ciaramella et al., 2014; D'Agaro et al., 2014; Wang et al., 2014; Albalat et al., 2019). However, there is little evidence that a concerted effort has been made to establish haematologic biomarkers or reference intervals for decapods, more specifically *Penaeus* spp., in captivity or under cultivation. Tu et al. (2010) trialled some measures – e.g., activities of glutathione-S-transferase, peroxidase and catalase – in *P. monodon* production sites along the Mekong River Delta (Vietnam) and could discriminate between animals reared in intensive systems versus those reared in integrated systems. Producing a suite of biomarkers for decapods is not straightforward as routine measures like cell (haemocyte) counts, protein levels or haemolymph enzyme activity (e.g., lysozyme, phenoloxidase) can fluctuate according to

season (Hauton et al., 1997) moult cycle (inter-moult, pre-moult, post-moult; Liu et al., 2004) or even lunar phases (Bautista-Covarrubias et al., 2020).

Rodríguez and Le Moullac (2000) reviewed health control measures of penaeid shrimp and cited 120 mg [protein] mL⁻¹ [haemolymph] for laboratory reared *P. vannamei* juveniles. Perhaps some inspiration can be taken from a marine invertebrate often misidentified as a decapod, the Atlantic horseshoe crab *Limulus polyphemus*. Horseshoe crabs are highly valued due to the sensitive endotoxin detection kit (Limulus Amebocyte Lysate) derived from the cellular fraction of their blood (haemolymph), which replaces the rabbit pyrogen test in pharmaceutical and vaccine development (Krisfalusi-Gannon et al., 2018). Because of this commercial sensitivity, reference intervals have been proposed for captive *L. polyphemus ex situ* (Smith et al., 2002) and free-ranging crabs *in situ* (Arnold et al., 2021). As horseshoe crabs suffer from a protein deficiency syndrome in captivity (Smith and Berkson, 2005), an acceptable range is considered ~34 to 112 mg [protein] mL⁻¹ [haemolymph] – with most of the soluble protein made up of haemocyanin (>90%; Coates et al., 2012). Haemocyanin comprises >60% of haemolymph protein in most decapods studied to date (e.g., *Nephrops norvegicus*, Coates and Nairn, 2013). Among the numerous cellular and biochemical indicators proposed across the literature, total protein levels and haemocyte counts represent the most likely candidates, in addition to lactate levels, as ‘welfare’ or ‘health’ indicators for decapods. These procedures are invasive, but not lethal, in the right hands. Some such measures have been used in an attempt to disentangle ‘captivity-associated stress’ in crabs (e.g., *Cancer pagurus*; Johnson et al., 2016) and lobsters (e.g., *Homarus gammarus*, Basti et al., 2010; Albalat et al., 2019). Haemolymph biochemical parameter assessment has been trialled with autoanalyzers (haemocytes) and handheld refractometers, e.g., crayfish (*Astacus leptodactylus*) and lobsters (*H. americanus*) (Sepici-Dinçel et al., 2013; Wang and McGaw, 2014).

A condition/welfare index based on both physical appearance and behaviour (e.g. ‘vigour’) as developed by Albalat et al. (2017) for trawl-caught langoustines *N. norvegicus*, combined with one or more haemolymph measures, would represent a major step forward.

3.2 Operational Welfare Indicators (OWIs): Individual and Group Based, Direct and Indirect and Invasive vs. Non-Invasive

If farmers want to detect welfare problems during the farming of crustaceans, they must have good feasible operational welfare indicators (OWIs) that can be measured quantitatively by staff based at the farms. OWIs must be species- and life stage-specific, minimally invasive, fit for purpose (for production systems and operations) and robust. In **Table 3**, we suggest a list of positive and negative survival and situation-related factors to be observed based on the 5 domains framework suggested by Mellor and Reid (1994) and based on animal (outcomes) and resource (inputs) measures applicable to the crustaceans welfare.

TABLE 3 | List of negative and positive survival and situation related factors to be observed in order to generate the best Operational Welfare Indicators (OWIs) for the species based on the 5 domains framework (adapted from Mellor and Reid, 1994 to crustaceans).

| PHYSICAL/FUNCTIONAL DOMAINS | | | | |
|---|--------------------------|---|--|------------------------------------|
| Survival related factors | | Situation-related factors | | |
| 1. Nutrition | | 2. Environment | 3. Health | 4. Behaviour |
| Negative | Positive | Negative | Negative | Positive |
| Restricted food | Enough Food | Uncomfortable environment | Disease, injury or functional impairment | Behavioural expression restricted |
| Bad quality food | Balanced and varied diet | Environmental requirements at suboptimal levels | Stress | Able to express natural behaviours |
| Diet requirements not met | No physical enrichment | Physical features are adequate | | |
| AFFECTIVE EXPERIENCE DOMAINS | | | | |
| 5. Mental states | | | | |
| Negative Experiences | | Positive Experiences | | |
| Overcrowding, Hunger, Malnutrition, Temperature out of ranges, Noise (hearing discomfort) | | Homeostasis, good diet, Satiety, Physical comfort, Vigour, Good Health/fitness, Reward, Calmness, | | |
| Repetitive stressors, Injury, Pain, Disease, Fear, Boredom, Anxiety, Panic, Exhaustion | | Sociability, Natural Behaviours, Enriched environment | | |
| WELFARE | | | | |

Each of the risk factors and stressors during the life cycle of the farmed crustaceans can be assessed by a welfare indicator closely related to the original stressor. For example, acute or unexpected changes in water temperature (the stressor) can lead to changes of the appetite level in the farmed crustaceans. Monitoring and evaluation of the feeding responses is a common OWI potentially related to an environmental stressor (Martins et al., 2012). Real time monitoring of the animals and the environmental parameters is key for the identification of environmental-related welfare issues.

In order to be able to measure the different welfare domains, we present in **Table 4** a list of potential direct (animal) and indirect (environment-based) welfare indicators for *P. vannamei*, that could also be used for other decapod crustaceans as appropriate. Direct individual based OWIs include both physical health and physiological parameters as well as behavioural measures. On the other hand, indirect or resource-based measures include environmental parameters related to water quality, husbandry procedures, type of food and other measures such as predator presence or enclosure design and substrate access (e.g. environmental enrichment such as the background colour of tanks, use of hides and substrates or different feeding methods).

OWIs must be used at different levels depending on the intensity of the intervention needed (Noble et al., 2018). OWIs at the *primary* level of assessment include environmental parameters, visual observations (behaviour and appearance) and sudden changes in relation to them. When this is not sufficient to evaluate the welfare status of the animals then it is necessary to move to a *secondary* level of assessment and use the more invasive handling and laboratory-based OWIs (indicated in orange in **Table 4**) that require physical manipulation of the animals (vigour index or close visual assessment of the damage or disease). If the welfare problem is not solved then the next stage would be the *tertiary* level of assessment where handling and sampling of blood and tissues is required (anaesthetics are recommended for this sampling procedure) with more detailed analysis need to be performed: PCR for disease diagnosis, histopathology, haemolymph sampling for stress parameters or other techniques might be required: behavioural assessment under controlled conditions, etc.

3.3 Other Emerging Operational Welfare Indicators

Biosensors, loggers and early warning systems are: cheap, user-friendly, non-invasive methods for early detection of critical alterations in the farming conditions. They can be automatic systems for behavioral observations or water quality sensors (Barreto et al., 2022). With the use of Artificial Intelligence (AI) and machine learning methods the monitoring of environmental parameters and behaviour can be done automatically, and the decision-making process becomes more accurate (O'Donncha et al., 2021) and autonomous. These methods of precision farming (PF) are being developed for finfish and molluscs with proven success. In shrimp farming, there are examples of monitoring systems to automatically

TABLE 4 | List of potential OWIs to be measured for welfare assessment and their use as a decision support method. Invasive OWIs are highlighted using an asterisk (*).

| Operational Welfare Indicators (OWI) | Group |
|---|---|
| Direct animal Based (outcome) | |
| Individual | |
| Vigour index (condition factor)* | Stocking density |
| Behaviour (startle response, feeding response, lethargy, thigmotaxis, activity, swimming) | Behaviour (feeding response, aggression, aggregation, panic response, group activity, swimming and dynamics, dominance) |
| Haemolymph (protein, stress parameters: glucose, lactate; immune measures: haemocyte counts, lysozyme and phenoloxidase)* | Growth (SGR, FCR)* |
| Life stage (juvenile, moulting, adult) | Disease and disease control* |
| Eye damage (eyestalk ablation or others) | |
| Antennae damage | |
| External coloration | |
| Melanosis | |
| White calcifications | |
| Milky abdomen | |
| Uropod/Telson degeneration | |
| Microbiome (gut)* | |
| Pathogen screening* | |
| Indirect environment based (input) | |
| Water Quality | Rearing Systems |
| Temperature | Water current |
| Salinity | Light and photoperiod |
| Oxygen | Noise |
| Carbon Dioxide | Stocking Density |
| pH | Tank shape and background colour, furniture, RAS systems |
| Ammonia/Nitrogen | Predator presence |
| Nitrite/Nitrate | Environmental Enrichment (positive welfare) |
| Pollution (heavy metals, organic matter, antibiotics) | |
| Total Suspended Solids | |
| Microbiome (water and substrate) | |
| Pathogens | |

record changes in physicochemical water parameters that are processed using AI (Borquez-Lopez et al., 2016). However, AI models could, for example, identify some of the animal-based OWIs in *P. vannamei*, such as body melanosis, antenna and eye damage, and could also monitor their behaviour and assess possible risk factors, based on individual and group behaviour, interaction with environment and social behaviour (aggression, aggregation, feeding responses, etc) (Huang et al., 2019).

Use of rapid on-site testing for disease diagnostics is another emerging health and welfare tool. In general, in aquaculture systems, animal mortality results from complex interactions between genetic background, environmental conditions, stress and opportunistic pathogens, making most laboratory approaches sub-optimal. The on-site testing makes it possible to obtain physiological and behavioural responses to the real environmental conditions and to assess their welfare in relation to those responses (Barreto et al., 2022). Some of those on-site diagnostics are cheap and affordable paper-based diagnostics, widely used for human health (Hu et al., 2014) and recently translated into fish farming². Other advances in welfare indicators are the development of smartphone based diagnostic

technologies (Rateni et al., 2017) as well as smartphone applications for visual based diagnostics and the use of cloud metadata³.

The importance of promoting positive welfare and the need to offer more natural environments for the cultured animals has increased in recent years. The use of environmental enrichment (EE) to increase the complexity on the environment e.g., hiding sites, tanks of various colours and shapes, flows and natural light regimes, life feeding or feeding methods that mimic their natural behaviours, are crucial to promote social interaction, to avoid maladaptive behaviours and to reduce stress related to captive conditions (Arechavala-Lopez et al., 2021). In decapod crustaceans, as observed in fish and other farmed vertebrates, the use of EE could be used to improve their welfare status. EE could be developed on temperature gradients (behavioural prophylaxis) based on thermal preferences and related to their internal emotional states (Rey et al., 2015). Other structural EE are already used for *P. vannamei* such as a dark background colour of the tanks or the rounded shape has been mentioned before.

3.4 Existing Aquaculture Standards Applied to the Farming Practices of *P. vannamei*: Gaps in Welfare Indicators

For the farming of decapod crustaceans and specifically for penaeid shrimp farming the key factors that need to be identified and if necessary revised include feed quality and

² <https://www.globalseafood.org/advocate/investment-firm-paper-based-disease-test-kits/>

³ <https://www.innovationnewsnetwork.com/ensuring-fish-welfare-in-aquaculture-with-new-digital-app/12855/>

delivery methods, water quality and flow, stocking density, transport methods and the number of transport events during shrimps' life cycle. The main management practices and interventions to be considered include alternatives to eyestalk ablation for broodstock, grading of juveniles and disease treatments up to point of slaughter. In this way, a good welfare assessment can be achieved for each of the potential stressors, leading to improved husbandry practices.

Most commercial shrimp aquaculture standards use different welfare indicators related to the environment and the health of the animals (input and outcome measures). Few use the 5th domain on the mental status of the animals that should be the main factor to determine the outcome of the welfare status of the animals (Table 3).

The Aquaculture Stewardship Council (ASC) Shrimp Standard⁴, for example, places a lot of emphasis on the effect of shrimp farming on biodiversity and impacts on the environment, as well as the use of wild fish as an ingredient for feed. Farms are required to measure water quality parameters, as well as, monitor diseases and report during auditing. The use of antibiotics is restricted, and they should be monitored in the water systems. Social aspects of the farming practices are also included. However, no direct animal-based welfare measures and targets are included besides from the routine health checks. No morphological, physiological or behavioural OWIs are required.

Other standards such as the Asian Seafood Improvement Collaborative⁵ (ASIC) shrimp standard or the Best Aquaculture Practice⁶ (BAP) standards for finfish and crustacean farms are based on best management practices with very general considerations regarding the use of OWIs (stocking densities, feeding, fasting, crowding, and dewatering, water quality, behaviour and condition and testing for disease outbreaks). Humane slaughter techniques are mentioned but with no specifics given. The main issues addressed are environmental, social and those concerning food safety (drug and chemical management, microbial sanitation, hygiene, harvest and transport, biosecurity and traceability). ASIC requirements for shrimp health status are limited to compliance to existing national standards and the stocking densities need to be recorded to monitor and estimate the number of escapes. Mortalities need to be recorded, as well as, the main environmental parameters (temperature, DO, salinity and ammonia). BAP contains a short section on shrimp-specific standards related also to environmental and effluent management (water exchange), as well as, to food safety during harvest and transport (e.g. the use of sulphite solutions as antimelanotic at harvest). It also specifies a maximum biomass limit based on performance measures for health and survival. BAP requires training for staff to provide appropriate levels of husbandry and awareness of these standards.

In Asia, there are also local government standards that address some health and welfare issues, and also focus on

animal welfare indicators⁷. These standards contain specific information on best practices and the recommended range of stocking densities for the different culture systems. However, the recommendations on animal welfare are still very general, with no specific animal-based OWIs required.

Therefore, most common shrimp standards are still using very few animal-based OWIs and recommendations are very general, with no guidance on ranges of tolerance or rates for the most common parameters provided. For example, standards should provide guidance on the rates of survival or mortality rates per life stages of shrimp production. Feeding should be focused on nutritional requirements and best feeding practices. Maximum fasting times should be provided, in addition to guidance on crowding prevention techniques and disease prevention and monitoring of relevant parameters. Regulation on humane stunning and slaughter methods and the use of drugs and chemicals, as well as handling standards should be provided. Most of those parameters have been studied and there is sufficient knowledge in the scientific literature to be used in farming protocols and standards. Our review is an example of the amount of knowledge that exists on shrimp farming and mitigation methods.

4 STUNNING AND SLAUGHTER PRACTICES IN RELATION TO SENTIENCE

4.1 Current Scientific Evidence for Sentience in Decapod Crustaceans

Sentience can be defined as having the capacity to feel (Kirkwood, 2006) or following Darwin's definition 'a sentient animal is one for whom feelings matter' (Darwin, 1872). Within the context of this review, we will more specifically align with the Animal Welfare Committee in the UK which has defined sentience as the capacity to experience pain, distress or harm, although as pointed out by other authors this definition lacks the consideration of animals being able to experience positive feelings (Mellor and Beausoleil, 2015; Birch et al., 2021). In any case, the capacity for a living organism to be capable to have feelings such as pain requires a level of awareness and cognitive ability (Broom, 2019). So far, arguments in support of the view that decapod crustaceans have the capacity to feel pain have been made by some authors (Conte et al., 2021; Passantino et al., 2021) while others support the view that the evidence is currently insufficient (Diggles, 2019).

Frameworks to provide scientific evidence of pain in vertebrate species have been proposed and generally agreed for several years (Bateson, 1991). The translation of such frameworks seeking to establish if invertebrates experience pain has been presented by a number of authors (Smith, 1991; Smith and Boyd, 1991; Birch et al., 2021) (Table 5).

Upon examining research performed in *P. vannamei*, current evidence of sentience in decapod crustaceans is mainly

⁴https://www.asc-aqua.org/wp-content/uploads/2019/03/ASC-Shrimp-Standard_v1.1_Final.pdf

⁵ ASIC: <http://www.asicollaborative.org/>

⁶ BAP: <https://bapcertification.org/blog/category/shrimp/>

⁷<https://asean.org/wp-content/uploads/images/archive/AMAF%2033%20Standard%20on%20ASEAN%20Shrimp%20GAP.pdf>

TABLE 5 | Examples of frameworks used to evaluate scientific evidence of sentience in animals.

| Criteria emphasis | Description of criteria | |
|-----------------------------|--|--|
| | Smith and Boyd, 1991 | Birch et al., 2021 |
| Neurological | Possession of receptors sensitive to noxious stimuli, located in functionally useful positions on or in the body, and connected by nervous pathways to the lower parts of a central nervous system | Possession of nociceptors |
| | Possession of brain centres which are higher in the sense of level of integration of brain processing (especially a structure analogous to the human cerebral cortex) | Possession of integrative brain regions |
| | Possession of nervous pathways connecting the nociceptive system to the higher brain centres | Connections between nociceptors and integrative brain regions |
| Cognitive/ Behaviour | Receptors for opioid substances found in the central nervous system, especially the brain. | |
| | An animal's response to stimuli that would be painful for a human is functionally similar to the human response | Motivational trade-offs that show a balancing of threat against opportunity for reward |
| | An animal's behavioural response persists, and it shows an unwillingness to resubmit to a painful procedure; the animal can learn to associate apparently non-painful with apparently painful events | Flexible self-protective behaviours in response to injury and threat |
| Mixed | | Associative learning that goes beyond habituation and sensitisation |
| | | Responses affected by potential local anaesthetics or analgesics |
| | Analgesics modify an animal's response to stimuli that would be painful for a human | Behaviour that shows the animal values local anaesthetics or analgesics when injured |

concentrated in what it is known for the largest decapods (crabs, lobsters and crayfish), which do not include penaid species. From a 'neurological perspective', three neuropil areas (terminal medullae, hemiellipsoid bodies and the accessory lobes) are candidates for higher integrative centers (Sandeman and Harzsch, 2016). The terminal medullae and the hemiellipsoid bodies are part of the lateral protocerebrum. Recent research on decapod crustacean brains (supraesophageal ganglia) (Strausfeld et al., 2020) indicate the presence of protocerebral centers in *P. vannamei*. Immunohistochemistry results showed the presence of a substantial lateral protocerebral and optic neuropils and a much reduced anti-DC0-immunoreactive centre, the location of which corresponds to the ancestral mushroom body of Stomatopoda and Caridea. This area, therefore, comprises an integrative centre that is associated with the hemiellipsoid body (homologous to insect mushroom bodies) linked to learning and memory.

From a 'cognitive and behavioural perspective', the little research on *P. vannamei* published to date has mainly focused on feeding behaviour, production practices such as stocking densities and mating behaviour (Yano et al., 1988; Bardera et al., 2020; Bardera et al., 2021). A review by Bardera et al. (2019) highlighted the importance of studying behaviour for the farming/production of *P. vannamei*, but studies on motivational trade-offs, flexible self-protective behaviours in response to injury, threat and associative learning that goes beyond habituation and sensitisation are currently missing. In this regard, recent studies performed in understanding agonistic behaviours in the cultured swimming crab (*Portunus trituberculatus*) (e.g. Su et al., 2021; Wu et al., 2021) and the interaction between animal behaviour and production practices (e.g. Zhang et al., 2021) highlight the importance to factor behavioural aspects in production systems for decapod species.

Finally, from a 'mixed perspective', Taylor et al. (2004) examined the role of a topical anaesthetic (lidocaine) and a

coagulation agent in minimising the stress response due to eyestalk ablation in *P. vannamei*. Their results indicate that the application of an anaesthetic significantly improves the elapsed time of feeding onset and their swimming behaviour. On the other hand, the effects of anaesthetics (clove oil/eugenol) have also been examined in a number of decapods, including *P. vannamei* (Parodi et al., 2012; Wycoff et al., 2018; Becker et al., 2021). Results from Wycoff et al. (2018) show that *P. vannamei* was very susceptible to stress and therefore measurements of heart rate, motor nerve activity and sensory rate activity were inconclusive or not possible to perform. However, the study showed that the animals were lethargic within 2 min, and they were inactive to tail pinches within 6 to 12 min when bathed at a eugenol concentration of 200 ppm.

As the number of studies is certainly limited, especially from a cognitive and behavioural perspective it is clear that more fundamental research in this area is needed. However, following the 'precautionary principle' the basic requirement to define current production practices from an animal welfare perspective is relevant. In the case of *P. vannamei*, the demand for increased welfare could potentially be driven not only by stakeholders and certification schemes but also by consumers based in key importing countries, since a very significant proportion of these animals are traded internationally. Therefore, in the next section we evaluate key challenges associated with the stunning and slaughter of *P. vannamei* from an animal welfare perspective.

4.2 Stunning and Slaughter Practices in *P. vannamei*

Partly due to the current lack of welfare protection for decapod crustaceans (see *Current Legislation Relevant to Decapod Crustaceans*); harvesting, stunning and slaughter practices for this group in general, and *P. vannamei* in particular, are not

regulated, or even standardised. Before entering into further detail, it might be relevant to indicate that within the context of this review we have used the terminology of Conte et al. (2021) whereby ‘stunning’ refers to rendering the animals insensible, and that this process can be reversible or irreversible (in the latter case it should be considered as ‘slaughter’). Methods for stunning and slaughter used in decapod crustaceans are varied and include cold shock in air, ice or slurry ice, boiling, freshwater or salt baths, carbon dioxide narcosis, high pressure, mechanical splitting or spiking of the nervous system, dismemberment and electrical stunning as reviewed by Conte et al. (2021). The variety of techniques used not only reflects the absence of legislation but also the fact that decapod species are very diverse in their anatomy and physiology. Decapod crustaceans are held and slaughtered for different ends, e.g., scientific research studies versus food production, and in the latter case, there may be the additional issue of dealing with very large numbers of animals, which is true for *P. vannamei*.

A requirement to use more humane methods of slaughter is now demanded by advocacy bodies, increasingly being mandated by legislative bodies and governments in several parts of the world, and is currently being debated in the UK (see *Current Legislation Relevant to Decapod Crustaceans*). When applied to decapod crustaceans, the majority of methods mentioned above are considered inhumane by the European Union’s Scientific Panel on Animal Health and Welfare (Animal Health and Animal Welfare Panel of the European Food Standards Agency, 2005) since most take some time to have an effect and hence have the potential to confer suffering or stress. Methods of rapid dispatch that minimise the potential to inflict suffering and stress, and thus improve welfare, continue to be sought by the crustacean fishery and aquaculture industries. Currently, there is no published information on the commercial slaughter practices for penaeid shrimps, and for that reason it is difficult to ascertain if these are applied correctly to minimise peri-slaughter stress of *P. vannamei* during the harvest process.

A previous study has shown that the use of slurry ice on *P. vannamei* (thought to be the most commonly used slaughter method) renders the animals sedated, accompanied by a reduced cardiac function within seconds (substantial reduction within 30 seconds) (Weineck et al., 2018). Despite a decrease in heart rate, specimens would tail flip within 1 min when exposed to cold water and more importantly, upon transfer to warm water the heart rate increased, indicating that the stunning is reversible. Therefore, although slurry ice proved to be a fast method for stunning, controlling the time and conditions between stunning and slaughter in commercial farms would be very important from a welfare perspective. Regarding electrical stunning, several references are available for the larger decapods (Neil, 2010; Roth and Øines, 2010; Neil, 2012a; Roth and Grimsbø, 2013; Fregin and Bickmeyer, 2016; Roth and Grimsbø, 2016). These studies have shown the effectiveness of electrostunning in rendering these larger decapods insensible rapidly (within 1 second reported) (Roth and Øines, 2010). The effectiveness of electrical stunning in *P. vannamei* was also evaluated recently (Weineck et al., 2018), and was found to be as effective, but less consistent than cold stun in ice slurry.

The choice of slaughter method will also impact post-harvest product quality (Neil, 2012b), and a study performed on *P. vannamei* has shown that the use of slurry ice is more beneficial for post-harvest quality than cold storage and flake ice (Wang et al., 2014). Inappropriate harvesting, stunning and slaughter trigger an acute stress response, which can impact the initial post-mortem metabolism and reduce product freshness (Albalat et al., 2009; Gornik et al., 2010). Furthermore, other stress-related consequences such as extensive autotomy (the casting off a part of the body by an animal under threat) has been reported when crabs were stunned using a low electrical field strength to the whole animal (Roth and Øines, 2010) an effect not reported with higher electrical field strengths (Neil, 2010; Neil, 2012a). Blackening or melanosis development can also be affected if the stunning/slaughter method causes an increase in temperature (Albalat et al., 2022).

5 CURRENT LEGISLATION RELEVANT TO DECAPOD CRUSTACEANS

Countries that protect decapods or place them under some degree of legal protection are Australia, Austria, New Zealand and Norway. Italy and UK do have some partial protection or changes in legislations are being contemplated (in UK).

Australia: In 2004, Australia adopted the Australian Animal Welfare Strategy (AAWS), which explicitly covered ‘all sentient animals—that is, those with a capacity to experience suffering and pleasure’. Crustaceans are only protected in some states and by some provisions. These provisions cover cephalopods (Australian Capital Territory) and crustaceans (Australian Capital Territory and New South Wales – for human consumption; Victoria – adult decapods since 1997). However, there is room for improvement in many domains related to animal welfare in general, Australia ranking D on the animal protection index⁸. Animal sentience is still not recognised at the Commonwealth level.

Austria: The Austrian Animal Welfare act of 2004⁹ protects crustaceans under national husbandry guidelines. It has been scored as B on the animal protection index¹⁰.

Switzerland: The Animal Welfare Ordinance from 2008¹¹ protects crustaceans during slaughter and during transport (stunning is required and no ice is allowed during transport)

New Zealand: Animal Welfare act in 1999¹² changed the definition of the NZ animal animal protection act (1960) to cover crabs, lobsters and crayfish.

⁸ API: http://api.worldanimalprotection.org/sites/default/files/api_2020_-_australia.pdf

⁹ The Federal Act on Animal Welfare (Tierschutzgesetz - TSchG), Federal Law Gazette I 2004/118

¹⁰ https://api.worldanimalprotection.org/sites/default/files/api_2020_-_austria_0.pdf

¹¹ <https://www.globalanimallaw.org/downloads/database/national/switzerland/TSchV-2008-EN-455.1-2011.pdf>

¹² <https://www.legislation.govt.nz/act/public/1999/0142/latest/DLM49664.html>

¹³ <https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571188/#:~:text=The%20intention%20of%20this%20Act,welfare%20and%20respect%20for%20animals.&text=The%20Act%20applies%20to%20conditions,squid%2C%20octopi%20and%20honey%20bees>

Norway: Legal protection for decapods by the Norwegian Animal Welfare Act (2010)¹³ including killing, confining and transport.

The UK government commissioned a report in 2021 to evaluate the current evidence on the welfare status of crustaceans and the potential for them to be sentient (Birch et al., 2021). Following the report DEFRA tabled an amendment to the Animal Welfare (Sentience) Bill was approved in April 2022 and now includes decapod crustaceans and cephalopod molluscs.

Other countries such as Italy have partial protection in some provinces/regions and since 2017 crustaceans cannot be kept on ice in restaurant kitchens (all regions) or boiled alive (in the province of Regno Emilia for example).

6 CONCLUDING REMARKS

Despite the wide knowledge on crustacean nutritional requirements, stress physiology and immunology as well as disease control, still little is known about some key parameters related to the five welfare dimensions, as they might be applied to penaeid shrimp, and some of these gaps have been identified in this review. The main challenges facing the aquaculture of penaeid shrimp such as *P. vannamei* today are still based on improving best management practices (BMPs) taking into consideration different regions/farming systems, the development of species-specific Codes of Good Aquaculture Practices (CAqP), the importance of staff training by continuous professional development courses (CPDs) and the improvement of the monitoring systems both for the aquatic environment and the behaviour, health and welfare of the farmed animals. A systematic integrated welfare assessment encompassing all the different aspects of their farming and life cycle up to slaughter should be implemented in all aquaculture systems to better understand the basic needs of these organisms, to reduce the stressors, which would minimise the effects of stress responses and improve productivity and quality of the final product. The development of the precision farming (PF), with real-time monitoring of the environmental parameters and the possibility of system modelling and machine learning is a step forward for aquaculture systems and the solution for many welfare/stress related issues. Direct and indirect species-specific

OWIs should be developed for all decapod crustaceans currently farmed, similar to the ones suggested in this review for *P. vannamei*. Positive welfare using environmental enrichment and better and less invasive husbandry procedures needs to be promoted at all life stages and husbandry practices, from broodstock to slaughter methods.

Understanding sentience in invertebrates is important from an animal and scientific perspective (Rowe, 2018). Moreover, as pointed out by Birch (Birch et al., 2021) evidence of sentience in invertebrates is crucial from a policy perspective. For instance, in the European Union decapod crustaceans were not included in the EU Directive 2010/63/EU on the protection of animals used for scientific purposes, nor other national directives such as the UK's Animal Welfare Act 2006. However, protection of decapod crustaceans has been argued for a number of years and therefore it is possible that the scope of current regulations will be expanded to decapod species. This has already happened in a number of cases such as in the New Zealand's Animal Welfare Act (1999), which includes crabs, lobsters and crayfish, while in Norway legislation includes all decapods as well as some insects (honeybees). Regarding the use of decapod species for scientific purposes, again while there are country- and even institution-specific differences, the requirement for ethical review has been firmly proposed (Rowe, 2018). More research related to crustacean welfare therefore needs to be developed to inform the industry. The new focus on welfare in crustaceans should be seen as an opportunity to boost the farming industry and address the needs of consumers.

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All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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