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Salinity Impacts on the Histology of the Muscle of Whiteleg Shrimp, *Litopenaeus vannamei*

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Abstract: The most significant species of shrimp in South American Aquaculture is the whiteleg shrimp, *Litopenaeus vannamei*, though in recent years it has been introduced to various Asian countries, who have taken over as its primary producers. Whiteleg shrimp, *L. vannamei*, were obtained from the different aquaculture farms under the different salinities (5, 20, 35 and 50 ppt). Muscle tissue of *L. vannamei* reared at 20 ppt revealed normal histoarchitecture. It is composed of elongated muscle fibers, each an individual muscle cell, held together by connective tissues. In the other salinities viz., 5ppt, 35ppt and 50 ppt the muscle histoarchitecture displayed severe alterations such as Highly Disintegrated Epidermis (HDE), Vacuolation (V), Gap Formation between the Muscle Bundles (GFMB), Sloughing of Epidermal Layer (SEL), Haemocyte Infiltration (HI), Broken Myofibrils (BMF), Fusion of Muscle Bundles (FMB), Congestion of Muscle Bundles (CMB), Rupture of Muscle Bundles (RMB) and Accumulation of Granular materials between the Muscle Fibers (AGMF).

Keywords: Muscle, Histoarchitecture, Salinity, Shrimp, *Litopenaeus vannamei*

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Introduction

One of the key environmental elements influencing marine shrimp culture is salinity, which is among the most significant environmental parameters in the distributions of living organisms (Chandra Mohan and Sreenivas, 1998). Aquatic organisms' physiological responses are altered, including their metabolism, growth, tolerance, life cycle, feeding, and connections between different species (Venkataramiah *et al*., 1974). Marine organisms that regulate the osmolality of body fluids encounter the dual problems of internal dilution at low salinities and concentration of body fluids at high salinities (Castille and Lawrence, 1981).

The yield caused a few epizootics of White Spot Diseases (WSD) due to the effects of climatic change on fluctuations in physicochemical parameters of the water ponds during shrimp culture, especially during the stocking phase and on the time of harvesting (Soltani *et al*., 2000; Kakoolaki, 2004). One of the most significant environmental elements that influences the prevalence of WSD is salinity (Liu *et al*., 2006; Kakoolaki *et al*., 2010). Few studies evaluated the effect of salinity on the occurrence of viral diseases of the shrimps (Annies and Rosamma, 2007; Kakoolaki *et al.,* 2010; Kumlu *et al.,* 2010; Yu *et al.,* 2010).

The most significant species of shrimp in South American aquaculture is the whiteleg shrimp, *L. vannamei*, though in recent years it has been introduced to various Asian countries, who have taken over as its primary producers (FAO, 2006). *L. vannamei* has gained popularity as a species for low salinity culture (Weihua *et al*., 2016). One of the main aspects that makes them a desirable species for aquaculture is their capacity to survive variations in salinity and temperature as well as their propensity to extend inland. Due to the high energy requirement for osmoregulation, the ideal salinity for *L. vannamei* growth is near 20 ppt, and large variations from this value have an adverse effect on growth and survival. Shrimp actively maintain a hyperosmotic internal environment at low salinities, along with the water flows required to maintain the creatures' homeostasis (Roast *et al*., 2001). The effect of salinity in histopathological changes in various tissues of red swamp cray fish, *Procambarus clarki* were reported (Yi xiao *et al*., 2022). In this study we have evaluated histological alterations in muscles of whiteleg shrimp *L. vannamei* procured from different aquaculture farms under the different salinities (5, 20, 35 and 50 ppt).

Materials and Methods

Sample preparation:

Whiteleg shrimp, *L. vannamei*, with the size of 60 shrimps kg^{-1}, were obtained from the different aquaculture farms under the different salinities (5, 20, 35 and 50 ppt). The shrimps were placed in ice with an ice: shrimp ratio of 2:1 (w/w) and transported to the research laboratory, within approximately 1 h and 4 h, respectively. Upon arrival to the research laboratory, the shrimps were washed with clean water and deheaded. The shells were then peeled off. The shrimps were deveined and the edible portions were ground to obtain uniformity. The samples were placed in polyethylene bags and kept in ice during the analyses.

Histological studies:

At the time of harvest in different salinities (5, 20 (used as a control), 35 and 50 ppt) shrimp samples from each salinity was picked out randomly. The collected animals were sacrificed and muscle tissue is excised out and make it in small pieces of 4-5 mm sizes and fixed in 10% buffered formalin for 24 h, dehydrated through a graded ethanol series and embedded in paraffin. Tissue sections (5 µm thick) were stained with haematoxylin-eosin for observation by the Nikon bright-field transmission microscope with Koehler illumination and an automatic exposure unit.

Results

Muscle is the tissue of motion and is widely distributed in various organs of the body. Muscle tissue of *L.vannamei* reared at 20 ppt revealed normal histoarchitecture. It is composed of elongated muscle fibers, each an individual muscle cell, held together by connective tissues. The photomicrograph of the muscle (Fig. 1A) depicted the presence of normal myotomes (MT) with equally spaced muscle bundles the fascicular arrangement of myofilaments (MF) with emarginated epimysium, binding to connective tissue and tendon at the extremities of the smooth muscles. The striated muscle fibres (SM) were tightly packed. The nuclei were arranged along the margins of the muscle bundles (Fig. 1 B, C).

In the other salinities viz., 5 ppt, 35 ppt and 50 ppt the muscle histoarchitecture displayed severe alterations. In 5 ppt the muscle histology revealed marked histological changes in the epidermis. In this salinity range, the muscle tissue showed highly disintegrated epidermis (DE) with marked

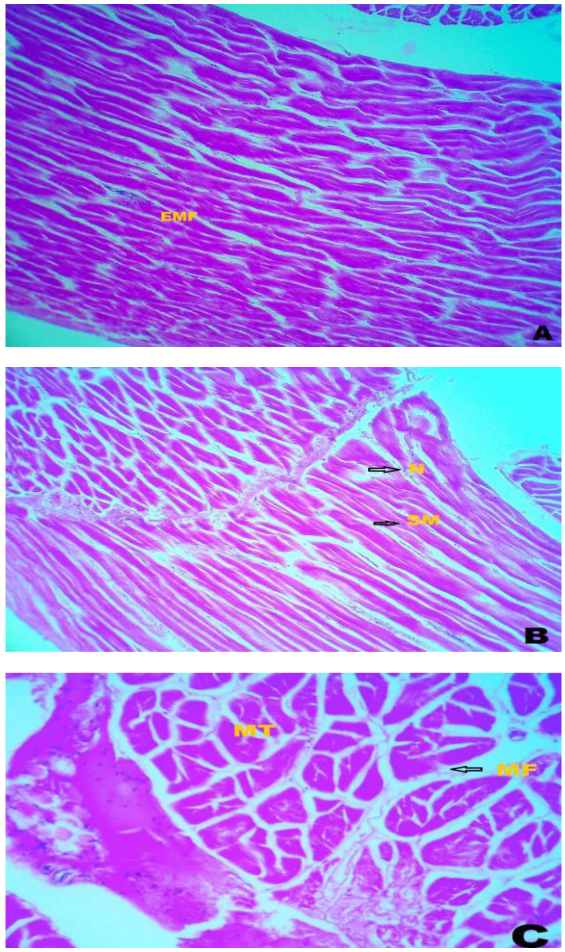

Fig. 1A, B, C: Muscle tissue in *L. vannamei* culture in 20 ppt salinity (Control). Heamatoxylin and Eosin (40X). EMF- Elongated muscle fibers, MT – Myotomes, MF – Myofilaments, SM – Striated Muscle, N- Nuclei.

thickening and separation of muscle bundle and pronounced intramuscular oedema with minor dystrophic changes (Fig. 2A, B, C). In 35 and 50 ppt, the muscle bundles were completely disrupted with discontinuity of striations and complete disappearance of nuclei. In some regions of muscle tissue at 35 ppt there was sloughing of epidermal layer (SEL) (Fig. 3A). Lesions (LN) and mild haemocyte infiltrations (HI) were the marked changes in 35 ppt followed by fusion of muscle bundles (FMB) (Fig. 3 B, C). In 50 ppt, the muscle tissue expressed significant changes like broken myofibrils (BMF), coagulative necrosis (CNE), congestion of muscle bundles (CMB) followed by rupture of muscle bundles (RMB). Severe haemocyte infiltration (HI) and accumulation of granular materials in between the muscle fibers (GMF) were also noted (Fig. 4A, B, C).

Discussion

Salinity is an important abiotic factor affecting the metabolism, growth, survival and osmotic capacity and possibly, the immune system of penaeid shrimp (Alvarez *et al.,* 2004; Wang and Chen, 2005). Although juvenile are competent hyper-hypo osmoregulators (Castille and Lawrence, 1981), salinity changes may cause stress to the animal, especially when associated with inadequate handling procedures and poor water quality (Brock and Main, 1994) and thus facilitate infections. Molecular and biochemical indicators are potentially suitable for evaluating stress, because stress classically leads to a rapid onset and a cascade of molecular and physiological response (Sanchez *et al.,* 2001). Histology acts as an integrated parameter, providing a more complete evaluation of the organism's health,

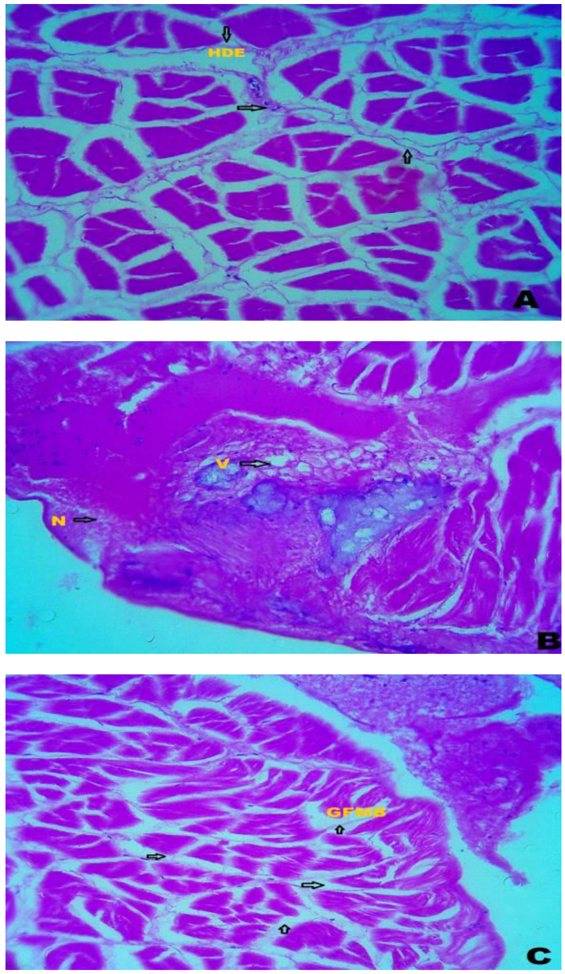

Fig. 2A, B, C: Histological changes of muscle tissue in *L. vannamei* cultured in 5 ppt salinity. Heamatoxylin and Eosin (40X). HDE- Highly disintegrated epidermis, V- Vacuolation, GFMB- gap formation between the muscle bundles, NE- Necrosis.

effectively monitoring the effects of exposure to environmental fluctuations (Van Der Oost *et al*., 2003).

In the present study, the histopathology of muscle shows progressive damage in the structure of muscle in different salinities. In 20 ppt salinity range the animal expressed normal muscle histoarchitecture whereas in 5, 35 and 50 ppt severe alterations were noticed. In 5ppt, the epidermal layer of muscle tissue was highly altered. This may be due to osmoregulation in low saline conditions. Carbajal- Sanchez *et al*. (2008) believed that lower salinity shows a severe trend of mortality in *L. vannamei* in comparison to the normal and greater levels normal condition. Low salinity may make shrimp susceptible to bacterial diseases (Jiravanichpaisal *et al.,* 2004) and high salinity can affect negatively on the growth of *L. vannamei* in challenging with infectious hypodermal and haematopoietic necrosis virus (IHHNV) (Bray *et al.,* 1994). Lower salinity or hardness could lead shrimp to susceptibility to White spot virus (Tendencia *et al.,* 2010).

According to Lightner *et al*. (1983), muscle necrosis appears after periods of severe stress (*e.g.* overcrowding, low dissolved oxygen levels, abrupt temperature or salinity changes, rough handling, etc.). The present study clearly depicted the muscular alterations in higher salinities. The significant changes are broken myofibrils, coagulative necrosis, and congestion of muscle bundles followed by rupture of muscle bundles. Severe haemocyte infiltration and accumulation of granular materials in between the muscle fibers

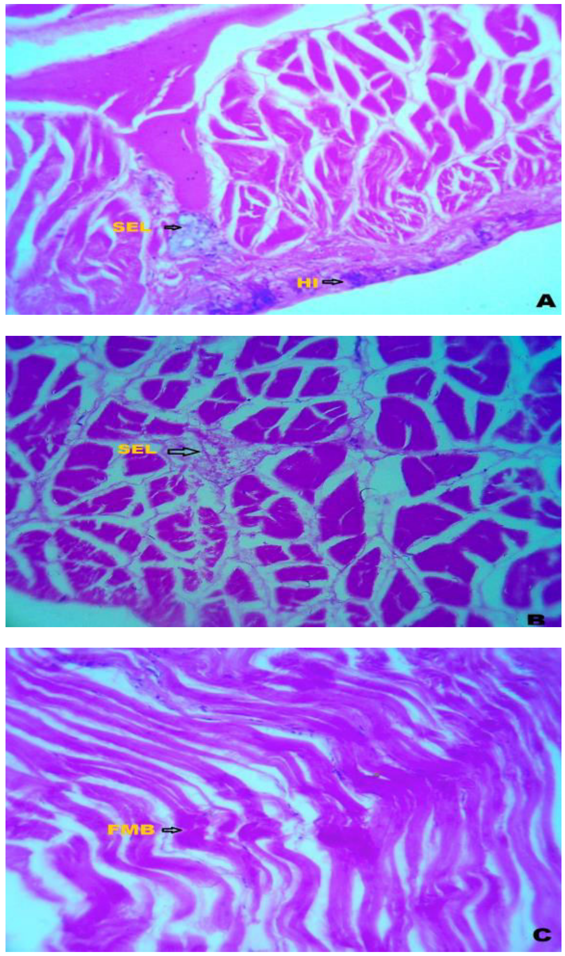

Fig. 3A, B, C: Histological changes of muscle tissue in *L. vannamei* cultured in 35 ppt salinity. Heamatoxylin and Eosin (40X). SEL- Sloughing of Epidermal layer, HI – Haemocyte infiltration, FMD – Fusion of Muscle bundle.

were also noted. The causes of non-infectious muscle necrosis was physiological stress in response to environmental factors, such as sudden changes in temperature, salinity or dissolved oxygen.

In past, some Ecuadorian shrimp farmers have reported several cases of muscle necrosis in *P. vannamei* grow out cultures, associated to low mortalities at harvest (Melena *et al*., 2012). This abnormal condition is characterized by focal to extensive necrotic areas in striated tail muscle tissues, displaying a white, opaque appearance. Furthermore, histological analysis from suspected samples with macroscopic lesions revealed a loss of sarcomeric structure accompanied by coagulative muscle necrosis along with hemolytic infiltration.

According to Diwan *et al.* (1989) adult *P. monodon* could osmoregulate well between salinities 3 and 45 ppt for 24 and 48 h duration with isosmotic points around l8.5% and 23.5%, respectively and a duration of 48 h is essential for the prawns to adjust to the new medium. According to Vijayan and Diwan (1995), the shrimps exposed to very low and high (5 and 50 ppt) salinity levels developed severe muscle necrosis and these reports are in accordance with our present study. Lakshmi *et al*. (1978) also observed muscle necrosis in *P. aztecus* exposed to sub-optimal and supra-optimal salinity ranges. They described the development of muscle necrosis as an indication of stress on the animal. In the present study the low feed intake in animals exposed to low and high saline conditions could be due to physiological stress and adaptation.

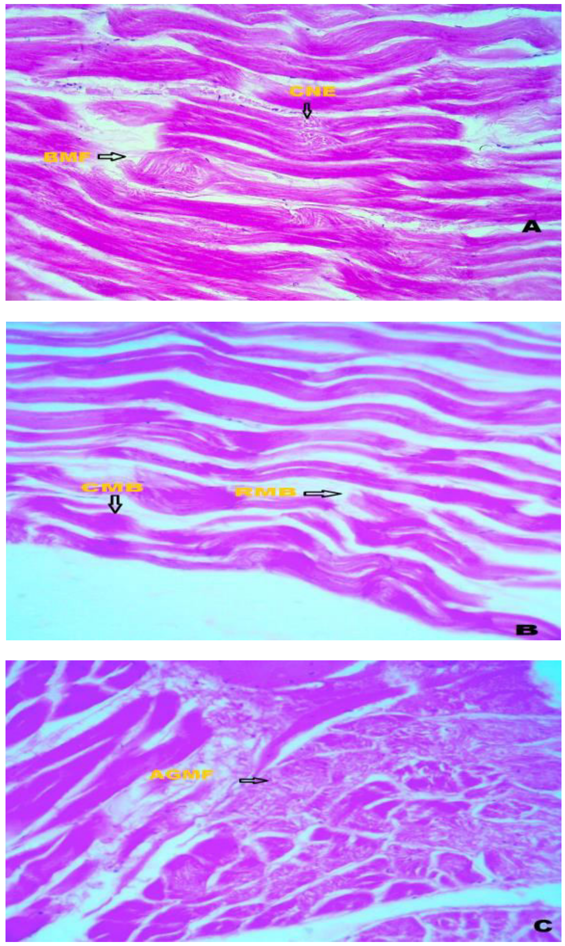

Fig. 4A, B, C: Histological changes of muscle tissue in *L. vannamei* cultured in 50 ppt salinity. Heamatoxylin and Eosin (40X). BMF: Broken myofibrils, CNE- Coagulative necrosis, CMB- Congestion of muscle bundle, RMB- Rupture of muscle bundle, HI- Haemocyte infiltration, AGMF- Accumulation of granular materials between the muscle fibers.

It is now a well established fact that most euryhaline prawn species are hyperosmo-regulators at low salinities and actively pumping ions from seawater into haemolyph (Mantel and Farmer, 1983). According to Pallavi *et al.* (2012) in comparison with hepatopancreas and gills, muscle is metabolically low active in relation to different salinities. In some species changes in salinity affected temperature tolerances. Studies in *Drosophila melanogaster* have shown that in hypertonic solutions, water from tissues drains into the hemolymph (Folk *et al.,* 2003). Mat and Potts (1985) have reported that at very high salinities, muscle extracellular space increases and water content of cells decreases. This reduction in volume of muscle fibers and increased space between fibers may lead to disorganization of the muscular tissue ultrastructure.

Lester and Pante (1992) pointed out that salinities below 5 and above 35 ppt usually had a negative impact on prawn growth. These observations showed that although these shrimps can survive in a wide range of saline fluctuations, best growth takes place only in optimal salinity. Outside the optimum range, the growth increment of the shrimp attained low and this was well documented by the present histological and biochemical observations of the muscle tissue of *L. vannamei*. In the extreme range of salinities, these animals must spend considerable energy for osmoregulation at the expense of other processes like growth. The feeding pattern observed in the present study is worth mentioning in this aspect that the biochemical and histological aspects of muscle varies considerably due to the effect of salinity. In such cases, induction of the 'stress cascade' can allow latent infections to manifest as disease via generalized host immunosuppression induced by the prevailing environmental conditions (Johnson, 1983). Various stresses have been associated with enhanced free radical generation causing oxidative damage (Siers, 1985). These changes finally affect the aquaculture potential.

Conclusion

The present study clearly suggested the pathological conditions of muscle in extreme saline ranges and these are the osmoregulatory adaptations due to severe stress. Stress has been implicated in the development of disease in aquatic animals, both in aquaculture and in natural conditions.

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