EFFECTS OF CO_2 DRIVEN ACIDIFICATION ON BIOLOGICAL AND PHYSIOLOGICAL RESPONSES OF THE SHRIMP *LITOPENAEUS VANNAMEI*

(01.02.2018 to 31.03.2020)

No. F.30-354/2017 (BSR); Date: 21.12.2017

Submitted to

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Submitted by

Dr. T. MURALISANKAR

ASSISTANT PROFESSOR

PRINCIPAL INVESTIGATOR

DEPARTMENT OF ZOOLOGY

BHARATHIAR UNIVERSITY

COIMBATORE – 641046

TAMIL NADU, INDIA.

EXECUTIVE SUMMARY

The marine environment provides enormous resources to humans including nutritional security and employment improving the socio-economic status of humans. However, marine environments are highly vulnerable to various pollutants including heavy metals, hydrocarbons, microplastics, greenhouse gases, etc. (Chitrakar et al., 2019). Among divergent pollutants, CO_2 plays a significant role in marine pollution in terms of changes in seawater chemistry and warming which affect the biology and physiology of marine organisms, followed by undesirable alterations in food webs (Brown et al., 2010; Hoegh-Guldberg, 2011). One third of atmospheric CO_2 has been absorbed by the oceans which leads to an increase in seawater temperature and acidification (Frolicher et al., 2015; NOAA, 2020). These small changes in pH of seawater are highly vulnerable to marine calcifiers species, such as mollusks, crustaceans, reef-forming corals, etc., which form skeletons or shells out of calcium carbonate (Hofmann et al., 2010). Crustaceans play a significant role in the world aquaculture trade due to their higher market value. India has contributed 35.99 lakh tonnes of crustaceans in 2016. On the other hand, crustaceans are very sensitive to biotic and abiotic factors including ocean carbon chemistry also known as ocean acidification (Whiteley, 2011). The alterations in carbon chemistry of the surrounding environment can produce fluctuations in regulations of acid-base balance, calcification, molting, and immunity which leads to poor survival, growth, and easily susceptible to various biotic and abiotic factors (Small et al., 2010; Whiteley et al., 2011; Roleda et al., 2012; Taylor et al., 2015). However, the studies on the effect of CO_2 driven ocean acidification on the biology and physiology of marine shrimps are limited. Hence, the present study was focused to investigate the possible detrimental effects of CO_2 driven seawater acidification on the Pacific white-leg shrimp *Litopenaeus vannamei* to understand the possible impacts of ocean acidification on the marine shrimp species.

The shrimp *L. vannamei* post-larvae (PL15) was obtained from Aqua Nova Hatcheries Private Limited, Chennai. The seawater pH manipulation system was designed according to Riebesell et al. (2010) with some modifications with six different pH, such as pH 8.20 (control), pH 7.8 (IPCC-predicted ocean pH by 2100), 7.6, 7.4, 7.2, and 7.0 using pure (99.9 %) CO_2. The changes in pH were monitored by individual pH meters which pre-connected with respective aquaria. Every four hours the pH of each aquarium was monitored and maintained manually. During the exposure experiment, water parameters (salinity, temperature, ammonia, dissolved oxygen, and total alkalinity) were analyzed as per the standard methods of APHA (2005) and the partial pressure of CO_2, bicarbonate ions (HCO^-_3), carbonate ions (CO_3^{2-}), calcium carbonate saturation state for aragonite and calcite (ΩCa and ΩAr), and total CO_2 were calculated using CO_2 calculator (Robbins et al., 2010). For each acidification treatment, the stocking density was 30 post-larvae (stage PL-20) of *L. vannamei* were assigned for each aquarium. The shrimps were fed (10 % of body weight) with commercial pellet feed (which was used during acclimatization) twice per day at 06:00 h and 18:00 h and the photoperiod was maintained as 12h: 12h light and dark. The molts, unfed feeds, and wastes of shrimps grown were collected daily in each aquarium during the seven weeks experiment while renewing 40% of seawater. At the end of the seven weeks experiment, the survival, growth, feed intake, muscle biochemical constitutions, composition of amino acids and fatty acids, minerals, chitin, haemocyte populations, antioxidants, metabolic enzymes, gut microbial population, and pathogen susceptibility against *Aeromonas hydrophila* were studied.

The present study revealed that survival, growth, feed index, biochemical constitutes (protein, amino acids, carbohydrate, and lipid), chitin and minerals (Na, K, and Ca), haemocytes populations, muscle amino acids, and saturated fatty acids of shrimps were found to be significantly decreased in CO_2 driven acidified seawater which indicates the negative impacts of acidified seawater on these parameters in *L. vannamei*. Moreover, the increased level of monounsaturated fatty acids and polyunsaturated fatty acids in the shrimps under acidified environment indicates that the shrimp *L. vannamei* can produce these essential fatty acids in extreme acidified environments to overcome the stress. Further, the level of antioxidants (superoxide dismutase, catalase, and lipid peroxidation) and activity of metabolic enzymes (glutamic oxaloacetic transaminase and glutamic pyruvate transaminase) were found to be significantly elevated in the muscle of shrimps exposed to acidified seawater suggest that the *L. vannamei* under free radical stress and metabolic stress. The reduction of probiotic and increase in pathogenic bacterial species in the shrimp exposed to acidified seawater indicate that shrimps had susceptible to pathogens by decreasing probiotic populations with evidence of poor immune response and increased mortality under acidified seawater against *A. hydrophila*. Hence, the present study indicated that the elevated level of seawater acidification can produce harmful effects on *L. vannamei* which lead to the potential threat to the culture of shrimp species.

Publication from the project:

Muralisankar, T., Kalaivani, P., Thangal, S. H., & Santhanam, P. (2020). Growth, biochemical, antioxidants, metabolic enzymes and hemocytes population of the shrimp *Litopenaeus vannamei* exposed to acidified seawater. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 239: 108843. doi:10.1016/j.cbpc.2020.108843 (**IF: 3.22**).

*****