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Research Article

# Effect of Osmotic Shocks on Sodium Regulation and Na-K-ATPase Activity of Pacific White Shrimp *(Litopenaeus vannamei* Boone, 1931)

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**Abstract:** Effects of Millieu exterieur osmotic shocks on sodium ($Na^+$)regulation and Na-K-ATPase activity of *Litopenaeus vannamei* (Boone, 1931) were investigated. Active uptake of sodium and Na-K-ATPase enzyme activity followed similar pattern; they decreased to minimum levels and became almost constant after the shrimp was reared in an isosmotic medium (875.40 mOsm $L^{-1}$ H2O ∞ 30 g $L^{-1}$). The research was conducted by experimental laboratory method using completely randomized design with four treatments and three replicates. This study showed that Na-K-ATPase activity was directly affected the active uptake of sodium. Cultivated shrimps which were reared in an isosmotic medium regulated their haemolymph osmotic concentration passively; thus, decreased their osmotic works and increased their potential energy needed for moulting process and growth. Active transport of $Na^+$ and the activity of Na-K-ATPase in the body fluids of L. vannamei shrimp followed similar pattern, at which they decreased to minimum levels and became almost constant after the shrimps were reared in and isosmotic medium (± 30 g $L^{-1}$ ∞ 875.40 mOsm $L^{-1}$ $H_2O$).

**Keywords:** *Litopenaeus vannamei* (Bonne, 1931), Na-K-ATPase activity, osmoregulation, osmotic work, sodium regulation

## 1. INTRODUCTION

Changes in the water salinity have a significant effect on aquatic animals, especially catadromic shrimps, with respect to their migratory behaviour. Life cycle of shrimps is subject to the change of waters salinity. In the mature, eggs and early larval stages, shrimp postlarvae and juvenile stages, they need lower salinity so that they move to the estuary or inhabit waters having high salinity (polyhaline or hyperosmotic medium). The opposite event occurs in littoral waters. The migration from medium high salinity to the lower one and vice versa will bring about serious problems with regard to the osmotic concentration of shrimp, and therefore this evidence need to be taken into account in the shrimps culture operation.

Shrimps cultured in an artificial environment (brackishwater ponds) are subject to some problems resulting from different osmotic concentration between shrimps and their surrounding water (millieu exteriour). In order to overcome this problem, shrimps have to maintain the osmotic balance by keeping up the stability of internal body fluids which is done in two ways. First, maintaining the osmolality of extracellular fluids without adjusting with the environment. Second, maintaining the osmolality of intracellular fluids so that it is similar to the environment. These two ways involve regulation of $H_2O$ volume in the extracellular fluids and controlling ion exchange, particularly $Na^+$ and $Cl^-$ , between intra and extracellular fluids [1; 12]. The uptake of ions is usually done by means of active transport [2], which

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involves oxidative metabolism and the activity of Na-K-ATPase. The active transport of ion necessitates energy (ATP), because the movement of ions (osmoeffectors) tends to run relevant to the electrochemical gradient. The uptake of $Na^+$ is always followed by the removal of $NH^{4+}$ and $H^+$ [3].

From biochemical point of view, Na-K-ATPase plays an important role in the active transport process of $Na^+$ and $Cl^-$ [4]. The enzyme acts in the hydrolysis of ATP and maintains the balance between concentration of $Na^+$ within extracellular fluids and concentrations of $K^+$ in the intracellular fluids. Anggoro and Subandiyono [5] found that active transport of $Na^+$ and Na-K-ATPase movement in Metapenaeus elegans (De Man, 1907) is linear in various level of salinity (hypo until hypertonic). This indicates that the enzyme is involved actively in the transportation of ions. Consequently, the amount of energy spent for osmo-ionic regulation process is very dependent upon the extent of the enzyme's activity.

This experiment aimed at examining the active transport mechanism of $Na^+$ within the intra and extracellular fluids of L. vannamei. Shrimp body was grown in hyper, hypo and isosmotic medium. In addition, this research determined the effect of Na-K-ATPase enzyme in hyper, hypo and isosmotic condition on the active transport process of $Na^+$ and $K^+$ ions.

## 2. MATERIALS AND METHODS

Pacific white shrimp [*L.* vannamei (Bonne, 1931)] used in this experiment were collected from the Jepara Brackishwater Shrimp Hatchery Center, Central Java, Indonesia. The density of tested shrimps in the tank was 10 juvenile per 100 L. Juvenile shrimps with initial weight of (914.66 ± 0.26) g were used. Water medium was prepared by mixing seawater and freshwater to make brackishwater at desired salinities. To obtain the desired level of salinity, dilution was done by adding pre-filtered deionized water into the testing chambers. The research was carried out at the Coastal Laboratory, Diponegoro University, Jepara from July to October, 2015.

Tested shrimps were fed with commercial feed obtained from a local dealer with the following composition: protein (40 % to 41 %), lipid (5 %), fiber (3 %), and water (1 %). Feed was given at 8 % of the average weight of tested animals in the afternoon (5 pm), night (11 pm), morning (4 am), and noon (12 am), Unused feeds were siphoned every afternoon 2 h prior to feeding time.

The research was conducted by experimental laboratory method using completely randomized design with four treatments and three replicates. The main treatment was medium with different salinity levels that consisted of: S1 treatment with salinity of 20 g $L^{-1}$ (578.73 mOsm $L^{-1}$ $H_2O$), representing the hypo-osmotic medium for fingerling vanamaei shrimp; S2 treatment with salinity of 25 g $L^{-1}$ (728.22 mOsm $L^{-1}$ $H_2O$), representing the hypo-osmotic medium; S3 treatment with salinity of 30 g $L^{-1}$ (875.46 mOsm $L^{-1}$ $H_2O$), representing the intermolt isosmotic medium; and S4 treatment with salinity of 35 g $L^{-1}$ (1 021.36 mOsm $L^{-1}$ $H_2O$), representing the hyperosmotic medium. The ambient temperature was maintained at ideal level suitable for the growth of fingerling tested shrimp, which was 30 °C to 31 °C, with Cole-Parmer Cooling-Heating Circulating Baths (GY-12122-12, Cole-Parmer, Illinois, USA).

To maintain temperature stability and reduce evaporation, each chamber was covered with dark plastics. The variables measured were: (i) haemolymph osmolarity and osmotic work level, (ii) the activity of Na-K-ATPase enzyme, (iii) concentration of inorganic osmo-effector (ions $Na^+$, $Cl^-$, $Ca^{2+}$, $Mg^{2+}$, $K^+$) in the water and within body fluid of shrimp (haemolymph). Variable (i) and (iii) were measured following the method of Anggoro and Subandiyono [5]

Shrimp haemolymph was taken as much as 0.10 mL from pericardium cavity with 23G syringe and osmolality was measured with Micro-Osmometer (Automatic Type 13/13DR-Autocal, Herman Roebling MESSTECHNIK, Berlin, Germany). The inorganic osmoeffectors ($Ca^{2+}$, $Mg^{2+}$, $Na^+$ and K+) were measured with flame photometers (Type GY-02655-15, Cole-Parmer, Illinois, USA); and Cl– was measured with Chloride Analyzer (Type GY-02656-20, Cole-Parmer, Illinois, USA). The Na-K-ATPase enzyme activity was also measured

**Table 1.** Osmo-ionic concentration medium and haemolymph, sodium and Na-K-ATPase activity of the Pacific white shrimp fingerling (*L. vannamei*).

| Parameters | Salinity Level (g $L^{-1}$) | | | |
|---|---|---|---|---|
| | 20 | 25 | 30 | 35 |
| Water osmolality (mOsm $L^{-1}$ $H_2O$ ) | 578.72 ± 0.00 | 726.20 ± 0.00 | 875.40 ± 0.00 | 875.40 ± 0.00 |
| B.F. osmolality (mOsm $L^{-1}$ $H_2O$ ) | 794.22 ± 2.33 | 840.53 ± 2.28 | 846,78 ± 2.07 | 848.11 ± 2.51 |
| Osmotic works (mOsm $L^{-1}$ $H_2O$ ) | 215.50 ± 1.83$^{bcd}$ | 1 14.33 ± 1.61$^{acd}$ | 28.62 ± 1.28$^{abd}$ | 178.29 ± 1.18$^{bc}$ |
| Water contents (%) | 84.20 ± 0.11 | 82.05 ± 0.11 | 80.65 ± 0.09 | 78.55 ± 0.09 |
| Influx $Na^+$ (µmol $L^{-1}$ $H_2O$ $h^{-1}$) | 2 396.8 ± 1.68$^{bcd}$ | 1 365.8 ± 1.72$^{acd}$ | 1 118.9 ± .48$^{abd}$ | 1 120.8 ± 1.73$^{abc}$ |
| Na-K-ATPase(µmol $Pig^{-1}$ $h^{-1}$) | 2 400 ± 9.44$^{bcd}$ | 1 540 ± 7.93$^{acd}$ | 1 120 ± 8.02$^{abd}$ | 2 580 ± 9.89$^{abc}$ |

following the method of Duncan et al. [2].

The enzyme activity assay was performed using 100 mmol $L^{-1}$ NaCl, 5 mmol $L^{-1}$ MgCl2, 13 mmol $L^{-1}$ KCl, 3 mmol $L^{-1}$ ATP and 30 mmol $L^{-1}$ imidazole and pH 7.4 at 25 °C for 30 min. Quabain (2 mmol $L^{-1}$) was added in duplicated to determine quabain sensitive ATPase activity. The inorganic phosphate in the haemolymph supernatant was measured spectrophotometrically at 620 µm (Type GY-83057-06, Cole-Parmer, Illinois, USA), and the Na-K-ATPase activity was expressed as µmol Pi $g^{-1}$ $h^{-1}$. The water content was determined based on the difference between wet weight and dry weight (after tested shrimp being dried in the oven for 24 h in temperature of 12 °C). Efflux determination was conducted after shrimp being soaked in the treated water for 48 h after 6 h of preparation. Efflux value (Ke) was calculated using empirical formula (1) [6], i.e.:

$$Ke = 1/t \cdot \ln(Ao/At) \quad (1)$$

Where, Ao = activity of $Na^{22}$ in shrimp body at time 0; At = activity of $Na^{22}$ in shrimp body at time t; t = time of contact with medium (48 h).

Influx value (Ki) was calculated using a formula (2) [6]:

$$Ki = 1/t \cdot \ln (C/C\text{-}Ct) \quad (2)$$

Where, C = activity of $Na^{22}$ in body fluids in the equilibrium time (24 h); Ct = activity of $Na^{22}$ in body fluids after t time.

The activity of Na-K-ATPase was analyzed using Duncan et al. [2] procedure. The rate of $Na^+$ and Na-K-ATPase active transport and its response were analyzed using variance analysis and response curve model [7].

## 3. RESULTS AND DISCUSSION

Osmotic concentration of medium is closely related to the level of osmotic work performed by shrimp. The level is equivalent to the difference of osmotic concentration between extracellular fluids and that at body fluids (haemolymph) of shrimp [8, 5]. The osmotic and ionic concentration of medium was higher at the higher salinity (Table 1). Particular ions which take a major role in determining the osmotic concentration were: $Cl^-$ , $Na^+$, $Mg^{2+}$, $Ca^{2+}$ and $K^+$.

The osmotic concentration of the medium had a significant effect on the active transport of $Na^+$ ($P < 0.05$). Total flux volume of $Na^+$ decreased with the increase salinity of the medium (Fig. 1) and it reached a minimum value in the isosmotic medium (30 g $L^{-1}$). The value decreased sharply in hyper (35 g $L^{-1}$) and hypo-osmotic medium (25 g $L^{-1}$ and 20 g $L^{-1}$). This was mainly due to the work of hyperosmotic regulation which was sufficiently strong and resulted in the increase of electro chemical gradient [9, 10, 2]. In this case, the differences of $Na^+$ content between haemolymph

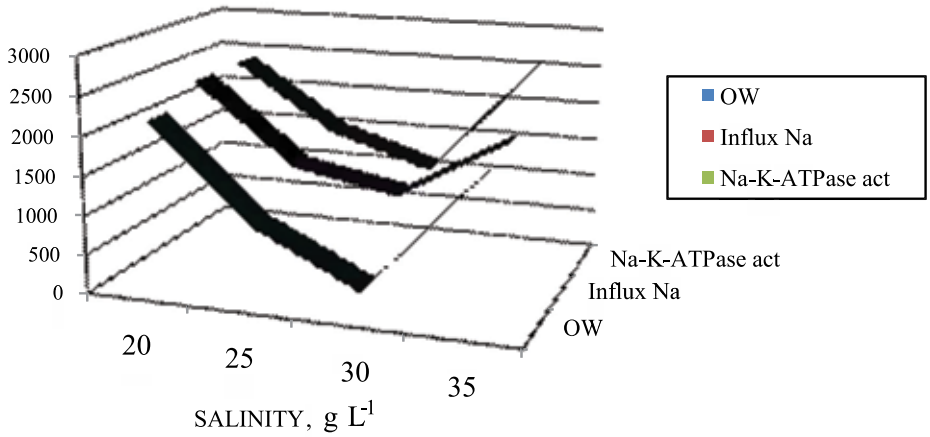


**Fig. 1.** Influx sodium ($Na^+$), Na-K-ATPase activity, and osmotic works (OW) in various levels of salinity (20 g $L^{-1}$, 25 g $L^{-1}$, 30 g $L^{-1}$, 35 g $L^{-1}$).
Notes: Sodium Influx ($Na^{22}$): µmol / L $H_2O$/h; Na-K-ATPase activity = µmol Pi $g^{-1}$ $h^{-1}$; OW = different osmolality medium and osmolality shrimp haemolymph (mOsm $L^{-1}$ $H_2O$).

and intracellular fluids increased the movement of active transport process of $Na^+$. The difference between influx and efflux values indicates the occurrence of active transport process of $Na^+$ [2].

Fig. 1 shows that the active transport of $Na^+$ tended to get smaller value in the almost isosmotic medium. This result supported the conclusion made by Anggoro and Subandiyono [5] that active transport of $Na^+$ and osmotic work in fine shrimp (*Metapenaeus elegans*) tended to increase in the hyper and hypo-osmotic medium.

The osmotic concentration of medium also had significant effect on the activity of Na-K-ATPase ($P < 0.01$). Fig. 1 shows that activity of the enzyme slowed down when approaching intermolt-isosmotic medium (30 g $L^{-1}$). The result of previous experiment by Duncan et al. [2] and Anggoro and Subandiyono [5] also suggested that activity of Na-K-ATPase in hyper and hypo-osmotic media was stronger than that in isosmotic medium. From the above discussion it can be stated that active transport of $Na^+$ has almost similar pattern in quadratic polynom response ($R^2 = 0.85$) with the activity of Na-K-ATPase enzyme. This fact indicates that the enzyme is directly involved in pumping ion in the process of $Na^+$ active transport. The experimental results conducted by Saoud and Davis [11] and Anggoro and Muryati [8] indicated that the utilization of food and shrimp growth were more efficient in a hypotonic medium which is close to isosmotic range. This is closely related to the osmotic work which may weaker when shrimps were cultured in an almost isosmotic medium range. In this condition, the energy spent for osmotic work can be saved, thus, it can be used for shrimp moulting and growth. When the osmotic work is weak, active transport of $Na^+$ and the activity of Na-K-ATPase enzyme will follow the similar pattern.

## 4. CONCLUSIONS

Active transport of $Na^+$ and the activity of Na-K-ATPase in the body fluids of vanamei shrimp followed similar patterns, decreased to minimum levels and became almost constant after the shrimp was reared in isosmotic medium (± 30 g $L^{-1}$ ∞ 875.40 mOsm $L^{-1}$ $H_2O$). The activity of Na-K-ATPase played important role in regulation of $Na^+$ in the intra and extracellular fluids of L. vannamei shrimp. In the condition of low osmotic work (approaching isosmotic medium), the activity of Na-K-ATPase and active transport of $Na^+$ decreased, however this condition increased the portion of energy used for moulting and growth.

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