Osmoregulation pattern and condition factor of Indian white shrimp (*Penaeus indicus*) in the mangrove eco-edutourism area of Tapak, Semarang

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Abstract. The mangrove eco-edutourism area of Tapak, Indonesia, has a high mangrove diversity and associated biota. One species of this biota is the Indian white shrimp (*Penaeus indicus* H. Milne Edward, 1837). *P. indicus* is a species that has not been cultivated widely in Semarang Coastal Area, Indonesia. Therefore, a domestication attempt is required. The study of osmoregulation pattern of Indian white shrimp (*P. indicus*) becomes very important in the domestication process, because the salinity of water facilitates the survival and growth of shrimp. This research aimed to recognize the osmoregulation pattern, condition factor and the relationship between them. Sampling was carried out by purposive sampling. The research method used was case study and correlation analysis for the relations between the osmotic work level and the condition factor. The results showed that a variation of the osmoregulation pattern between the 2 study stations exists. Indian white shrimp in station 1 had a hyperosmotic osmoregulation pattern, while in station 2 it was hypoosmotic regulation. The average condition factor of Indian white shrimp in station 1 was 1.6, and in station 2 it was 1.85. The osmotic work level was negatively correlated with the condition factor, a higher osmotic work level incurring a lower condition factor.

Key Words: electrolyte, growth pattern, Na^+/K^+- ATPase, osmotic work level.

Introduction. Semarang City administratively has 4 coastal districts: Tugu (2985.99 ha), West Semarang (2247.97 ha), North Semarang (1168.94 ha) and Genuk (2708.38 ha). It also has 14 coastal sub-districts. The development of Semarang City leading to a metropolis is indicated by the high increase of the number and population density that lives in the village/sub-district area in the coastal Semarang City (Ambariyanto & Sugianto 2012). Semarang City has coastline of 13.6 km and 62.5% of its region is coastal or lowland area.

The Semarang City area has wide mangrove forests. The mangrove forest in the coastal area of Semarang has a vital role in the ecology and economic growth of Semarang City (Hillary et al 2019). Mangroves have an ecologically important role, as spawning, nursery and feeding grounds. The economic additional value can be obtained from the mangrove ecotourism sector, among others (Sachin et al 2016). A well-known form of mangrove ecotourism in Semarang City is mangrove eco-edutourism in Tapak, Tugurejo Sub-district, Tugu District, Semarang City. Eco-edutourism ctivities in the region include recreational and educational activities, while trying to preserve the coastal mangrove ecosystem.

According to Handayani et al (2016), in the Tapak mangrove ecosystem, there were some biota species from Grabsidae, Sesarmidae, Varunidae, Portunidae, Ocypodidae, Squillidae, and Penaidae families. One species of shrimp from the Penaidae family found in the mangrove area of Tapak is the Indian white shrimp (*Penaeus indicus* H. Milne Edward, 1837). Juvenile Indian white shrimp is abundant in mangrove and coastal waters with muddy or sandy-muddy substrate (Vance & Rothlisberg 2020). *P.*

indicus is a fishery commodity with the potential to be cultivated, and an increasing production from fisheries.

The Ministry of Marine Affairs and Fisheries of Republic Indonesia targets the increase of national shrimp production from 1.05 million tons in 2020 to 1.2 million tons in 2021. One shrimp species that will become a target of cultivation and will be mass-produced is *P. indicus*. Currently, the Ministry of Marine Affairs and Fisheries is developing a domestication process of *P. indicus,* given that it is a wild shrimp not widely cultivated. For successful domestication, adjustments in the cultivation media are required. The adjustment of pond water parameters and feed quality are key factors of domestication. Incompatible salinity will cause the shrimp to use more energy in the osmoregulation process. The high osmoregulation energy consumption will decrease the energy allocated for growth (Rusdi & Jompa 2020). Therefore, research regarding the osmoregulation pattern, condition factor and the relationship of both is required. This research aims to determine the osmoregulation pattern, growth pattern, condition factor, and the relationship between osmotic work level and condition factor of *P. indicus* in the mangrove eco-edutourism area of Tapak, Semarang.

Material and Method

Description of the study site. This research was conducted in the water area of the mangrove eco-edutourism Tapak, Tugurejo Sub-district, Tugu District, Semarang City, Indonesia (Figure 1). In 2015 the mangrove area of Tapak eco-edutourism was 19.27 ha, 6.27 ha were with rare density mangroves, 1.22 ha with medium density mangroves and 11.78 ha of high density mangroves (Perdana et al 2016). Mangrove ecosystem degradation increases every year, so in 2020 the community of Tapak Village planted more than 50000 mangrove plants through collaboration programs with the government (Septiarani & Handayani 2020). The mangrove area of Tapak was dominated by *Rhizopora mucronata* and *Avicennia marina*. According to Putro et al (2020) the substrate was dominated by silt, with 4.3% gravel, 25.16% coarse sand, 12.82% fine sand, 45.72% silt, and 12% clay. The pond area in Tapak reached 50 ha, mostly being used for the aquaculture of milkfish (*Chanos chanos*) or polyculture with various species of shrimp (Irsadi et al 2017).

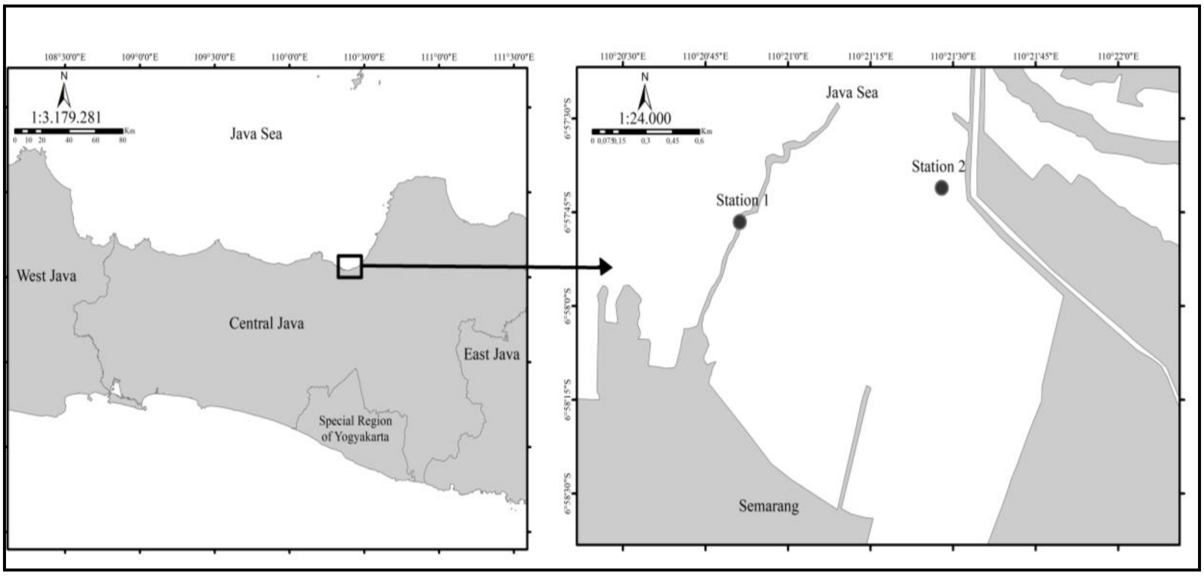

Figure 1. Sampling location.

Sampling and sample handling. Sampling of *P. indicus* and water was conducted in 2 stations. Station 1 was in the water of the mangrove area, which was a transportation route for fishing boats and recreational boats. Station 2 was in the area around a fish aggregating device and directly adjacent to Ahmad Yani Semarang Airport and the sea of

Tugu District. In each station, 30 *P. indicus* of various sizes were collected. *P. indicus* were placed in a zipper bag with water from the sampling sites and in a cool box. The water quality parameters measured *in situ* were temperature, pH, dissolved oxygen (DO) and salinity. Temperature and pH were measured with a water quality tester EZ-9908, DO was measured using a DO meter jpb-70a and salinity was measured using a hand refractometer S/Mill-E Atago.

Identification and observation were conducted in Fisheries Resource Management Laboratory, Aquatic Resources Management Study Program, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Indonesia. The media osmolarity and haemolymph of *P. indicus* were determined using an automatic Micro-Osmometer Roebling. The measurement of electrolyte content was conducted using an electrolyte counter osmometer. The total length was determined using a caliper (0.01 mm precision) and the body weight was determined by digital scales (0.01 g accuracy).

Data analysis. The Osmotic Work Level (OWL) was determined through the measurement of media osmolarity and haemolymph osmolarity of *P. indicus*. The computation of OWL used the formula previously used by Anggoro et al (2018a):

$$OWL = [P\ Osm\ haemolymph - P\ Osm\ media]$$

Where: P Osm haemolymph is the body liquid osmotic pressure and P Osm media is the osmotic pressure of its second external media, in mOsm L^{-1} H_2O. After the osmolarity value of *P. indicus* and its osmotic work level were known, the conversion was conducted to determine the range of the isoosmotic salinity value by using the following equation (Anggoro 1992):

$$Osm = -5.4081 + 29.3489\ S$$

Where: Osm - biota osmolarity; S - isoosmotic salinity.

The length-weight relationship was obtained by using the equation $W=aL^b$, which was transformed into the linear equation:

$$\log W = \log a + b \log L$$

Where: W - weight (g), L - total length (mm), a - intercept, b - slope. The b value shows the growth pattern: negatively allometric ($b<3$), isometric growth ($b=3$), and positively allometric ($b>3$) (Omar et al 2020).

The Fulton condition factor was measured based on Okgerman (2005):

$$K = W\ L^{-3} \times 100$$

Where: K - condition factor; W - total weight (g); L - total length (mm).

Statistical analysis. Data was tabulated and analyzed at a confidence level of 95%, using SPSS 16.0® software. In the analysis of the length-weight relationship, the t-test was conducted to determine the significant differences of the b value ($b=3$ or $b\neq3$) (Firdaus et al 2020). The relationship between the condition factor and OWL was analyzed using a linear correlation (Pearson test) to find out whether variables correlated positively or negatively (Suzuki et al 2020).

Results and Discussion

Osmoregulation. The OWL and osmoregulation pattern of *P. indicus* at station 1 is presented in Table 1 and at station 2 is presented in Table 2. After OWL values were obtained, the conversion was executed and the isoosmotic salinity of Station 1 is presented in Figure 2 and of station 2 in Figure 3.

Table 1

Haemolymph osmolarity, media osmolarity, osmotic work level and osmoregulation pattern of *Penaeus indicus* from station 1

Osmolarity (mOsm L^{-1} H_2O)		*OWL (mOsm L^{-1} H_2O)*	*Osmoregulation pattern*
Haemolymph	*Media*		
886	880	6	Hyperosmotic
886	880	6	Hyperosmotic
887	880	7	Hyperosmotic
887	880	7	Hyperosmotic
887	880	7	Hyperosmotic
887	880	7	Hyperosmotic
887	880	7	Hyperosmotic
888	880	8	Hyperosmotic
887	880	7	Hyperosmotic
888	880	8	Hyperosmotic
888	880	8	Hyperosmotic
889	880	9	Hyperosmotic
889	880	9	Hyperosmotic
890	880	10	Hyperosmotic
889	880	9	Hyperosmotic
890	880	10	Hyperosmotic
888	880	8	Hyperosmotic
893	880	13	Hyperosmotic
890	880	10	Hyperosmotic
894	880	14	Hyperosmotic
892	880	12	Hyperosmotic
900	880	20	Hyperosmotic
900	880	20	Hyperosmotic
911	880	31	Hyperosmotic
910	880	30	Hyperosmotic
912	880	32	Hyperosmotic
913	880	33	Hyperosmotic
910	880	30	Hyperosmotic
914	880	34	Hyperosmotic
914	880	34	Hyperosmotic

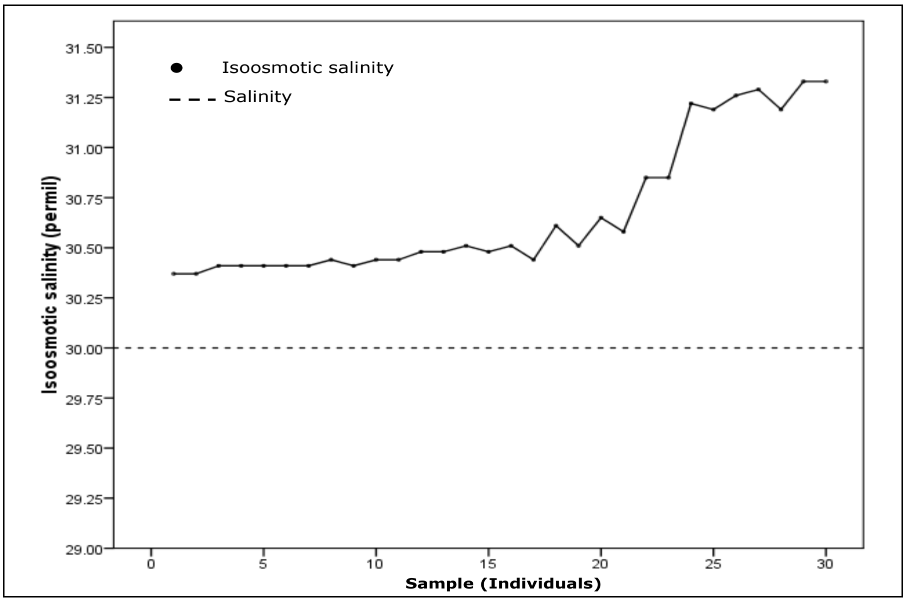

Figure 2. Isoosmotic salinity of station 1.

Table 2

Haemolymph osmolarity, media osmolarity, osmotic work level and osmoregulation pattern of *Penaeus indicus* from station 2

Osmolarity (mOsm L $^{-1}$ H_2O)		*OWL*	*Osmoregulation pattern*
Haemolymph	*Media*	*(mOsm L $^{-1}$ H_2O)*	
879	909	30	Hypoosmotic
879	909	30	Hypoosmotic
880	909	29	Hypoosmotic
880	909	29	Hypoosmotic
880	909	29	Hypoosmotic
880	909	29	Hypoosmotic
879	909	30	Hypoosmotic
879	909	30	Hypoosmotic
880	909	29	Hypoosmotic
880	909	29	Hypoosmotic
888	909	21	Hypoosmotic
915	909	6	Hyperosmotic
915	909	6	Hyperosmotic
883	909	26	Hypoosmotic
880	909	29	Hypoosmotic
885	909	24	Hypoosmotic
879	909	30	Hypoosmotic
881	909	28	Hypoosmotic
882	909	27	Hypoosmotic
880	909	29	Hypoosmotic
880	909	29	Hypoosmotic
881	909	28	Hypoosmotic
885	909	24	Hypoosmotic
888	909	21	Hypoosmotic
915	909	6	Hyperosmotic
916	909	7	Hyperosmotic
916	909	7	Hyperosmotic
915	909	6	Hyperosmotic
915	909	6	Hyperosmotic
916	909	7	Hyperosmotic

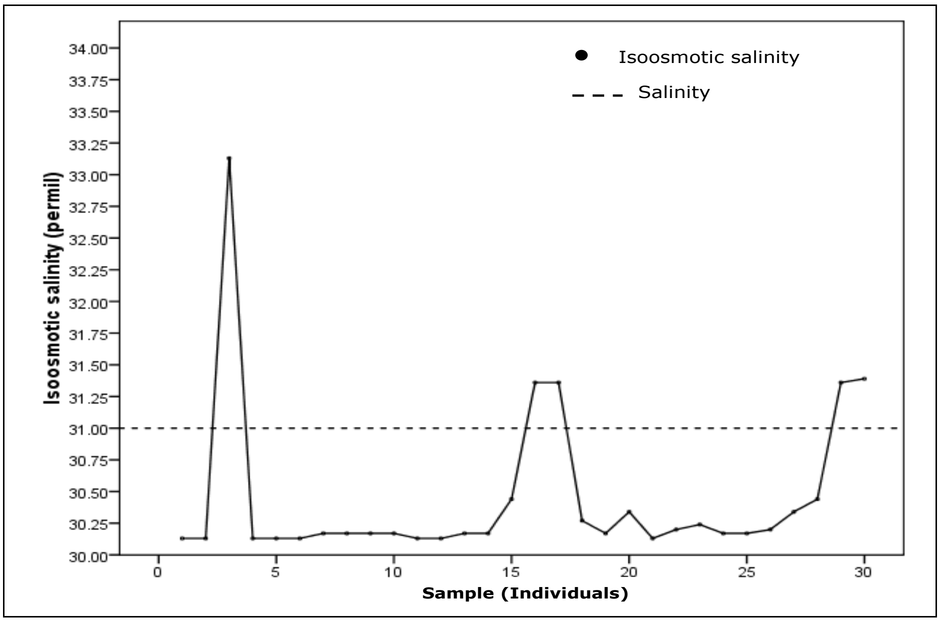

Figure 3. Isoosmotic salinity of station 2

Length-weight relationship and condition factor. *P. indicus* had an isometric growth pattern (b=3) in station 1, and a positive allometric growth in station 2 (b>3) (Figure 4).

The condition factor of Indian white shrimp in the water area of mangrove eco-edutourism Tapak ranged between 0.57–2.17 in station 1 and 1.2–2.28 in station 2 (Table 3).

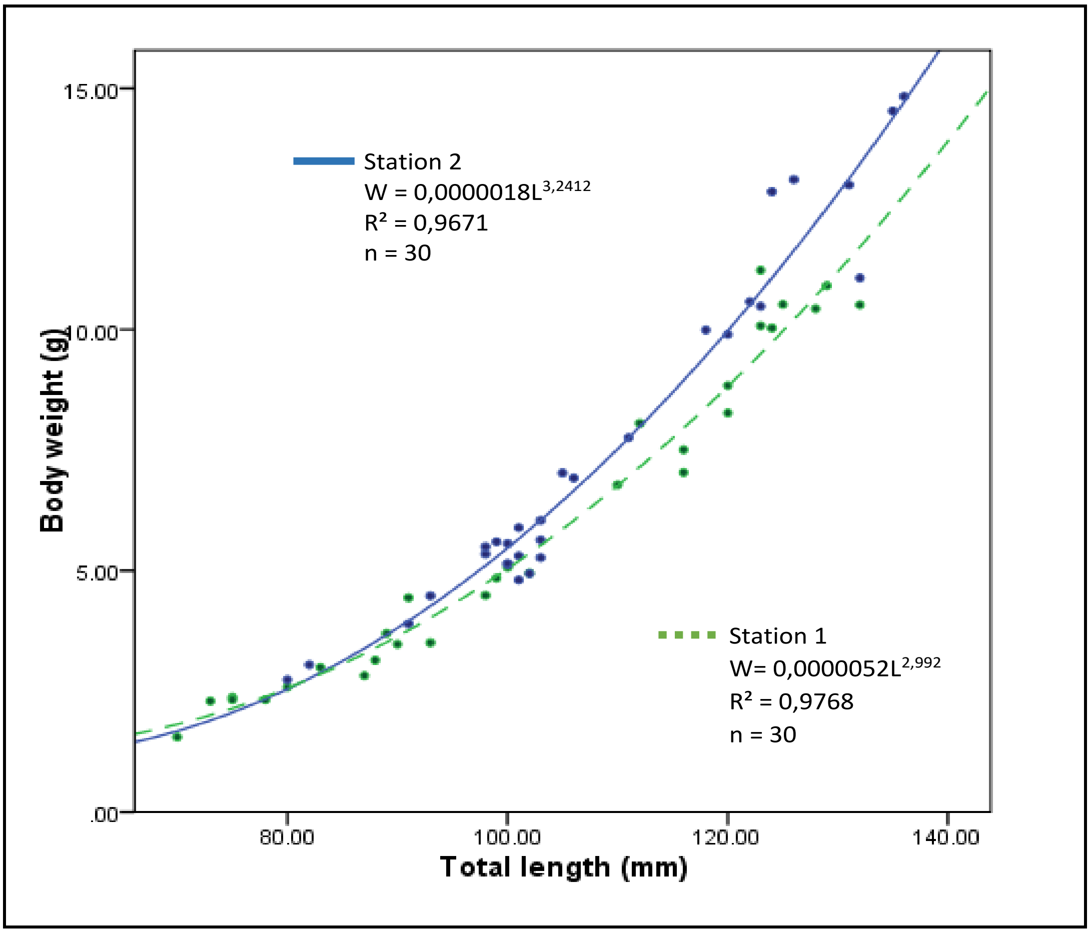

Figure 4. Length-weight relationship of *Penaeus indicus* in the study location.

Table 3

Condition factor of *Penaeus indicus* from the two stations

Station	*n*	*Condition factor*		
		Minimum	*Maximum*	*Mean±SD*
1	30	0.57	2.17	1.6±0.425
2	30	1.2	2.28	1.85±0.287

Condition factor relationship with OWL. The results of the linear correlation of the condition factor and OWL, *P. indicus* showed a linear negative relationship ($p<0.05$). A higher OWL lowers the condition factor of *P. indicus* (Figures 5 and 6). Based on the correlation degree test (Pearson), the value of 0.9 was obtained in station 1 and 0.8 in station 2; therefore, there is a very strong correlation between the condition factor and OWL.

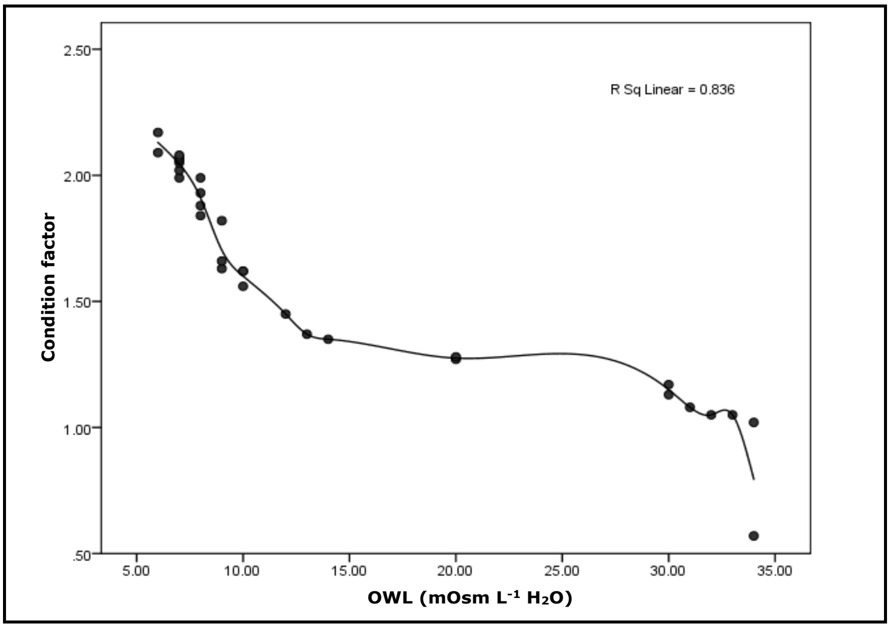

Figure 5. Relationship between condition factor and osmotic work level (OWL) of *Penaeus indicus* in station 1.

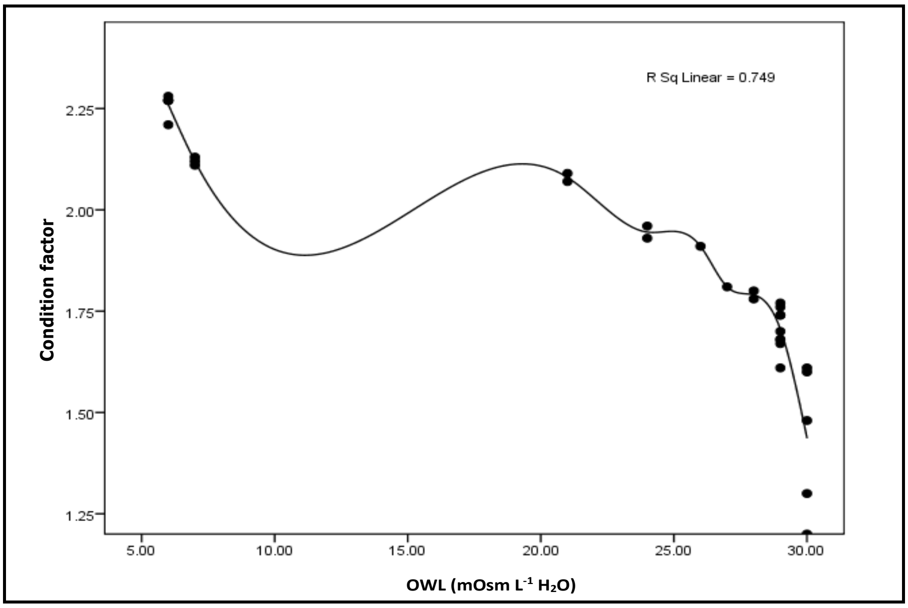

Figure 6. Relationship between condition factor and osmotic work level (OWL) of *Penaeus indicus* in station 2.

Water quality. The results of water quality obtained are presented in Table 4.

Table 4

Water quality in area of mangrove eco-edutourism Tapak Semarang

Variable	*Station*		*Optimum value*	*Source*
	Station 1	*Station 2*		
Temperature (^oC)	32.1	31.1	26-32	Supriatna et al (2020)
pH	6.72	6.79	6.5-9	Anand et al (2019)
Dissolved oxygen(mg L^{-1})	8	8,4	>5	Lalramchhani et al (2020)
Salinity ($^o/_{oo}$)	30	31	15-25	Suwarsih et al (2016)

Electrolytes. Media electrolyte content in stations 1 and 2 can be seen in Table 5.

Table 5

Electrolyte content in external media

Electrolyte	*Station 1 (gL^{-1})*	*Station 2 (gL^{-1})*
Na^+	8.82	9.12
Cl^-	16.05	16.60
Mg^{2+}	1.07	1.11
Ca^{2+}	0.34	0.35
K^+	0.31	0.33

OWL represents the performance response of *P. indicus* to preserve the balance of internal body liquid osmolarity with external media. Indian white shrimp is able to survive in a wide range of salinity, being resistant to salinity fluctuations (Lalramchhani et al 2020). The haemolymph osmolarity value of shrimp was higher than the external media osmolarity. Thus, the Indian white shrimp in station 1 had a hyperosmotic osmoregulation pattern. In hyperosmotic condition, the media water will enter the body parts with thin layers, such as gills, intestines and skin (Lopez-Mananes et al 2002). The lower media osmolarity will enter the body, causing ion diffusion outward from the body of shrimp, and internal liquid will be deionized (Iskandar 2021). To solve this excessive deionization, shrimp will osmoregulate by increasing ion absorption from the media through gills and intestines, then kidney will respond the condition by excreting hypoosmotic urine (Jaffer et al 2019; Upling 2020).

Osmolarity measurement results of Indian white shrimp and media in station 2 showed a significant difference ($p<0.05$) compared to the results from station 1. Haemolymph osmolarity in station 2 was hypoosmotic towards the external media osmolarity, due to the haemolymph osmolarity being lower than the external media osmolarity. In a hypotonic environment, the body liquid tends to move outward from the body through gills, digestive tract and skin by osmosis (Esbaugh & Cutler 2016; Riza et al 2020). In order to maintain homeostasis conditions, shrimp will ingest more water to obtain free ionic water to produce urine (Anggoro et al 2018a).

The differences in the pattern of osmoregulation at stations 1 and 2 are influenced by the location of the intake station. Station 1, which is a mangrove eco-edutourism recreation center, was heavily influenced by human activities. During the COVID-19 pandemic, many antiseptics and disinfectants were provided for visitors, and there was an increase in the frequency of washing hands with soap in the recreation area. The possibility of this waste could have polluted the water. When exposed to pollutants, shrimp osmoregulation may encounter physiological stress (Inman & Lockwood 1977). Several studies have already showed that there is disruption of normal osmotic and ionic balance after exposure to pollutants such as stressing physico-chemical conditions in aquatic biota (Lin et al 1993).

Station 2 is the migration route for *P. indicus* from the sea to the mangrove area and the other way around. 8 individuals of *P. indicus* that have hyperosmotic osmoregulation pattern tended to be larger in size, so it is possible that these shrimp are adults of *P. indicus* from the estuary that will migrate to the sea or *P. indicus* from the surrounding area that migrate through station 2. This is in accordance to Vance & Rothlisberg (2020), who stated that adult *P. indicus* will migrate towards the sea to spawn, after going through several phases, while juvenile *P. indicus* will return to the mangrove area.

The difference in OWL between stations was caused by salinity difference. Station 1 had a salinity of 30‰, whereas station 2 had 31‰ salinity. Optimal salinity for shrimp ranges between 15-25‰. External media osmotic pressure is directly proportional to electrolyte concentration, so a water with high salinity has a high osmotic pressure. Besides affecting external media osmolarity, salinity also affects haemolymph osmolarity of Indian white shrimp. According to Sobirin et al (2014) and Anggoro et al (2018b), a higher external media salinity lowers the OWL. The decreasing OWL value due to increasing salinity incurs higher energy needs of the Indian white shrimp for osmoregulation. Isoosmotic salinity measurement was carried out for Indian white shrimp in stations 1 and 2. The average of isoosmotic salinity in station 1 was 30.67‰ and 30.52‰ in station 2. The smaller difference in salinity toward isoosmotic salinity will reduce osmotic stress and preserve energy used for osmoregulation, so the growth of shrimp will be better (Politis et al 2018; Anggoro et al 2018b).

The high water salinity of the mangrove area is caused by high ion content, mainly Na^+ and Cl^- ions. A higher electrolyte content will increase salinity. The shrimp's body will try to balance electrolytes in haemolymph and media, therefore, the OWL of shrimp will increase (Rachmawati et al 2017).

Other electrolytes were also found in the media, like Mg^{2+}, Ca^{2+} and K^+ (Table 5). Na^+ and Cl^- affect water salinity, which is directly affecting OWL; K^+ and Mg^{2+} can also affect the osmoregulation process. K^+ takes part in Na^+/K^+ -ATPase enzyme activation that further takes part in crustaceans osmoregulation processes when salinity fluctuations occur (Kaligis 2016). Mg^{2+} functions as cofactor in the ion pump work mechanism. Na^+/K^+ -ATPase activity tends to increase proportionally toward external salinity (Anggoro et al 2018b; Sellami et al 2020; Zaffar et al 2020). Na^+/K^+ -ATPase helps in excreting NaCl and facilitates the Na^+ and K^+ ions pass through the cell membrane; it also adjusts osmolarity, membrane permeability and cell volume (Atli et al 2015). Different types of ATPase, such as Na^+/K^+ -ATPase and Mg^{2+} -ATPase are enzymes with important roles in the osmoregulation system of shrimps. Na^+/K^+ -ATPase and Mg^{2+} -ATPase preserve the balance of Na^+, K^+ and Mg^{2+} ions in the biota's body (Canli et al 2016). Therefore, ion transportation is an important component in the osmoregulation process (Niu et al 2020).

The length-weight relationship in this study has a high determination coefficient (r^2) (Figure 4). It showed that the increase in weight was closely related with the increase of body length. The growth pattern is affected by multiple factors, like sampling time, sampling location, food availability, reproduction period and catching device used (Mahadevan et al 2020). Fulton's condition factor (K) is an essential parameter which represents fitness of fish, heavier fish at particular length being considered to be in better condition (Singh & Serajuddin 2017). The condition factor illustrates the interaction between abiotic and biotic factors related with the physiological functions of biota (Komi & Francis 2017). A higher K value shows a better physiological condition (Getso et al 2017; Oktaviani et al 2020). The condition factor of *P. indicus* in both stations is higher than 1, meaning that the shrimp have a good condition, with nutritional requirements met and good quality parameters of the water (Komba et al 2020). The average value of the condition factor in station 2 was higher compared to the condition factor of shrimp in station 1 ($p<0.05$), showing that water parameters and food availability could have been better in station 2.

Station 2 is represented by water with a fish aggregating device owned by local people, so, in addition to natural food, there are also remains of man-made feed that could attract shrimp. Food availability is affected by water condition. Thus, the tide causes nutrients in the sediment to rise in the water column (Long et al 2018). The water of station 2 faces directly the beach of Tugu District, so the food supply from the mangrove area and sea is more abundant than in station 1.

Based on the results of the correlation analysis (Figures 5 and 6), the condition factor was correlated linearly negatively with OWL. A higher condition factor will lower the OWL value. If OWL value is low, the energy required by shrimp for osmoregulation is also low. A surplus of energy will be used for growth, and a better development will increase the condition factor.

Conclusions. Based on the results osmotic work level (OWL), the osmoregulation of *P. indicus* in the water area of mangrove eco-edutourism Tapak varied in the two stations. Indian white shrimp in station 1 had a hyperosmotic osmoregulation pattern and in station 2 a hypoosmotic osmoregulation pattern. Indian white shrimp had an isometric growth pattern in station 1 and a positive allometric growth in station 2. The condition factor was 1.6 in station 1, and 1.85 in station 2. The regression showed that OWL was negatively correlated with the condition factor.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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