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Osmotik Word Level *Teredo navalis* Linnaeus 1758 in the Mangrove Root Habitat in East Halmahera, Indonesia

Yumima Sinyo[1*], Suparman Suparman[2], Asmar Hi Daud[3], Tamrin Robo[4]

[1] *Department of Biology Education, Universitas Khairun, Indonesia, sinyoyumima@gmail.com*
[2] *Department of Biology Education, Universitas Khairun, Indonesia, suparman@unkhair.ac.id*
[3] *Department of Aquaculture, Universitas Khairun, Indonesia, asmarhidaud75@gmail.com*
[4] *Department of Geography Education, Universitas Khairun, Indonesia, Tamrinrobo.geoling18@gmail.com*

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ABSTRACT

This study aims to analyze the osmotic work level and pattern of osmoregulation at mollusc *Teredo navalis* Linnaeus 1758 in mangrove root habitats from different salinities. Case study was performed with the purposive sampling method. The research data were analyzed descriptively and quantitatively. The sampling technique is based on tidal conditions of seawater. Two locations was designated, namely station I and II in the north and the south respectively. *Teredo navalis* samples were taken from 2 types of mangrove roots. Analysis of the level of osmotic work using Automatic Micro Osmometer Roebling Type 13/13 DR. Salinity was measured using a refractometer. The results showed that the osmotic level of *Teredo navalis* in the root habitat of *Rhizophora* sp. and *Avicennia* sp. at high and low tide conditions varied, specifically 523 mOsm/l H_2O, 123 mOsm/l H_2O, and 32 mOsm/l H_2O. This varying level of osmotic action results in three patterns of osmoregulation, viz. hyperosmotic, hypoosmotic, and isosmotic. The highest salinity was found in *Avicennia* sp. mangrove at station II and the lowest in *Rhizophora* sp. at low tide at station II, with the value was 31‰ and 10‰ respectively. Differences in salinity determine the osmoregulation pattern of *Teredo navalis* L in different habitats.

Keywords: *Teredo navalis* L, Osmotic work level, Root mangrove habitat, East Halmahera

ABSTRAK

Penelitian ini bertujuan untuk menganalisis tingkat kerja osmotik dan pola osmoregulasi pada moluska *Teredo navalis* Linnaeus 1758 di habitat akar mangrove pada salinitas yang berbeda. Jenis penelitian ini adalah studi kasus dengan menggunakan metode purposive sampling. Data penelitian dianalisis secara deskriptif kuantitatif. Teknik pengambilan sampel didasarkan pada kondisi pasang surut air laut. Lokasi penelitian terdiri dari dua stasiun yaitu stasiun I di Utara dan stasiun II di Selatan. Sampel *Teredo navalis* diambil dari 2 jenis akar mangrove yang digunakan sebagai habitat. Analisis tingkat kerja osmotik menggunakan *Automatic Micro Osmometer Roebling Type* 13/13 DR. Salinitas diukur menggunakan refraktometer. Hasil penelitian menunjukkan bahwa tingkat kerja osmotik *Teredo navalis* di habitat akar *Rhizophora* sp. dan *Avicennia* sp. pada kondisi pasang dan surut bervariasi, yaitu 523 mOsm/l H_2O, 123 mOsm/l H_2O, dan 32 mOsm/l H_2O. Tingkat kerja osmotik yang bervariasi ini menghasilkan tiga pola osmoregulasi, yaitu hiperosmotik, hipoosmotik, dan isosmotik. Hasil analisis salinitas tertinggi terdapat pada mangrove *Avicennia* sp. di stasiun II dengan nilai 31‰ dan terendah pada *Rhizophora* sp. pada kondisi surut di stasiun II dengan nilai 10‰. Perbedaan salinitas tersebut menentukan pola osmoregulasi *T. navalis* di habitat yang berbeda.

Kata kunci: *Teredo navalis* L, Tingkat kerja osmotik, Habitat akar mangrove, Halmahera Timur

INTRODUCTION

East Halmahera Regency is one of the districts in North Maluku Province which is located in the Eastern Region of Indonesia and has mangrove forests covering an area about 5.751.51 hectares and most of it is protected forest (Widiyanti et al, 2018; Sinyo et al, 2019a). Mangrove forest is a natural resource that is unique and has a special ecological role with a very high stock for nutrient storage (Sinyo et al, 2019b). Mangrove forests include natural resources that can be recovered so that they can ensure the preservation of organisms and can maintain the existence of habitats for sustainability in the future (Alwidakdo et al, 2014). The mangrove forest ecosystem is a plant community that grows on the coast and experiences a natural maturity phase. Mangroves grow and develop in tidal areas and are tolerant of salinity and time of inundation (Abubakar et al, 2021). The mangroves in this area are experiencing a phase of natural maturity and weathering. The productive mangrove forest area is one of the grounds for aquatic biota and always gets a supply of salt and fresh water and receives and accommodates nutrients that are beneficial to aquatic organisms (Izzah & Roziaty, 2016; Swain & Rao, 2017).

Teredo navalis Linnaeus 1758 is an aquatic organism belonging to the Phylum Mollusca class Bivalvia Teredinidae which is also called the shipworm or mangrove worm because it lives in fragile mangrove roots (MacIntosh et al, 2014; Lippert et al, 2017). *Teredo navalis* can perforate fragile mangroves and make them a habitat (Appelqvist & Havenhand, 2016; Sinyo et al, 2019a) Morphologically, *Teredo navalis* has a small shell on its head in the form of a drill that functions to make a hole, its body is naked while its head is covered with a shell, its body surface is smooth and soft like a worm and its body length reaches 30 to 70 centimetres (Voight, 2015; Eriksen et al, 2017). *Teredo navalis* can perform physiological adaptations by destroying and digesting wood assisted by cellulose and nitrogen binding bacteria and other morphological functions and utilizing wood as food (Evans et al, 2011; Appelqvist et al, 2015). *Teredo navalis* body contains protein (Setyabudi et al, 2020), and is a mangrove resource that humans need to meet the body's protein needs (Rosaini, Heni, 2015). *Teredo navalis* is highly dependent on salinity as an ecological component that plays an important role in the osmoregulation process (Appelqvist & Havenhand, 2016; Perez-alvaro, 2016), zoning factors, and tides (Yulianda et al, 2013). *Teredo navalis* maintains its osmolarity stability by carrying out an osmoregulation process that aims to increase salt ions and water media absorption, which is carried out through the gills (Weigelt et al, 2017). Salinity is an important ecological factor that affects the physiology of an organism's body (Temmy et al., 2018). *Teredo navalis* osmoregulation is the main variable that affects the osmolarity of the media and the osmolarity of haemolimph/body fluids (Yuliani et al, 2018).

Salinity that often changes causes differences in the pressure of the media and the body of the organism (Temmy et al., 2018), so if there is a difference in the pressure of the osmotic media, the organism's body undergoes a process of osmotic regulation, this process is called osmoregulation. Maulana et al, (2013) state that if the salinity level rises and is in the high range, the osmoregulation process of the organism's body will experience pressure and experience environmental osmotic stress and even cause death. Conversely, if the salinity decreases, the organism will again experience pressure to undergo the osmotic control process so that it has to expend large amounts of body energy (Pamungkas, 2012). Therefore, the osmolarity of body fluids (haemolimph) and the osmolarity of the media must always be in normal conditions because changes in salinity levels cause *Teredo navalis* to experience environmental osmotic stress. *Teredo navalis* needs osmoregulation to carry out physiological processes in the body (Romano & Zeng, 2012). Osmoregulation plays a role in the storage of substances that will be used by *Teredo navalis* and serves to balance the osmotic concentration of intracellular and extracellular fluids (Pati et al., 2014). The growth of *Teredo navalis* in mangrove habitats requires an osmotic work rate (OWL) by regulating the osmolarity and hemolime osmolarity media (Samudra et al., 2020).

The mangrove area in East Halmahera is dominated by mangrove species *Rhizophora* sp. and *Avicennia* sp. Based on the survey results in water conditions during high tide and low tide, the researchers found that the mangrove roots of *Rhizophora* sp. and *Avicennia* sp., which were already fragile, were inhabited by *Teredo navalis* in large numbers, as evidenced by the formation of many gaps in the mangrove roots. Based on the survey results, the researchers are interested in researching *Teredo navalis*, which is inhabited by mangrove roots, the level of osmotic work, and the effect of salinity because until now no research has been conducted on the osmotic work rate of *Teredo navalis* in the mangrove roots habitat of *Rhizophora* sp. and *Avicennia* sp. in Indonesia, especially in the mangrove area of Wailukum East Halmahera. Based on this description, the purpose of this study was to analyze the osmotic work level and the effect of salinity on *Teredo navalis* in the mangrove root habitat in Wailukum waters, East Halmahera.

METHODOLOGY

Time and Location

The research was case study and conducted from September 2019 to June 2020 in the mangrove waters of Wailukum, East Halmahera. The sampling method was purposive. Collecting sample of *Teredo navalis* , stem, and seawater samples was carried out in September, October, and November 2019. Measurement of salinity and osmotic work level (OWL) and data testing were carried out from March to April 2020. The sampling location consists of two stations as shown in Figure 1.

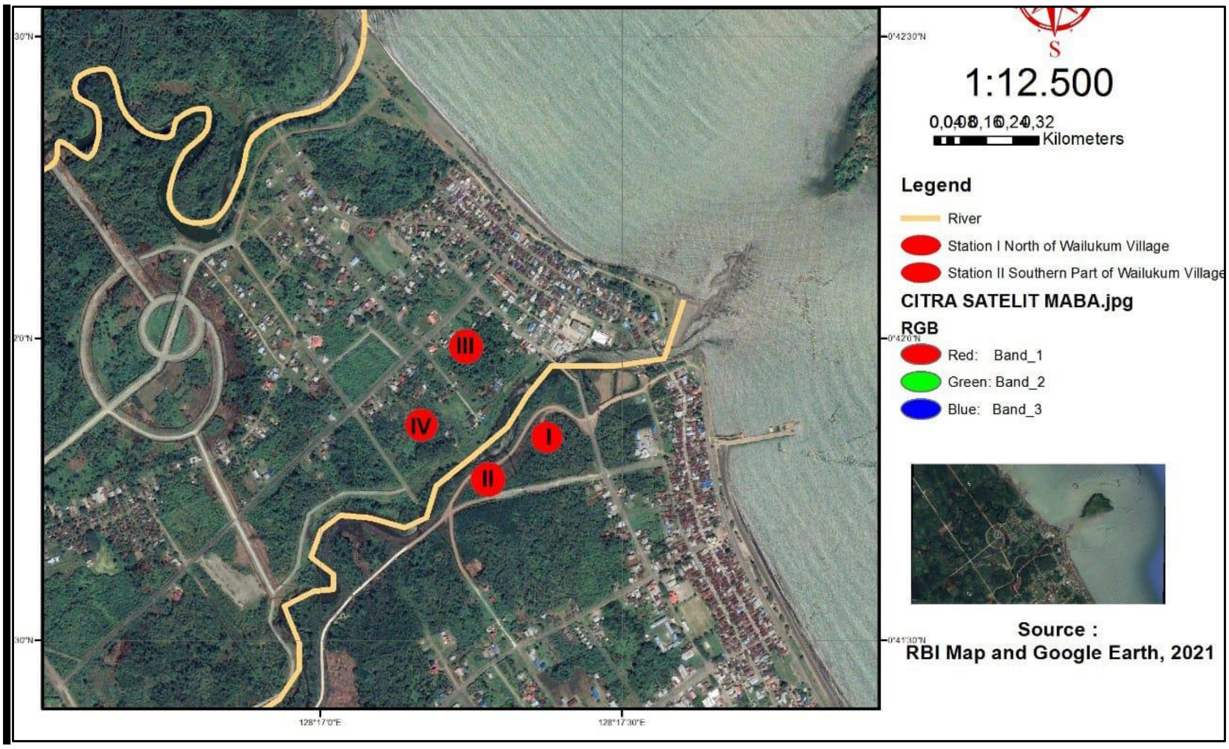

Figure 1. Sampling locations in the mangrove forest of East Halmahera

The location of the observation station consists of two stations starting from the northern part, namely station I, and the southern part designated as station II. Both are mangrove forest that was submerged by sea water as tidal area and become a center of community. The sampling area consist of 50 square meters. The tools used at the time of sampling were axes, swords to cut mangrove stalks, buckets to hold samples, tweezers to catch and clamp samples, coolboxes to accommodate cleaned samples, and refractometers to measure salinity, plastic and duct tape to glue samples, scissors for cutting material. PE bottle container for storing water samples, syringe to collect body fluids *Teredo navalis.* A Pipette is used to collect reagents.

Field Data Measurement and Sampling
The first stage of sampling was carried out at station I under high tide and low tide conditions. Samples of *Teredo navalis* were collected about 12 individuals for each type of mangrove root. Then proceed with the sampling of 12 pieces of mangrove stems measuring 8 cm and 2 bottles of water samples. All the samples were cleaned with distilled water. The second stage of sampling was carried out at station II with the same procedure and all were packed and put in a coolbox and brought to the laboratory, as shown in Figure 2.

Figure 2. A sampling of *Teredo navalis* L in mangrove root

Osmoregulation Analysis
Measurement of osmoregulation through the stem osmolarity and hemolymph (body fluid) test of *Teredo navalis* was carried out at the MSDP UNDIP Semarang laboratory using Roebling Type 13/13 DR Automatic Microsmometer based on the method (Temmy et al., 2018). The liquid sample was taken using a 0.1 ml microtube and then put into Roebling's Autocal Micro-osmometer and the tool would work and display the numbers on the glass screen as the measurement result. The results of the measurement of media osmolarity and hemolymph are presented in Figure 3.

Salinity Analysis
The ecological factors tested in this study were salinity levels in *Teredo navalis* biota and salinity in the mangrove root of *Rhizophora* sp. and *Avicennia* sp. The samples were analyzed using a refractometer.

Figure 3**.** Sample hemolymph osmolarity and media osmolarity

Data analysis

Osmoregulation is determined by analyzing the osmotic work level (OWL) which is calculated based on the difference between the osmolarity value of hemolymph *Teredo navalis* and the osmolarity of the media. The calculation of the osmotic work rate (OWL) uses the equation from (Samudra et al., 2020), as follows:

OWL = [P Osmo hemolymph - P Osmo media] (1)

Information,

OW : Osmotic working pressure (mOsm/l H_2O).
Osmo hemolymph : The osmotic pressure of body fluids (mOsm/l H_2O)
Ordinary media : Media osmolarity/osmolarity pressure.

RESULTS AND DISCUSSION

Osmotic Work Level and Osmoregulation Pattern

The results of measurements of hemolymph osmolarity of *Teredo navalis* (H) and osmolarity of media (plasma cells/B) at tidal and low tide conditions at stations I and II for mangrove species *Rhizophora* sp. and *Avicennia* sp. obtained osmotic work level (OWL) namely *Rhizophora* sp. ranged from 244 to 246 mOsm/l H_2O, and the osmoregulation pattern was hyperosmotic, while the osmotic work level value of *Avicennia sp.* ranged from 282 to 285 mOsm/l H_2O with the same osmoregulation pattern. namely hyperosmotic. Furthermore, the osmotic work level of *Rhizophora* sp. at station II ranged from 403 to 505 mOsm/l H_2O with a hyperosmotic osmoregulation pattern, while the osmotic work level value of *Avicennia* sp. ranged from 521 $_2O$ to 523 mOsm/l H_2O with a pattern hyperosmotic osmoregulation. The osmotic work level at low tide at station I for mangroves *Rhizophora* sp. ranged from 122 to 123 mOsm/ l H_2O and the osmoregulation pattern was hypoosmotic. The osmotic work level of *Avecenia* sp. ranged from 36 to 58 mOsm/l H_2O, the osmoregulation pattern is isosmotic. *Rhizophora* sp. osmotic work level values for station II ranged from 32 to 34 mOsm/l H_2O with an Isosmotic osmoregulation pattern, while the osmotic work level values for *Avicennia* sp. ranged from 200 to 201 mOsm/l H_2O with a hyperosmotic osmoregulation pattern. Based on the value of the osmotic work level of the two types of mangroves above shown that there are differences in osmoregulation patterns both in tide and low tide conditions. This can be expressed through the value of the osmotic work level which varies from each type of mangrove in each water condition. At high tide, the level of osmotic work is higher than at low tide in Figure 4.

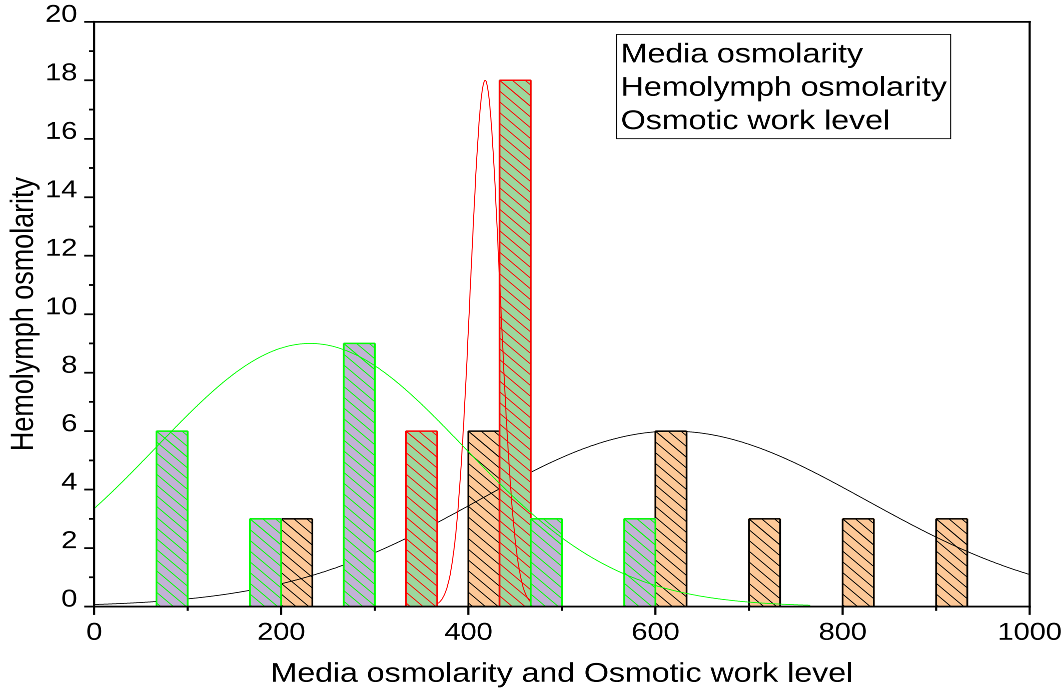

Figure 4. The results analysis of the osmotic work level (OWL) value of *Teredo navalis* in the root habitat

Based on the graph above, it is stated that the *value* of the osmotic work level (OWL) is categorized based on the habitat (media) of *Teredo navalis*, namely the root habitat of *Rhizophora* sp. and *Avicennia* sp. which is at high tide and low tide conditions. The results showed that the highest osmotic work level was seen in the water conditions at high tide at station II, namely, 523 mOsm/l H_2O found in *Avicennia* sp. with a hyperosmotic osmoregulation pattern. While the moderate osmotic work level value is found in the *Rhizophora* sp. type at station I at low tide, namely 123 mOsm/l H_2O with a hypoosmotic osmotic pattern, then the lowest osmotic work level value is also found in the mangrove *Rhizophora* sp. at low tide conditions at station II, which is 32 mOsm/l H_2O with isoosmotic osmoregulation pattern. Based on these results it can be said that there are differences in the value of the osmotic workload and the osmoregulation pattern at each research station both at high tide and low tide conditions. These results also indicate that the osmolarity value of the hemolymph of *Teredo navalis* is higher than the osmolarity value of the media. This suggests that *Teredo navalis* can regulate the concentration of body fluids against the concentration of the medium (Sinyo et al, 2020b). Each organism manages an osmoregulation mechanism to balance the osmotic pressure inside and outside the body. To maintain a relatively constant body fluid, aquatic animals perform osmotic regulation which is called osmoregulation (Samudra et al, 2020). Based on the value of the osmotic work rate in Figure 2, this shows that *T. navalis* has an osmoregulatory pattern, because if the aquatic animal is said to have an osmoregulatory osmoregulation pattern if the OWL value is >500 m Osm/1 H_2O, and is said to be an osmoconformer if the OWL value range is <500 mOsm/l H_2O, and if there is a high difference in the osmotic pressure of the body with the environment, the more energy required by aquatic animals to carry out the osmoregulation process until it reaches the tolerance limit.

The environmental osmotic pressure that is close to the osmotic pressure in the body will produce relative ions with a balanced concentration. The value of the osmotic work level serves to evaluate the osmotic response based on the need for osmoregulation in the Eco physiological mechanisms of aquatic animals (Temmy et al., 2018). Osmoregulation is the regulation of fluid concentration in living things which plays an important role in the process of osmotic balance between intra-cell fluid (CIS) and extracellular fluid. Aquatic organisms need an osmoregulation process, especially aquatic biota, Phylum Mollusca type *Teredo navalis* which is permeable to the environment and salt solutions. The definition of osmoregulation for *Teredo navalis* is the regulation of the osmotic pressure of body fluids that is suitable for the life of *Teredo navalis* so that the body's physiological processes work normally or homoeostasis (Maulana et al, 2013; Temmy et al., 2018). The value of the osmotic work rate (OWL) was obtained by reducing the osmolarity of the medium and the osmolarity of the haemolymph. *Teredo navalis* requires an iso-osmotic environment to live a normal life. The pattern of osmoregulation is determined based on the value of the osmotic Work Level. the OWL value of 0 is iso-osmotic, and the greater the OWL value, the osmoregulation pattern is hyperosmotic (Yuliani et al, 2018).

The varying workload values for mangroves *Rhizophora* sp. and *Avicennia* sp. at the tide and low tide conditions both at stations I and II produce three patterns. osmoregulation namely hyperosmotic, hypoosmotic and isosmotic. It is said that the hyperosmotic osmoregulation pattern is if the media osmolarity and the increase in salinity affects the osmolarity of the medium. The higher the salinity value, the higher the osmolarity of the medium (Samudra et al, 2020).

Salinity

The salinity test (salt content) of the mangrove roots of *Rhizophora* sp. and *Avicennia* sp. using a refractometer was carried out at the MSDP Laboratory of Diponegoro University Semarang, and the results are stated of testing the salinity value (salt content) of mangrove roots *Rhizophora* sp. and *Avicennia* sp. obtained varying results in each water condition, both at high tide and at low tide on stations I and II. The range of salinity values in *Rhizophora* sp. at high tide is between 23 to 30 ‰, and at low tide is in the range of 10 to 15 per mil (‰). While the salinity value of *Avicennia* sp. mangroves at high tide ranges from 24 to 31 per mil (‰) and at low tide it ranges from 15 to 22 per mil (‰). At high tide, the highest salinity value was found in the *Avicennia* sp. mangrove at station II, which was 31 per mil (‰) and the lowest salinity value was found in the *Rhizophora* sp. mangrove at low tide at station II with a salinity value of 10 per mil. (‰). This value has a relationship with the osmotic work level of *Teredo navalis* in Figure 5).

Based on Figure 5 above, it can be said that the difference in the physical properties of the environment causes the osmoregulation process between *Teredo navalis* and its habitat. This can be seen through the osmosis process which shows that the salt content in the body is higher than the salt content of the root media (Idrus, et al., 2014). However, when the salt level becomes normal, this process is said to be balanced (Maulana et al., 2013). In addition, the salinity of the root medium (Habitat) also affects the salinity balance process. The high and low salinity of the media greatly affects the osmotic workload. If the osmotic workload is high, the organism will absorb more energy to carry out the osmoregulation process (Pamungkas, 2012).

Teredo navalis performs an osmoregulation process to maintain its osmolarity stability by increasing the absorption of salt ions and water media through the gills. The hyperosmotic environment is a regulatory adaptation to avoid water loss from the body (Paalvast & Velde, 2013). Loss of water from the body occurs through the gills. When *Teredo navalis* absorbs large amounts of seawater, the salt content will also be absorbed and enter the body in large amounts, causing the body to experience excess salt (Wang et al., 2013). The excretion of large amounts of excess salt is also carried out through the gills because the gills contain specialized cells called chlorite cells. Chlorite cells are cells that function to remove NaCl from plasma into seawater. If *Teredo navalis* has a large osmotic workload, other physiological processes such as growth and reproduction will be disrupted (González-Duarte et al., 2018).

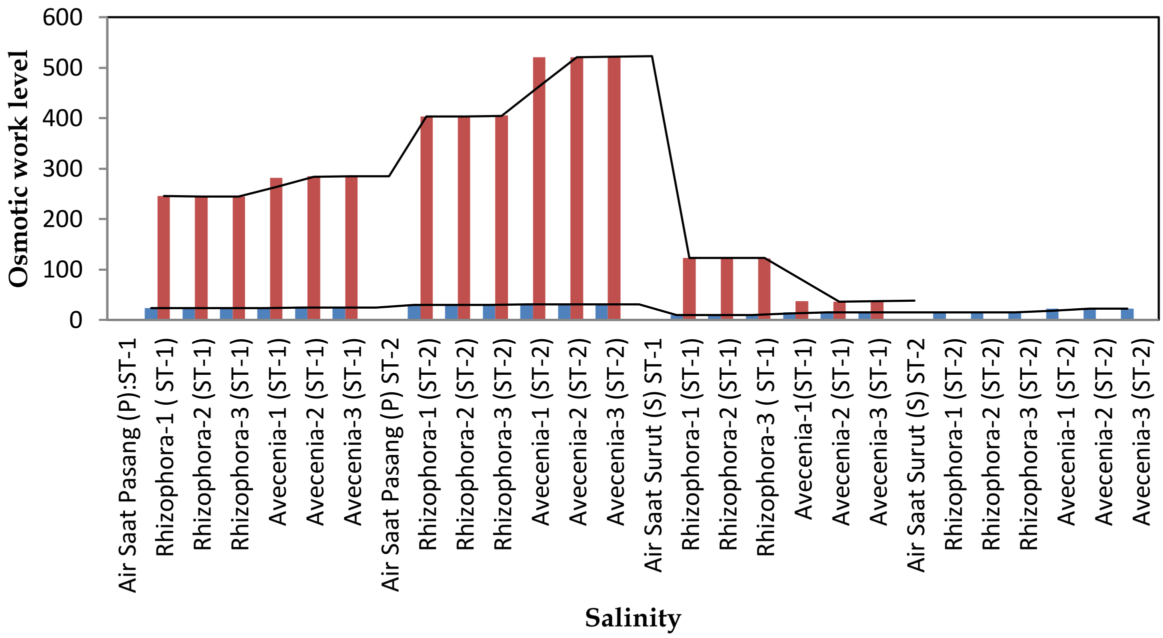

Figure. 5. Media salinity and the osmotic work level i *Teredo navalis* in the root habitat

CONCLUSION

The level of osmotic action of *Teredo navalis* in the mangrove root habitat of *Rhizophora* sp. and *Avicennia* sp. in the mangrove waters of Wailukum, East Halmahera varied, namely 523 mOsm/l H_2O, 123 mOsm/l H_2O, and 32 mOsm/l H_2O. Variations in the workload values of *Rhizophora* sp. and *Avicennia* sp. mangroves at high and low tide both at stations I and II resulted in three osmoregulation patterns, namely hyperosmotic, hypoosmotic, and isosmotic. The results of the salinity test (salt content) in the highest root habitat were found in the *Avicennia* sp. mangrove at station II, which was 31 per mil (‰) and the lowest salinity value was found in the *Rhizophora* sp. mangrove at low tide at station II. with a salinity value of 10 per mil (‰). Differences in environmental physical properties (salinity) cause an osmoregulation process between *Teredo navalis* and its habitat so that the salt content in the body is higher than the salt content in the root media (habitat).

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