Pertanika J. Trop. Agric. Sc. 41 (3): 1511 - 1521 (2018)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Short Communication

Study of the Tolerance of Black Sea Cucumber *Holothuria leucospilota* to Hypoxia Stress

Neviaty P. Zamani, Khoirunnisa Assidqi* and Hawis H. Madduppa

Department of Marine Science and Technology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University (IPB), Bogor, Indonesia

ABSTRACT

The aim of this study was to investigate the survival rates and the behavioural responses of *Holothuria leucospilota* under hypoxia stress condition. A total of 15 *H. leucospilota* were collected from Rambut Island, in Thousand Islands, Jakarta, Indonesia. They were exposed to three different dissolved oxygen concentrations (i.e. 2 mg O_2 L^{-1}, 3 mg O_2 L^{-1}, >6 mg O_2 L^{-1}). Mortality was measured at the 4th and 6th day by adding 2 mg O_2 L^{-1} and 3 mg O_2 L^{-1} of dissolved oxygen concentrations respectively. The survival rates of *H. leucospilota* significantly decreased by 80% at 2 mg O_2 L^{-1} and 40% at 3 mg O_2 L^{-1} compared to > 6 mg O_2 L^{-1} in which total survival was 100% at the end of 22 days of exposure to hypoxia. The behavioural responses of *H. leucospilota* to hypoxia stress were shown by decreased metabolic activity by releasing the contents of their stomach described as cuvierian tubules. The lower dissolved oxygen concentrations at 2 mg O_2 L^{-1} and 3 mg O_2 L^{-1} showed a higher metabolic rate in 20% and 50 % of individual organisms during 18 days hypoxia exposure. If their metabolic rate is high, they will need more energy reserves to compensate it. The result of this study revealed that the lowest dissolved oxygen at 2 mg O_2 L^{-1} can exert a stress on *H. leucospilota* which can lead to a high mortality and an impairment of behavioural responses.

Keywords: Behavioural responses, cuvierian tubules, hypoxia, invertebrate, *Holothuria leucospilota*

ARTICLE INFO

Article history:
Received: 26 July 2017
Accepted: 28 June 2018
Published: 29 August 2018

E-mail addresses:
npzamani@gmail.com (Neviaty P. Zamani)
nisaassidqi@gmail.com (Khoirunnisa Assidqi)
madduppa@gmail.com (Hawis H. Madduppa)
* Corresponding author

ISSN: 1511-3701
e-ISSN: 2231-8542

INTRODUCTION

Black sea cucumber *Holothuria leucospilota* (Brandt, 1835) is a tropical shallow reef holothurian species. *H. leucospilota* as well as other benthic organisms have important roles in the benthic ecosystem. At least seven species, including *H. leucospilota*, are found in Thousand Islands, Jakarta, Indonesia (Madduppa et al., 2017). Holothurian can be used as biotubators, the number of bacterial abundance and the exchange of nutrients and dissolved oxygen in water or marine coastal sediments (MacTavish, 2012). Some bacteria communities in holothurian guts are observed as having the potential to keep the balance in nutrient cycling such as microbes (Amaro et al., 2009; Zhang et al., 2012). Schneider et al. (2011) recorded holothurian that has a role in the coral reef ecosystem to balance $CaCo_3$. Holothurians are currently used as food supplement, food sources and medicine. Mohammadizadeh et al. (2013) found bioactive compounds from *H. leucospilota*, as having antibacterial and antifungal properties. Hence, holothurian is not only commercially valuable, but also has an ecological function for sustaining marine life.

Currently, the major problem affecting the marine ecosystem is related to threats from anthropogenic activities. Nutrients and pollutants enter the marine environment, not only as dissolved solids, but some of them are suspended solids which will be deposited in the sediment. This situation will potentially lead to sedimentation leading to oxygen depletion. Sedimentation of suspended solids may have an effect on holothurian who may not be able to adapt to high levels of sedimentation. Gray et al. (2002) reported that marine life cannot survive when concentration of dissolved oxygen in the water is below 2 mg O_2 L^{-1} to 0.5 mg O_2 L^{-1}. The primary effect on the marine environment is eutrophication, as a result of dissolved oxygen concentration, due to over-enrichment by nutrients. Hypoxia also can be built up under this condition. Consequently, it will lead to the loss biodiversity in the benthic ecosystem services, since the marine organisms will not survive in heavy sedimentation (Worm et al., 2006).

There are internal and external disturbance factors which cannot be predicted in the marine environment (Vilnäss et al., 2012). The internal factor is related to global warming whereby the increasing temperature can lead to reduce oxygen levels in the ocean. The external factor relates to anthropogenic activities which can lead to a drastic decrease in oxygen level. Thus, it would impair the performance of holothurian. Hypoxia phenomenon affects the survival of marine organisms in the ecosystems. Some studies have been reported that hypoxia has become a stressor and has a negative impact on the sustainability of marine organisms leading to reduction in growth and reproduction (Huang et al., 2012; Loddington, 2011; Vaquer-Sunyer & Duarte, 2008).

There is a connection between the physical and biological processes in the marine coastal environment. Holothurian is a deposit feeder which respires with all tentacles around their skin and consumes a lot of water and absorbs dissolved oxygen. Therefore, this study was conducted to investigate the survival rates and the behavioural of *H. leucospilota* exposed to hypoxia stress tolerance with *ex situ* observations in different dissolved oxygen concentrations. Studies have investigated the cause of hypoxia can lead to negative effect which reverses the loss of biomass on benthic organisms and changes behavioural and physiological responses as a feedback mechanism (Diaz & Rosenberg 1995; Diaz & Rosenberg, 2008; Huo et al., 2018). Since hypoxia condition can constitute a stress for sea cucumbers, we hypothesise the survival rates and behavioural responses to decrease with decreasing concentration of dissolved oxygen.

MATERIAL AND METHODS

Study Site and Sea Cucumber Collection

Individuals of the black sea cucumber *H. leucospilota* were collected in Rambut Island, Kepulauan Seribu, Jakarta, Indonesia (5°58’30.9”S 106°41’42.9”E) during low tide (Figure 1). *H. leucospilota* were found fine-grained sediments just below the low tide line. The length range of sea cucumbers used for the experiment were between 10 cm and 15 cm. The sea cucumbers were kept in cooled insulation box with seawater. During transportation into the laboratory, half of the seawater in cooled insulation box was exchanged every two hours with new stock of the seawater supply. This is to provide an oxygen circulation inside the box. A total of 15 individuals was used in

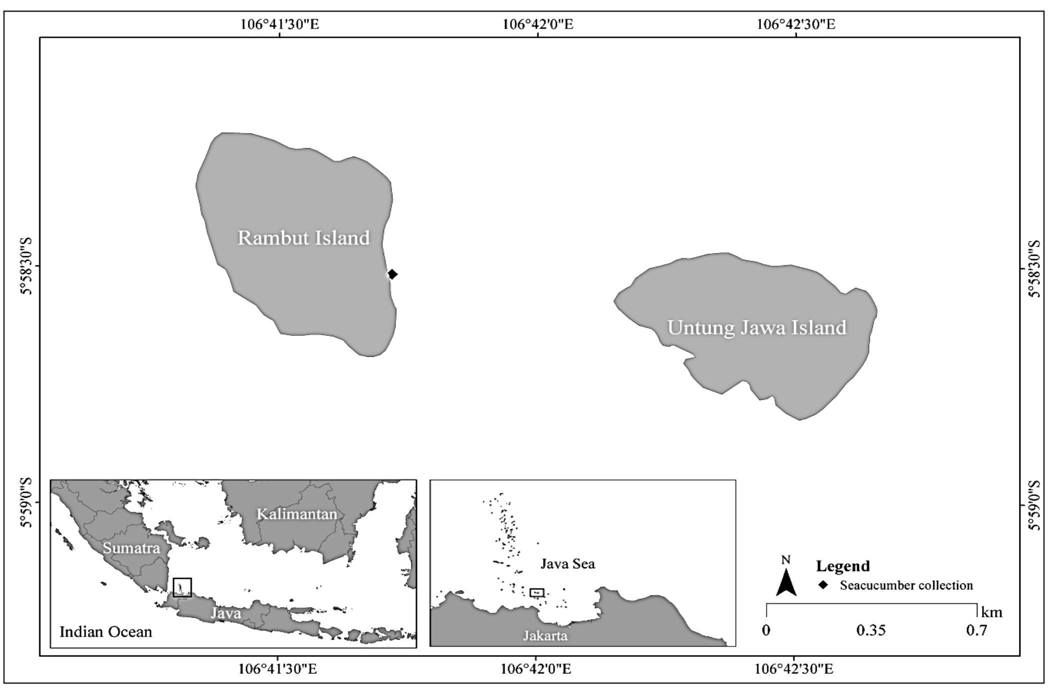

Figure 1. Map of Rambut Island, Jakarta, Indonesia showing the position of sampling *H. leucospilota* with black diamond bullet

the experiment, where each concentration levels used 5 individuals; there were three groups of 5 sea cucumbers each, all having similar length size. The low number of individuals was due to limited availability of sea cucumbers in the field. The sea cucumbers were acclimatised to laboratory conditions, every 5 sea cucumbers were transferred into a glass aquarium with 20 litres of seawater with constant aeration. During post acclimatisation in laboratory conditions for 10 days, 20% of sediment was added from the total volume. The seawater was changed daily to prevent accumulation of metabolic waste.

Experimental Setup

The hypoxia circulation system comprised three different dissolved oxygen (DO) concentrations (2 mg O_2 L^{-1}, 3 mg O_2 L^{-1}, >6 mg O_2 L^{-1}1) (Table 1). Each treatment group consisted of 5 individuals kept in individual containers with 2 l of seawater. The DO concentrations were measured using a digital oxygen meter, the sensor type is CellOx 325 for WTW Oxical 3205, Weilheim, Germany. The hypoxic water collected in the header tank were placed in containers. The inflow was connected to the seawater header tank which had one hole on the top nitrogen inflow. The concentration of dissolved oxygen in the seawater header tank was reduced by pumping bubbling nitrogen gas into it (Cheung et al., 2008; Long et al., 2008; Seitz et al., 2003). According to Vaquer-Sunyer & Duarte (2008), the threshold for hypoxia for echinoderms is ≥ 2 mg O_2 L^{-1}. The target DO concentrations were reached at 2 mg O_2 L^{-1}, 3 mg O_2 L^{-1}, >6 mg O_2 L^{-1}. The DO concentrations of >6 mg O_2 L^{-1} was chosen as the normal condition. The outflow distributed the seawater to fill in the experimental containers (Figure 2).

Table 1
Treatment groups for hypoxia stress tolerance

Treatment groups	Concentration levels of DO	Number of individuals
X	2 O_2 mg L^{-1}	5 individuals
Y	3 O_2 mg L^{-1}	5 individuals
Z	>6 O_2 mg L^{-1}	5 individuals

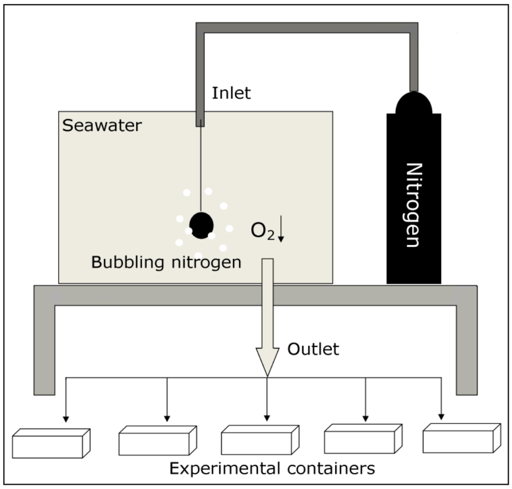

Figure 2. Design of hypoxia circulation system with one bottle of nitrogen immersed in the tank and later stored in the container

Each animal was placed in a tight closely to prevent re-oxygenation of the water. The seawater in the containers was exchanged daily. When changing the water, the containers and the top of the header tank had to be opened until the water body inside the containers was entirely replaced with hypoxic water from the header tank. The normoxia condition was applied >6 mg O_2 L^{-1} as control and aerated. However, presumably for all DO concentrations did not feed the sea cucumbers during the hypoxia test (Cheung et al., 2008).

In the laboratory study, the lighting condition was simulated like in the marine environment which had a day light on and off (12:12 h). During the experiment, the room temperature at 25 - 27°C and the salinity at 31 - 32‰ was controlled. 3 levels of dissolved oxygen concentration were applied (Table 1). Their behavioural responses under hypoxia condition and the surviving individual was observed twice a day. The saline and the ammonia concentration was measured at the beginning and at the end (death revealed) to ensure it was not caused by increasing salinity and ammonia concentrations (Table 2).

Table 2
Water quality checking data during hypoxia stress tolerance

Treatment groups	Water quality (mean ± SD)	
	Salinity (‰)	Ammonium ($NH4^+$) (mg L^{-1})
X	31.10 ± 0.22	< 0.05 ± 0
Y	31.10 ± 0.22	< 0.05 ± 0
Z	31.10 ± 0.22	< 0.05 ± 0

Statistical Analysis

The survival rates and the behavioural responses of *H. leucospilota* data were analysed using Cox proportional hazard test (Bewick et al., 2004; Goel et al., 2010). Data was analysed using as Kaplan Meier curves which showed the probability for the number of individuals to median time. All statistical tests and curves were calculated and produced using the free R-statistic software (version 3.1.2 (2014-10-31) “Pumpkin Helmet”). The significance result was assumed if p-value was lower than 0.05.

RESULTS AND DISCUSSION

H. leucospilota was exposed to hypoxia stress for 22 days, and it was found that behavioural responses as well as survival rates were low percentages due to oxygen depletion (Figures 3 and 4). Diaz and Rosenberg (1995) had studied three types of oxygen concentrations, the normoxia condition, the moderate hypoxia and severe hypoxia. As shown in Figure 3, 3 mg O_2 L^{-1} affected behavior of the specimens by 50% in 18 days compared with 2 mg O_2 L^{-1}. The different oxygen concentrations had a significant influence on the behavior of *H. leucospilota* which ejected their cuvierian tubules. 20% of the individual organisms showed the behavior by ejecting their cuvierian tubules upon exposure to 2 mg O_2 L^{-1} during 18 days (Figure 3). It was proven by Cox-ph test: Chisq = 8.1249, df = 2, p = 0.01721*. Therefore, that holothurian is more susceptible to 2 mg O_2 L^{-1} concentration.

The line drop of the survival rates in all oxygen concentrations indicated the death phase of holothurians. The survival rates of *H. leucospilota* even showed a decrease of 18% on day 4 at 2 mg O_2

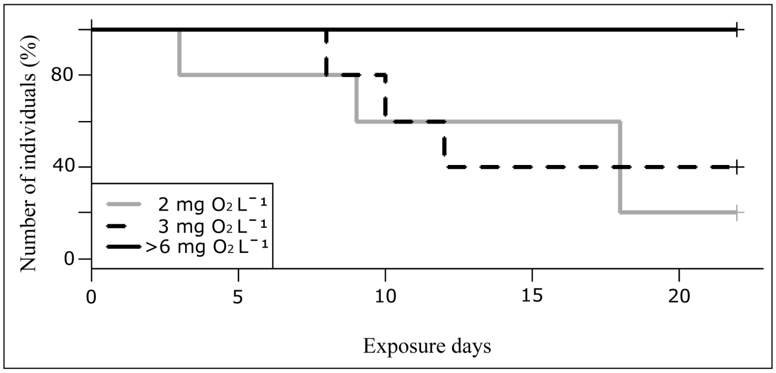

Figure 3. The behavioural responses of *H. leucospilota* showed by ejection of cuvierian tubules during hypoxia stress tolerance with different oxygen concentrations for 22 days (n=5)

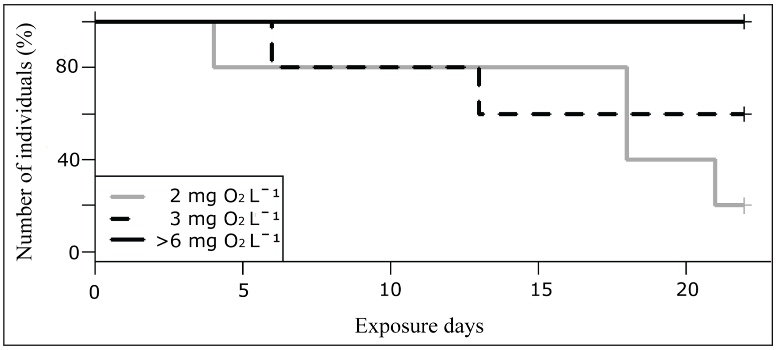

Figure 4. The survival rates of *H. leucospilota* showed by declining curves during hypoxia stress tolerance with different oxygen concentrations for 22 days (n=5)

L^{-1}. The lowest concentration (2 mg O_2 L^{-1}) had only 20% of initial individual organisms (Figure 4). This study revealed the median survival, individual organism in hypoxia concentration decreased with the lowest hypoxia concentration with a strong significant trend (Figure 4, Coxph-test: Chisq = 7.1477, df = 2, p = 0.02805*). The lowest concentration (2 mg O_2 L^{-1}) has a faster death rate compared with the higher concentration (3 mg O_2 L^{-1}) and normal concentration (>6 mg O_2 L^{-1}). At the end of this study, metabolic rate was not measured as it was not available due to the damaged body which was dissolved in the seawater.

H. leucospilota are deposit feeders and under normal conditions, they will use their peltate tentacles to push sediment into their mouths. The behavior responses of Holothurians were examined under hypoxia condition. These behavioural responses were very slow movement with a big open mouth (Figure 5A), and the colour of their tentacles changed from black to white (Figure 5B). This is shown when they eject their cuvierian tubules (Figure 5C). This confirms that the behavioural responses of *H. leucospilota* were strongly affect concentration. Oxygen concentration. Oxygen consumption indicates that holothurian can be very

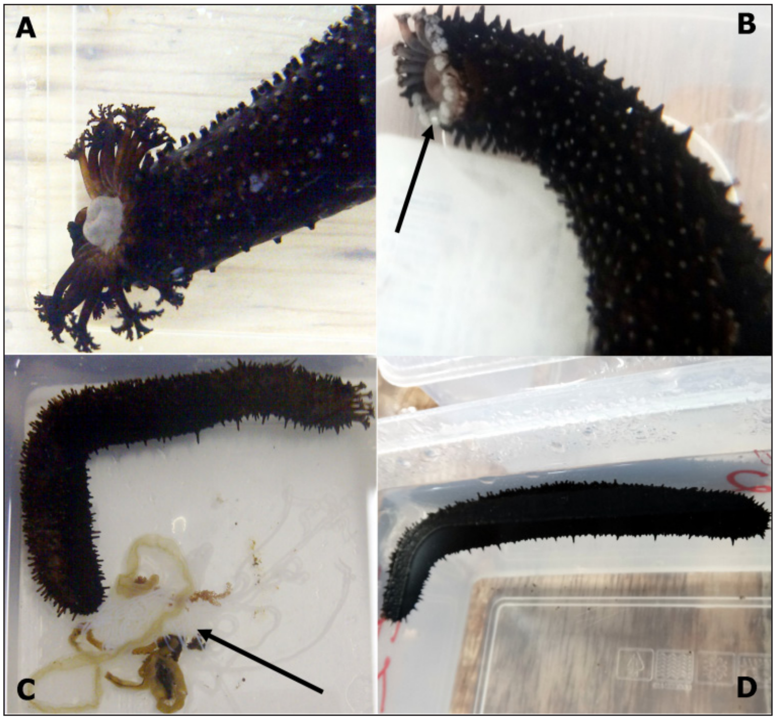

Figure 5. The different appearances under hypoxia (2 mg O_2 L^{-1}; 3 mg O_2 L^{-1}) and normoxia condition (>6 mg O_2 L^{-1}) (A) The anterior body hanging on the top and with a big open mouth (B) The colour of the tentacles changed from black to white (C) *H. leucospilota* ejected part of cuvierian tubules (D) *H. leuscopilota* under normoxia condition.

opportunistic and reduce energy expenditure to a minimum when oxygen is in short in supply (Siikavuopio et al., 2008).

The highest percentages were seen under normoxia condition (>6 mg O_2 L^{-1}), as holothurians did not eject their cuvierian tubules. Under normoxia condition, *H. leucospilota* moved around from one part to another part and looked healthier than in hypoxia condition (Figure 5D). This indicates that the holothurians did not receive any stressor under normoxia condition. The relationship between hypoxia and holothurians behavior is strongly affected by oxygen depletion concentrations.

Astall and Jones (1991) said that holothurians reduced their metabolic rate by ejecting the cuvierian tubules under hypoxia condition. It was relevant to our result which showed the lower dissolved oxygen concentrations led to holothurians ejecting their cuvierian tubules during the experiment. It was observed the lower oxygen concentrations showed a higher metabolic rate, if their metabolic rate is high, they needed more energy reserves to compensate it. They saved their energy with aestivation activity and they ejected their cuvierian tubules to keep their energy levels high.

Specifically, Holothurians ejected their cuvierian tubules as a defence mechanism (Becker & Flammang, 2010; Demeuldre et al., 2014). If they cannot produce cuvierian tubules, their predators can spot them easily and thus, increasing the risk of biodiversity loss. According to Cheung et al. (2008) prolonged reduced oxygen level at 1.5 mg O_2 L^{-1} decreased food consumption in marine scavenging gastropods *Nassarius festivus*. Hypoxia reduces growth of marine organisms and it increases their mortality rate (Rabalais et al., 2002).

There is a relationship between decrease in dissolved oxygen concentrations to holothurians behavioural responses and impact on their mortality. Hypoxia has consequences for living resources and an impact on benthic ecosystem services. The holothurians tried to survive during hypoxia, but unfortunately, some of them died, because they failed to aestivate. There were no significant differences in behavioural responses between under 2 mg O_2 L^{-1} and 3 mg O_2 L^{-1}, except for faster ejection of cuvierian tubules and followed by the mortality. 2 individuals survived until the end of the experiment during hypoxia condition in 3 mg O_2 L^{-1}, and therefore it can be assumed that they succeeded to aestivate themselves (Yang et al., 2006). The threshold for the hypoxia of holothurians was estimated at 3 mg O_2 L^{-1}. This study revealed that holothurian is susceptible to reduction of dissolved oxygen. Holothurians always eject parts of their cuvierian tubules when the environmental conditions do not support their lives.

Holothurian as well as *H. leucospilota* is an important species in benthic ecosystem services. They play an important functional role in the ecology of coastal ecosystem and coral reef ecosystem and a marker of eutrophication within the marine environment. The presence of *H. leucospilota* has an ecological role and in the trophic levels. Holothurian has been considered as one of the marine organisms which recycles nutrient in the coral reefs and seagrass beds ecosystem (Uthicke, 2001; Wolkenhauer et al., 2009). Therefore, Timmerman et al. (2012) concluded nutrient loading had a positive support to the biomass of benthic macrofaunal communities, but it also had potential to increase hypoxia which affected benthic biomass. Hypoxia condition has a negative impact on the marine life and the diversity of marine organism. If one of the marine species in an ecosystem cannot be sustained, this could create new problems for the food web in the ecosystem. This was proven by the Long et al. (2008), and Long and Seitz (2008) that the prey species would be easily detected by their predators.

CONCLUSION

The study showed survival rates of *H. leucospilota* due to hypoxia exposure had declined significantly. The behavioural responses of *H. leucospilota* to hypoxia stress were shown by their decreased metabolic activity by releasing the contents of their stomach described as cuvierian tubules. The results revealed that *H. leucospilota* was susceptible to a low dissolved oxygen less than (2 mg O_2 L^{-1}).

ACKNOWLEDGEMENTS

This study was conducted by GEOMAR in Germany and IPB in Bogor as part of GAME (Global Approach by Modular Experiment) project, as an international collaborative research programme. The authors are grateful to Dr. Mark Lenz for his advice and guidance. The study was funded by Kieler Nachricten. The authors are also grateful to Sinja Elena Rist and Directorate General of Forest Protection and Nature Conservation – Natural Resources Conservation Centre of Jakarta for approving their request for a sample specimen from Rambut Island.

REFERENCES

Amaro, T., Witte, H., Herndl, G. J., Cunha, M. R., & Billett, D. S. M. (2009). Deep-sea bacterial communities in sediments and guts of deposit-feeding holothurians in Portuguese canyons (NE Atlantic). *Deep-Sea Research Part I*, *56*, 1834-1843.

Astall, C. M., & Jones, M.B. (1991). Respiration and biometry in the sea cucumber *Holothuria forskali*. *Journal of the Marine Biological Association of the United Kingdom*, *71*, 73-81.

Bewick, V., Cheek, L., & Ball, J. (2004). Statistics review 12: Survival analysis. *Critical Care, 8*(5), 389–394.

Becker, P., & Flammang, P. (2010). Unravelling the sticky threads of sea cucumbers — a comparative study on cuvierian tubule morphology and histochemistry. *Biological Adhesive Systems*, 87-98.

Cheung, S. G., Chan, H. Y., Liu, C. C, Shin, P. K. S. (2008). Effect of prolonged hypoxia on food consumption, respiration, growth and reproduction in marine scavenging gastropod *Nassarius festivus*. *Marine Pollution Bulletin*, *57*, 280-286.

Demeuldre, M., Chinh Ngo, T., Hennebert, E., Wattiez, R., Leclère, P., Flammang, P. (2014). Instantaneous adhesion of cuvierian tubules in the sea cucumber *Holothuria forskali*. *Biointerphases*, *9(2)*, 029016.

Diaz, R. J., & Rosenberg, R. (1995). Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and Marine Biology: An Annual Review, 33*, 245–303.

Diaz, R.J., & Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science, 321*, 926-929.

Goel, M. K., Khanna, P., & Kishore, J. (2010). Understanding survival analysis: Kaplan-Meier estimate. *International Journal of Ayurveda Research*, *1*(4), 274–278.

Gray, J.S., Wu, R.S., & Ying Ying O. (2002). Effects of hypoxia and organic enrichment on the coastal marine environment. *Review Marine Ecology Progress Series*, *238*, 249–279.

Huang, L., Nichols, L. A. B., Craig, J. K., & Smith, M. D. (2012). Measuring welfare loses from hypoxia: The case of North California brown shrimp. *Marine Resource Economics*, *27*(1), 3-23.

Huo, Da., Sun, L., Ru, X., Zhang, L., Lin, C., Liu, S., Xin, X., & Yang, H. (2018). Impact of hypoxia stress on the physiological responses of sea cucumber *Apostichopus japonicus*; respiration, digestion, immunity and oxidative damage. *PeerJ*, *6*, 1-24.

Loddington, R. (2011). Marine invertebrates in hypoxia: Developmental, behavioural, behavioural and fitness responses. *The Plymouth Student Scientist, 4*(2), 267-277.

Long, W. C., & Seitz, R. D. (2008). Trophic interactions under stress: hypoxia enhances foraging in an estuarine food web. *Marine Ecology Progress Series, 362*, 59-68.

Long, W.C., Brylawski, B.J., & Seitz, R.D. (2008). Behavioural effects of low dissolved oxygen on the bivalve *Macoma balthica*. *Journal Experimental Marine Biology and Ecology, 359*, 34-39.

MacTavish, T., Stenton-Dozey, J., Vopel, K., & Savage, C. (2012). Deposit-feeding sea cucumbers enhance mineralization and nutrient cycling in organically-enriched coastal sediments. *Journal Plos One, 7*(11), e50031.

Madduppa, H., Subhan, B., Taurusman, A. A., Anggraini, N. P., Fadillah, R., & Tarman, K. (2017). DNA barcoding reveals vulnerable and not evaluated species of sea cucumbers (Holothuroidea and Stichopodidae) from Kepulauan Seribu reefs, Indonesia. *Biodiversitas, 18(3)*, 893-898.

Mohammadizadeh, F., Ehsanpor, M., Afkhami, M., Mokhlesi, A., Aida Khazaali, A., & Montazeri, S. (2013). Antibacterial, antifungal and cytotoxic effects of a sea cucumber *Holothuria leucospilota*, from the north coast of the Persian Gulf. *Journal of the Marine Biological Association of the United Kingdom*, *93*(5), 1401–1405.

Rabalais, N.N., Turner, R.E., Wiseman, W.J. (2002). Gulf of Mexico hypoxia, aka ‘‘the dead zone’’. *Annual Review of Ecology and Systematics*, *33*. 235–263.

Seitz, R. D., Marshall Jr, L. S., Hines, A. H., & Clark, K. L. (2003). Effects of hypoxia on predator-prey dynamics of the blue crab Callinectes sapidus and the Baltic clam Macoma balthica in Chesapeake Bay. *Marine Ecology Progress Series*, *257*, 179-188.

Schneider, K., Silverman, J., Woolsey, E., Eriksson, H., Byrne, M., & Caldeira, K. (2011). Potential influence of sea cucumbers on coral reef $CaCo_3$ budget: A case study at one tree reef. *Journal of Geophysical Research*, *116*, G04032.

Siikavuopio, S. I., Mortensen, A., & Christiansen, J. S. (2008). Effects of body weight and temperature on feed intake, gonad growth and oxygen consumption in green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture*, *281*, 77-82.

Timmerman, K., Norkko, J., Janas, U., Norkko, A., Gustafsson, B.G., & Bonsdorff, E. (2012). Modelling macrofaunal biomass in relation to hypoxia and nutrient loading. *Journal of Marine System*, *105-108*, 60-69.

Uthicke, S. (2001). Nutrient regeneration by abundant coral reef holothurians. *Journal of Experimental Marine Biology and Ecology*, *265*, 153-170.

Vaquer-Sunyer, R., & Duarte, C.M. (2008). Thresholds of hypoxia for marine biodiversity. *Proceedings of the National Academy of Sciences*, *105*, 15452-15457.

Villnäs, A., Norkko, J., Lukkari, K., Hewitt, J., & Norkko, A. (2012). Consequences of increasing hypoxic disturbance on benthic communities and ecosystem functioning. *Journal Plos One*, *7*(10), e44920.

Wolkenhauer, S.M., Uthicke, S., Burridge, C., Skewes, T., & Pitcher, R. (2009). The ecological role of *Holothuria scabra* (Echinodermata: Holothuridea) within subtropical seagrass beds. *Journal of the Marine Biological Association of the United Kingdom*, *90*(2), 215-223.

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., Jackson, J. B. C., Lotze, H.K., Micheli, F., Palumbi, S. R., Sala, E., Selkoe, K. A., Stachowicz, J. J., & Watson, R. (2006). Impacts of biodiversity loss on ocean ecosystem services. *Science*, *314*, 787–790.

Yang, H., Zhou, Y., Zhang, T., Yuan, X., Li, X., Liu, Y., & Zhang, F. (2006). Metabolic characteristics of sea cucumber *Apostichopus japonicas* (Selenka) during aestivation. *Journal Experimental Marine Biology and Ecology*, *330*, 505– 510.

Zhang, X., Nakahara, T., Miyazaki, M., Nogi, Y., Taniyama, S., Arakawa, O., ... Kudo, T. (2012). Diversity and function of aerobic culturable bacteria in the intestine of the sea cucumber *Holothuria leucospilota*. *Journal Genetic Application Microbiol*, *58*, 447-456.