Cortisol levels in Asian sea bass (*Lates calcarifer*) as response to stress in different transportation systems using rubber seed (*Hevea brasiliensis*) anesthetic

[1]Dhita M. Saputri, [1]Megananda Paradewi, [2]Laksmi Sulmartiwi, [3]Prayogo, [3]Hari Suprapto

[1] Aquaculture study Program, Faculty of Fisheries and Marine, Airlangga University, Surabaya, Indonesia; [2] Department of Marine, Faculty of Fisheries and Marine, Airlangga University, Surabaya, Indonesia; [3] Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Airlangga University, Surabaya, Indonesia. Corresponding author: L. Sulmartiwi, laksmi-s@fpk.unair.ac.id

Abstract. Transportation is carried out as a process of distributing Asian sea bass (*Lates calcarifer*) juvenile. Transportation methods are divided in two transportation systems that can be used for transportation of live fish, such as wet transportation and dry transportation. Transportation leads to stress on fish based on the cortisol level. The anesthetic effect of extracts of rubber seed extract, were tested on *L. calcarifer* as an alternative to reduce stress. Cortisol levels between wet and dry transportation system showed that the rubber seed extract as an anesthetic material significantly affected *L. calcarifer*.
Key Words: biochemistry, natural ingredient, transportation system, stress in fish.

Introduction. Asian sea bass (*Lates calcarifer*) is one of the leading marine aquaculture species in Indonesia. Transportation in seabass seed production activities is conducted as the seed distribution process. Transportation methods are divided in two systems that can be used for transportation of live fish, such as wet system and dry system. Close transportation system is reported to be more profitable due to the efficiency of the volume, resulting in carrying more seeds and covering longer distance (Junianto 2003). But wet transportation also has disadvantages such as higher costs, is impractical and requires a lot of space. The use of anesthesia in the public wet transportation system was carried out such as on botanical fish transportation for 18 hours using citronella oil with 80% survival rate (Hasan et al 2016) and the use of clove oil in Nile tilapia (*Oreochromis niloticus*) seed transport for 10 hours with 97% survival rate (Haditomo et al 2014). Therefore, this study compares the cortisol levels of fish transported on different transportation systems. Dry system transportation is expected to be an alternative for live fish distribution because it is more efficient, practical and cheaper despite the risk of high mortality (Pratama et al 2017). Examples of the use of dry system transportation are in the soot fish using banana stem liver extract for 4 hours of transportation with a 50% survival rate (Pratama et al 2017) and transportation of freshwater lobster using tuba root extract for 24 hours of transportation with a 100% survival rate (Hendra 2012).

According to Ross & Ross (2008) problems encountered in transportation is the occurrence of fish stress caused by temperature and water chemical composition's variation. Martínez-Porchas et al (2009) confirmed that fish stress can be observed by measuring the hematological parameters such as serum cortisol level. Cortisol is easily measured and the key in allostatic mechanisms, homeostatic control, and stress physiology (McEwen & Wingfield 2010). Increased plasma cortisol can cause an increase in plasma glucose level. Elevations in plasma glucose are generated initially by catecholamine-mediated glycogenolysis and at later stages, cortisol-mediated gluconeogenesis, and lactate concentrations rise as muscle lactate formed during anaerobiosis is released to the plasma (Begg & Pankhurst 2004).

In aquaculture, anesthesia is commonly used to minimize the level of sensitivity and reduce the physical injury to fish during transport (Pramod et al 2010). This obstacle occurred can be managed using fish anaesthetical technique. Until now, for anesthesia in transporting fish used chemicals as MS-222 (Trichain methasulfat) and Quinaldine (2-4 Methycynolon) (Ross & Ross 2008). While there are natural materials that are easy to be used such as clove oil (Velisek et al 2005). But there is another natural ingredient that are potentially for usage as an anaesthetic substance, called the rubber (*Hevea brasiliensis*) seed. Selmar et al (1987) mentioned that the major component found in *H. brasiliensis* was linamarase (17.14%). The presence of alkaloid compounds in rubber seeds has pharmacological properties as anesthetics (Madziga et al 2010). Research on the use of rubber seed extract on biochemistry of *L. calcarifer* during transportation has never been done before.

The present study was conducted to determine the biochemical effect of rubber seed extract as an anesthetic material and to know the optimal concentration that can be used during transportation.

Material and Method

Rubber seed extract. Rubber seed (*H. brasiliensis*) were obtained from CV. Alam Lestari. Ten kg of rubber seeds were dried and blended, obtaining 568 grams of dry rubber seed powder. The rubber seed powder used in this study was 500 grams which was macerated with one liter of ethanol for 24 hours with several stirring. The supernatant from the maceration process were evaporated with a rotary vacuum evaporator using a temperature of 70^{o}C with a speed of 50 rpm. This way between rubber seed extract was separated by ethanol (Wildan et al 2012).

Concentration determination. Concentration determination used in the preliminary research was based on range finding test that consisted of rubber seed extract 100 ppm, 150 ppm, 250 ppm, 350 ppm, 450 ppm, 550 ppm, 650 ppm and 750 ppm. The concentration on the main study was obtained by using the safe concentration from this preliminary research. Based on the results from the preliminary examination, the fish survival rate at rubber seed extract 100 ppm is the highest concentration and survival rate was 100%. So the concentrations used in the main study were rubber seed extract RS 100 ppm, RS 90 ppm, RS 80 ppm and RS 70 ppm. According to Hanggono (2006) safe concentration of clove oil was under CO 5 ppm. All treatments were done by triplicates.

Fish preparation. *L. calcarifer* size 10 cm and 18.46±2.16 g were retrieved from Brackish Water Cultivation Fishery (BPBAP) of Situbondo. Fish were obtained and selected based on the desired size for transportation process. Fish health status was determined by intact morphology and free from any other diseases by direct observation (clinical examination). Fish were fasted for one day before being transported in the specific media.

Wet transport preparation. Ten *L. calcarifer* were placed in a plastic bag filled with seawater and extracts of cloves seed according to treatment (5 ppm clove oil, 70 ppm, 80 ppm, 90 ppm and 100 ppm extract of rubber seeds) by 2 L. The oxygen was given with the ratio 1:3 according to plastic bag volume. This transport uses as many as two styrofoam that consists of one styrofoam sampling blood and one styrofoam survival rate on each observation time. Plastic bags were tied using rubber band and placed in styrofoam box, which was filled with ice cubes to stabilize the temperature between 21-22°C (Lim et al 2003).

Dry transport preparation. Ten *L. calcarifer* were placed tub with seawater and extracts of cloves seed according to treatment (5 ppm clove oil, 70 ppm, 80 ppm, 90 ppm and 100 ppm extract of rubber seeds) by 4 L until they become anesthetized. This transport uses as many as two styrofoam with size of 120 x 40 x 33 cm that consisting of one styrofoam for observing blood samples and one styrofoam for observing survival rates. The dry transportation system used styrofoam that has stuffed with ice cubes and chaff. Previously, calico fabric was sorted according to randomization and moistened to keep it in a humid atmosphere when transportation takes place. The packaging composition was carried as follows: (1) The base of the styrofoam box was given shaved ice cubes with a height of 4 cm and a volume of 19.200 cm^3 evenly wrapped in plastic bags; (2) Cold straw was placed on ice with a height of 7 cm and a volume of 38.400 cm^3 evenly. The arrangement packaging of transportation can be seen in Figure 1. Transport lasted 12 hours. Observations of fish behavior during exposure consisted of fish movements, equilibrium of the body and stimulus response. According to McFarland (1959) stunning takes place to the stage of total loss of balance (deep anesthesia) demonstrated by total loss of consciousness and balance, giving reaction only to strong stimulants. The process of transport was carried out in the afternoon to avoid a significant temperature change at noon.

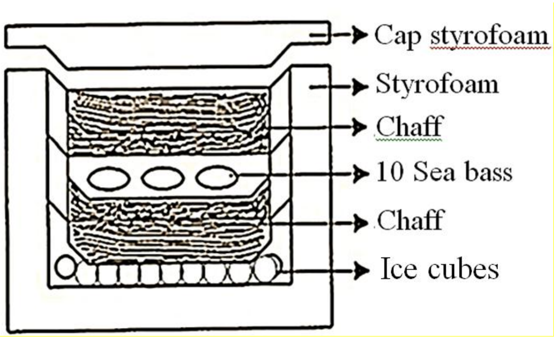

Figure 1. The packaging composition in dry transportation.

Blood sampling. Blood samples were taken from the caudal fin (Hanley et al 2010) using syringe of 1 mL. Each blood sample was placed in vacuum tube plain 5 mL and left at room temperature for an hour until separating the serum and the red blood cells. The blood serum was stored at the temperature -20^{0}C (Sink et al 2008).

The assessment parameters

Cortisol level. Cortisol level in *L. calcarifer* blood at pre-transportation, 6 hours transportation, 12 hours transportation, and post-transportation were measured by Non-competitive type Enzyme-Linked Immunosorbent Assay (ELISA), (Mouse Cortisol ELISA Kit Cat.No. E1066Mo; BT Lab, Shanghai Crystal Day Biotech Company, China).

Survival rate. *L. calcarifer* survival rate was measured at 6 and 12 hours transportation after being refreshed. Percentage of survival rate were counted based on Effendi (1997) who stated that survival rate is the ratio of total alive fish during transportation against total fish at the beginning of transportation and multiply by 100.

Statistical analysis. The effect of different concentration of extracts rubber seed on cortisol level with different system transportation were compared among each other with a comparative test (Independent T-test; α= 0.05).

Results. The use of different transportation system using extract of rubber seeds in *L. calcarifer* resulted different cortisol levels. In the wet transportation system, the swimming movement of the fish seemed calm at the bottom of the plastic bags. The stress reaction of *L. calcarifer* could be seen by changes at the cortisol levels. The highest corticol levels was observed in the case of 6 hours wet transportation in treatment RS 100 ppm (238.29±11.85 μg dL^{-1}) and the lowest was found in treatment RS 90 ppm (207.23±2.88 μg dL^{-1}) which is not significantly different from the treatment CO 5 ppm (221.64±1.92 μg dL^{-1}). While the highest corticol levels sea bass 6 hours dry transportation in treatment RS 70 ppm (620.99±9.86 μg dL^{-1}) and the lowest found in treatment RS 100 ppm (444.53±27.95 μg dL^{-1}) which is significant different from the treatment CO 5 ppm (503.78±13.73 μg dL^{-1}). Cortisol levels of *L. calcarifer* for 6 hours of transportation showed significant differences ($p<0.05$) between wet and dry transportation. The highest corticol levels of *L. calcarifer* in 12 hours wet transportation was found in treatment RS 100 ppm (377.28±2.53 μg dL^{-1}) and the lowest was found in treatment RS 90 ppm (324.12±3.37 μg dL^{-1}). Whereas at 12 hours dry transportation occurs overall death, so the cortisol data was not obtained (Figure 2). Cortisol levels in the 6 hour dry transportation was highest than in wet transportation. Cortisol level in 6 hour dry transportation different significant with addition of clove oil, while cortisol level in 12 hour transportation showed no significant different with clove oil supplement.

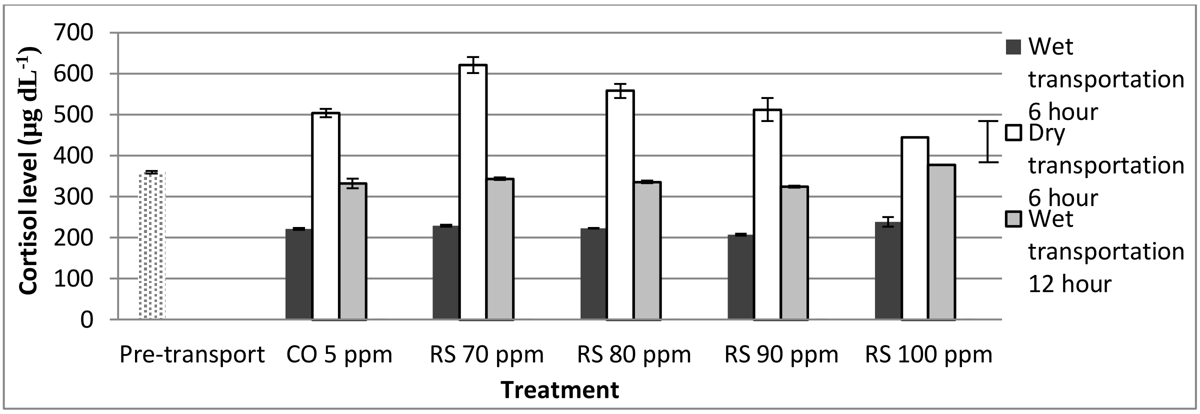

Figure 2. Cortisol levels of *Lates calcarifer* for 6 and 12 hours of transportation using rubber seed extract. Values represent the mean ± SD of three replicas. Clove Oil (CO); Rubber seed (RS).

Survival rate. The effect of using rubber seed extract in wet transportation ensured 100% fish survival for 6 and 12 hours transportation. While in dry transportation, the effect of rubber seed extract showed the highest survival (43%) in treatment RS 100 ppm and the lowest survival (7%) in treatment RS 70 ppm for 6 hours of transportation. The rubber seed extract supplement showed an increase in survival rate in each treatment (Figure 3).

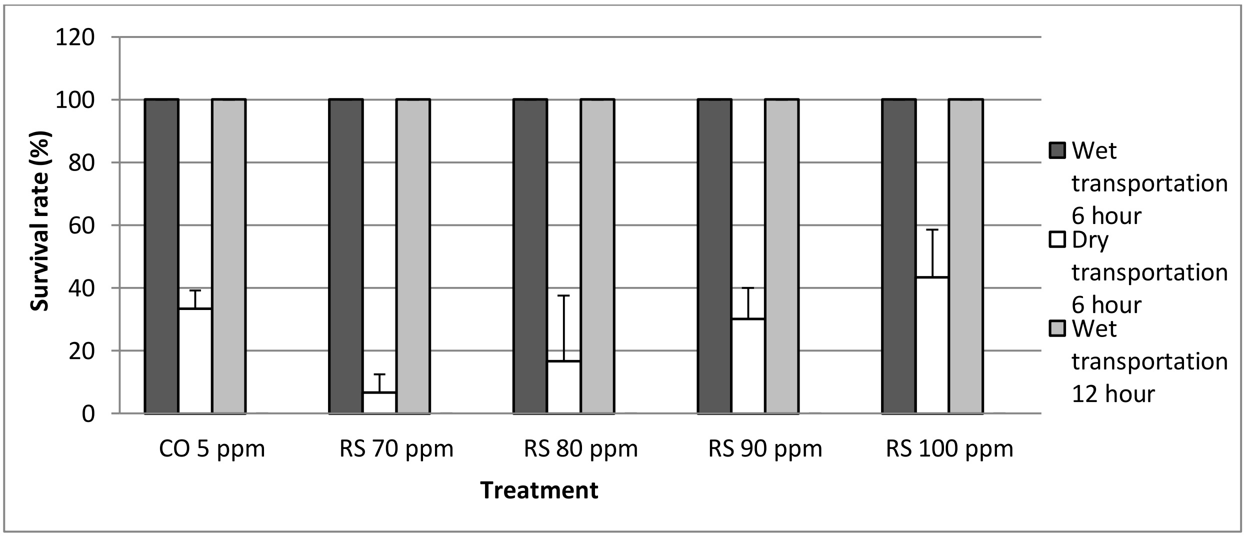

Figure 3. Survival rate of *Lates calcarifer* for 6 and 12 hours of transportation using rubber seed extract. The rubber seed extract supplement showed an increase in survival rate in each treatment. Values represent the percentage ± SD of three replicas. Clove Oil (CO); Rubber seed (RS).

Discussion. Improved efforts to cultivate abundantly in the marketing of fresh seeds and fish must be offset by the proper treatment process. The proper and kind handling of transportation provides the basis for the high value of selling products and reduces the mortality rate of fish (Gomes et al 2003). In the wet transportation system, the fish survive in a low metabolic state that is assisted by the available oxygen. So that the use of wet transportation systems could be used successfully for higher quantity and longer distances (Junianto 2003). As for the dry transportation, the fish survive by relying on media and environmental humidity (Sufianto 2008). Dry transportation is used either in a commodity that has an added respiratory apparatus such as the lobster have a labyrinth (Sandrayani et al 2013) and eels that have breathing apparatus of a thin, slimy skin located in the oral cavity (Topan & Riawan 2015).

In general, live fish transportation causes fish to experience high levels of stress caused by many factors which consist of mechanical changes in temperature and water chemistry fluctuations. Lisdiana (2012) stated that the stress level improve ACTH (adrenocorticotropin hormone) secretion, elevating cortisol level production which is a stress hormone that begins with the release of CRF (corticotropin releasing factor). CRF will stimulate the pituitary gland to release a variety of adrenaline hormones. Strong stressors are received by hypothalamus which will increase CRF releasing, pituitary stimulating and secreting cortisol by adrenal gland.

According to Little (2002) changes in physical behaviors and activities of fish are common indicators of environmental stressors as changes in the environment may accelerate metabolic rates. The findings of Little (2002) was in accordance with Cerdá-Reverter et al (1998) that *L. calcarifer* cortisol level is influenced by several environmental factors such as temperature and water quality. According to Ortuño et al (2001), the normal cortisol level of *L. calcarifer* is approximately 358 µg dL^{-1}. In the present study, cortisol level in dry transportation (444.53 µg dL^{-1}) was higher than cortisol levels in wet transportation (207.23 µg dL^{-1}). Due to the application of rubber seed extract with a given concentration of RS 90 ppm was affected the nervous system of fish and implicit cortisol level. Rubber seed extracted as anesthetic material during transportation will inhibit the release of catecholamine and cartecosteroid hormones so that there is a decrease in cortisol and glucose levels in fish because rubber seed is one of the natural anesthetics that have pharmacological properties due to its alkaloid function as an anesthetic. This condition was in accordance with Septiarusli et al (2012) who reported that the use of anaesthesia was aimed to suppress the metabolism activity as well as reducing stress risk.

The applied rubber seed extract in 12 hour wet transportation showed an increase in cortisol levels in each treatment. It is possible, because rubber seed extract has several compounds that have a negative impact as an anesthetic material. So if the concentration is too high, it will cause fish stress due to the inability to tolerate compounds that enter the body. This condition was corresponded with Selye (1936) who also reported that chemical changes in blood happen 6-48 hours after fish getting anaesthesized, such as cortisol level will increase in 12 hours.

In dry transportation systems, cortisol levels increase 2 times higher than in the wet transportation, which indicates that dry transportation, causes excessive stress. High cortisol levels of *L. calcarifer* during transport are thought to be due the possibility that concentration used on dry transportation is less effective, so fish become conscious fast and *L. calcarifer* don’t have breathing apparatus that can help when the environment oxygen level is low. The metabolism of aware fish will increase and oxygen demand for respiration will also increase, so that if the oxygen availability for fish is low, then the fish will gradually weaken and then death will occur (Abid et al 2014). Oxygen consumption is an indicator of respiration that also shows energetic metabolism. The rate of *L. calcarifer* oxygen consumption is high 0.71 mg O_2/g hour (Kadek 2013).

Conclusions. Studies have shown that dry transportation has a lower survival rate than wet transportation, which can be seen from high cortisol levels during transportation. Therefore, dry transportation requires attention to the species of fish used.

Acknowledgements. Thanks to Government research center for marine aquaculture Situbondo (BPBAP) for fish and facilities provided, and to Laboratory Anatomy and Laboratory Microbiology Faculty Fisheries and Marine University Airlangga Surabaya for facilities provided.

References

Abid M. S., Masithah E. D., Prayogo, 2014 Potensi senyawa metabolit sekunder infusum daun durian (*Durio zibethinus*) terhadap kelulushidupan ikan nila (*Oreochromis niloticus*) pada transportasi ikan hidup sistem kering. Jurnal Ilmiah Perikanan dan Kelautan 6:1-7.

Begg K., Pankhurst N. W., 2004 Endocrine and metabolic responses to stress in a laboratory population of the tropical damselfish *Acanthochromis polyacanthus*. Journal of Fish Biology 64:133-145.

Cerdá-Reverter J. M., Zanuy S., Carrillo M., Madrid J. A., 1998 Time-course studies on plasma glucose, insulin, and cortisol in sea bass (*Dicentrarchus labrax*) held under different photoperiodic regimes. Physiology and Behavior 64(3):245-250.

Effendi M. I., 1997 Metode Biologi Perikanan. Yayasan Pustaka Nusantara. Yogyakarta, Indonesia, 112 p.

Gomes L. C., Araujo L. C. A. R. M., Roubach R., 2003 Effect of fish density during transportation on stress and mortality of juvenile tambaqui *Colossoma macropomum*. Journal World Aquaculture Society 34:76-84.

Haditomo A. H. C., Rejeki S., Ardiansyah M. F., 2014 Kajian pemberian minyak cengkeh pada kepadatan yang berbeda terhadap kelulushidupan dan kadar glukosa darah benih nila. Seminar Nasional Hasil Perikanan dan Kelautan 2:2-9.

Hanggono B., 2006 Aplikasi Minyak Cengkeh Sebagai Obat Bius Ikan Kakap Putih (*Lates calcarifer* Bloch). Jurnal perikanan Indonesia 8:9-16.

Hanley C. S., Clyde V. L., Wallace R. S., Paulmurphy J., Patterson T. A., Keuler N. S., Sladky K. K., 2010 Effects of anesthesia and surgery on serial blood gas values and lactate concentrations in yellow perch (*Perca flavescens*), walleye pike (*Sander vitreus*), and koi (*Cyprinus carpio*). Journal of the American Veterinary Medical Association 236:1104–1108.

Hasan H., Raharjo E. I., Zamri S., 2016 Respon Pemberian Dosis Minyak Sereh (*Cymbopogon citratus*) untuk Anestesi Ikan Botia (*Chromobotia Macracanthus* Bleeker) dengan Metode Transportasi Tertutup. Jurnal Ruaya 4(2):3-9.

Hendra S. N., 2012 Pemingsanan lobster air TAWAR (*Cherax quadricarinatus*) dengan ekstrak akar tuba (*Derris elliptica* Roxb. Benth) dan kelulusan hidupnya selama penyimpanan dalam media serbuk gergaji. Thesis, Bogor Peertanian Institute, Bogor, Indonesia, 56 p.

Junianto, 2003 Teknik Penanganan Ikan. Penebar Swadaya. Jakarta, Indonesia.

Kadek R. N., 2013 Pengaruh Salinitas terhadap Kadar Glukosa Darah dan Laju Metabolisme pada Pendederan Ikan Kakap Putih (*Lates calcalifer*). Thesis, Bogor Peertanian Institute, Bogor, Indonesia, 35 p.

Lim L. C., Dhert P., Sorgebos P., 2003 Recent developments and improvements in ornamental fish packaging system for air transport. Aquaculture Research 34:923-935.

Lisdiana, 2012 Regulation of cortisol in stress and addiction conditions. Biosantifika 4(1):20-22

Little E. E., 2002 Behavioral measures of environmental stressors in fish. In: Biological indicators of stress in fish. Adams S. M. (ed), 2nd edition, American Fisheries Society, Bethesda, 431 p.

Madziga H. A., Sanni S., Sandabe, 2010 Phytochemical and elemental analysis of *Acalypha wilkesiana* leaf. Journal of American Science 6:510-514.

Martínez-Porchas M., Martínez-Córdova L. R., Ramos-Enriquez R., 2009 Cortisol and glucose: Reliable indocators of fish stress? Pan-American Journal of Aquatic Sciences 4(2):158-178.

McEwen B. S., Wingfield J. C. 2010 What's in a name? Integrating homeostasis, allostasis and stress. Hormones and Behavior 57(2):105-111.

McFarland W. N., 1959 A study of the effects of anaesthetics on the behaviour and physiology of fishes. Publication of the Institute of Marine Science 6:22–55.
Ortuño J., Esteban M. A., Meeguer J., 2001 Effects of phenoxyethanol on the innate immune system of gilthead seabream (*Sparus aurata* L.) exposed to crowding stress. Veterinary Immunology and Immunopathology 89:29-36.
Pramod P. K., Ramachandran A., Sajeevan T. P., Thampy S., Pai S. S., 2010 Comparative efficacy of MS-222 and benzocaine as anaesthetics under simulated transport conditions of a tropical ornamental fish *Puntius filamentosus* (Valenciennes). Aquaculture Research 41(2):309-314.
Pratama A., Buchari D., Sumarto, 2017 Uji Transportasi Sistem Kering Ikan Jelawat (*Leptobarbus hoevenii*) dengan menggunakan ekstrak hati batang pisang. Jurnal Online Mahasiswa 1:6-19.
Ross L. G., Ross B., 2008 Anesthetic and sedative techniques for aquatic animals. 3rd edition, Blackwell Publishing, United Kingdom, 222 p.
Sandrayani, Salnida Y. L., Ayu A. D., 2013 Pengaruh Media Pengisi terhadap Kelangsungan Hidup Udang Vaname (*Litopenaeus vannamei*) pada Transportasi Sistem Kering. Jurnal Perikanan Unram 1:1-8.
Selmar D., Lieberei R., Biehl B., Voigt J., 1987 *Havea* Linamarase: A Nonspecific β-Glycosidase. Plant Physiology 83:557-563.
Selye H., 1936 A syndrome produced by diverse nocuous agents. Nature vol. 138, p. 32.
Septiarusli I. E., Haetami K., Mulyani Y., Danar D., 2012 Potensi Senyawa Metabollit Sekunder dari Ekstrak Biji Buah Keben (*Barringtonia asiatica*) dalam Proses Anestesia Ikan Kerapu Macan (*Ephinephelus fuscoguttatus*). Jurnal Perikanan dan Kelautan 3:295-299.
Sink T. D., Lochmann R. T., Fecteu K. A., 2008 Validation use and disadvantages of enzim-linked immunosorbent assay kits for detection of corticol in channel catfish, largemouth bass, red pacu and gold shiners. Fish Physiology and Biochemistry 34:95-101.
Sufianto, 2008 Uji Transportasi Ikan Mas Koki (*Carrasius auratus* L.) Hidup Sistem Kering Dengan Perlakuan Suhu dan Penurunan Konsentrasi Oksigen. Thesis, Sekolah Pasca Sarjana, Bogor Pertanian Institute, Bogor, Indonesia.
Topan M., Riawan N., 2015 Budidaya Belut dan Sidat. Available at: https://books.google.co.id/books?idbudidaya+belut+dan+sidat. (Accessed 16-04-2019).
Velisek J., Svobodova Z., Piackova V., 2005 Effects of clove oil anaesthesia on rainbow trout (*Oncorhynchus mykiss*). Acta Veterinaria Brno 74:139-146.
Wildan A., Devina I. A., Indah H., 2012 Oil Extraction process from solid waste rubber seed by soxhletation and extraction solvent by stirring methods. In: International Conference on Chemical and Material Engineering 12–13 September 2012, ISBN: 978-602-097-281-7, Grand Candi Hotel, Semarang Indonesia.

Received: 01 May 2019. Accepted: 29 August 2019. Published online: 26 September 2019.
Authors:
Dhita Maulida Saputri, Airlangga University, Faculty of Fisheries and Marine, School of Aquaculture, Indonesia, 60115 Surabaya, e-mail: maulidadhita@gmail.com
Megananda Paradewi, Airlangga University, Faculty of Fisheries and Marine, School of Aquaculture, Indonesia, 60115 Surabaya, e-mail: meganandaparadewi@gmail.com
Laksmi Sulmartiwi, Airlangga University, Faculty of Fisheries and Marine, Department of Marine, Indonesia, 60115 Surabaya, e-mail: laksmi-s@fpk.unair.ac.id
Prayogo, Airlangga University, Faculty of Fisheries and Marine, Department of Fish Health Management and Aquaculture, Indonesia, 60115 Surabaya, e-mail: prayogo@fpk.unair.ac.id
Hari Suprapto, Airlangga University, Faculty of Fisheries and Marine, Department of Fish Health Management and Aquaculture, Indonesia, 60115 Surabaya, e-mail: hari-s@fpk.unair.ac.id

How to cite this article:
Saputri D. M., Paradewi M., Sulmartiwi L., Prayogo, Suprapto H., 2019 Cortisol levels in Asian sea bass (*Lates calcarifer*) as response to stress in different transportation systems using rubber seed (*Hevea brasiliensis*) anesthetic. AACL Bioflux 12(5):1524-1530.