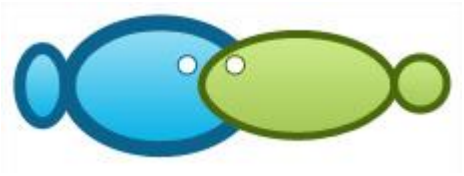

The effect of *Cosmos caudatus* extract on the survival rate of *Litopenaeus vannamei* post larvae against salinity

Ahmad Romadhoni, Sri Subekti, Kismiyati

Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia.
Corresponding author: S. Subekti, sriisubektiisrii@gmail.com

Abstract. Differences of salinity in fishponds could influence the survival rate of *Litopenaus vannamei* in the post larval life stage. They can cause stress, inhibit growth, and can even lead to the death. However, this condition can be overcame by using *Cosmos caudatus* extract, which has the potential to reduce oxidative stress in *L. vannamei*. The aim of this study was to determine the effect of *Cosmos caudatus* extract on the survival rate of *L. vannamei* post larvae. The effects were observed under salinity stress with the purpose of knowing the optimal dose of the extract that can be used. This study used a completely randomized design. There were 5 treatments with 4 replications of each treatment. The treatments consisted of several doses of *C. caudatus* extract, namely 7 mL, 14 mL, 21 mL, negative control without *C. caudatus* extract, and positive control with vitamin C. The data was analyzed using the ANOVA test. The results show that a dose of 14 mL of *C. caudatus* extract was the best dose to improve the survival of *L. vannamei* post larvae. It can be concluded that *C. caudatus* extract could improve the survival rate of *L. vannamei* post larvae under salinity stress.
Key Words: extract, maintenance, salinity, shrimp, stress.

Introduction. *Litopenaeus vannamei* is one of the shrimp species commonly used in the aquaculture industry. This species has some advantages including rapid growth, easy maintenance, high economic value, and availability for intensive cultivation (Bachère 2000). In *L. vannamei* farming in Latin America, the health of seeds is a major factor in the production of post larvae with a better survival rate (FAO 2003). These healthy seeds can be selected by their ability to survive in poor environmental conditions and to withstand changes in salinity.

Indonesia is a country with a diverse topography. This characteristic causes each region to have a water source with a variety of salinity conditions, including water sources used in ponds for vannamei shrimp cultivation. Ponds close to the sea tend to have higher salinity, whereas areas far from the sea tend to have lower salinity. Among all physico- chemical factors of water, salinity has a large influence on the metabolism, growth, and survival of vannamei shrimp (Ponce-Palafox et al 1997).

Changes in the salinity of the water where *L. vannamei* is farmed could induce stress, thereby stimulating the increase of free radical formation (ROS). Oxidative stress occurs when the formation of free radicals exceeds the ability of the body to protect itself against free radicals (KKP 2013). If the oxidative stress is not immediately overcame, it can cause the death of *L. vannamei*. Probiotics are used to overcome stress issues. Provision of probiotics in the maintenance of vannamei shrimp post larvae (PL) can increase the survival rate (SR), growth rate (GR), and the beneficial microbes in the body of the shrimp (Nimrat et al 2011). No PL died when *L. vannamei* were reared in water with a temperature lowered from 30 to 2°C, at a rate 0.1°C min^{-1}. PL had significantly lower mortality rates compared to the controls, when suddenly placed from fresh water in salt water with 60 ppt salinity (Liu et al 2010).

The high differences in salinity between ponds and selective rearing cause PL to experience stress, which results in slow growth or even death. Therefore, for improving the quality of *L. vannamei* PL, it is necessary to provide additional nutrients in order to

improve the immune system, so that they can withstand significant changes in salinity in the cultivation environment. Nowadays, there are many types of plants that are used to improve the immune system (Liu et al 2011) and food conversion ratio (FCR) of shrimp. Immunostimulants can neutralize free radicals and other polluting factors in the cultivation environment (Yang et al 2015). In addition, the use of extracts can cause shrimp to become resistant to *Vibrio alginolyticus* (Fu et al 2007). One type of plant that can be used as an additional nutrient source is *Cosmos caudatus*.

C. caudatus originates from the tropical region of Central America and has spread to almost all tropical countries in the world (Shui et al 2005). *C. caudatus* contains flavonoids, ascorbic acid (vitamin C), phenols, and carotenoids (Andarwulan et al 2012). These substances are a source of antioxidants that can reduce oxidative stress, and potentially inhibit pathogens (Shui et al 2005).

Herbal plants were recommended as alternative foods that promote growth, health and defense systems of fish, being safer, effective, and biodegradable (Syahidah et al 2015). *C. caudatus* contains some types of antioxidants that can increase the immunity of *L. vannamei* PL, further increasing the SR when exposed to salinity stress after being stocked into larger ponds. *C. caudatus* is also a plant that is easy to obtain and has a cheap price, so it can be easily used by farmers. Therefore, this study aimed to determine the effect of *C. caudatus* extract on the SR of *L. vannamei* PL under salinity stress and to determine the optimal dosage to be used.

Material and Method. This research was conducted from July 1, to July 20, 2015, in the laboratories of the Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia. The experiment used a completely randomized design with 5 treatments and 4 replications. The treatments consisted of several doses of *C. caudatus* extract, with a concentration of 10%. The treatments are as follows: P0 (without administration of *C. caudatus* extract); P1 (7 mL of extract per 1 kg of commercial feed); P2 (14 mL of extract per 1 kg of commercial feed); P3 (21 mL of extract per 1 kg of commercial feed); and P4 (73 mg of vitamin C per 1 kg of commercial feed).

The tools used in this study were 20 glass aquariums (50x30x40 cm), aerator, fishing nets, thermometer, pH meter, dissolved oxygen (DO) meter, refractometer, drop pipette, and a measuring cup. The materials used in this study were *C. caudatus* extract, *L. vannamei* PL, and PL feed.

The *C. caudatus* extract used in this study was obtained from leaves with a wet weight of 500 g. Lyophilization was carried out to obtain dry *C. caudatus*. 125 g of dried leaves were measured. The drying was conducted to prevent damage to substances sensitive to heat. The dried leaves obtained from the lyophilization process were crushed and sieved (mesh size from 200 to 300 μm). The small particles of the leaves can maximize the dissolution process during extraction. For each 50 mg of dry powder, 2.5 mL of 95% ethanol were added (Andarwulan et al 2010) and left to soak for 12 hours. The immersion process was carried out 3 times, so that all substances in the leaves could be entirely dissolved. The liquid from the soaking *C. caudatus* is concentrated using a rotary evaporator. 10 mL of concentrated leaf extract was mixed with distilled water, until it reached a volume of 100 mL and could be sprayed on the feed provided to the shrimp. The determination of the extract dose was based on its vitamin C content, of 108.83 mg/100 g of wet weight of *C. caudatus* (Andarwulan et al 2010).

L. vannamei post larvae was obtained from loggers in Sidayu Village, Gresik Regency, who acquired the larvae from the hatchery in Paciran Village, Lamongan Regency. The transportation to the study site was carried out at the time the vannamei shrimp were 20 days old, then acclimatization was done for another day. The study was conducted from the age of PL of 21 days to 30 days.

The feed used in this study was standard commercial feed with a crude protein content of 35% (Center of Marine and Fisheries Extension 2011) and particle size of 200-300 μm (FAO 2003).

The water was first placed in a reservoir and aerated, so it decanted and the DO level increased. To obtain the salinity needed for research, seawater was mixed with

fresh water, then measured using a refractometer to check the salinity concentration before use.

This study was carried out in several stages, namely producing the extract and adding it to the feed, feeding and maintenance, and the salinity stress tests. The research variables consisted of independent variables, dependent variables, and control variables. The independent variable in this study was the dose of *C. caudatus* extract. The dependent variable in this study was the survival rate during the maintenance period, when salinity stress occurred. The control variables in this study were the age, species of shrimp, and water salinity.

The main parameter observed in the study was the SR during salinity stress. The supporting parameters observed were water quality parameters, such as DO, pH, salinity, temperature, ammonia levels, and the number of PL that were stressed. Stressed shrimp were characterized by changes in body color to pale white and swimming near the surface of the water. Salinity stress tests were carried out on *L. vannamei* PL 21, with an initial salinity of 20 ppt, water salinity being reduced by 2 ppt per day until reaching 2 ppt at the end of the study. The fresh water source at the study site had a salinity of 2 ppt. The SR calculations were conducted daily before the salinity was lowered, from the beginning to the end of the maintenance period. Water quality measurements were carried out every day at 08.00 AM (Indonesian Western Standard Time).

The data were analyzed using ANOVA. Duncan's Multiple Distance test was carried out (95% confidence level) to find out the difference between treatments (Kusriningrum 2008).

Results and Discussion. The administration of *C. caudatus* extract produces significant differences in the SR of vannamei shrimp PL at 2 ppt, 14 ppt and 16 ppt salinity, when compared with controls (Table 1).

Table 1

Results of ANOVA calculation of *Cosmos caudatus* extract

Age (days)	*Salinity (ppt)*	*p value*
PL21	20	0.438
PL22	18	0.129
PL23	16	0.002*
PL24	14	0.048*
PL25	12	0.192
PL26	10	0.293
PL27	8	0.364
PL28	6	0.341
PL29	4	0.104
PL30	2	0.000*

Note: * - significantly different ($p<0.05$); PL - post larva.

The administration of *C. caudatus* extract had a significant effect on the survival of PL at salinities of 2 ppt, 14 ppt and 16 ppt, compared to control.

The content of vitamin C in the 14 mL extract with a concentration of 10% of 500 g of *C. caudatus* is equivalent to the vitamin C requirement of shrimp larvae, which is 73 mg kg^{-1} of feed (Moe et al 2004).

At salinities of 20 ppt, 18 ppt, 12 ppt, 10 ppt, 8 ppt, 6 ppt, and 4 ppt, there were no significant effects of the extract on the SR of *L. vannamei* PL. The SR differences between treatments are presented in Table 2.

At the end of the study, when the salinity was lowered to 2 ppt, the highest SR (51%) was obtained from the treatment with a 14 mL extract dose. The highest SR was observed in the treatment with 14 mL extract added to the feed, the extract dose increasing the SR of *L. vannamei* PL.

Table 2

Average survival rate of *Litopenaeus vannamei* post larvae

Age (days)	*Salinity (ppt)*	*Control (-)*	*7 mL extract per kg feed*	*14 mL extract per kg feed*	*21 mL extract per kg feed*	*Control (+)*
PL21	20	99^{a}	100^{a}	100^{a}	100^{a}	100^{a}
PL22	18	92^{a}	97^{ab}	98^{b}	98^{b}	95^{ab}
PL23	16	80^{a}	94^{b}	95^{b}	96^{b}	86^{a}
PL24	14	74^{ab}	88^{b}	75^{ab}	88^{b}	72^{a}
PL25	12	60^{a}	74^{a}	69^{a}	77^{a}	64^{a}
PL26	10	53^{a}	62^{a}	60^{a}	65^{a}	54^{a}
PL27	8	52^{a}	59^{a}	56^{a}	56^{a}	52^{a}
PL28	6	50^{a}	54^{a}	51^{a}	53^{a}	51^{a}
PL29	4	50^{ab}	53^{b}	51^{ab}	53^{b}	47^{a}
PL30	2	37^{a}	48^{c}	51^{c}	50^{c}	42^{b}

Note: PL - post larva; different superscript letters in the same row show significant differences ($p<0.05$).

In a previous study, it was observed that the SR of *L. vannamei* PL1 in a salinity of 18 ppt after 30 minutes was 64%, with limits between 54.2% and 67.6%. A salinity of 3 ppt for PL20 produced a SR between 32.7% and 42.3%, in a period of 30 minutes (Racotta et al 2004).

C. caudatus extract has a significant effect in increasing the SR of *L. vannamei* PL under salinity stress. This occurs due to various kinds of antioxidants, such as the total flavonoid content of 52.19 mg per 100 g wet weight, total phenol content of 342.06±0.37 mg gallic acid equivalents (GAE) per 100 g wet weight, total anthocyanins (0.78±0.05 mg per 100 g wet weight), β-caroten (1.35±0.03 mg per 100 g wet weight) and total carotenoid (9.55±0.27 mg β-carotene equivalents per 100 g wet weight) (Andarwulan et al 2012). The total ascorbic acid (vitamin C) is 108.83±0.50 mg per 100 g wet weight (Andarwulan et al 2012).

Various kinds of antioxidants will bind and decompose free radicals produced because of decreasing salinity, thus preventing the occurrence of oxidative stress in the body of the shrimp. Phenols, especially flavonoids and phenylopropanoids, can be oxidized by peroxidase and play a role in the decomposition of hydrogen peroxide (Michalak 2006). In addition, the phenolic hydroxyl group is a good H donor, clearing the reactive oxygen species and breaking the cycle of new free radical formation. Antioxidants can inhibit the oxidation of free radicals to lipids, proteins, and DNA, which can later cause abnormalities. Phenols also act as antioxidants by preventing the entanglement of enzymes in the formation of free radicals (Castellano et al 2012).

Carotenoids also have an antioxidant effect, by reacting with free radicals so that the resulting product is harmless, or by destroying the chain of free radical reactions (Dutta et al 2005). In addition, vitamin C and β-carotene are often referred to as antioxidant vitamins that can reduce oxidative damage to organisms (KKP 2013). However, in this case, the real effect only occurred at salinities of 16 ppt, 14 ppt, and 2 ppt, while at other salinities no differences were found.

Salinity has a large influence on metabolism, growth, and survival in cultured shrimp (Ponce-Palafox et al 1997). In this study, each level of salinity shows its respective influence on the survival of *L. vannamei*. Moreover, PL are more sensitive to environmental changes compared to adults.

On the first day of maintenance, there were no significant differences between treatments in terms of SR because the PL had not experienced salinity stress yet, so there were no mortalities. On the second day, the salinity was reduced from 20 ppt to 18 ppt. This caused stress, resulting in the death of PL, but the number was still very small, so there were no significant differences between treatments (Table 2). The low mortality is due to the antioxidants in the shrimp body, which are able to process the formed free radicals. In most animals, carotenoids are broken down to provide raw materials for the biosynthesis of vitamin A (deficiency can cause blindness) and has many important

physiological functions (antioxidant activity, immunostimulants, nutrition of embryos, protection against light, reduced molecular degradation of the eye) (Cazzonelli 2011).

When the salinity was reduced to 16 ppt, there were significant differences between positive and negative controls and the treatments. The administration of extract produced a higher SR. The positive control with vitamin C presented a higher mean value of SR when compared to the control. *L. vannamei* with more vitamin C can last longer than those without or with small doses of vitamin C, when the stressor is formalin, but the results are not significantly different (Moe et al 2004).

When the salinity was reduced from 12 ppt to 4 ppt, there were no significant differences between controls and treatments. This was due to the effect of stress, because a decrease in salinity produced more free radicals, which exceeded the antioxidant capacity of the *C. caudatus* extract. Oxidative stress occurs when the formation of free radicals exceeds the ability of the body to protect itself against free radicals (KKP 2013). If oxidative stress is not immediately overcome, it can cause the death of shrimp.

When the salinity is low, the mineral content of water also decreases, and minerals are needed for the development and survival of *L. vannamei*. As a result, vannamei PL administered mineral supplements will have a higher SR than those without mineral supplements (Roy & Davis 2010). The application of fertilizers in the form of K and Mg in the maintenance media can significantly increase the SR and GR in vannamei cultivation. Minerals that play a role in the osmoregulation processes, such as potassium (K), magnesium (Mg), sodium (Na) and chloride (Cl) have been recommended as supplements for the maintenance of *L. vannamei* in low salinity. In water with low salinity, the presence of minerals is not adequate and does not meet the mineral needs of *L. vannamei* (Roy & Davis 2010).

In addition, the decrease in salinity will cause the disruption of the osmoregulation system. This is because the concentration of body fluids is more hypertonic than external fluids. To overcome this condition, the external fluids will enter the body, thus causing excess fluid in the body. To remove excess fluid from the body of *L. vannamei*, the kidneys will work harder, so the energy needed for the osmoregulation process will be higher. If *L. vannamei* is unable to remove the excess water, it can cause lysis in the cells and result in death.

All the results suggest that the SR of *L. vannamei* PL in this study was strongly influenced by the salinity level. A decrease in salinity affects the mineral content, osmoregulation system, and antioxidant capacity. Reactive molecules in animals that arise due to the effect of decreasing salinity will cause very serious cell damage if the antioxidants in the body are unable to process the free radicals (Fang et al 2002).

In this study, most water quality parameters were generally in optimal condition for the maintenance of the *L. vannamei* PL: less than 0.1 mg L^{-1} ammonia; DO levels higher than 3 mg L^{-1}; 15-25 ppt salinity; 28.0-31.5°C temperature; and pH between 6.5-8 pH (Center for Marine and Fisheries Extension 2011). However, there were some parameters that were not suitable at all times. For example, the water temperature was sometimes low, reaching even 26.2°C. The low water temperature was caused by the influence of weather conditions on the location of the study, but efforts to stabilize the temperature were made, so the temperature was low only for a short time. The pH of the water was sometimes high, reaching 9.1, although most of the time it was in optimal ranges.

Conclusions. *C. caudatus* extract can improve the survival rate of *L. vannamei* post larvae under salinity stress conditions. This is due to various kinds of antioxidants found in *C. caudatus* extract that could bind and decompose free radicals caused by a decrease in salinity. The optimal extract dose that can be used to improve the survival of post larvae under salinity stress conditions was 14 mL.

Acknowledgements. We are grateful to the staff at the laboratory of the Faculty of Fisheries and Marine, Airlangga University, Surabaya city, Indonesia, for giving us permission to utilize the research facilities, advice, guidance, and support.

References

Andarwulan N., Batari R., Sandrasari D. A., Bolling B., Wijaya H., 2010 Flavonoid content and antioxidant activity of vegetables from Indonesia. Food chemistry 121(4):1231-1235.

Andarwulan N., Kurniasih D., Apriady R. A., Rahmat H., Roto A. V., Bolling B. W., 2012 Polyphenols, carotenoids, and ascorbic acid in underutilized medicinal vegetables. Journal of Functional Foods 4(1):339-347.

Bachère E., 2000 Shrimp immunity and disease control. Aquaculture 191(1-3):3-11.

Castellano G., Tena J., Torrens F., 2012 Classification of phenolic compounds by chemical structural indicators and its relation to antioxidant properties of *Posidonia oceanica* (L.) Delile. MATCH: Communications in Mathematical and in Computer Chemistry 67:231-250.

Cazzonelli C. I., 2011 Carotenoids in nature: insights from plants and beyond. Functional Plant Biology 38(11):833-847.

Dutta D., Chaudhuri U. R., Chakraborty R., 2005 Structure, health benefits, antioxidant property and processing and storage of carotenoids. African Journal of Biotechnology 4(13):1510-1520.

Fang Y. Z., Yang S., Wu G., 2002 Free radicals, antioxidants, and nutrition. Nutrition 18(10):872-879.

Fu Y. W., Hou W. Y., Yeh S. T., Li C. H., Chen J. C., 2007 The immunostimulatory effects of hot-water extract of *Gelidium amansii* via immersion, injection and dietary administrations on white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish and Shellfish Immunology 22:673-685.

Kusriningrum R. S., 2008 [Design of experiments]. Universitas Airlangga, Surabaya, 82 p. [In Indonesian].

Liu K. F., Chiu C. H., Shiu Y. L., Cheng W., Liu C. H., 2010 Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. Fish and Shellfish Immunology 28:837-844.

Liu X. L., Xi, Q. Y., Yang L., Li H. Y., Jiang Q. Y., Shu G., Wang S. B., Gao P., Zhu X. T., Zhang Y. L., 2011 The effect of dietary *Panax ginseng* polysaccharide extract on the immune responses in white shrimp, *Litopenaeus vannamei*. Fish and Shellfish Immunology 30:495-500.

Michalak A., 2006 Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies 15(4):523-530.

Moe Y. Y., Koshio S., Teshima S., Ishikawa M., Matsunaga Y., Panganiban A. J., 2004 Effect of vitamin C derivatives on the performance of larval kuruma shrimp, *Marsupenaeus japonicus*. Aquaculture 242(1-4):501-512.

Nimrat S., Boonthai T., Vuthiphandchai V., 2011 Effects of probiotic forms, compositions of and mode of probiotic administration on rearing of Pacific white shrimp (*Litopenaeus vannamei*) larvae and postlarvae. Animal feed science and technology 169(3-4):244-258.

Ponce-Palafox J., Martinez-Palacios C. A., Ross L. G., 1997 The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture 157(1-2):107-115.

Racotta I. S., Palacios E., Hernandez-Herrera R., Bonilla A., Perez-Rostro C. I., Ramirez J. L., 2004 Criteria for assessing larval and postlarval quality of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931). Aquaculture 233(1-4):181-195.

Roy L. A., Davis D. A., 2010 Requirements for the culture of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters : water modification and nutritional strategies for improving production. Avances en Nutricion Acuicula XI-Memorias del Decimo Primer Simposio Internacional de Nutricion Acuicola, pp. 61-78.

Shui G., Leong L. P., Shih P. W., 2005 Rapid screening and characterisation of antioxidants of *Cosmos caudatus* using liquid chromatography coupled with mass spectrometry. Journal of Chromatography B: Analytical Technologies in the

Biomedical and Life Sciences 827(1):127-138.
Syahidah A., Saad C. R., Daud H. M., Abdelhadi Y. M., 2015 Status and potential of herbal applications in aquaculture: a review. Iranian Journal of Fisheries Sciences 14(1):27-44.
Yang Q. H., Tan B. P., Dong X. H., Chi S. Y., Liu H. Y., 2015 Effects of different levels of *Yucca schidigera* extract on the growth and nonspecific immunity of Pacific white shrimp (*Litopenaeus vannamei*) and on culture water quality. Aquaculture 439:39-44.
***Center of Marine and Fisheries Extension, 2011 [Vannamei shrimp cultivation (*Litopenaeus vannamei*)]. Pusat Penyuluhan Kelautan dan Perikanan, 60 p. [In Indonesian].
***FAO, 2003 Health management and biosecurity maintenance in White shrimp (*Penaeus vannamei*) hatcheries in Latin America. Inland Water Resources and Aquaculture Service, FAO Fisheries Technical Paper No 450, Rome, 69 p.
***KKP, 2013 [East Java Province's marine and fisheries profile to support kp industrialization]. Center for Statistics and Information Data Secretariat General, Ministry of Maritime Affairs and Fisheries, 139 p. [In Indonesian].

Received: 12 September 2019. Accepted: 17 September 2019. Published online: 03 July 2020.
Authors:
Ahmad Romadhoni, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, 60115 Surabaya, Indonesia, e-mail: ahmad-ro-11@fpk.unair.ac.id
Sri Subekti, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, 60115 Surabaya, Indonesia, e-mail: sriisubektiisrii@gmail.com
Kismiyati, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, 60115 Surabaya, Indonesia, e-mail: kismiyati@fpk.unair.ac.id

How to cite this article:
Romadhoni A., Subekti S., Kismiyati, 2020 The effect of *Cosmos caudatus* extract on the survival rate of *Litopenaeus vannamei* post larvae against salinity. AACL Bioflux 13(4):1820-1826.