IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 10 Ver. I (Oct. 2015), PP 74-78
www.iosrjournals.org

The Dosage of Saline Water Dilution on The Osmoregulatory Capacity of Clownfish (*Amphiprionocellaris)*

Mauli Kasmi[1], SamsuAdi Rahman[2]
[1)]*StateAgricultural PolytechnicPangkep–Indonesia*
[2)]*Lecturerat theFaculty of Fisheries, University ofMuhammadiyahLuwuk-Indonesia*

Abstract,*The study aims to find out osmoregulatory capacity of clownfish (A. ocellaris) at different dilution to saline water media. The study will be beneficial as information source for ornamental fish farming and breeding. It is expected that the clownfish will be capable of tolerating a further environment (euryhaline). The study takes place from June to August, 2014 in Fish Quarantine Station Installation, Fishery Product Quality and Safety Control Class II Luwuk, Banggai Regency, Central Sulawesi Province. The data is analyzed in descriptive on fish's osmoregulatory capacity and behaviour. The findings indicate that clownfish in treatment A with salinity level at 7 ppt and treatment B with salinity level at 6 ppt, in the fourth week, maintains osmoregulation. Mortality, however, is found to clownfish in treatment C with saline water dilution at 5 ppt in the fourth week and treatment A and B in the fifth week (at 0 ppt). Water quality in final findings is considered appropriate for clownfish (Amphiprionocellaris) life where the temperature ranges from 26-28^{o}C and pH 7.5-8.*
Keywords:*Osmoregulation, Salinity, Clownfish, Growth, Survival rate*

I. Introduction

Clownfish is one of coral reef fishes that are overall reddish color, and many show white bars. It is known by its widespread distribution, especially in Indian and Pacific oceans. In general, it lives in rocky lagoons around coral reefs, or shallow coastal area of less than 50 meter of depth and in clear water [1].

Considering ornamental fish export that makes a very good prospect for Indonesia's economic development, it shall be built simultaneously by intensive pattern. Intensive culture combines high technology and higher use of inputs [5], [10]. Besides, ornamental fish rearing is not built well yet as the rearing is complicated. One of the effort to cope with the issue is by manipulating rearing media, i.e. through a process of making saline water concentration weaker or less where the ornamental fish or clownfish will be able to adapt under different conditions. Environmental manipulation is an alternative to preserve aesthetics of clownfish for timeless pleasure [8].

Fish adaptation to environmental effects refers to homeostasis where fish maintains stable condition in an active process against the effects. Homeostasis is organism's tendency to control and adjust fluctuation of its internal environment [21.

Salinity or brininess is amount of solid content of a kilogram of seawater. In such condition, all carbonate has been transformed into oxide, brom and yodium has been made equivalent to chlorine and oxidized organic matter. Directly, salinity of a media will affect osmotic pressure of fish's body fluids. Accordingly, the study aims to find out fish's adaptive capacity by osmoregulation process toward environmental change which is manipulated to some decreasing treatment in salinity [7]. Fish's adaptive capacity in particular salinity are categorized into stenohaline (fish that cannot tolerate a wide fluctuation in the salinity of water) and euryhaline (fishes are able to adapt to a wide range of salinities). The findings, in expectation, will find a way where clownfish can tolerate a wider range of salinities (euryhaline) [8].

According to previous study on clownfish's osmoregulation process, it found that the highest osmoregulation occurs at salinity level 5 ppt under salinity level decrease 100 ml fresh water/liter in one-day saline water. The study is tailed by salinity level decrease and increase by half-value 100 ml/liter [6].

To achieve fish farming goals or raise aesthetic value in *ornament*, varied fish farming and rearing aspects becomes directive absolute points to observe. One of the most important aspects is clownfish's osmoregulation process into fresh water [10]. The study aims to find out clownfish's (*A. ocellaris)*osmoregulatory capacity to different dilution of saline media. The study will be beneficial as information source for ornamental fish farming and breeding. It is expected that the clownfish will be capable of tolerating a further environment (euryhaline) [9].

II. Research Method

The study takes place from June to August, 2013 in Fish Quarantine Station Installation, Fishery Product Quality and Safety Control Class II Luwuk, Banggai Regency, Central Sulawesi Province. Research

instruments for the study are aquarium measuring 40x30x30 cm^2, aerator, aeration hose, aeration rocks, salinometer, pH meter, thermometer, infusion tube, scale, spotting pipette and measuring cylinder. Research materials consist of clownfish (*A. ocellaris)*, saline water, fresh water and pellet.

Test animal is clownfish (*A. ocellaris*) 1.5-2 cm in lenght and 0.9 gram in average weight caught from coral reef fishing area in Banggai Islands Regency waters.

Pellet is provided by "ad libitum feeding" referring to a condition when fish eats the pellet reluctantly after consuming the pellet, and feeding time frequency is twice per day.

Experimental design applied in the study is Completely Randomized Design (CRD) in 3 treatments: treatment A: dilution with 50 ml fresh water/liter, B: dilution with 100 ml fresh water/liter, C: dilution with 150 ml fresh water/liter and every treatment are repeated 3 times. Placement of every experimental unit is made randomized, and below is the layout after randomisation of experimental unit:

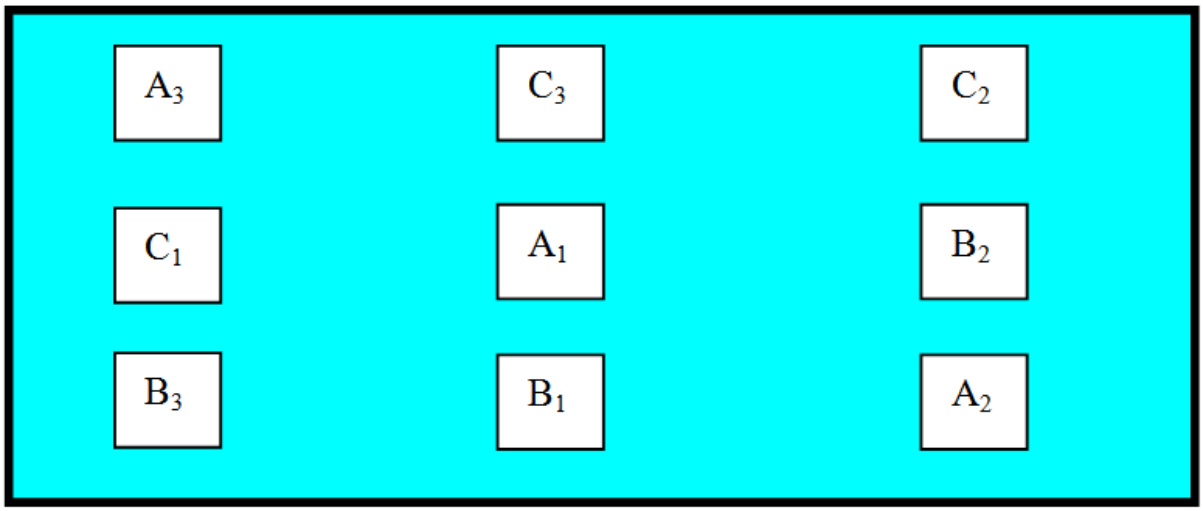

Figure 2. Placement of Experimental Units

Observed Variables

The study observes fish's osmoregulatory capacity and behaviour on daily basis. The dataof water qualitycovers temperature, salinity and pH measurements. Temperature and salinity measurements are carried out every morning and afternoon, while pH measurement is made weekly.

Test organism used in the treatment is acclimatized in aquarium containing water where the salinity is made equal, and this acclimatization is carried out for 3 days.

After test organism is adapted to the saline-equalized media, it is put into aquarium containing same population density, i.e. 5 fishes/10 liter. Feed wastage and fish's feces cleaning are carried out in so far as needed to maintain water quality in recirculation system. Pellet is provided by *ad libitum*, and feeding time frequency is made twice per day. Addition of water is conducted every day based on the dosage of fresh water for every treatment, and this addition of fresh water is by unlocking infusion tube slowly. The study lasts for 35 days, and during the days, data records are made. Data collection is acquired from fish' behavior and survival rate observation as listed in Table 1. To find out fish's osmoregulatory capacity and behavior, the data is analyzed in descriptive [11].

III. Research Findings And Discussion

Osmoregulatory Capacity of Clownfish

Table 2. Weekly Saline Water Dilution

Treatment	Initial Salinity Level 0	Salinity Level in Week 1	Salinity Level in Week 2	Salinity Level in Week 3	Salinity Level in Week 4	Salinity Level in Week 5
	Ppt	Ppt	ppt	ppt	Ppt	Ppt
A (50 ml fresh water/liter)	35	28	21	14	7	0
B (100 ml fresh water/liter)	35	27	20	13	6	0
C (150 ml fresh water/liter)	35	26	19	12	5	0

Based on the Table 2, salinity level decreases every week as the consequence of addition of fresh water. In initial fish distribution, salinity level of each treatment is 35 ppt. This salinity level setting aims to

observe fish's osmoregulatory capacity on the dosage of fresh water added on daily basis. Salinity level of 35 ppt is an optimal salinity level for marine aquatic organism, fish is in active motion or swim freely, operculum opens and closes normally. In week 1, dosage of salinity declines in each treatment; salinity in treatment A (50 ml fresh water/liter) is 28 ppt, B (100 ml fresh water/liter) is 27 ppt and treatment C (150 ml fresh water/liter) is 26 ppt. Declining salinity is the consequence of addition of fresh water subject to the dosage of each treatment. Salinity level in each treatment is still in normal level or tolerable for fish's life. In week 2, salinity level is declining in each treatment (A: 21 ppt, B: 20 ppt, C: 19 ppt) where these level is below clownfish tolerance. In a lower salinity level, clownfish begins osmoregulation process by hypotonic or hyperosmotic regulation pattern, i.e. an active regulation when the body fluids concentration is lower than concentration in media. In week 3, the salinity level declines into 14 ppt in treatment A, 13 ppt in treatment B and 13 ppt in treatment C. In the third week, salinity level is considered extreme for clownfish, but the fish is still able to adapt to such environment. According to [16] fish adaptation to such effects indicates homeostasis where fishes are maintaining stable condition through an active process against the effects. Homeostasis is organism's tendency to control and adjust fluctuation of its internal environment.

In week 4, salinity level in treatment A is 7 ppt, 6 ppt in treatment B and 5 ppt in treatment C. In treatment A and B, clownfish is still alive, but in treatment C, most of clownfishes are dead due to water osmotic pressure. In such salinity level, fishes are incapable of regulating osmotic pressure. In week 5, salinity level reaches 0 ppt, and all fishes in all treatments are dead.

According to [3] fish tends to live in media where the salinity level is feasible for fish body osmotic pressure. Fish's osmotic pressure is different from the environment so that this organism shall prevent water deficiency and abundance to keep biological processes in the body normal.

Fish's Behavior

Table 3. Clownfish (*A. ocellaris)*Behaviour in Every Treatment

Treatment	Initial Salinity Level 0	Salinity Level in Week 1	Salinity Level in Week 2	Salinity Level in Week 3	Salinity Level in Week 4	Salinity Level in Week 5
A (50 ml fresh water/liter)	Active	Active	Slow motion and fading colour	Up- and downward motion	Fishes are motionless and getting together	Died
B (100 ml fresh water/liter)	Active	Active	Slow motion and fading colour	Up- and downward motion	Fishes are losing their balance	Died
C (150 ml fresh water/liter)	Active	Active	Slow motion and fading colour	Up- and downward motion	Died	Died

Based on the table above, it displays fish's behavior in each treatment on weekly basis. All in treatment A (50 ml fresh water/liter), B (100 ml fresh water/liter) and C (150 ml fresh water/liter) refers to initial fish distribution where salinity level is still optimal, i.e. 35 ppt so that fish's swimming behaviour is normal and active.

In the first week, fish is still active in each treatment as such salinity level is tolerable for fish's life. According to [15] clownfish lives in salinity level ranging from 24 to 40 ppt. In week 2, clownfish shows psychological symptom, i.e. slow motion and fading colour. In week 3, fish's behavior in treatment A, B and C indicates up- and downward motion and weak swimming movement, and in the fourth week, most fishes are getting together in treatment A, fishes are losing their balance in treatment B and the worst, all fishes are dead in treatment C. And in week 5, fishes in treatment A and B are all dead. In short, fishes in treatment A and B survive until week 4 only, and they are all dead in week 5 when salinity level is 0 ppt as the consequence of fish' incapability to osmotic pressure they live within and intolerable to the water or media if it is zero saline. This survival rate depends on when the fish takes osmoregulation process, i.e. a condition where fish makes osmotic pressure inside and outside of the body balanced. Osmolarity scaling up relates to osmoregulatory mechanisms taking place within clownfish's body. The fish takes osmotic process for osmoregulation resulting in high energy spending for high-level osmoregulation process. High energy spending will consequently cause dead and impaired function of body organ due to high osmotic pressure.

According to [3], osmolarity of a medium constitutes determinant of osmotic performance in clownfish body. Osmolarity of a medium turns higher in accordance with salinity increase or decrease. It relates to imbalance concentration of dissolved ions between ions in the body and the medium. If ions dissolved in the body or medium turns higher or lower, osmotic concentration of the solution will turn higher so that it can cause higher osmotic pressure.

Clownfishes in each treatment are all dead if it is zero saline, i.e. in week 5 for treatment A and B, but the fishes are all dead as well in treatment C even if the salinity level is still 5 ppt. Accordingly, clownfish capacity limit to process osmoregulation ranges from 5 to 0 ppt. In such condition, fish's kidney is enlarged and

fish gills turns pale that it affects bodywork function of other tissue, and worse, disfunction of the tissue. According to [2], organism has different capacity to go through osmoregulation issue as a response toward osmotic changes in external environment. Concentration change tends to disrupt a stable internal condition. To deal with such issue, animal regulates osmotic pressure by decreasing osmotic gradient, like body fluids and fluids in environment. Fish is incapable of adapting to osmotic pressure difference between body fluids and environment. Consequently, it causes degradation or mortality.

Water Quality Parameter

Water quality measurement range during the study is made in list in Table 3.

Table 3. Range of Water Quality in Each Treatment during the Study

Treatment	Water quality	
	Temperature (^oC)	pH
A (50 ml fresh water/liter)	26 – 28	7.5-8
B (100 ml fresh water/liter)	26 – 28	7.5-8
C (150 ml fresh water/liter)	26 – 28	7.5-8

Table 3 indicates that water quality range in each treatment is still feasible for clownfish life where the temperature ranges 26-28^{o}C, and pH 7.5-8. It affirms statement of [12] that feasible temperature range for clownfish (*A. ocellaris*) rearing and ornamental fish ranges 20-29 °C. According to [14] feasible temperature for marine aquarium ranges 24-27^{0}C.

Further, [13] states that saline water is usually base with pH level above 7 as it contains base salt. The water with a lot of CO_2 generally contains pH below 7 and base. The degree of acidity (pH) in water ranging from 6.5-9.0 is feasible for fish life. And, according to Cust and Cox (1978), optimal pH for clownfish (*A. ocellaris*) growth is ranging from 7.2 to 8.3 or 8-8.3.

IV. Conclusion And Suggestion

Conclusion

According to the findings about the dosage of different saline water dilution on osmoregulatory capacity of clownfish (*Amphiprionocellaris*) are made in conclusion as follows:

1. Clownfish in treatment A with salinity level 7 ppt and treatment B with salinity level 6 ppt in week 4 is still able to process osmoregulation, but in week 4 in treatment C where the saline water dilution is 5 ppt, clownfish is dead. In week 5 or 0 ppt for both treatment A and B, all fishes are being mortal.
2. Water quality in research findings is still in feasible range for clownfish (*Amphiprionocellaris*) life where the temperature ranges from 26 to 28^{o}C and pH level is 7.5-8.

V. Suggestion

According to the findings, it requires further study on a lower saline water dilution, under 50 ml fresh water/liter to find out osmoregulatory capacity with a lower osmotic.

References

[1]. Affandi, T.K, Manik, B., Rosadi, M., Utomo., M., Senge., Adachi, dan Oki., 2002. Soil Erosion UnderCoffe Trees with Different Weed Management in Humid Tropical Hilly Area of Lampung. South Sumatera, Indonesia.

[2]. Agriwawasan, S., 2004. MarineOrnamental FishIndonesia. Penebar Swadaya. Jakarta.

[3]. Carlie, B., 2004. Amphiprion The Clownfishes. University of Texas at Austin.

[4]. Colt, J. 2006. Water quality requirements for reuse systems. Aquacultural Engineering 34 (2006) 143–156

[5]. Effendi, M.L., 1997. FisheriesBiology. Yayasan Pustaka Nusantara. Jakarta.

[6]. 11. Efendie, M. I., 1979. BiologicalMethodsIIFishingFishPopulation Dynamics. Faculty of Fisheries, Bogor Agricultural University. Bogor.Gaspersz, V., 1991. Metode Perancangan Percobaan. CV. Armico. Bandung.

[7]. Grouther, M. 2004. AmphiprionPercula (Lacepe, 1802) Http://Www. Australian museum.Com.(Nopember 2004).

[8]. Gufran, 2007. Water QualityManagementAquaculture. RinekaCipta. Jakarta.

[9]. Karim, M. Yusri. 2007. The Effect of Osmotic at Various Medium Salinity on Vitality of Female Mud Crab (Scylla olivacea). Vol. 14 No. 1. Th. 2007

[10]. Marshall, W.S., and Grosell, M. 2006. ion transport, osmoregulation, and acid-base balance. In the Physiology of Fishes, Evans, D.H., and Claiborne, J.B. (eds.). taylor and Francis Group. 601 pp

[11]. Poernomo, A. 2003. ShrimpAquacultureShrimpDitambak. Research ProjectPotentialEnergy Resources. Osealogiinstitutions. NationalLipi, Jakarta.

[12]. Putro dan Sunaryat, 1995. Tigergrouperhatchery. Agriculture department.The DirectorateGeneral of Fisheries. HallMarine Aquaculture. Lampung.

[13]. Randall, D., W. Burggren, & K. French. 2001. Animal Physiology: Mecahnisma and Adaptations. W.H. Freeman and Company. New York.

[14]. Setiawati, K.M., Wardoyo, D. Kusumawati, T. Ahmad., 2006. MaintenanceClownfish, Amphiprionocellarisin the FrameworkEffortsMarineOrnamentalFish Culture. Proceedings ofthe NationalConference onAquaculture 2005, Undip, 2006, Hal. 245-249.

[15]. Sidik, A.S., 1996. HydroponicsutilizationinWaterRecirculationAquacultureSystemClosed. ResearchinstitutionsMulawarman, Samarinda.
[16]. Spotte, S., 1992. Captive Seawater Fishes. University of Connecticut.Connecticut.
[17]. Steffens. 1989. Principles of Fish Nutrition. Homboldt. Universitas Berlin.
[18]. Susanto, H. 2003. AgriwawasanseriesOrnamental FishSeawater(Revised Edition Ke-3).PenebarSwadaya.
[19]. Susanto, H., 1990. OrnamentalfishSeawaterOrnamentalfishSeawater. Penebar Swadaya. Jakarta.
[20]. Takei dan Hirose, 2001. The Natriuretic Peptide System in Eel: A Key Endocrine System for Euryhalinity.
[21]. Tissera, K., 2010. Global trade in ornamental fishes. Souvenir Ornamentals Kerala-2010 Dept. of Fisheries Govt. of Kerala, pp. 35–38.
[22]. Tullock, J. H., 1998. Clownfishes and Sea Anemones. Barron's. Wireless Boulevard.
[23]. Zonneveld, N., E. A. Huisman dan J. H. Boon., 1991. Principles ofAquaculture.Gramedia Utama. Jakarta.