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Contributed Paper

Marine Fish Acclimatization Technique at Industrial Scale

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Abstract

The low survival rate is the main issue on marine ornamental fish trading due to improper acclimatization. So far, the drip-line method is the most practical method. However, the approach is not efficient and harmful for fish close to a threshold of death. This research introduced a new acclimatization method, combining pH and carbonate hardness regulation, allowing direct and safe fish transfer from contaminated packing water to the water system. The objective of this research was to increase the survival rate through pH and carbonate hardness regulation at an industrial scale. This research was conducted by factorial design. The low pH method was performed by lowering the pH of new and clean water, combined with two levels of carbonate hardness. Six species representing marine ornamental fish types were tested. There was no significant difference ($p>0.05$) on the survival rate among the species. The difference was somewhat correlated to the acclimatization method and the carbonate hardness level. The highest survival rate was performed by the low pH method (99%) and significantly higher ($p=0.000$) compared to a drip-line method (78.91%) and conventional method (44.07%). Regarding carbonate hardness, high carbonate hardness showed a significantly higher survival rate ($p=0.000$) than the normal carbonate hardness. Thus, the best method is the combination of low pH method and high carbonate hardness.

Keywords: low pH, carbonate hardness, ornamental fish, industrial scale, survival rate

1. Introduction

Indonesia is the most potential country in exporting marine ornamental fish. Despite the high volume (in number), the export value of Indonesian marine ornamental fish is low compared to other countries, like Singapore or Thailand. One of the most prominent problems in Indonesian products is the low survival rate at the destination. It is then important to ensure the safety and the good condition of the fish at the destination because dead fish at the destination would have accumulated all the additional costs [1]. The highest cost of commodities is actually not on the fishes itself but additional cost, especially the freight and documents charges.

Dead risks of marine ornamental fish are very high as the chain of custody in marine aquarium trading is very long [2]. One of the most important points that must be considered along the chain of custody is the acclimatization at each displacement. Inappropriate acclimatization method could lead to more loses of fish. In Indonesia, the loss of fish from the fishers to the fish exporters can reach 15%, even 50% when disabled or sick fish are counted [3].

Various acclimatization methods had been applied to overcome the fish mortality at the destination. The most updated and frequently used method is the drip-line method [4]. Compared to the conventional method, drip-line could decrease fish mortality [4]. However, this method needs a prolongation in the packing water until the water quality, equal to one of the waters of the system. The fish need to be taken out from the contaminated water packing, as most of the fish are normally dying (close to the threshold of death) because of the long transport. Therefore this method only suits the relatively fresh fish. Low pH adjustment method could overcome those problems because it allows fish to be taken out from the polluted packaging water and transferred directly to the new and clean water but acid. The fish would not experience any shock as the acidic or low pH environment will guide the nitrogen compound toward ionized ammonium (NH_4^+) which is relatively non-toxic and avoid the formation of non-ionized ammonia (NH_3) [5].

Low pH method has been tested at laboratory scale [6]. However, it was only limited to the individual test of 4 fish species, and the variable measured was only individual endurance. The experiment needs to be scaled up in number and species to achieve the industrial level that reflects the reality and represents all group of ornamental fishes. Besides, the method needs to be combined with stabilizing the environment treatment during the acclimatization process. The survival rate is the variable that describes more the reality compared to individual endurance due to extensive number and variability in marine environmental conditions as described in the previous research [6].

As mentioned above, besides the toxic ammonia compound, the fish need a stable environment during the acclimatization process, especially in terms of stable pH. The fluctuation of pH could harm the living organisms and could even kill the fish by an event known as the 'pH shock' [7]. High carbonate hardness treatment gives a more stable pH environment that will prevent the pH fluctuation. As with any buffer system, the pH is balanced by the presence of both a weak acid and its conjugate base, so that excess acid or base introduced to the system is neutralized.

The objective of this research was to improve acclimatization method by guiding harmful compound such as non-ionized ammonia (NH_3) shifted toward relatively nontoxic ionized ammonium (NH^{4+}) by stabilizing the environment of fish through pH adjustment by avoiding its fluctuation through increasing carbonate hardness.

2. Materials and Methods

2.1 Sample Preparation

The main materials used in this research were six species ornamental marine fish representing the six main groups of marine ornamental fish, ie. *Chromis viridis* representing damsel, *Halichoeres chloropterus* representing wrasse, *Aeoliscus strigatus* representing razorfish, *Amphiprion ocellaris* representing anemone fish, *Pomacanthus imperator* representing angel fish, and *Synchiropus splendidus* representing gobies, respectively. The number of fish was 30 per species per repetition. So the total number of fish used was 480 fishes per species or 2880 fishes for all species. They were small (S) size (according to the exporter standard), 2-3 cm for *Chromis viridis* and *Amphiprion ocellaris,* 4-6 cm for *Halichoeres chloropterus,* 6-7 cm for *Aeoliscus* *strigatus* 5-6 cm for *Pomacanthus imperator* and 3-4 cm for *Synchiropus splendidus*. The fish were collected from Pulau Seribu (Java Sea), Bali (Lombok Strait), Banyuwangi (Bali Strait), Garut (Indian Ocean), and Lampung (Sunda Strait). The fishes were collected using a net trap and scoop. The main tools used were the whole system for acclimatization procedure, i.e., conventional drip-line, and low pH adjustment owned by CV. Cahaya Baru, a fish and coral exporter, located in South Jakarta, Indonesia.

2.2 Experimental Design

Factorial design with two treatments was applied. The first treatment was acclimatization technique with three factors, i.e. conventional (temperature adjustment), drip-line (gradual adjustment), and low pH method (pH adjustment). The second treatment was the carbonate hardness with two factors (7^{o}dH unit, considered as a normal and 14^{o}dH unit, considered as high carbonate hardness). Carbonate hardness regulation was made by adding sodium bicarbonate ($NaHCO_3$) and arranged minimal one hour before the acclimatization procedure. After the arrangement, the carbonate hardness value was measured by a carbonate hardness test kit (test kit). For the normal carbonate hardness treatment, there was no arrangement of the system because the normal system's carbonate hardness was normally seven dKH.

2.3 Fish Acclimatization

The fishes received at the site were the fishes packed individually in a plastic bag in origin (Bali, Lampung, etc.) with a volume proportion of 1/3 water and 2/3 oxygene. The volume water in the bag was adjusted until 1 cm above the dorsal fin of the fish so the fish can swim freely. At the arrival, all the fishes (160 fishes per repetition per species) were conditioned at the site by keeping at the packing bags for approximately 2-3 days until fish achieved the treshold of death. At this stage, the fish looked passive and swam diagonally or stayed on the bottom. After the conditioning, the 180 fishes were distributed randomly according to the six treatments (3 acclimatization methods x 2 kH levels).

For the conventional method treatment, the bags with fish inside were floated (10-15 minutes) in the system of an aquarium containing new water (the series of 60 aquariums with size = 10 x 50 x 60 cm^3) to adjust the temperature. Then, the bags were opened, and the fishes were directly placed into the water system (pH: 8.0-8.1). For the drip-line method treatment, the bags were opened, and the new water from the aquarium was added slowly until the condition became the same as the system condition, especially in terms of pH (8.0-8.1). For the acclimatization with low pH treatment, first, the pH on the packing water (the dimension of packing water = 40 x 80 cm^2 containing 1/3 water and 2/3 O_2) was measured by pH meter and then, the pH of the system was lowered according to the pH level of packing water (common pH within 6.1-6.5). In lowering the pH, CO_2 gas was flowed to the water system. When the pH level on the acclimatization system was equal to the one of packing water, then fish (without water) from the packing bags was directly moved to the system. When the fish transfer was finished, the pH of the system was then slowly increased by adding O_2 gas until the pH system became normal (8.0-8.1).

2.4 Measurement of Survival Rate

The main observed variable was the survival rate (SR) and interpreted into pourcentage. The survival rate was the inverse of DOA (*Death on Arrival*) and DAA (*Death after Arrival*).

$$SR = \frac{N-(DOA+DAA)}{N} \; x100\%$$

Where:
SR: the survival rate
N: number of fish per treatment per repetition

(30 pieces)
DOA: Death on arrival (observed during three hours ofter the treatments)
DAA: Death after arrival (observed by the next day for three days). The other observation was done by monitoring the behaviours and physical conditions of fish during the acclimatization process.

3. Results and Discussion

The survival rate of fish was significantly different (p = 0.000) both acclimatization method treatments and carbonate hardness level treatments (Figure 1). The interaction between the two treatments was significant (p = 0.000), but at the tested level, the acclimatization method and carbonate hardness did not cross each other as depicted in Figure 2. Thus, the two treatments can be interpreted separately. For the acclimatization method, the low pH method showed that the highest survival rate, and was significantly different (p = 0.000) higher than the drip-line method (78.91%) and the conventional method (44.07%). The survival rate for the low pH method almost reaches 100%, especially at the high carbonate hardness level tested (99.44%), meaning that nearly all the fish survived.

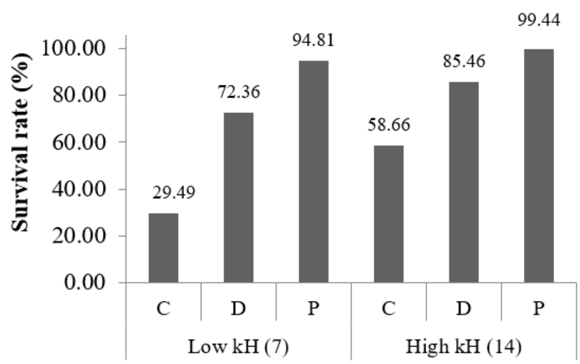

Figure 1. The survival rate of fish according to the acclimatization method (C: conventional; D:drip-line; P:low pH) and carbonate hardness degree (low KH: 7; high KH: 14).

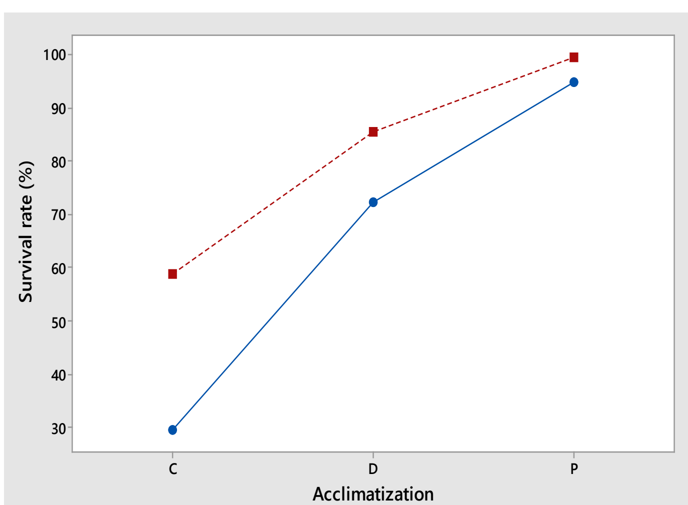

Figure 2. Interaction between acclimatization method treatments and carbonate hardness treatments (square: high carbonate hardness, dot: normal carbonate hardness, C: conventional, D: drip-line, and P: low pH).

According to carbonate hardness treatments, the overall results showed that the high carbonate hardness gave a higher survival rate compared to the normal carbonate hardness. High carbonate hardness increased 30% of the fish survival rate for the conventional method and 13% for the drip-line method. These results showed that the significant difference between the two carbonate hardness treatments.

In terms of fish species, all six fish types showed the same survival rate patterns, but a slight difference could be observed. For the low pH adjustment method, *Amphiprion ocellaris* held the lowest survival rate (Figure 3d), followed by *Pomacanthus imperator* (Figure 3e), and by *Halichoeres chloropterus* (Figure 3b), then by *Aeoliscus strigatus* (Figure 3c). *Chromis viridis* and *Synchiropus splendidus* held the highest survival rate for the

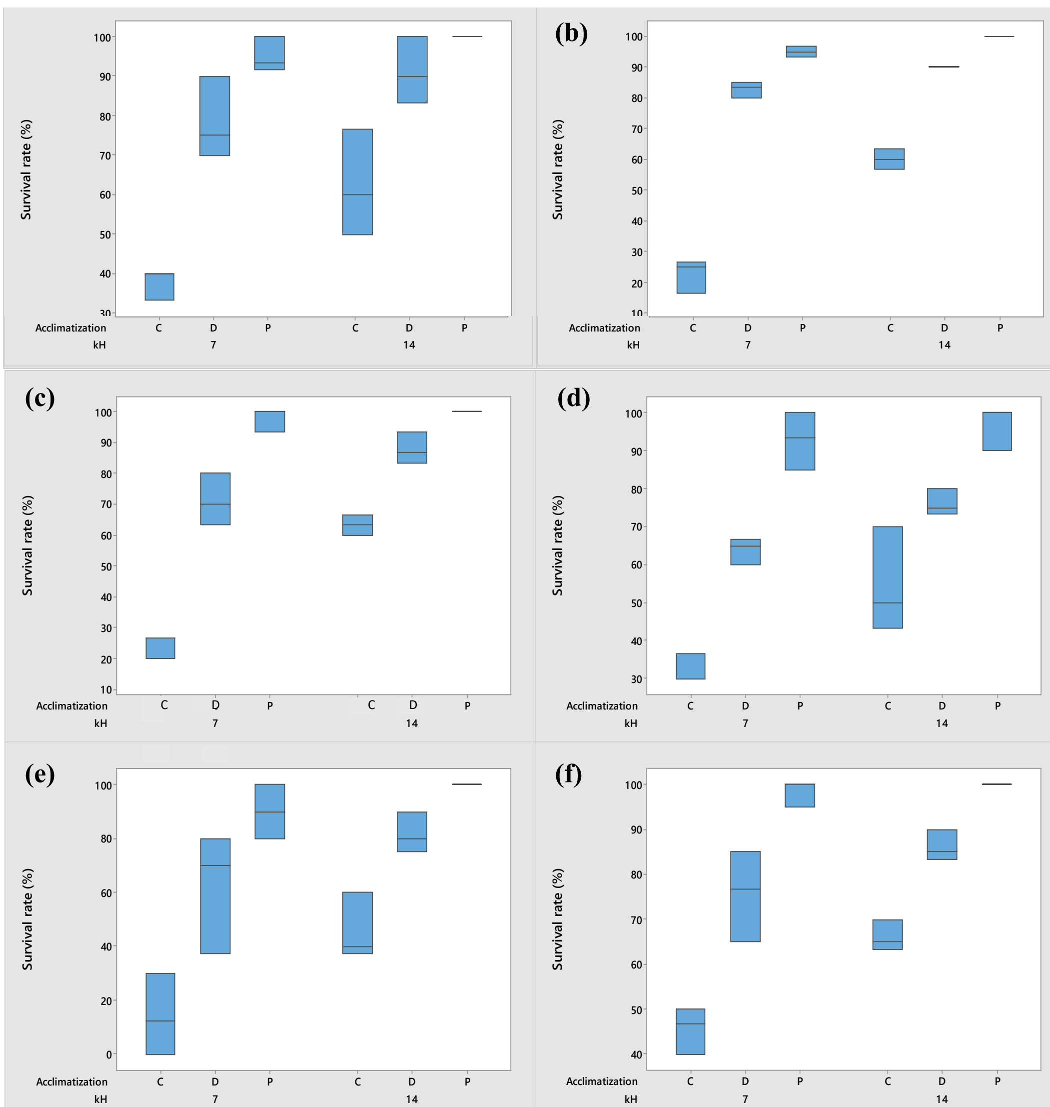

Figure 3. Survival rate graphic for both acclimatization and carbonate hardness treatment (6 species of fish): (a) *Chromis viridis*; (b) *Halichoeres chloropterus*; (c) *Aeoliscus strigatus*; (d) *Amphiprion ocellaris*; (e) *Pomacanthus imperator*; (f) *Synchiropus splendidus*.

low pH adjustment method (Figure 3a and 3f). When the low pH method was combined with high carbonate hardness, the survival rate of all species was 100%, as shown in Figure 3, except for *Amphiprion ocellaris* (Figure 3d). The same pattern showed by *Pomacanthus imperator* when the carbonate hardness is low (Figure 3e).

The highest survival rate of fish was performed by the low pH treatment, significantly different from drip-line and the conventional method. This was probably related to the environment, especially the nitrogen compound. By lowering the pH, the formation of toxic ammonia (NH_3) compound can be avoided. Literally, at low pH environment, most of the nitrogen compound formed is in the form of ammonium (NH_4^+) which is relatively safer than the toxic ammonia (NH_3). This latest compound was formed at the high pH [5, 8]. So the fish transferred to low pH and clean water were freed from the polluted packing water without experiencing any shock in terms of pH and logically freed from toxic NH_3.

The drip-line method was better than the conventional method. However, this method is only suitable for relatively fresh fish but not suitable for the dying fish. These latest had to spend extra time in the contaminated packing water. Extension of time may not be harmful to the relatively fresh fish but would be very dangerous to the dying fish. Although the environmental changes in the drip-line method were not drastically different, the addition of new high pH water to the water packing automatically shifted the equilibrium to the formation of toxic ammonia (NH_3). This problem did not occur at the low pH method. By using the low pH method, fish can be transferred immediately from the polluted packing water to the new and clean water in the system without experiencing any shock because the pH of the system water has been reduced in accordance to the pH of the packing water around 6.2.

The lowest survival rate was shown by the conventional method. The conventional method solved the temperature shock problem because basically, the principle of this method is the temperature adjustment, which is adjusting the packing water temperature with the system temperature. In fact, the main problem is not the temperature shock, but the formation of the toxic ammonia. Even though the temperature had been adjusted, the fishes were shocked due to the drastic pH changes because the pH of the packing water was low around 6.2, while the pH of the new system water was high (8.0-8.1). When fish was moved from the packing water to the system, they were stressed and eventually died because of the formation of the toxic ammonia compound [9]. Ammonia compound blocked the oxygen transfer from the gills to the blood that causes the hyperplasia of gill, which further reduces the levels of hemoglobin in the blood [10]. When ammonia exposed to the fish, it will inhibit the oxygen binding to the hemoglobin because the affinity of ammonia is higher than the oxygen. The interaction formed was Hb-NH_3 rather than Hb-O_2 [11].

The low pH method not only increased the survival rate but also provides a more simple and efficient operational system compared to the drip-line method. On the contrary, the drip-line method requires a longer time to operate and needs a complicated system, especially when it is applied individually (bag per bag).

In terms of carbonate hardness, the overall results from each species of fish showed that the high carbonate hardness treatment was better than the normal carbonate hardness. It proved that the survival rate of fish was certainly related to the buffer system. At high carbonate hardness, there was less pH fluctuation because the high carbonate hardness stabilized the pH following equation below.

$$\mathbf{CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows HCO_3^- + H^+}$$

The buffer system balances both a weak acid and its conjugate base so that any excess acid or base introduced was then neutralized. The combination the low pH with the high carbonate hardness is ideal for fish transfer as the pH of the environment is low (the formation of toxic NH_3 can be avoided), and the pH is stable due to buffer system [12].

In general, there was no difference in the survival rate patterns between each type of fish. A slight difference was observed on clownfish and *Pomacanthus imperator*. The clownfish (*Amphiprion ocellaris*) were the most sensitive species compared to all species tested. Clownfish is territorials that need wide space. Besides the territorial problem, they tend to form the mutualistic symbiosis with the anemone. The anemone protects the fish, and similarly, the clownfish protects the anemone from the butterfly fish. Therefore, the clownfish becomes more sensitive without the existence of the anemone [13]. Indeed, the clownfish survival rate was the lowest among the six fishes tested. Angelfish (represented by *Pomacanthus imperator*) is also a sensitive species because basically, angelfish is difficult to maintain in captivity because they have their territory and could prey on each other [14]. The cannibal problem was surmounted by special handling. They were packed and treated on the individual bag. The territorial problem was solved, but the other problem appeared by using the cup system. Placing fish in the cup can cause the limitations for fish to move or swim during the acclimatization process. On one side, the cup system was good to solve agonistic and predatory behaviour problems, but on the other side, it could limit the movement of fish during the acclimatization process. Under stress condition, fish need a certain altitude, especially for freely swim in order to open their gills for the respiratory process [15].

Species *H. chloropterus* and *A. strigatus* showed almost similar by displaying the schooling behaviour. Fish with this behaviour tend to swim in the same direction in a coordinated way [16]. For the goby fish group, the problem appeared was somewhat related to the toxic problem. The first result showed that the fish packed in a group all dead. By the group packing, fish cannot survive because if they are stressed, they will spread toxic compound like mucus that will affect one another inside the same packing [17]. The problem was then solved by the individually packing (one by one) so that it will not spread the poison to the other fish.

The damsel's group showed the most varied results, but this group was the most efficient for the low pH method because *C. viridis* was the type that lives on the surface of the water. There were plenty of waters on the surface, so they will be easier to take the oxygen if they lack it to replenish the condition [18]. Depends on the behaviour, it was also the one that stood out the most and the easiest species to be observed for the differences because at the normal condition, the fish were very active, so they can be observed easily when they were weak.

4. Conclusions

The interaction between the two factors (acclimatization and carbonate hardness) was significant (p = 0.000), but at the level tested, the acclimatization method and carbonate hardness did not cross each other. For the acclimatization method, the highest survival rate held by the low pH method (99%) and significantly (p = 0.000) higher than the drip-line method (78.91%) and the conventional method (44.07%). In terms of carbonate hardness, high carbonate hardness (14 dKH) was 40% higher than the normal carbonate hardness (7 dKH). There was no significant difference between the six types of fish tested. The low pH method combined with high carbonate hardness was the best combination for the fish acclimatization, whose survival rate was 99%. It is suggested to the stakeholders, especially exporters and importers, to apply the low pH acclimatization method integrated with high carbonate hardness.

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