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Rory Anthony Hutagalung
Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jalan Jenderal Sudirman, Jakarta, Indonesia

Vivitri Dewi Prasasty
Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jalan Jenderal Sudirman, Jakarta, Indonesia

Ignatius Sakti Pranandya
Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jalan Jenderal Sudirman, Jakarta, Indonesia

Correspondence
Rory Anthony Hutagalung
Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jalan Jenderal Sudirman, Jakarta, Indonesia

Ecological approach on marine hermit crab (*Calcinus* spp.) packing technique

Rory Anthony Hutagalung, Vivitri Dewi Prasasty and Ignatius Sakti Pranandya

Abstract
Marine hermit crabs is one of marine organisms that interest aquarium hobbyist, due to its coloration, and especially to their function in aquarium ecosystem. Marine hermit crabs are called as tank cleaner for their capability on feeding detritus and cadavers that keep the aquarium clean. Therefore, hermit crabs are exported overseas. Mortality is the main issue in marine hermit crab shipment due to poor packing method. The purpose of this research was to lower mortality of marine hermit crabs during shipment through ecological approach. A 2x3 factorial design was applied. The first treatment was dormancy mecanism with three factors (solitary, non-dormant group, forced dormant group) and the second treatment was temperature with three factors (25 ºC, 20 ºC, 15 ºC). Significant survival rate difference (p: 0.002) among the dormancy factors was observed. The solitary packing method resulted the highest survival, followed by forced dormancy group. Despite the higher survival rate on the solitary method, forced dormancy group method was more practical as the packing was in group. In terms of temperature, the middle temperature (20 ºC) showed the highest survival rate and it was significantly different (p:0.0034), compared to the lower temperature (15 ºC) and the higher temperature (25 ºC).

Keywords: marine hermit crabs, *Calcinus spp.*, dormancy, packing, temperature

1. Introduction
Marine hermit crab is one of the principal organisms in marine aquarium ecosystem. Beside their distinct colorations, their contribution to the marine aquarium environment is considerable. Marine hermit crabs, specifically, are detritivores and have opportunistic tendencies, and thus scavenge on dead organisms while being reef-safe (do not damage any other species in tank). Hermit crabs are in demand from various countries because of their unique role in aquarium habitats. The main issue with the shipment of marine hermit crabs is mortality as the custody chain is frequently long. Long-term shipment risks death related to accumulation of stress in hermit crabs during shipment. The longer shipping time might be detrimental to the health of the hermit crabs, resulting in damaged or dead specimens at destination called death on the arrival (DOA).

Stress in hermit crab shipment might be caused by poor handling or subpar packing method. During shipment, hermit crabs excrete ammonia and others metabolic wastes which are toxic to hermits crabs. So far, researches on hermit crabs packing method are very limited. The most current and common practiced to relatively close organism (fishes) was the use of additives in order to reduce the organism activity that, in turn, lower the metabolic rate and minimize the stress. The common additives used is the application of anesthetics agent [4]. The method succeeded in reducing mortality of certain fishes. However, the use of anesthetics agent is unpractical, unstable, and especially harmful due to unwanted residues. Therefore, safer method is needed and ecological approach is one the ways to solve the problem.

Ecologically, the metabolic rate of organism can be lowered by inducing dormancy mechanism or by lowering the temperature. The application of dormancy to hermit crabs through packing methods might ensure a lower metabolic rate and therefore reduces mortality during shipment. The reduction of metobolic rate by dormancy mecanism can be combined with temperature adjustment. Some studies have been done on hermit crabs to test the limits of hermit crab's tolerance against temperature [6]. So the premise of altering temperature to regulate the metabolic rate could be another option for marine hermit crabs packing method.

The aim of this research was to lower the mortality of marine hermit crabs during shipment through ecological approach, ie. dormancy mechanism and temperature adjustment.

2. Materials and Methods

This research was conducted in the Aquaculture Laboratory of Atma Jaya University located in Central Jakarta since January 2017 to May 2017. The main materials of this research were marine hermit crabs ie. Dwarf Zebra Hermit Crabs (*Calcinus laevimanus*) and Gaimard's Hermit Crabs (*Calcinus gaimardii*) originated from Garut, West Java. The specimens were acclimated to the aquarium system within the laboratory using heavy aerations to ensure the optimal living conditions of the hermit crabs within captivity. They were feeded by dead fishes two times a day (in the morning and afternoon) during at least 2 weeks before they used for the treatment and were fasted 24 hours before packing.

The experiment was preceded by preliminary research to determine the lethal temperature for the hermit crabs packing and to determine the number of specimens per bags for group treatments. For the preliminary research, the specimens were stored in Styrofoam boxes that they have significantly higher water temperature due to metabolisms and the ambiance temperature within the box itself. To homogenize the ambiance temperature, the Styrofoam boxes were then filled with water and the temperature was lowered to respective targets. For the dormancy treatment, the experiment was tested at different quantities of hermit crabs in one bags ie. 3 individuals per bag, 5 individuals per bag, 10 individuals per bag, and 20 individuals per bag. For the main experiment, a factorial design was applied in this experiment with 2 treatments i.e., dormancy with 3 factors (solitary, non-dormant group, forced dormant group) and temperature with 3 factors (25 ºC, 20 ºC, 15 ºC). The experiment was replicated into ten times.

In the solitary treatment, a single hermit crab was inserted individually in packing plastics, while the non-dormant group was packed with five hermit crabs inserted into the packing plastics. Forced dormant groups treatment was done by inserting 5 hermit crabs into fabric filter bags, packed tightly, then put in packing plastic bag and resumed to conventional packing method (Fig 1). Conventional packing method included adding water (50cc/hermit crabs), oxygen until fully saturated, and tying the packing plastic bag.

As for the temperature treatment, the plastic bags were kept in refrigerator to lower the water temperature to respective target conditions (25 ºC, 20 ºC, and 15 ºC), and stored in Styrofoam boxes for insulation. The specimens were held in Styrofoam boxes for three days (36 hours) to simulate shipping time. The variable mesured was the survival rate that was observed afterwards.

Fig 1: Method of packing for dormancy treatment (left to right: Non-dormant group, Forced dormant group)

The normality of the data was tested to verify the use of parametric statistics (analysis of variance - ANOVA). If the data was not normally distributed, the non-parametric statistics in this case, Kruskal-Wallis test was used to test the significant difference among the treatments.

3. Results and Discussion

The preliminary experiment showed that the lethal temperature for marine hermit crabs was 13 ºC. While in group treatment, the maximum number of specimen was 5 individuals per bag. No mortality was observed until this level (5 individuals per bag). Mortality started when the crabs were packed in 10 or more individuals per bag. Five individuals per bag was therefore used for dormancy group treatment in the main experiment.

For the main experiment, since the data was not normally distributed ($p < 0.010$), the non-parametric method (Kruskal-Wallis) was then applied to analyze the differences among the treatments. On the dormancy treatments, the survival rate was significantly difference among the treatments (p: 0.002). The highest survival rate was performed by solitary method (97 ± 3.8%), followed by forced dormant group method (88 ± 3.9%) and finally by non-dormant groups (85 ± 5.8%) (Fig. 2).

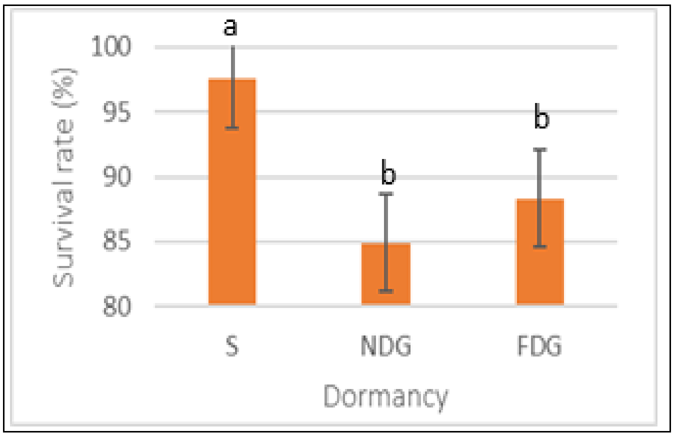

Fig 2: Survival rate according to dormancy treatments (± se) (S: Solitary, NDG: Non-dormant groups and FDG: Forced Dormant groups)

A significantly different survival rate (p: 0.0034) was also observed on the temperature treatments. The highest survival rate was the hermit crabs packed in water temperature of 20 ºC (95 ± 2.4%), followed by those packed in 25 ºC (92 ± 3.5%), and 15 ºC (84 ± 3.3%) (Fig. 3).

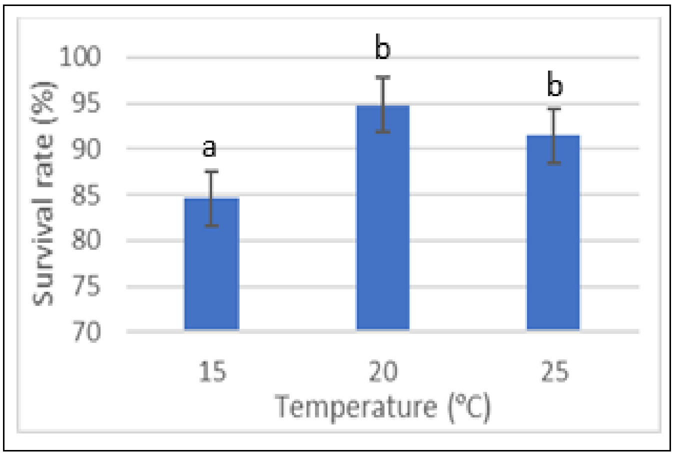

Fig 3: Survival rate according to temperature treatments (± se)

The survival rate of hermit crabs packed individually was the highest. The less crowded it is in the bag, the less stressed the hermit crabs will be. This is due to smaller amount of metabolic waste in the bag. However, this method is costly and impractical for shipping large numbers of hermit crabs. The forced dormancy group was rather considered to be more advantageus despite its lower survival rate compared to solitary treatment.

The average survival rate of solitary method was 97%, while the average survival rate of forced dormant group method was 88%. For 1000 hermit crabs, the mortality difference between the forced dormant group method and the solitary method was 90 pieces (970 pieces subtracted by 880). The price per hermit crab was US $0.5. When multiplying the mortality difference with the price per hermit crab, the loss due to mortality of the forced dormant group for 1000 hermit crabs would be US $45. While packing 1000 hermit crabs individually would take 2 hours of work for 5 personnel, which cost US $10/hour/person. Overall packing fee for solitary method cost US $80. Mortality could be compensated for time consuming method and extra cost for packing. Therefore, the forced dormant group is considered to be better, despite the lower survival rate.

Higher survival rate of forced dormant group compared to non-dormant groups might be related to the motoric activity of hermit crabs. Increasing activity of hermit crabs will cause more oxygen consumption, more energetic cost, and more metabolic waste; and the environment resources were limited to an extent. Excess movement of the hermit crabs would deplete the oxygen quickly and provoke the metabolic waste build up that is harmful to the hermit crabs. This phenomenon did not occur on the forced dormant groups because the activity was minimized (hermit crabs stayed inside the shell). This will force the hermit crabs to only maintain the basic function such as respiration [6], and thus limit unnecessary energy and metabolic waste.

Researches regarding the use of dormancy mechanism on hermit crab shipment are yet to be done. Similar research has been done on ornamental fishes using anesthetic agent during shipment to lower metabolic rate. Using anesthetic agents slowed down the motoric activity of the fishes [5]. Anesthetic agent MS-222 reduced the heart rate of zebrafishes and caused high mortality under long-term sedation [3]. Other agents such as 2-phenoxyethanol impaired ventilation, lowered blood O_2 levels and blood pH, and reduced immune system function of the animal [5]. This method, however, posed some risks to the fishes, as side effects might occur. Anesthetic agents must be used with caution as different agents on non-validated species have unexpected side effects [12]. Therefore, the use of anesthetic agents to pack hermit crabs would not be beneficial.

In the experiment, the middle temperature (20 ºC) resulted the highest survival rate compared to the higher (25 ºC) and lower temperature (15 ºC). This might be caused by the nature of marine hermit crabs themselves. Most of hermit crabs inhabits the coastal zone in warmer climate, where water temperature on daytime ranges between 24-30 ºC on reefs and water temperature in intertidal pool ranges up to 40-45 ºC [7].

Marine hermit crabs generally reside in tropical climate regions that have a higher temperature so they might not be able to cope with such low temperature [4]. Having higher ambiance temperature also did not help the survival of the hermit crabs, because the metabolic rate is not suppressed, leading to more metabolic waste. Crustaceans have high concentrations of Mg^{2+} in the hemolymph, combined with low temperature might induce an anesthetic property in crustaceans. If the temperature is too low, it might hinder water circulation through the gill, resulting in inadequate gas exchange and oxygen deficit at cellular level [7]. The lethal temperature that of the hermit crabs tested was 13 ºC, so naturally the hermit crabs would not survive the long-duration packing while being kept at such low temperature.

There has been a research where hermit crabs were subjected to low temperature and high pressure to observe any change in behavior and metabolic rate. Hermit crabs that were kept in low temperature have lower oxygen consumption, and limiting metabolic activities. When exposed to low temperature and high pressure, hermit crabs would remain withdrawn rather than trying to escape from such conditions. The shells of hermit crabs act as a microhabitat, allowing them to separate themselves from extreme conditions and increase tolerance [6]. Another research studies how salinity and temperature affects the oxygen consumption of intertidal crabs *Hemigrapsus oregonensis* and *H. nudus* [1]. When exposed to both high and low water temperature in separate experiments, the crabs show high oxygen consumption in both temperature. The high oxygen consumption in low temperature is due to the increase work to maintain osmotic balance of the crabs, not because of increasing motoric activity.

4. Conclusion

Ecological approach through dormancy mechanism and temperature adjustment succeeded to lower the mortality of hermit crabs during shipment. Despite the highest survival rate on the solitary treatment, resulted the highest survival rate, forced dormancy group treatment was considered to be the most practical packing method. In terms of temperature, 20 ºC was considered to be the optimum as the survival rate at that degree was higher than 15 ºC and 25 ºC. The combination of dormancy group treatment and the temperature of 25 ºC performed the best result. Shareholders, especially importer and exporter are recommended to use the combination to ensure the better survival and quality of marine hermit crabs shipment. Further research is needed to ensure both survival and quality of the hermit crabs, by taking into account the physical quality of hermit crab after shipment.

5. Acknowledgements

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