THE EFFECTS OF pH ON SURVIVAL, GROWTH PERFORMANCE AND CHEMORECEPTION OF *Macrobrachium rosenbergii* POSTLARVAE

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Abstract

Acid rain causes acidification of freshwater resources which can be harmful to all aquatic organisms. Water acidification is difficult to reverse due to continuous development and emission of anthropogenic carbon dioxide. This will lead to ecosystem damage and unsustainable aquaculture especially crustaceans production. It is hypothesized that acidic water might damage the chemoreception of giant freshwater prawn (*Macrobrachium rosenbergii*) subsequently lead to poor growth performance and survival. The survival, growth performance and chemoreception of the postlarvae (PL) were examined in water with pH 6, 5, 4 and control (pH 7.5) in laboratory. All treatment groups which contained 250 PL per treatment were slowly acclimatized to respective pH. Mortality was monitored throughout 27 days of rearing. Feeding trials were conducted every evening using agar pellets containing different concentration of chemo-attractants. The touch frequency and duration of contact the PL made with the pellet were recorded. Body weight and total length were measured after 27 days of rearing. From the results obtained, touch frequency and duration of contacts show no tendency of decreasing or increasing with reducing water pH. It was also found that vision is one factor that affecting the feeding trial. Thus further analysis could not be completed. Even with poor chemoreception, the PL were able to locate the agar and make contact. However, it was known that the PL at pH 4 have damaged chemoreception based on duration of contact the PL make with control agar pellets which did not contain chemo-attractant. The PL from pH 4 took extra one second to release the control agar compare to control, pH 6 and 5. The slow response could indicate damaged chemical sensitivity. Based on survival and growth performance, pH 6 is the best for rearing of giant freshwater prawn PL since the survival was highest (83.6 %) and the size variation was homogenous. Based on length-weight relationship analysis, the PL growth at pH 6, 5 and control have similar growth pattern and were metamorphosed into early juveniles after 27 days. Length-weight relationship of PL at pH 4 shows that they were split into two groups with different body ratios and could not turn into early juveniles after 27-day rearing. It can be concluded that pH 4 is extremely harmful to PL of giant freshwater prawn and pH 6 is recommended for PL production.

Abstrak

Hujan asid telah menyebabkan sumber air tawar menjadi semakin berasid dan ini amat membahayakan semua organisma akuatik. Pengasidan sumber air tawar susah dikembalikan ke asal disebabkan pembangunan yang berterusan dan pelepasan karbon dioksida antropogenik. Ini akan merosakkan ekosistem dan menyebabkan akuakultur semakin tidak mampan terutamanya pengeluaran krustasia. Hipotesis kajian tersebut adalah air yang menpunyai pH rendah akan merosakkan kemoresepsi udang galah (*Macrobrachium rosenbergii*) post-larva (PL) dan seterusnya menurunkan prestasi tumbesaran dan kemandirian. Kemandirian, prestasi tumbesaran dan kemoresepsi *postlarvae* (PL) udang galah dalam air pH 6, 5, 4 dan set kawalan (pH 7.5) telah diperiksa dalam makmal. Penyesuaian udang ke air pH rendah telah dijalankan dengan perlahan untuk menyesuaikan PL ke air pH rendah mengikuti kumpulan rawatan masing-masing. Kematian PL telah diperhatikan selama 27 hari eksperimen. Ujian perbandingan makanan telah dijalankan setiap petang mengunakan pelet agar yang mengandungi kepekatan pengkemotarik yang berbeza. Kekerapan sentuhan dan tempoh sentuhan PL terhadap pelet agar telah dikira dan direkod. Berat badan dan kepanjangan badan PL telah diukur selepas 27 hari peliharaan di air pH rendah. Keputusan kajian tentang kekerapan sentuhan dan tempoh sentuhan tidak menunjukkan kecenderungan berkurangan atau meningkat dengan pengurangan pH air. Kajian juga mendapati penglihatan adalah salah satu faktor yang mempengaruhi ujian perbandingan makanan. Ini telah menyebabkan analisis lanjut tidak dapat dilakukan. Walaupun kemoresepsi PL menjadi teruk, mereka dapat mengesan pelet agar menggunakan penglihatan dan buat sentuhan. Walau bagaimanapun, didapati bahawa kemoresepsi PL di air pH 4 telah rosak berdasarkan tempoh sentuhan PL membuat dengan pelet agar kawalan yang tidak mengandungi pengkemotarik. PL di pH 4 mengambil masa yang lebih 1 saat untuk melepaskan pelet agar kawalan berbanding dengan PL di set kawalan, pH 6 dan 5. Reaksi perlahan menunjukkan sensitiviti PL terhadap kimia telah rosak. Berdasarkan kemandirian dan prestasi tumbesaran, pH 6 merupakan pH yang terbaik untuk pemeliharaan PL udang galah disebabkan kemandirian yang tertinggi (83.6 %) dan variasi saiz yang homogen. Berdasarkan analisis hubungan panjang-berat, PL di pH 6, 5 dan set kawalan mempunyai pola pertumbuhan yang sama dan telah berubah menjadi juvana awal selepas 27 hari peliharaan. Hubungan panjang-berat PL di pH 4 menunjukkan bahawa mereka telah berpecah kepada dua kumpulan dengan nisbah badan yang berbeza dan tidak dapat bertukar menjadi juvana awal selepas 27 hari. Kesimpulan kajian tersebut ialah pH 4 adalah sangat berbahaya kepada PL dan pH 6 adalah disyorkan untuk pengeluaran PL udang galah.

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%	Percentage
PL	Postlarvae
BW	Body weight
TL	Total length
RM	Ringgit Malaysia
ppt	part per thousand
°C	Degree celcius
cm	Centimetre
cm^2	centimetre square
DO	Dissolved oxygen
HCl	Hydrochloric acid
g	Gram
mL	Millilitre
n	Number
σ	Standard deviation
SE	Standard error
ACV	Anomaly coefficient of variance
mg	Milligram
log	Logarithm
mm	Millimetre
Ca	Calcium

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Acidification of Water Resources

Carbon dioxide released into atmosphere naturally will be absorbed by forest in exchange of oxygen and land which have buffering capacity. However, since the beginning of industrial revolution, increase in release of anthropogenic carbon dioxide is causing rising of carbon dioxide concentration in the atmosphere too fast in a rate that cannot be compensated by the nature anymore. Part of the carbon dioxide gas is then absorbed by the ocean, leading to formation of more hydrogen ions in the seawater. This subsequently lowers the seawater pH which can threaten all aquatic animals.

Other than that, gaseous waste discharge contains high concentration of nitrogen oxide and sulphur dioxide from anthropogenic sources like burning of fossil fuels in industrial areas, increase in number of vehicles and large scale forest fires leads to acid rain problem being gradually more serious than before. These air pollutants carried by wind travels long distance and settle in other continents or locally in the form of dry or wet deposition onto land. Wet deposition is usually in the form of acid rain, snow and fog while dry deposition refers to dust and smoke. Acidic rainwater which falls down to land combines with land water and thus slowly reducing the freshwater pH. Areas with soil having low buffer capacity facing the problem of neutralizing acidified water and thus causing the water become dead zone where no aquatic organisms can survive.

Mangrove area where the ocean water mixes with freshwater to produce brackish water with varying salinities is also one essential environment to sustain the food production capacity of the nature. This is because mangrove area plays an important role as the breeding ground of most fish and crustaceans as well as the nursery ground for many species of larvae. Freshwater and seawater acidification automatically results in brackish water being acidified.

1.2 Effects of Acidic Water on Aquaculture and Fisheries Management

Aquaculture activities depend greatly on water resources from nature. High technology indoor aquaculture system may be able to treat water to control the water quality parameters. However, to overcome this issue, water use in hatcheries will have to be treated to optimize the pH. This will subsequently increase the cost of operation for fish production. Traditional culture systems like cage culture, pen culture and pond culture which directly use water resource without treatment will be affected significantly in a negative way.

Low water pH might affect the well-being of the farmed fish or crustacean. Generally, calcium carbonate will dissolve in acidic water. There have been many researches done to investigate the effects of acidic water on aquaculture animal welfare, for examples by Chen & Chen (2003), Cheng & Chen (2000), Klaprat *et al.* (1988) and Leduc *et al.* (2004). There has also been some research done on how acidification of water affect the seafood production by Branch *et al.* (2013).

Other than that, water acidification in the environment is able to jeopardize sea or river ranching activity for fisheries management purpose. Fish or prawn fries released will not survive due to unsuitable water pH. This can lead to low recapture quantity and unprofitable ranching.

1.3 Problem Statement

Despite, acidic water is mostly likely to post huge threat to crustacean culture industry since crustaceans possess calcium carbonate exoskeleton. Exoskeleton acts like scale or skin to house the animals. Thus, acidic water might damage their exoskeletons which further might lead to physiological damage to the crustaceans. Physiological damage includes damage of vision, chemoreception which is divided into olfaction and gustation as well as mechanoreception.

Crustacean depends on chemical sense to look for food, and finding suitable mating partner. Fail in looking for food will reduce their food intake and growth subsequently leads to low survival. This happens in both captivity and natural environment.

Low survival and poor growth are the main issues in aquaculture business. This phenomenon will pressurize the aquaculture industry and seafood production worldwide. Production of expensive and high demand crustaceans like lobster, crayfish, abalone and prawn like giant freshwater prawn may become difficult in the future. Therefore, this study is important to determine whether acidic water can harm the crustacean production industry. In addition, the results obtained in this study serve as fundamental information for development of solution to this issue.

1.4 Hypothesis

It is hypothesized that acidic water can damage the chemoreception of freshwater giant prawn. Damage in chemical sense is likely to influence the food intake of the prawn, leading to poor growth performance and survival. Acidification of water is likely to affect this aquaculture species.

1.5 *Macrobrachium rosenbergii*

Macrobrachium rosenbergii was the target species of this research as it is one of the crustacean species that is affected by acidic water. It is commonly known as giant freshwater prawns, giant river prawn or Malaysian prawn. It is one of the most desirable candidates for freshwater aquaculture due to high market demand and relatively easier production.

This species is widely cultured in Asian countries and has contributes to high income on aquaculture sector. However, problems arise when the sustainable culture of this species is affected by the global water acidification especially the freshwater which is being acidified by acid rain and industrial waste discharge.

Macrobrachium rosenbergii is a catadromous species which migrate to brackish water for spawning. Sometimes, they are found in marine environment as well. This species hatch from eggs as zoea which are planktonic, living in brackish environment. After gone through 11 sub-stages of zoea, they metamorphosed into postlarvae (PL). As they grow from zoea to PL, the prawns changes physically, physiologically as well as ecologically. They slowly migrate up stream to freshwater environment. During zoea stage, they feed primarily on zooplanktons whereas in PL stage, they become omnivorous benthic feeders. In hatchery, artificial sinking pellet are given once zoea turn into postlarvae.

According to Moller (1978), the feeding behavior pattern of *M. rosenbergii* larvae and postlarvae resembles those of adults. Although the food capture by larvae and postlarvae is largely unselective, their ingestion appears to be dependent on chemical cues (Moller, 1978).

In this research, *M. rosenbergii* of postlarvae stage (Photo 1.1) was studied instead of the earlier stage, zoea. Postlarvae is the first stage in life cycle which are able to control their own locomotion. Zoea is not able to control their own swimming and are not able to swim as they are planktonic. The feeding experiment designed is more suitable for postlarvae which can control their movement toward or away from the taste substance. This orientation behavior of *M. rosenbergii* postlarvae was further explained by Moller (1978).

Photo 1.1 Postlarva of *Macrobrachium rosenbergii* viewed under dissecting microscope. The pleopods are not visible in this photo as it was clung onto the prawn's body due to absent of water.

1.6 Chemoreception Test

There are two popular methods to detect the damage of chemoreception of aquatic animals, namely electrophysiology and behavioural method. In this research, behavioural method based on feeding trial was used instead of electrophysiology technique because this method is simple and inexpensive. Besides, size of PL is too small to be used in electrophysiology which requires connection of the heart to a machine using wire yet test subject needs to be alive.

In the present study, prawn specimens were subjected to different water pH. To investigate whether the acidic water are able to damage the chemo-sense of the prawn postlarvae, a feeding experiment was designed. A medium was used to deliver the chemo-attractant to the prawn specimens at different pH treatments. Agar gel pellet was chosen to be the medium to deliver the chemo-attractant to the prawn specimens. Agar gel pellet is made from agar cut into suitable pellet size. Agar is a tasteless and odourless substance. Thus, its taste or smell will only be determined by the chemical or chemo-attractant incorporated into it.

Chemo-attractant refers to chemical agent, either organic or inorganic which induce movement of organisms toward it. If the chemo-sense of the prawns is still normal, they will be attracted to taste or eat the agar gel pellet. Natural chemo-attractants for the giant freshwater prawns were selected based on previous studies done by Harpaz (1997), Kawamura *et al.* (1995) and Pavadi *et al.* (2012). It is expected that prawns with damaged chemo-sense will not react the same compare to prawns in control group.

1.7 Objective

The objective of this study was to investigate the effects of pH on chemoreception of giant freshwater prawn (*Macrobrachium rosenbergii*) postlarvae. Other than that, survival and growth performance of the prawns in acidic water was also examined.

CHAPTER 2

LITERATURE REVIEW

2.1 Previous Researches on Ocean Acidification

According to research done by Caldeira and Wicketts (2003), the ocean has already taken up carbon dioxide from 280 ppm to 396 ppm from the year 1800 until now. In the future, it is predicted that this phenomenon continue to increase the CO_2 concentration in ocean up to 800 ppm in the year 2100 (Caldeira & Wickett, 2003).

Through a series of chemical reaction, water acidification can alter the carbonate chemistry consequently hinders the formation of shells in planktons, crustaceans and corals (Mustafa, 2009). According to Riebesell *et al.* (2000), increase in concentration of CO_2 is able to lower the calcite production and thus slow down production of calcium carbonate in the ocean surface. This is extremely important as it affect shell formation in all calcifying organisms.

2.2 Literature Review on Acid Rain and Acid Deposition

Acid rain is one of the acid deposition phenomena. Acid rain has been an environmental issue for decades and now it is speeding up the rate of water acidification in the world. Naturally, rainwater is slightly acidic due to dissolve of carbon dioxide in it and the acidity will then be neutralized by land (Environmental

Protection Agency, 2004). Normal pure rainwater usually has a pH of about 5.3 (Environmental Protection Agency, 2004). In Peninsular Malaysia, it was reported by Norela *et al.* (2009) that pH of rainwater at Nilai Industrial Park in Negeri Sembilan ranged from 5.34 to 3.86. It was also reported in Danum Valley, Sabah pH of rainwater at there ranged from 5.90 to 4.89 (Sumari *et al.*, 2009).

Subsequntly, acid rain can lead to acidification of freshwater sources. The water of Tasik Chini's Feeder River in Pahang, Malaysia was reported to have pH ranged from 3.20 to 6.32 (Muhammad-Barzani *et al.*, 2007). In Langat River in Selangor, Malaysia, the water pH was reported to be ranged from 3.5 to 6.7 (Juahir *et al.*, 2009). Another river in Selangor, the Semenyih River was recorded to have pH ranged from 4.62 to 6.59 (Al-Badaii *et al.*, 2013). Freshwater acidification is not a local issue but it happens globally. In the United Kingdom, the Upper River Wye in Plynlimon, the lowest water pH recorded was 4.21 (Reynolds *et al.*, 1997). In Joetsu district in Niigata prefecture of Japan, natural and artificial lakes in ridge crests was reported to have lowest pH of 4.96 (Sato, 2004). According to Ikuta *et al.* (1999), rain water with pH 4 often precipitated throughout Japan.

In New York city, acid deposition occurance affect the water quality by lowering its pH level, reducing the acid-neutralizing capacity (ANC) and increasing the aluminium concentration in the water (Driscoll *et al.*, 2002). There were 64% (388 lakes) of the acid-sensitive lakes in New York had a chemical composition which suggests that their acidity was largely resulted from sulfate associated with acidic deposition (Driscoll *et al.*, 2002).

Acidification of natural water resources can lead to two consequences. One of the effects is deterioration of ecosystem. The acidification of lakes, marshes and rivers by the inflow of the acid rain can definitely lead to the decrease of fish in those waters, thus having grave impacts on the ecosystem (Ministry of the Environment Japan, 1990). Second, aquaculture activities very much depend on water from natural environment. Deterioration of quality in natural water can lead to poor farm production and higher investment is needed to treat the water.

2.3 Target Species for this Research

The species of prawn chosen to be studied in this final year project was *Macrobrachium rosenbergii.* Morphologically, freshwater prawn is easily distinguished from other shrimps by the presence of a pair of huge chelipeds possess by males (Holthuis, 2000). The planktonic larvae of this species move in tail first direction with ventral side uppermost while postlarvae have same physical appearance of adult prawns (Ismael & New, 2000).

Giant freshwater prawn is a catadromous crustacean. In natural environment, they live in tropical freshwater that is linked to estuarine due to the reason that spawning takes place in brackish water (Ismael & New, 2000). Larva hatchs as zoea, living in brackish water of 8 to 12 ppt and slowly migrate upstream to freshwater as they reach postlarva stage (Ismael & New, 2000).

Water quality of known water resources have seen to be degraded over time due to pollution. Water acidification cannot be reversed because development cannot be slow down and production of anthropogenic waste is too much that the natural environment cannot compensate with it. In the near future, aquaculture activity will be affected so badly that culture of pH sensitive aquatic organisms especially crustaceans will be reduced or gone.

Culture of giant freshwater prawn is one of the expensive aquaculture business that will be affected by degradation of water pH. Malaysia, US, Mexico, Peru, Brazil, Iran, India, Thailand, China, Taiwan, Vietnam and Indonesia have been the major producing countries of the giant freshwater prawn (FAO, 2013). In 2011, Malaysian produced 334.34 tonnes of freshwater giant prawn and sold at price around RM 26 to 30 per kg (Department of Fisheries Malaysia, 2013). Therefore, Malaysian aquaculturists earned about RM 9,343,000.28 and RM 13,770,000 by wholesale and retail respectively. This amount might decrease instead of rising due to unsustainable culture of this prawn cause by acidified water.

2.4 Effects of Acidic Water on Crustaceans Welfare

Nonetheless, water acidification discussed earlier is a huge treat to the culture of crustaceans species. Previous research done by Chen & Chen (2003) revealed that acidic water significantly lower the survival, body weight, total length, molting frequency and feeding rate of *Macrobrachium rosenbergii* juveniles. Another research done by Cheng & Chen (2000) found that very acidic water can decrease hemocytes count and phenoloxidase activity of *M. rosenbergii*.

Despite, crayfish (*Cambarus bartonii bartonii*) in southern Appalachian streams are found to be able to tolerate acidity up to pH 2.43, 2.56 and 2.85 for adults, advanced juveniles and early juveniles respectively (DiStefano *et al.*, 1991). But the tolerance of these crayfish toward acidic water is affected by the ages or sizes of the crayfish, molting or intermolt and water temperature. Observation by DiStefano (1987) reveals that molting crayfish is less resistant to severe acid stress compare to crayfish in intermolt stage. The crayfish can tolerate acidic water better at stream water temperature of 20.2 to 13.3 °C (DiStefano *et al.*, 1991). Even though they can tolerate such low pH, their reproduction activity will be altered and this could lead to damage of population in long term (DiStefano *et al.*, 1991).

Other than than, it was also found that the tolerance of freshwater crayfish (*Orconectes virilis*) toward water with low pH increase from hatchlings to adults (France, 1984). Nonetheless, episodic acidification of natural water could result in extinction of the whole population eventually due to mortality of crayfish larvae.