FEEDING AND RESPIRATION OF POST-LARVAL MUD CRABS (*Scylla serrata*) IN AN RECIRCULATING CELLULAR SYSTEM (RCS)

FINAL REPORT

Partial fulfilment for the degree of Master in Marine Studies

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PART ONE

Crab post larval feeding in an RCS system

Abstract

A study of food and feeding of post-larval mud crabs was undertaken to describe and compare feeding habits and also to establish whether growth rate is influenced by feeding frequency and the stage where the crabs enter the system (harvested and transferred from ponds to RCS system). The recirculating cellular system (RCS) is designed to stop cannibalism by keeping the larvae individually when they are still in infancy. The research result shown that with this RCS, the losses due to the mortality are very low (survival rate almost 100%). Growth experiments were conducted for nine weeks started with eight weeks old mud crab instar. Crab feeding three times a day gained the highest growth rate and final body weight. Compared to megalopae and C4 stage, C2 stage is the best time to harvest and to transfer the crabs from ponds to RCS. The relationship between body weight and carapace width was founding as equation $CW = 24.58e^{0.50W}$ ($R^2 = 0.88$; CW=carapace width, W=body weight) or $CW = 10.22Ln(W)+15,44$ ($R^2 = 0.91$).

1. Introduction

Mud crabs occurs throughout tropical to warm temperate zones (Heasman, 1980) and considered as a delicacy in Australia and Southeast Asia. There are four species of mud crabs within the genus Scylla, i.e *Scylla serrata, S. olivacea, S. tranquebarica, S. paramamosain* (Keenan, Davie, & Mann, 1998).

Several countries such as Indonesia, The Philippines, Vietnam, and Australia have investigated farming of these mud crabs. Recently, some interest in

commercial mud crab farming has developed in Australia, especially the Northern Territory and Queensland (Allan & Fielder, 2004). However, lack of regular seed supply has hindered the growth of the mud crab industry. Therefore mud crab breeding and seed production is being actively undertaken to promote its future expansion.

Hatchery protocols developed in Australia basically similar to those in Indonesia and Philippines. In Queensland, research on mud crabs is held by Queensland DPI through The Bribie Island Aquaculture Research Centre (BIARC). BIARC protocols still produce inconsistent yields (Allan & Fielder, 2004). For that reason, until recently in BIARC, the scientists are conducting several experiments to develop technology for a consistently high yield mud crab seed production.

In communal growing, cannibalism is a major problem to productivity in early stages of crab culture. The problem usually encountered in the nursery stage is the low survival rate of megalopa and early stage crab instars. Cannibalism is continuous throughout the nursery and grow-out cycle. In BIARC, some research is conducted to mitigate the cannibalism. The recirculating cellular system (RCS) is designed to stop cannibalism by keeping the larvae individually when they are still in infancy. In addition, RCS system, in which organisms are cultured and water is serially conditioned and reused, offers good result in maintaining water quality.

Water quality has several parameters, for example: temperature, pH, salinity, dissolved oxygen, and ammonia level. Amongst these parameters, ammonia level is very critical for mud crab aquaculture. Like other aquatic

crustaceans, mud crabs are ammononotelism, where ammonia makes up 60% to 100% of the total excreted nitrogen (Renault, 1997). The major source of nitrogen compounds is the protein contained in the feed. Therefore, the rate of ammonia production is proportional to the feeding rate.

The quality and quantity of the diet in the RCS is very critical as there is no other source of supplementary source of nutrition for the crabs. The quantity of diet should be observed as the quantity of waste derived directly from the diet lead to pollution problems. Therefore, present research conducted in BIARC is related with quantity of formulated diet in RCS.

One of the important things of grow-out mud crabs is find suitable feed. Feed is one of the major costs for aquaculture operations, typically making up between 30% and 60% of the total operating costs, depending on the intensity of the operation (Southgate, 2003). In recirculating system, feed cost constitutes 30% (Newman, 1999). Lawrence and Lee (1997) also added labour associated with feeding as another major production cost.

Grow-out stage requires some basic understandings of nutrient requirement of the species to achieve optimal growth, production efficiency, and maximising economic return, the most important thing in every business. Nutrition knowledge deals with process on ingestion, digestion, absorption, and metabolism.

Unfortunately, feeding habits of mud crabs are poorly known. Williams (1979) indicated that feeding and choice of food by crustaceans is influenced by many factors including their behaviour, morphology, and energetics. Nutritional research with crustacean species is relatively new and the amount of information

on specific nutritional requirements is quite limited compared to that of fish or terrestrial species (D'Abramo, 1997; Cuzon, et. al., 1994).

Artificial feeds for grow-out periods for mud crabs are mainly based on commercially available prawn feeds. Pelleted prawn diets, especially those available for *Penaeus japonicus*, the kuruma prawn, have given acceptable growth (Mann and Paterson, 2003b). The feed contains 50% of crude protein, 8% crude fat, and 19% crude ash (Pavasovic et. al., 2004). The nutrient composition of the feed matches to nutrition requirements for mud crabs. Catacutan et. al., (2003) found that protein are highly digestible to mud crab. A dietary lipid level ranging from 5.3 to 13.8% appears to meet the lipid requirements for juvenile mud crabs (Sheen and Wu, 1999). For cholesterol, the optimal dietary requirement was found to be approximately 0.51% (Sheen, 2000).

The experiments of crab post larval feeding in an RCS system was conducted to compare feeding habits and growth rate of *S. serrata* over nine week grow-out trial. This experiment used two different regimes of feed and three different stages of mud crabs when they were entering the systems from the ponds. In addition, the experiment also describes the survival rate of the crabs in RCS system and how this system maintaining water quality.

2. Material and method

The growth experiment was conducted at the Bribie Island Aquaculture Research Centre using a RCS system. The experiment was started with eight-week-old mud crabs (age was calculated since they were harvested from the pond), started from December 2004. The average initial weight of crabs in all groups was

about 1.72 – 2.05 gram. Three different groups of mud crabs exist when they were entering the RCS system. The groups comprising the megalopa stage, C2 stage, and C4 stage. In addition, there were two different frequencies of feeding (twice and three times feeding per day) during the experiment. In total, there were six different groups of mud crabs in the RCS system, defined as 2M, 3M, 2C2, 3C2, 2C4, and 3C4. Each group consists of 36 crabs.

Table 2.1 six group of crabs in the experiment

Stage at harvesting time (transferred from ponds to RCS)	Feeding frequency (per day)	Label/ group
Megalopae (M)	2	2M
	3	3M
C2	2	2C2
	3	3C2
C4	2	2C4
	3	3C4

The water quality was checked everyday. The parameters were temperature and pH. A random sample was chosen to detect the ammonia level using indophenol test for 28 crabs. During the experiment, to maintain the water quality, the system was cleaned daily.

To understand the effects of feeding frequency on growth rate and survival rate, the amount of feed was recorded daily. Mud crabs were fed EBI star prawn feed at a rate approximately 4% body weight (twice feeding) and 6% (three time feeding) per day. Once every week, the mud crabs were weighed. In addition, in

week 4, the carapace width was measured to establish the relationship between weight (W) and carapace width (CW).

Average values with error estimates given in the text or in figures and tables, represent arithmetic mean values ± standard deviation (SD). Differences in diet between different groups have been examined by analysis of variance and 95% confidence intervals of the mean with Duncan test from SPSS 10.5 software. It means that for all analyses, a significant level was considered for $p < 0.05$.

3. Result and discussion

3.1 Water quality and survival rate in RCS system

During the experiment, the water quality seems at acceptable level for optimal growth of mud crabs. Temperature was ranging from 26-28ºC, and pH from 7.8 to 8.1. The ammonia level was ranging from 0.000 to 0.012 mg/L.

A characteristic of aquatic crustacean is ammononotelism where ammonia makes up 60% to 100% of the total excreted nitrogen in Crustacea. Ammonia can be toxic if accumulate, and even at low levels can inhibit grow. Ammonia is harmful, therefore, ammonia level must be carefully controlled.

Ammonia is the major waste product of protein or nitrogenous metabolism in aquatic organisms. The major source of nitrogen compounds is the protein contained in the feed. Therefore, the rate of ammonia production is proportional to the feeding rate. It is excreted primarily across the gills, and in urine and faeces. Ammonia is also produced during the aerobic decomposition of organic matter by bacteria.

The high survival rate of crabs (almost 100%) shows that RCS is an excellent system for grow-out stage, particularly for mud crabs. Cuzon and Guillaume (1997) stated that survival rate of more than 80% is considered good in crustacean studies. From week 3, some crabs escaped from the containers. This is the reason why the total number of samples is not 216. The decreasing number did not relate to mortality but escaping.

One of the important issues for growing mud crabs is cannibalism (Mann & Paterson, 2003a; Pavasovic, et. al., 2004). The RCS system is designed to stop cannibalism by keeping the larvae individually when they are still in infancy. There are at least three benefits gained from growing individual crab in an individual container in RCS system. First, it reduces the cannibalism between crabs. Second, RCS conserves water, permits high-density culture where space and water are limiting. Third, RCS also minimises effluent, increased control, exclude predators, and there is a possibility of waste recovery.

RCS offers high level of animal husbandry and management in all inputs feeding, water, and animal density. In turn, in also promises maximum output of product in minimum area, water, and time. This system seems to be suitable for high value species. The main problem with RCS is the high establishment cost. Newman (1996) also stated, unfortunately, that material and labour costs in developed country tend to increase. In addition, Mann & Paterson (2003a) supposed that this system is relatively complex and labour intensive unless automation is employed.

Within an excellent system, however, in week five and six, some crabs were died, probably due to bacterial or fungal infection. The staff at BIARC did

excellent jobs when they excluded the unhealthy crabs from the system before the disease affected other crabs.

3.2 Growth rate

Crabs grew at different rates and reached different final weights. Mean weight of crabs ranging from 1.72 gram (2M group) to 2.05 gram (3M group) in the first week. After nine week experiment, the weight ranging from 20.58 gram (2C4 group) to 24.02 gram (3C2 group).

Table 3.2.1 outlines the initial and final body weight, and the SGR (specific growth rate), where SGR = 100% * {[Ln (BW9) – Ln (BW1)]/ days}. In the first week, there were already differences in mean weight between groups, where 2M was the lowest (1.72) and 3M was the highest (2.05). After 9 week experiment, the differences were significant only for 3C2 group (mean weight = 24.08). Specific growth rates (%SGR) varied between 4.21%/day (2C2) and 4.47%/day (2M).

Table 3.2.1 Initial and final body weight (in gram) and SGR (in % per day)

Groups	BW1	BW9	Ln (BW9) – Ln (BW1)	SGR (% per day)
2M	1.72 ± 0.43^{a}	21.04 ± 4.28^{c}	2.50	4.47
3M	2.05 ± 0.73^{b}	21.40 ± 6.25^{c}	2.35	4.19
2C2	2.05 ± 0.47^{b}	21.61 ± 3.30^{c}	2.36	4.21
3C2	2.03 ± 0.35^{b}	24.08 ± 5.23^{d}	2.47	4.42
2C4	1.89 ± 0.40^{ab}	20.58 ± 4.84^{c}	2.39	4.26
3C4	1.84 ± 0.34^{ab}	20.74 ± 4.68^{c}	2.42	4.33

Note: BW1= body weight week 1 (initial weight)
BW9= body weight week 9 (final weight)
Total days = 56 days
Mean body weight with different superscript letters are significant different ($p<0.05$)

Feeding frequency affected growth. Three time feeding per day resulted in higher body weight compared to twice feeding per day (3M higher than 2M; 3C2 higher than 2C2; and 3C4 higher than 2C4). This finding is different from

observation for other species. Catacutan et. al. (2003) did experiment on digestibility of freshwater prawn and found that feeding rate and feeding frequency have no effects on protein digestibility and feeding behaviour in general.

If we combined the effects of feeding frequency and harvesting time, ANOVA shows that the differences were significant only for 3C2 group (three time feeding per day, transferred to RCS system at C2 stage). It can be concluded that the best stage to transfer (harvest) mud crabs from the ponds to RCS system is C2. To gain the highest body weight, three time feeding per day (approximately 6% of body weight of feed) is the appropriate frequency for crabs.

Figure 3.2.1 illustrates the body weight during 9 week experiment. From this figure, it seems that after the initial weighing (week 1), significant growth (probably due to moulting), happened in week 3, 5 and 9. This estimation is based on the fact that mud crab almost doubles its weight every moulting.

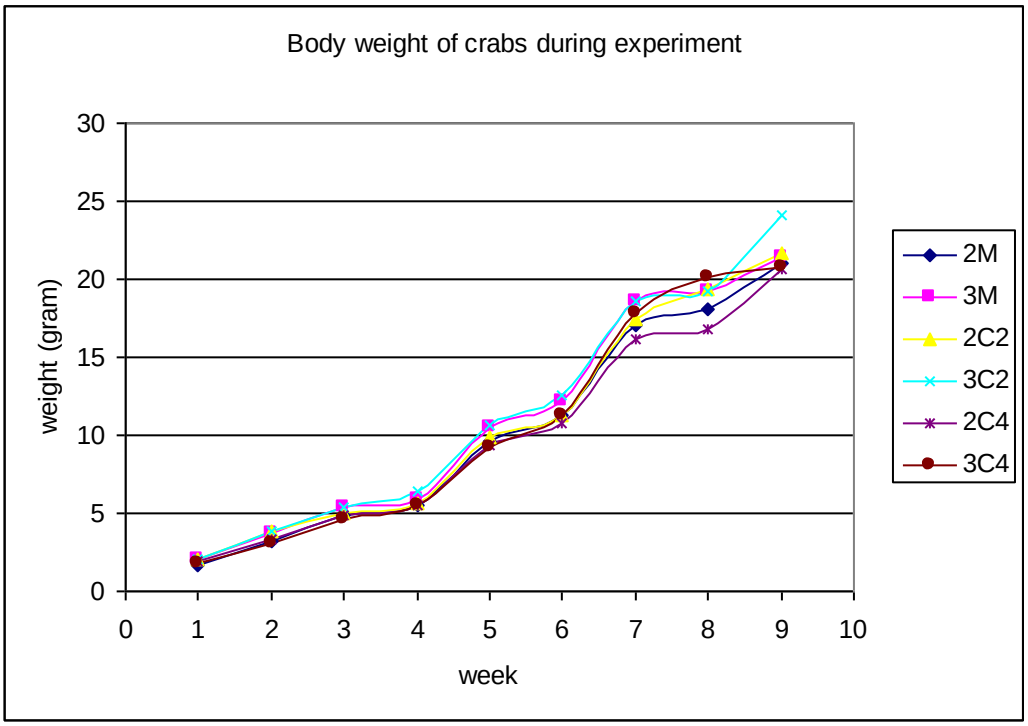

Figure 3.2.1 Body weight of crabs in 9 week experiment

Fielder et. al. (1994) and Wright (1998) summarized that growth of crustacean depends on the frequency of moulting and the growth increment at each moult. Like other crustaceans, growth of mud crabs relates to an increase in weight gain after moulting. Crustacean growth pattern is discontinuous, which consists of ecdysis (actual moult), premoult (period immediately before moult, lime stored from old skeleton, new skeleton forms beneath old), following by intermoult (stable weight period, water mass converts to muscle mass), and postmoult (period of swelling/water intake immediately after moult.

The observation revealed that after moulting, mud crabs not only consume the feed but also their exuvia. Sheen (2000) found that cholesterol gained from the feed, which is a metabolic precursor for ecdysone, plays important role in moulting.

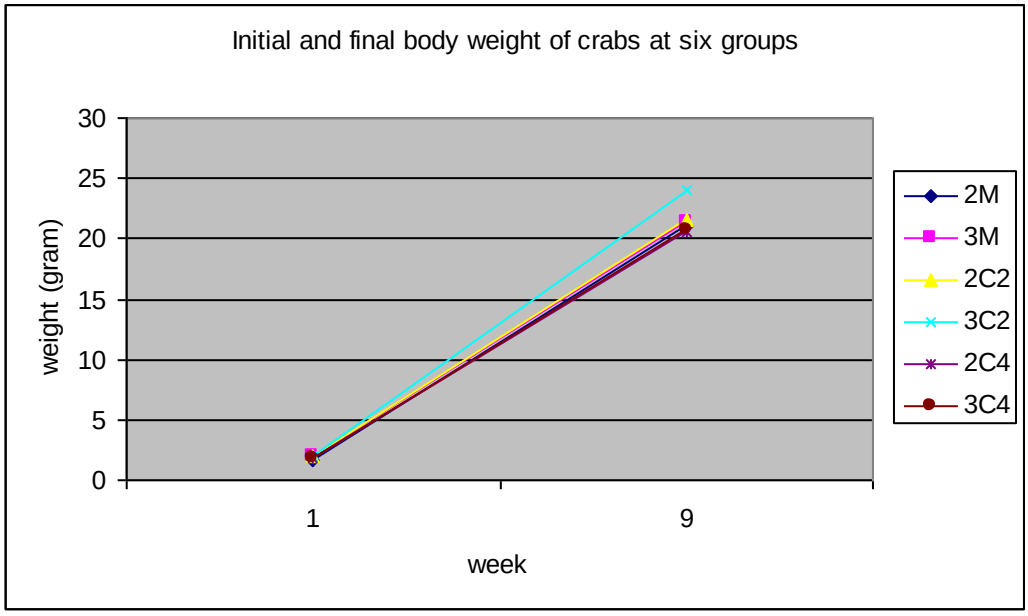

Figure 3.2.2 Initial and final body weight of crabs

Figure 3.2.2 (only plotted the initial and the final body weight) shows clearly that 3C2 group has the highest body weight compared to other groups. This group was always larger than other groups. Compared to megalopae and C4

stage, C2 stage has the best adaptation to a new environment/system. In other words, it is too early to transfer mud crabs from ponds to RCS when they are still in megalopae stage. During megalopae stage, crabs need more feed, which is more abundant in ponds, compared to in the RCS system where feed is always washed away together with the recirculated water. In the contrary, it is too late to transfer crabs if they have already in C4 stage. It seems that they have been adapted to abundant feed in the ponds. Harvested from the ponds and then transferred these C4 stage crabs to an RCS system will reduce the growth rate of the crabs. If we compared the growth performance of megalopae and C4 groups, even though the differences are not significant (Table 3.2.1), 2M and 3M groups have relatively higher final body weights than 2C4 and 3C4 groups.

Observation also revealed that mud crabs are active feeder. Not long after being fed, mud crabs consume the feed quickly. This is probably the main reason why three times feeding gave better growth performance in all groups (3M better than 2M; 3C2 better than 2C2, and 3C4 better than 2C4).

Even though mud crabs are active feeders, there is an exception during the time around moulting. Fielder et.al., (1994) stated that during premoult period, the crabs will not be able to feed, and its vision and its ability to respire will be impaired.

The moult cycle has major effects on quantity and types of foods eaten by crustaceans. Moult stage must be considered in any investigation of diet in crabs because feeding is very closely associated with the moult cycle. In particular, the period during and about the time of ecdysis with its accompanying rapid and complex physiological and biochemical changes is intimately and importantly

linked with feeding habits in crabs. Study by Williams (1979) suggest that portunid crabs fill their gastric mills with shell and other calcareous material immediately after they moult and while they are still soft. The physiological role of this food intake is probably to provide calcium for the formation of the new shell.

Being active feeders, in contrary, mud crabs also show a high survival rate even though being unfed for particular time (in the observation, twenty crabs being starved up to three days). After starvation, the crabs eat quickly when they realized that the feed are already for them.

3.3 The relationship between body weight and carapace width

Figure 3.3.1 demonstrates the relationship between body weight and carapace width. The equation is $CW = 24.58e^{0.50W}$ ($R^2 = 0.88$; CW=carapace width, W=body weight) or CW=10.22Ln(W)+15,44 ($R^2 = 0.91$)

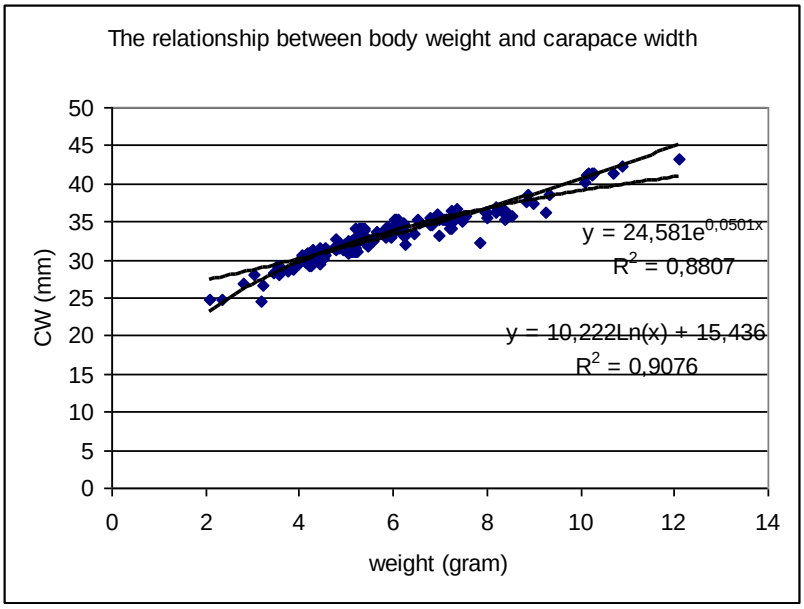

Figure 3.3.1 Scatterplot for the relationship between body weight and carapace width

Growth of swimming crabs is usually expressed as carapace width or length increment. In this experiment, the weight is ranging from 5.42 gram to 6.35 gram (based on mean for each group). It is true that mud crabs show a high survival rate (almost 100%) in an RCS system. But, in terms of growth rate (based on carapace width and weight gained), it seems that crabs in the wild and in other systems (e.g ponds) grow faster.

Research by Ali, et. al (2004) found that different equation applied for bigger crabs (Log W = -3.73 + 3.06 LogCW). Using a random sample of 168 mud crabs, Fortes (1999) found that for average weight 45.81 gram, the average carapace width is 62.42 mm. Crabs can increase their size to approximately 65 mm CW within 60 days of moulting from a megalopae (Catacutan, 2002). Christensen, et. al. (2004) started their experiment with 8.69 gram of individual crabs in the pond. After 70 days, the crabs reached about 100 gram in weight.

Those findings show that mud crabs grow slower in an RCS system. RCS system improves survival rates during megalopae and early stage of crabs (C1) as this system can avoid cannibalism and predation. After they show a well ability to survive, it is better to transfer the crabs to ponds so that they can grow faster as the feed is more abundant and is not washed away.

5. Conclusion and Future direction

Harvesting time and feeding frequency are both important in determining the differences in growth rate of mud crabs. Being active feeder, mud crabs response to feeding quickly. Three time feeding per day gave better result in growth performance than twice feeding. There could be significant benefits for

producers by applying three times feeding per day. In terms of harvested time, the best stage to transfer juvenile mud crabs from pond to RCS system is C2 stage. RCS shows an excellent promise to maintain the water quality. RCS seems to be a suitable system for grow-out mud crabs and results in high survival rates but low growth rates compared to other system. The problems being encountered with this system are the establishment cost and labour cost (for feed the crabs and clean the system). Another problem is how to achieve uniform growth rates which is important for industrial scale.

During the experiment, grow-out feeds are based on commercially available prawn feeds. Pelleted prawn diets, especially those available for *Penaeus japonicus*, the kuruma prawn, have given acceptable growth but are very expensive. Finding cheaper feeds would significantly affect cost of production. The pellet size, shape, buoyancy and hardness were listed as important issues and prawn pellets are deficient especially with regard to size and shape.

Catacutan, et. al. (2003) found that the digestibility of lipid in protein-rich animal feedstuffs was low compared to carbohydrate-rich plants feedstuffs. Pavasovic et. al (2004) observed that diets containing 47% carbohydrate resulted in high activities of amylase, cellulase, and xylanase. These two findings suggested that diet containing carbohydrate-rich plant stuffs probably an alternative to kuruma prawn diet.

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PART TWO

The effects of body size and feeding activity on oxygen consumption of mud crabs (*Scylla serrata*)

Abstract

The effect of crabs size and feeding activity on the oxygen consumption was studied with the aim of estimating the individual oxygen consumption of crabs based on their size and feeding activity. Measurements were performed at 27 ± 0.5ºC for 16.81 - 348.01 gram of intermolt mud crabs. In general, individual rate of weight specific oxygen consumption decreased with increasing body mass. The rate of oxygen consumption for mud crabs between 16.81 – 43.47 gram varied from 0.04 to 0.33 mg/g/h for unfed crabs and from 0.18 to 0.75 mg/g/h for the fed crabs. The relationship between oxygen consumption and crab live weight was found as Log VO_2 (mg/h) = 0,705 Log BW – 0,286 (R^2 = 0.77) or Log MO_2 (mg/g/h) = -0.3069Log BW – 0.2659 (R^2 = 0.39). This equation will give predicted oxygen consumption for every body size.

1. Introduction

Respiratory metabolism has been considered as a good indicator of the activity of aquatic organism in relation to the energy required for feeding, migration, reproduction, and escape from predators. In crustacean, this can be seen as reflection of the energy utilized for general muscular movement and heart activity, as well as the internal regulation and movement (Penkoff & Thurberg 1982).

In order to respire efficiently, an animal must possess means of moving the respiratory medium across the gas exchange surfaces, be able to take up

oxygen from the medium, and transport oxygen to the tissues and the carbon dioxide produced to the gas exchange surface (Waldron, 1991 in Forteath, 1998).

As in other aquatic animals, the oxygen consumed by crustacean, is used as the last acceptor of electrons in the respiratory process, which usually begins with the degradation of carbohydrates in the glycolysis cycle (Chang and O'Connor, 1983 in Rosas et. al., 1992).

Buchanan (1999) concluded that oxygen consumption is an indicator of basic metabolic rate and energy expenditure. It provides an indirect measure of heat production and can be used to determine the relationship between live weight and metabolic activity. It can also provide insight into metabolic response to diet. As well being a key indicator of metabolic rate, the estimate oxygen consumption has important practical for the design of holding facilities. The prediction of oxygen consumption would allow operators to design holding tanks and transport systems to meet the oxygen demand of the culture animals. It is essential water flow and aeration are sufficient to provide adequate oxygen for the number of animals being held.

Some factors influence the oxygen consumption rate, particularly temperature, a major environmental determinant of metabolic rate of animals (Armitage, 1982; Belman & Childress, 1973). Other factors, such as salinity, light, oxygen concentration, size, sex, food, and activity has also been studied (Ansell, 1973; Armitage, 1982; Carvalho & Phan, 1997; Davenport & Wong, 1987; Du Preez, 1983; Issartel et. al., 2005; Sabourin & Stickle, 1980). Moulting stage also has a major affects on the oxygen consumption of crustaceans (Alcaraz & Sarda, 1981; Penkoff & Thurberg, 1982).

A number of studies on oxygen consumption of mud crabs have been done. Veeranan (1972) reported that 10g of mud crabs at 27ºC consume 2.020 ml/h. Chen& Chia (1996) showed that the oxygen consumption of 0.5g mud crabs at 30ppt and 22ºC is less than 0.1 $mgO_2/g/h$. Research by Valarmathi (2002) found that 3-4 g of crabs *Sesarma quadratum* consumed 0.9 mgO_2/L. Unfortunately not much research has been done on other warm water crabs to compare with. Davenport & Wong (1987) found that mud crabs show a well developed ability to exploit the rich source of oxygen available in the air.

The objective of this study was to define the oxygen consumption rate of individual mud crabs in relation to crab's size, feeding, and to establish if daily/diurnal pattern is present.

2. Materials and methods

Fifty-four mud crabs ranging size from 16.81 - 348.01 grams were used to measure oxygen consumption. Oxygen consumption by organism was determined using a flow-through respirometer based on the method described by Marais et. al. (1976). Before the start of the experiments, the crabs were allowed to remain 15 minutes in the chambers in open water circulation, in order to avoid any immediate activity associated with handling.

The water is pumped from the sump up to the heated and aerated header tank. This is kept at a constant level to maintain a constant head with respect to the respiration chambers below. A constant flow (12 L/h) of 27 ± 0.5 ºC sea water was allowed to flow by gravity from the header tank into a manifold and from there to different sizes of watertight plastic chambers, depending upon the crab's size (0.75 L for crabs below 50 gram and 4L chambers for crabs above 50 gram). The salinity of the seawater was 30ppt and aerated to saturation.

The oxygen electrode, solenoid array and the oxygen meter were used for the experiment. Small non-corroding solenoid valves were used to divert water from each chamber in turn to the oxygen sensor, using a computer program controlling a "Basic Stamp" processor BS1 (Parallax Inc, California, USA) located in a water-proof enclosure. The oxygen electrode therefore received a continuous water flow alternating from chamber to chamber at intervals of 5 minutes.

To ensure that flow rate was uniform during switching, the flow rate was checked before, during, and after measurement in each chamber at the beginning and at the end of experiments. There were two directions of small plastic pipes out from each chamber, one is connected to the oxygen meter and another one goes to the sump. Flow rate was measured using the pipe that goes to the sump (out-flowing water). For example, when the electrode is measuring the oxygen level of chamber number two, the out-flowing water from pipe number three was counted for one minute in a measuring glass and then converted to L/h unit. When the electrode switches to chamber number three, the out-flowing water from pipe number three was determined again to ensure that flow rate is uniform and crabs

did not realize whether they are being measured or not. When the electrode switches to chamber four, the out-flowing water of pipe number three was determined once again to ensure that the flow-rate is about the same before, during, and after the measurement. During the experiment, the flow-rate was found as (12 ± 0,12) L/h. Maintaining flow rate is necessary since rate of flow affects oxygen meter recordings and because flow rate past the probe and through by-passes must be equal.

Oxygen concentrations were measured by a precision oxygen meter (HQ20 Hach Portable LDO^{TM}). The oxygen meter was programmed to log the oxygen measurements at 5 minute intervals, and was timed so that the record was taken in the middle of the "open" period for each channel. Replicates of oxygen consumption were recorded every 30 minutes during the 24-hour period to establish if daily oxygen consumption pattern/rhythm was present. After every experiment, all the excess of water was removed from the animal which was then weighed. Respiration rate (mgO_2/gBW/h where BW represents wet body weight) was calculated from the logged oxygen concentrations.

Initial oxygen concentration of the water was determined from the blank chamber as a reference for the inlet water in the other chambers. Then, a series of crabs (consist of 5 crabs and blank) in six chambers was introduced into the chamber individually and allowed to respire for 30 minutes. The amount of oxygen used by each crab was determined by subtracting the mean value of the blank (control) from each reading.

To understand the effect of feeding on oxygen consumption rate, a series of crabs was used twice. In the first day, the crabs were starved for 24 hour prior

to the experiment and were not fed during the experiment period. In the second day, the experiments were started by introducing feed into chambers using the same crabs as day one.

Statistical analyses (paired student t-tests) were used to evaluate differences between unfed and fed crabs. The analyses were performed on the SPSS statistical package with the α set at 0.05 (confidence interval 95%).

3. Results and discussion

3.1 Standard oxygen consumption

Standard oxygen consumption is defined as the minimum oxygen consumption for unfed, resting fish (Fry 1971 in Forteath, 1998). Therefore, it is important to determine the specific time during 24-hour experiment which is consider as the time for standard oxygen consumption.

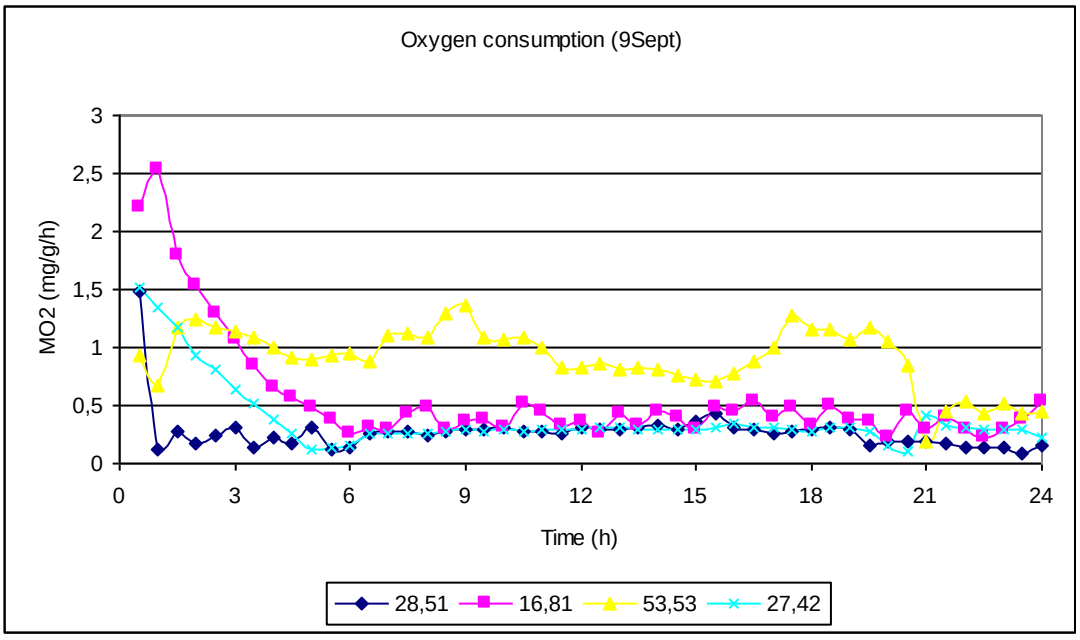

Figure 3.1.1 Weight specific oxygen consumptions of small crabs

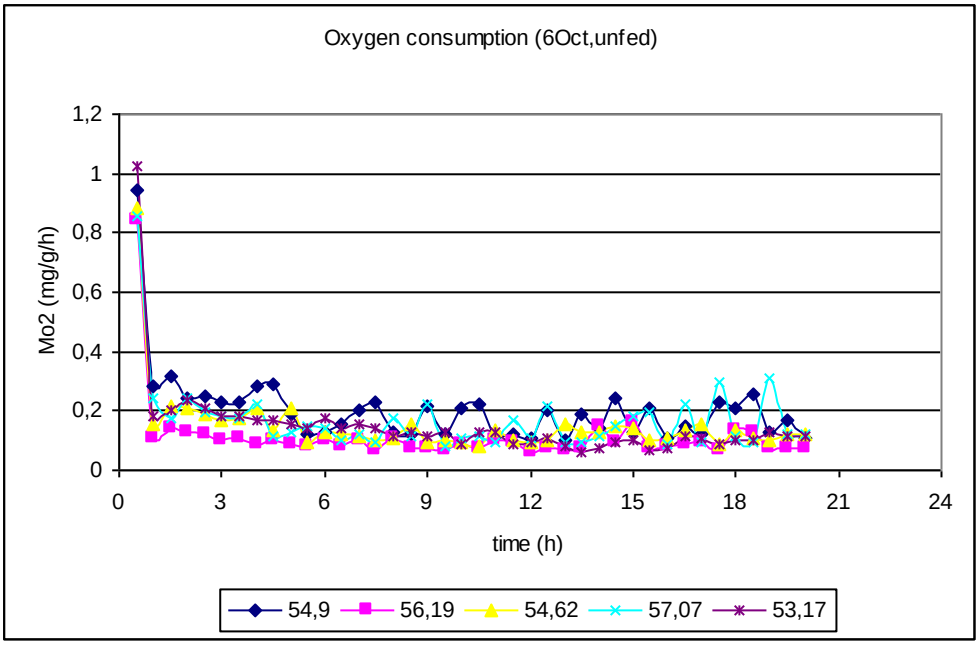

Figure 3.1.2 Weight specific oxygen consumption of medium crabs

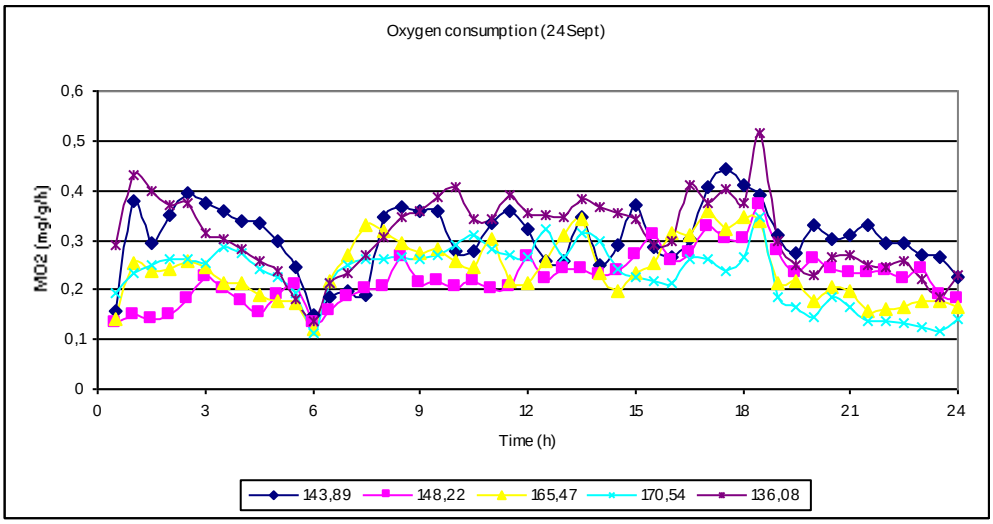

Figure 3.1.3 Weight specific oxygen consumption of medium-large crabs

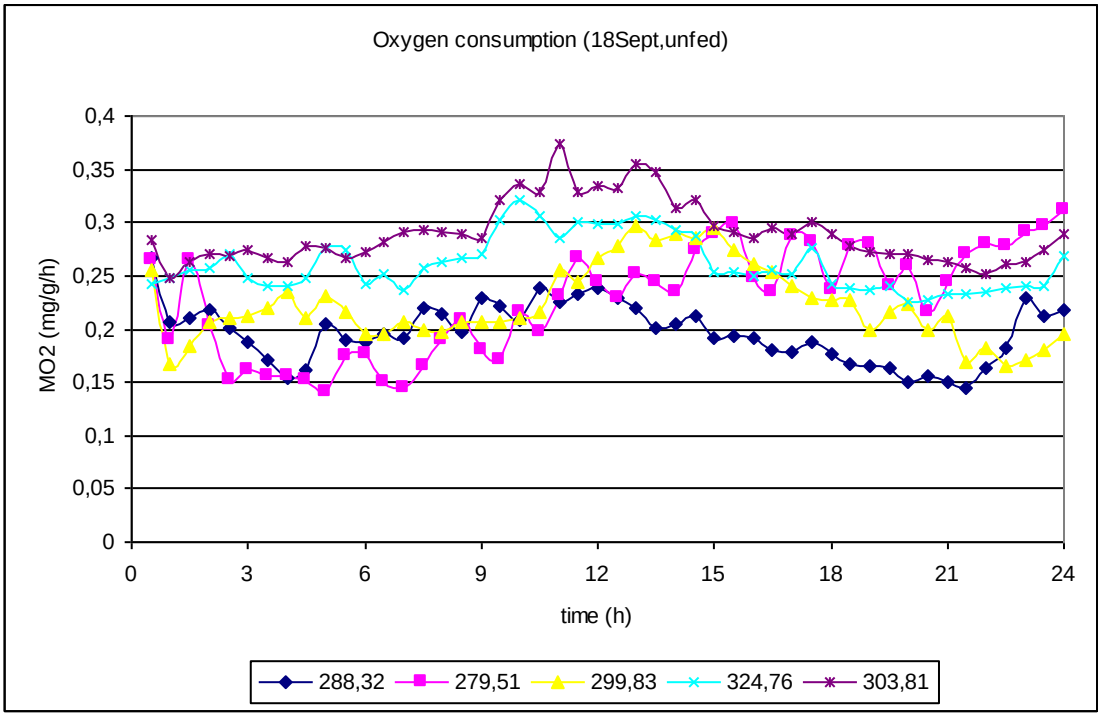

Figure 3.1.4 Weight specific oxygen consumption of large crabs

Compared to other crustaceans, mud crabs take a longer time (6 hours) to return to their standard oxygen consumption. Research by Buchanan (1999) found between 1-3 hours for crayfish (*Cherax quadricarinatus*). Crear and Forteath (2000) concluded between 4 - 4.5 hours for lobster (*Jasus edwardsii*) to recover after a period of handling and emersion.

3.2 The effect of body size on oxygen consumption

Figure 3.2.1 describes the standard oxygen consumption for crabs ranging from 16.81 to 348.01 gram. The oxygen consumption rates were plotted against the size of the crabs. It is important to establish the relationship between oxygen consumption and live weight. The equation which describes the relationship, will

give predicted oxygen consumption for every body size. In this experiment, the relationship between total oxygen consumption and body weight was found as Log VO_2 (mg/h) = 0,705 Log BW – 0,286 (R^2 = 0.77). Figure 3.2.2 describes the specific oxygen consumption while figure 3.2.3 illustrates the equation for the relationship between weight specific oxygen consumption and body weight, which is Log MO_2 (mg/g/h) = -0.3069 Log BW – 0.2659 (R^2 = 0.39).

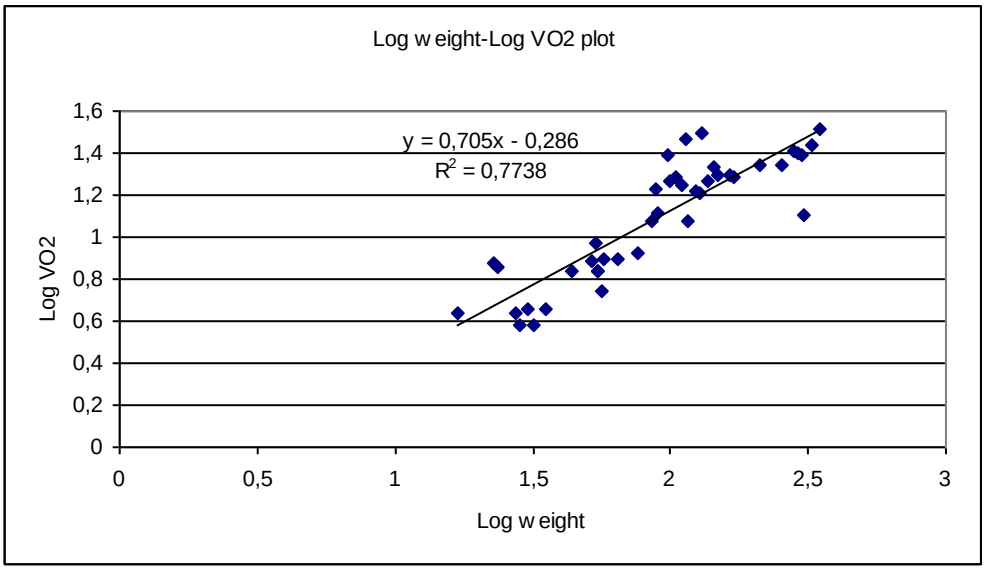

Figure 3.2.1 Log weight – log VO2 plot (VO2 in mg/h)

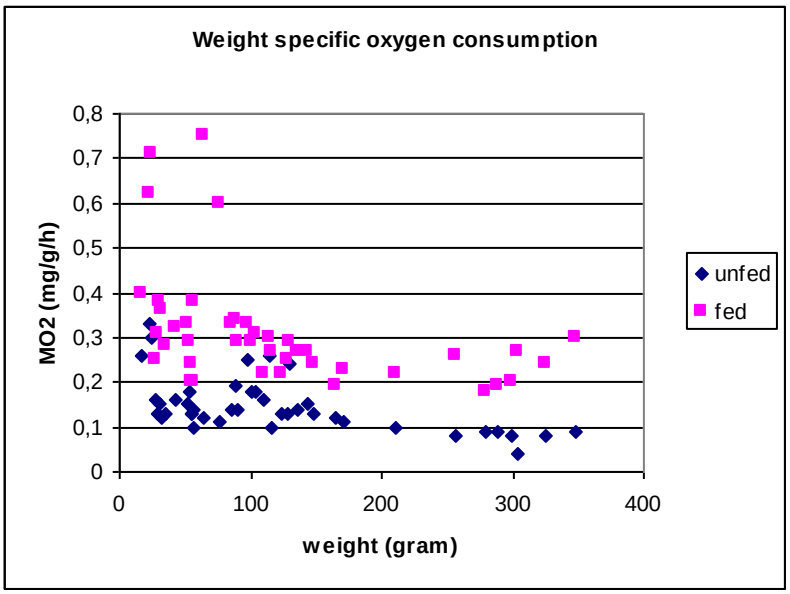

Figure 3.2.2 Weight specific oxygen consumption

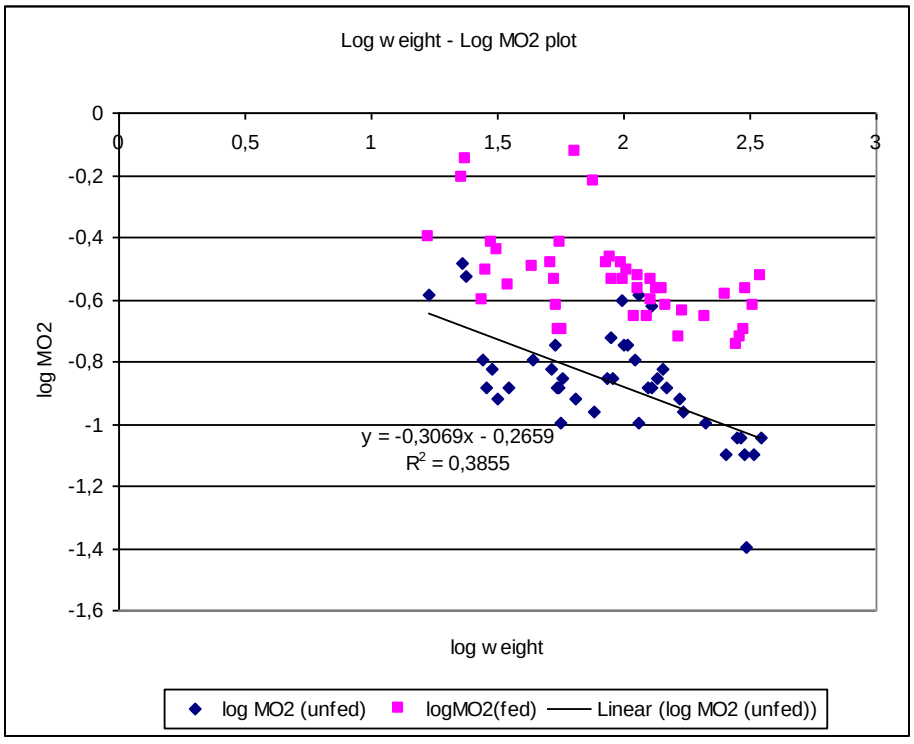

Figure 3.2.3 Log weight – log MO_2 plot

Total oxygen consumption (VO_2 in mg/h) increases with body weight. However, oxygen consumption per gram of tissues (MO_2 in mg/g/h) decreases as

live weight increases. In other words, per weight basis, larger crabs consume less oxygen than smaller crabs. This is the general pattern for animals but the rate of the decline varies between species. Based on oxygen requirements, larger animals can be maintained at a higher stocking rate than smaller ones (Forteath, 1998). As oxygen consumption is a function of metabolic rate, the metabolic rate of crabs could be assumed following the oxygen consumption pattern.

Compared to previous research by Veeranan (1972) and Chen & Chia (1996), the oxygen consumption found in this experiment seems different. Those two research only deal with small crabs while in this experiment, the size range is wider. The differences may also have been caused by differences in the measurement procedures.

Observation during the experiment reveals that crabs did not move very often. Larger crabs tend to move their legs while the smaller crabs were only sitting on the bottom. Lack of activity probably the major cause why crabs only consume small amount of oxygen compared to other crustaceans. In addition, the crabs used for this experiment has been reared in an RCS system for almost one year. Whether or not the crabs behave in a manner different to wild stock is relatively unknown. Probably, the crabs have been adapted to the conditions where feed is always supplied for them. This does not apply in the wild where crabs have to be active to find food.

3.3 The effect of feeding on oxygen consumption

Being starved for 48 hours in total, crabs show a well ability of survival. When the feed was introduced into the chamber, smaller crabs responded faster

compared to bigger crabs. Here, smaller crabs have a higher feeding activity as they can actually move more successfully.

Figure 3.2.2 compared the oxygen consumption between fed crabs and unfed (starved) crabs. Feeding increased oxygen consumption to about two times the unfed level. Mean oxygen consumption for unfed crabs is (0.15 ± 0.06) mg/g/h while for fed crabs is (0.31 ± 0.13) mg/g/h. Here, starvation resulted in a decrease in oxygen consumption. Paired student t-test shows that the differences between the fed and the starved crabs are significant ($t = 9.21$ $p = 0.02$). In this experiment, we should expect such differences to be due primarily to differences in feeding activity as there is no other major activity being done by crabs. Feeding activity consist of approaching, handling, eating, and digesting the food.

In natural environment, whether or not starvation occurs will depend on food availability and the behaviour of the animal in foraging (Ansell, 1973). Quality and quantity of food also directly affect metabolic rate. Food intake is divided between metabolism and growth. As moulting is the only actual growth for crustaceans, during intermolt stage, the major energy is spent for metabolism.

In an RCS system, mud crabs not only has less movement but also has a lower feeding activity compared to crabs in the wild. The amount of food introduced to the chamber is no more than 1% of body weight. In fact, after 24 hours, sometimes the food is still left.

3.3 The daily oxygen rhythm of crabs

According to Dall (1986), it is necessary to make an estimate of animals 24-h metabolic rate to develop a reliable energy budget for the animals. Unfortunately, it is difficult to establish if daily oxygen consumption rhythm is

present for mud crabs as the pattern from this study varied widely. The observation started between 12-3pm and finish 24 hours later. In general, the specific oxygen consumption decreases considerably in the first three hours and continue to 6h. After 6 hours, the consumption is slightly fluctuated.

From the observation, mud crabs probably opportunistic animals. They can be active during the day or the night, particularly if they realized that sufficient food is supplied for them. According to Hill (1978), mud crabs are more active during the night but not for all the night. Mud crabs are also more active in the presence of food.

It takes about 6 hours for crabs to reach their standard oxygen consumptions. Bigger crabs need longer time to recover compared to smaller crabs. After 24 hours of observation, smaller crabs have the same or similar oxygen consumption with the initial oxygen consumption.

Davenport & Wong (1987) added that culture system for mud crabs should feature shallow areas to allow crabs to exploit atmospheric air in the event of failure to artificial aeration. In the laboratory, confinement of RCS probably makes the crabs did not respond to biorhythms which relate to natural environment such as tides, moon, etc.

4. Conclusion and future direction

Individual rate of weight specific oxygen consumption decreased with increasing body mass. Feeding activity affect significantly (two times) on the oxygen consumption of crabs. It is difficult to establish the oxygen consumption pattern of crabs from 24 hour experiment. Probably, it needs more than 24 hours to pick up the pattern as in the lobster which needs 48 hours (Forteath, 1998). In addition, confinement of RCS probably makes the crabs did not respond to biorhythms which relate to natural environment such as tides, moon, etc.

Apart from body size and feeding, it is important to understand the oxygen consumption of crabs during molting. This kind of experiment has been done for other crustaceans but not yet for crabs. It is also interesting to compare the oxygen consumption of cultured and wild crabs.

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