Searching for Nature Story 2014

# The Amazing Creature with Appendages Around the Mouth

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Group 3/ Team A

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# The Amazing Creature with Appendages attached to the mouth

**Abstract**: Juvenile horseshoe crabs were used to study the effects of presence of light on their activity, the types of foods they preferred and the effect of water waves on their turnover activity. Through observation and simple experimental work, the obtained data suggested that they were responsive to light, selective feeders on bivalves(clams), squid and brine shrimps and could make good use of their tail spine(telson) and wave action to help them to turnover.

## Introduction

Horseshoe crab is an eminent living fossil creature that appeared in the Geological history. They have not altered their structure of body irrespective of the changeable environment. Then, how can they adapt to the changeable environment?

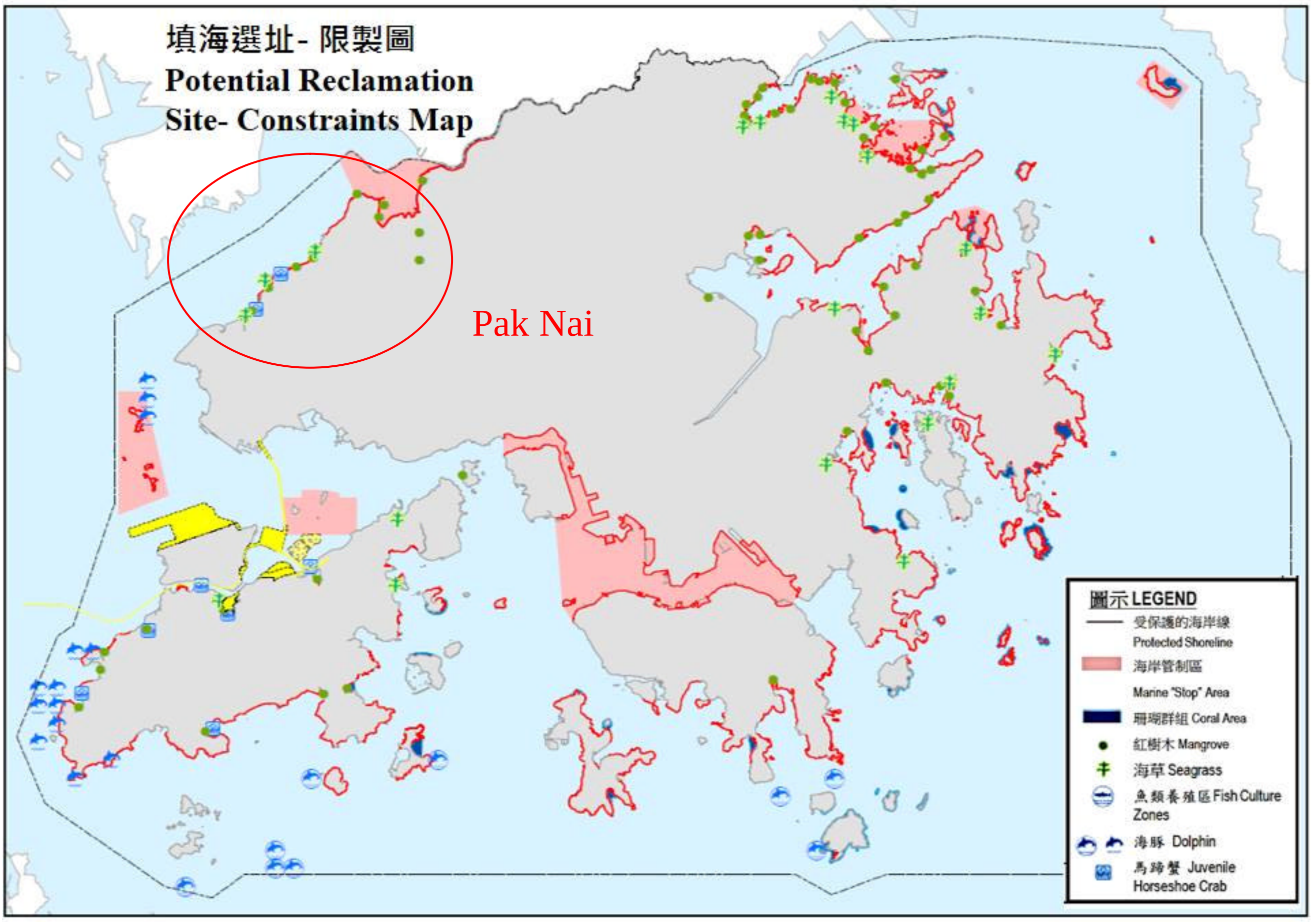
Horseshoe crabs belong to the phylum of Arthropods, which consists of animals having an articulated body and jointed limbs**.** The horseshoe crabs belongs to its own class called Merostomata, which means "legs attached to the mouth". Though they are called "crabs", but they are not.

From some research papers, we found that they were once abundant in the shores of Hong Kong such as Pak Nai, San Tau and Shui Ha. But recent data shows that their population is decreasing drastically due to the loss of breeding grounds, habitat destruction and marine pollution. They are in the danger of becoming extinct. So we are interested in looking at some factors that related to their survival. Besides, we also joined a Juvenile Horseshoe Crab Rearing programme, we have some juvenile horseshoe crabs in our biology laboratory and we decide to take this golden opportunity to investigate the effects of numerous factors on the activity of horseshoe crabs together with the observation from the natural habitat. The factors that we studied are presence of light on their activity, the types of foods they preferred and the effect of water waves on their turnover activity. If we know more about them, we may help conserve them in a better way.

The type of juvenile horseshoe crab is *Tachypleus tridentatus* in about 3-4 instar stage.

## A. Observation of *Tachypleus tridentatus*

**i.** Locations that we could find *Tachypleus tridentatus* in Hong Kong




**ii. Field study**

1. Date: 27th December, 2013 Time: 11:00-13:00 Venue: Ha Pak Nai

Work: observed the habitat of horseshoe crabs and seek for horseshoe crabs.

**2.** Date: 18th February, 2014 Time: 12:00-16:00 Venue: Ha Pak Nai

Work: observed the habitat and stucture of *Tachypleus tridentatus.*

**3.** Date: 22nd March, 2014 Time: 2:00 – 17:00 Venue: Ha Pak Nai

Work: observed the habitat and stucture of *Tachypleus tridentatus*.

**iii. Observation of External structure of *Tachypleus tridentatus:***

***Why is horseshoe crab a crab but not a crab?***

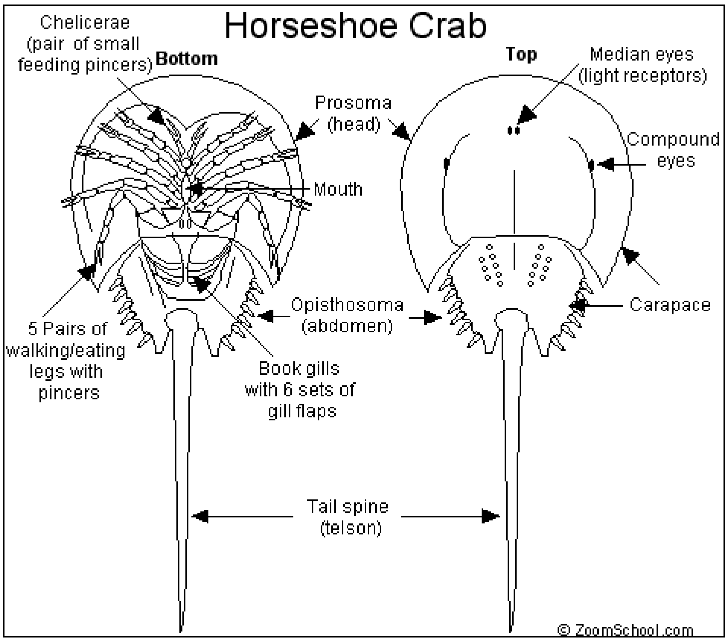



*Fig 1 shows the basic structure of a horseshoe crab*

*Comparison between a horseshoe crab and a true crab*

| | *A Horseshoe Crab* | *A True Crab* |
|---|---|---|
| *2 pairs of antennae* | *No* | *Yes* |
| *A pair of mandibles* | *No* | *Yes* |
| *No. of pair of appendages* | *7* | *5* |
| *No. of pair of appendages with claws* | *5* | *1* |

*Base on the simple morphology, we found that Tachypleus tridentatus was not a crab.*

## A. 1.1 Experiment 1: To study the effect of light on the activity of Juvenile *Tachypleus tridentatus*

**A.1.1.1 Aim** :To study the preference of Juvenile *Tachypleus tridentatus* for brightness and darkness

**A. 1.1.2 Hypothesis:** Hungry Juvenile *Tachypleus tridentatus* will move to the area with light in order to search for food.

**A. 1.1.3 Materials and Apparatus:**

| |
|---|
| 1 tank with water |
| 2 desk lamp |
| 1 piece of black cardboard |
| 8 Juvenile *Tachypleus tridentatus* |

**Key:**

| | |
|---|---|
| Zone A | Area with light |
| Zone B | Area without light |

**A. 1.1.4 Observation:**
Juvenile *Tachypleus tridentatus liked to move to area with light and they moved their appendages rapidly under lighted condition as well.*

**Fig 1.1 shows the set-up of the experiment**

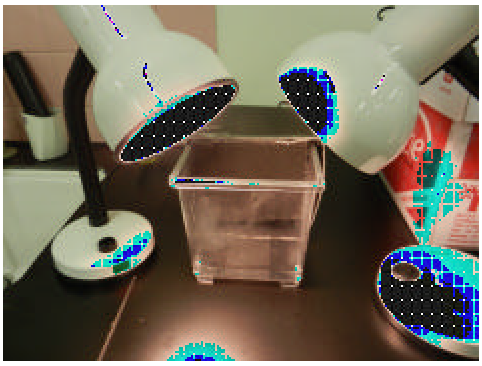
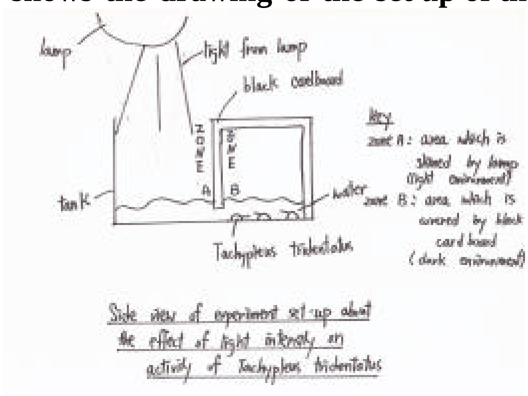


**Fig 1.2 shows the drawing of the set-up of the experiment**



### A. 1.1.5 Method:

1.Put all hungry Juvenile *Tachypleus tridentatus* in the dark area of the tank.

2.Observe Juvenile *Tachypleus tridentatus*' movement.

3.Count the number of Juvenile *Tachypleus tridentatus* which move out to the bright area every 5 minutes.

4.Repeat the above steps 1 – 3 with Juvenile *Tachypleus tridentatus* which are full.

### A. 1.1.6 Variables:

**The following table shows the variables of this experiment:**

| Independent variables | Dependent variables | Controlled variables |
|---|---|---|
| √ Presence of light or absence of light | √ the number of Juvenile *Tachypleus tridentatus* which exited from the dark area | √ Temperature of the atmosphere<br>√ Amount of water in the tank<br>√ Size of bright area and dark area |

**Assumption:** The more Juvenile *Tachypleus tridentatus* coming out to the bright area, the higher preference for the light as they prefer to carry out activities in the area with light

Table 1.1 shows the results of the number of Juvenile *Tachypleus tridentatus* which moved to the bright area counted in different time intervals

| Number of 5-minute time interval | 1st trial | 2nd trial | Average rate |
|---|---|---|---|
| 1st time interval | 3 | 3 | 37.5% |
| 2nd time interval | 6 | 4 | 62.5% |
| 3rd time interval | 5 | 5 | 62.5% |
| 4th time interval | 3 | 4 | 43.75% |
| 5th time interval | 6 | 6 | 75% |
| 6th time interval | 6 | 7 | 81.25% |
| **Overall rate** | | | **60.42%** |

Table 1.2 shows the results of the number of full Juvenile *Tachypleus tridentatus* counted in different time intervals

| Number of 5-minute time interveral | 1st trial | 2nd trial | Average rate |
|---|---|---|---|
| 1st time interval | 7 | 2 | 56.25% |
| 2nd time interval | 5 | 4 | 56.25% |
| 3rd time interval | 5 | 3 | 50% |
| 4th time interval | 4 | 5 | 56.25% |
| 5th time interval | 4 | 6 | 62.5% |
| 6th time interval | 4 | 6 | 62.5% |
| **Overall rate** | | | **57.29%** |

### A. 1.1.7 Discussion:

It was found that the hypothesis was not supported by the obtained data as Juvenile *Tachypleus tridentatus* were attracted by light no matter they were full or hungry. This was reflected by the result of similar overall rate of both types of Juvenile *Tachypleus tridentatus*. We first predicted that only hungry Juvenile *Tachypleus tridentatus* were attracted by light because they wanted to forage. After we have repeated the experiment several times, the obtained result suggested that they moved to bright area no matter they were hungry or full. In most of the cases, they came out from the dark side to the light side which meant that they were very sensitive to the light. Most of the Juvenile *Tachypleus tridentatus* were more active under light condition with their moving appendages. Besides, we also found that they actively moved along the boundary of the tank.

*Tachypleus tridentatus* had structures to detect light. Each *Tachypleus tridentatus* has ten eyes. They were two large compound eyes on the top of the shell for finding mates as shown in Fig 1. The compounded eyes composed of about 1000 ommatidia which could determine brightness and color. Besides, it also had a pair of median eyes that were able to detect visible light and UV-light. Apart from these, it had numerous smaller eyes called photoreceptors on its top shell and along its tail. Being sensitive to light, they helped synchronize the crab's internal clock with daily cycles of light and darkness to find food or for navigation. The ability to detect UV-light was important to them as they could see well at night as they did in the day since they lived in benthic habitat where the condition was mainly dark.

### A. 1. 1.8 Summary:

The ability of detecting light was very important to Juvenile *Tachypleus tridentatus* as they had a lot of eyes (ten) all over their body. They use their eyes to find mates, to find food and for navigation. This was important for them to survive in the benthic habitat.

**A.1.1.9 Limitations:** There were some limitations in this experiment. First, the result we obtained was not very reliable due to the number of Juvenile *Tachypleus tridentatus studied*. We only studied the behavior of 8 Juvenile *Tachypleus tridentatus*. Time was another factor that we could only try out the experiment for several times. For example, we only studied the effect of light on horseshoe crabs within 30 minutes. It might take a longer time to response. We should study for a longer time and more trials so that a more reliable result would be obtained.

### A. 1.1.10 Conclusion:

Juvenile *Tachypleus tridentatus* showed a higher preference on brightness than that of darkness.

# A. Experiment 2 .1: To study the Preference of Juvenile Horseshoe Crabs for Different Kinds of Food

**B.2.1.1 Aim:** To study the preference of Juvenile *Tachypleus tridentatus* for different kinds of food (*Artemia*, blood worms, clams and squids)

**B.2.1.2 Materials and Apparatus:**

| | |
|---|---|
| - 8 Juvenile *Tachypleus tridentatus*<br>- 1g of *Artemia* (brine shrimp)<br>- 1g of blood worms<br>- 1g of clams<br>- 1g of squids | - 1 measuring cylinder<br>- 1 electron balance<br>- 4 beakers (250mL)<br>- 1 razor blade<br>- 1 spatula<br>- 1 white tile<br>- 50mL of salt water |

Fig 2.1 shows the materials for this experiment

**B.2.1.3 Variables:**

The following table shows the variables of this experiment:

| Independent Variables | Controlled Variables | Dependent Variable |
|---|---|---|
| ➢ The types of food (*Artemia*, blood worms, clams and squids). | ➢ The weight of food<br>➢ The volume of water<br>➢ The temperature of water<br>➢ The number of Juvenile *Tachypleus tridentatus*<br>➢ The time for observation on preference of Juvenile *Tachypleus tridentatus* for different food | ➢ The movement of Juvenile *Tachypleus tridentatus'* appendages. |

**B.2.1.4 : Method:**

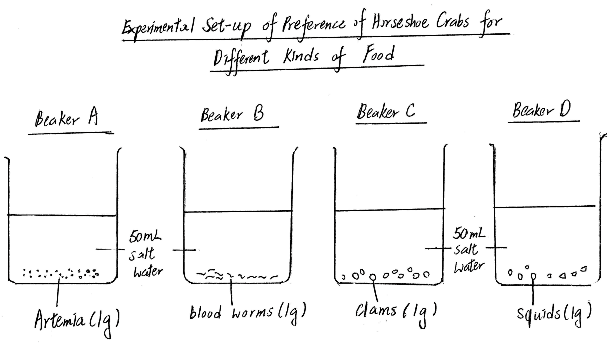
1. 50mL of salt water was measured by a measuring cylinder and poured into beaker A.
2. *Artemia* was cut into small pieces on a white tile.
3. 1g of *Artemia* was weighed using an electron balance.
4. Steps(1) - (3) were repeated to blood worms(B), clams(C), squids (D).
5. The following table shows the contents of each beaker.

| Beaker | *Kinds of food used* |
|---|---|
| Beaker A | *Artemia* |
| Beaker B | Blood worms |
| Beaker C | Clams |
| Beaker D | Squids |

5. Two Juvenile *Tachypleus tridentatus* were put into each beaker.
6. Juvenile *Tachypleus tridentatus*' preference was observed for two hours.

**Fig 2.2 shows the set-up of this experiment**

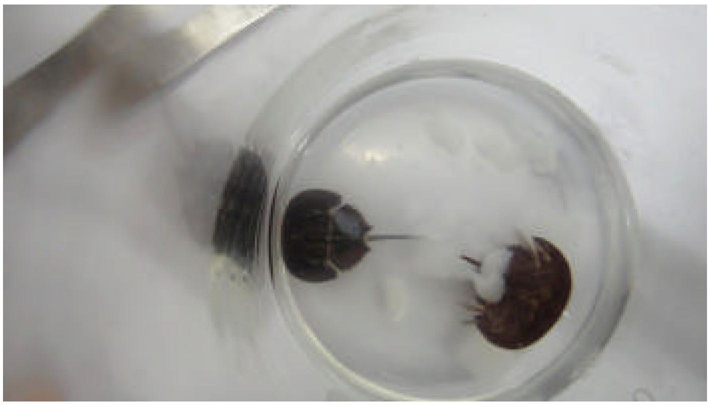
**Fig 2.3 shows the drawing of the set-up**

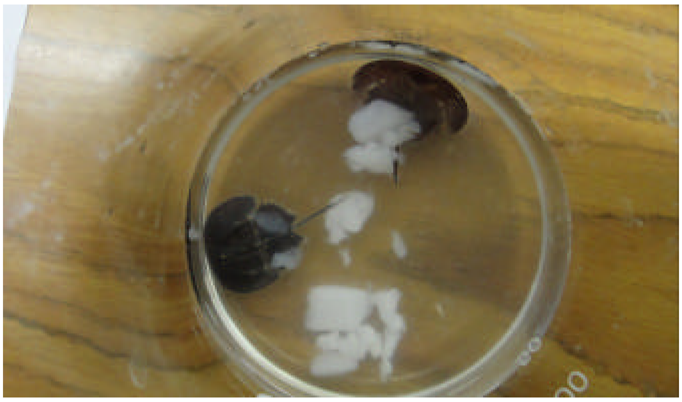



**B. 2.1.5 Observation :**

Whenever food was put into the beaker, the appendages of Juvenile *Tachypleus tridentatus* showed some degree of movement. There was the movement of flebellum which was a large spatulate organ that acted as a "pusher leg". Besides, jointed appendages especially the hind appendages also moved rapidly to create current to draw the food towards the mouth of Juvenile *Tachypleus tridentatus* and then they used some spiny projections around the mouth to crush the food particles.

**Fig 2.4 and 2.5 show the movement of appendages of Juvenile *Tachypleus tridentatus when food is provided***





**Assumption:** The more rapid movement of Juvenile *Tachypleus tridentatus*' appendages, the higher preference for the food since they used their hind appendages to help break up food.

**B. 2.1.6 Result:**

Table 2.1 shows the results of the relative preference of Juvenile *Tachypleus tridentatus* for four types of food

| Relative degree of movement of appendages | | 1st trial | 2nd trial | 3rd trial | Average(+) |
|---|---|---|---|---|---|
| A | *Artemia* | + + | + + | + + + | 2.3 |
| B | Blood worms | - | - | + + + | 1 |
| C | Clams | + + + + + | + + + + + | + + + + + | 5 |
| D | Squids | + + + + | + + + + | + + + + + | 4.3 |

Key:

**-** **:** No movement of Juvenile *Tachypleus tridentatus*' appendages

**+** **:** Movement of Juvenile *Tachypleus tridentatus*' appendages

(**+** **:** the least rapid ; **+ + + + +** **:** the rapidest)

**B. 2.1.7 Discussion:**

From the above results, Juvenile *Tachypleus tridentatus* showed relatively highest preference for clams, followed by squids, Artemia and blood worms respectively. It was found that there were a lot of peg sensillum and pit sensilla over the entire surface of the *Tachypleus tridentatus* and some were located on the surface of prosoma, opithosoma and especially appendages. They could let the Juvenile *Tachypleus tridentatus* 'feel'. Pit sensilla were especially important for 'feeling the water'. The taste organs in the coxal spurs were widely developed in the appendages of Juvenile *Tachypleus tridentatus* which came in frequent contact with food and taste could easily be detected by them using their chemoreceptors on appendages and even the whole body. It was found to be more astonishing that each sensilla contained between three and fifteen sensory cells. Much of it came from the appendages, with an estimated 3-4 million fibers from the chelae (pincers), 1 million from the flabellum and more. Adding the 1 million neural fibers from the peg sensilla, each Juvenile *Tachypleus tridentatus* had about 7 million axons that allowed it to feel and sense the world around it. As clams and squids had stronger taste than *Artemia* and blood worms, Juvenile *Tachypleus tridentatus* preferred the former more.

The obtained results were different from that suggested by some references that they liked to feed on *Artemia*. Perhaps, they may not have a strong taste as we used frozen food mainly and they were juvenile but not adult. There were abnormal results in the third trial. Juvenile *Tachypleus tridentatus*' preference for each kind of food increased basically, especially for blood worms. No movement of their appendages was observed at the first and second trial but they showed preference for blood worms at the third trial. It was suggested that Juvenile *Tachypleus tridentatus* were feeding on food whatever accessible. Since Juvenile *Tachypleus tridentatus* had not been fed for days, they ate whatever we gave them.

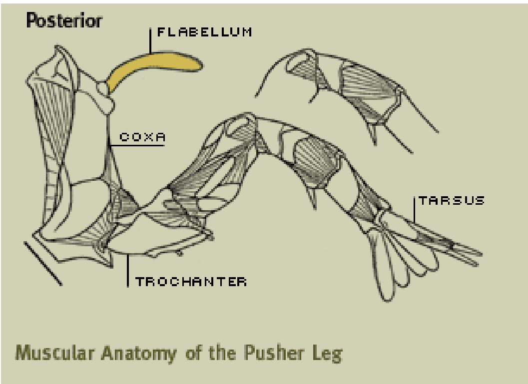



*Fig 2.6 shows the structure of coxa on the appendages*

**B. 2.1.8 Conclusion:**

Horseshoe crabs showed a higher preference on clams, followed by squids, *Artemia* and blood worms respectively.

## Experiment 2.2 : To study the feeding preference of the Juvenile Horseshoe Crab for frozen clams and fresh clams

**B. 2.2.1 Reason for carrying out the experiment**:
Base on the previous experiment, we discovered that the preference for clams is slightly higher than *Artemia*, blood worms and squids. Therefore, we carried out a progressive experiment on frozen clams and fresh clams to observe their preferences.

**B. 2.2.2 Aim:** To study the preference of Juvenile *Tachypleus tridentatus* for frozen clams and fresh clams.

**B. 2.2.3 Materials and Apparatus:**

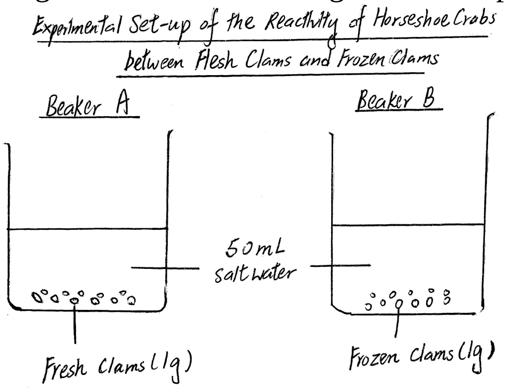
| | |
|---|---|
| - 4 Juvenile *Tachypleus tridentatus*<br>- 1g of frozen clams<br>- 1g of fresh clams | - 1 measuring cylinder<br>- 1 electronic balance<br>- 2 beakers (250mL)<br>- 1 razor blade<br>- 1 spatula<br>- 1 white tile<br>- 50mL of salt water |

**B. 2.2.4 Variables:**
The following table shows the variables of this experiment:

| Independent Variable | Controlled Variables | Dependent Variable |
|---|---|---|
| ➢ Frozen clams or fresh clams | ➢ The weight of clams (frozen and fresh)<br>➢ The volume of the salt water<br>➢ The temperature of salt water<br>➢ The number of horseshoe crabs<br>➢ The time for observation on preference of Juvenile *Tachypleus tridentatus* for different food | ➢ The movement of Juvenile *Tachypleus tridentatus' appendages* |

**B. 2.2.5 Methods:**
1. 50mL of salt water was measured by a measuring cylinder and poured into Beaker A.
2. Fresh clams were cut into small pieces.
3. 1g of fresh clam was weighed and put into the beaker.
4. Steps (1) - (3) were repeated to frozen clam (B).
5. Juvenile *Tachypleus tridentatus*' preference was observed for 5 minutes.

Fig 2.7 shows the drawing of the set-up



**B. 2.2.6 Observation:**

We observed the use of flebellum, as a "pusher leg", to test the composition of water to the gill chamber. There was also the movement of jointed appendages especially the hind appendages to ' feel the food' .

**Assumptions:**

The more rapid movement of Juvenile *Tachypleus tridentatus*' legs, the higher preference for the food since they use their appendages to help break up food.

**B. 2.2.7 Results:**

Table 2.2 shows the results of the preference of Juvenile *Tachypleus tridentatus* for two types of clams as food

| | | Relative degree of movement of appendages |
|---|---|---|
| A | Fresh clams | + + + + |
| B | Frozen clams | + + |

Key :

**- :** No movement of Juvenile *Tachypleus tridentatus' appendages*

**+ :** Movement of Juvenile *Tachypleus tridentatus' appendages*

(**+ :** the least rapid ; **+ + + + + :** the rapidest)

**B. 2.2.8 Discussion:**

From the above results, Juvenile *Tachypleus tridentatus* showed a higher degree of movement of appendages toward fresh clams comparing frozen clams. It was suggested that the relative amount of juice inside the fresh clams was more than frozen clams. When two kinds of clams were added into the beakers containing 50mL of salt water, the relative concentration of juice per $cm^3$ inside Beaker A was relatively higher than Beaker B. The taste organs in the coxal spurs located on the appendages, Juvenile *Tachypleus tridentatus* easily detected the chemical of juice of the food. The higher the concentration, the easier the horseshoe crabs detected. Besides, Olfactory ogans leading to olfactory nerves at the top of the brain gave the sensation of smell from surrounding. As a result,
they preferred fresh clams than that of frozen clams.

**B. 2.2.9 Conclusion:**

Juvenile *Tachypleus tridentatus* showed a higher preference on fresh clams to frozen clams.

**B. 2.3 Limitations :**

1. Since we could do a few trials, we hoped to obtain more readings and observation to make the result more reliable, for at least 3 to 5 times.
2. Each time we randomly selected two to three Juvenile *Tachypleus tridentatus* to study but they may be subjected to different conditions before.
3. It would be better if we could dissect the organisms to observe their gut contents to find out the food they really took in. As Juvenile *Tachypleus tridentatus* are in danger of becoming extinct and we needed to rear them, we could not dissect them.
4. We only tried fresh/frozen clams and a few varieties of food so we could try more types of food.

### B.2.4 Summary

As a summary, Horseshoe crab is also called the organism with ‘ legs attached to the mouth’ . We found that the mouth was located in the centre of its body on the ventral side. They used their hind appendages to hold food and to break up food. Instead, there were also small front appendages, chelicera, which placed food into its mouth. With the help of receptors and nervous system, they can detect the chemicals in water to know the food is around them and moved the food towards them. We found that they preferred fresh clams than frozen clams and they had a preference of food in the order of clams, squid, *Artemia* and blood worms.

## C. 3.1 Experiment: To study the effect of water waves on turning over of Juvenile *Tachypleus tridentatus*

**C. 3.1.1 Aim**: To study the effect of water waves on turning over of Juvenile *Tachypleus tridentatus*

**C.3.1.2 Materials and apparatus** :

| |
|---|
| 1 tank with water |
| 1 water pump |
| Eight Juvenile *Tachypleus tridentatus used* |

**Fig 3.1 shows the set-up of this experiment**

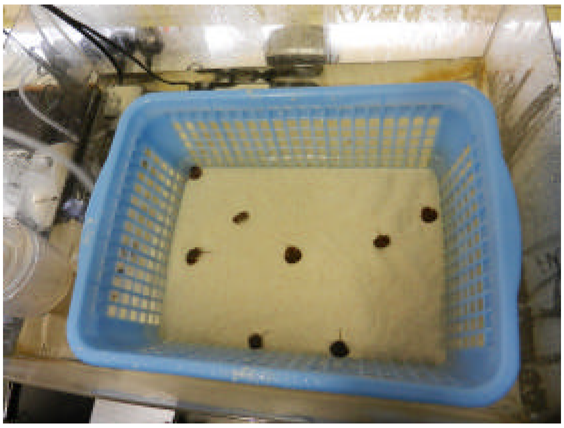
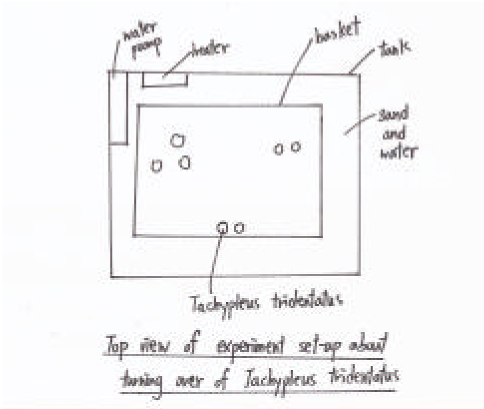


**Fig 3.2 shows the drawing of the set-up**




**C. 3.1.3 Method**:

1.Put Juvenile *Tachypleus tridentatus* into the tank.

2.Use water pump to make water waves for 20 minutes.

3.Observe the movements of Juvenile *Tachypleus tridentatus*.

4.Record the number of Juvenile *Tachypleus tridentatus* which had turned over after 20 minutes.

5.Repeat the above steps without water waves.

**C. 3.1.4 Observation :**

It was observed that the tail spine was used by Juvenile *Tachypleus tridentatus* as a rudder underwater. It also helped a stranded and vulnerable Juvenile *Tachypleus tridentatus* right itself when overturned by a wave onto the beach. First, it bent its hind part forward. Then it thrusted the telson' s point downward into the sand. Kicking its legs started a rocking motion which eventually allowed it to roll back over to its underside.

**Assumption**: The more Juvenile *Tachypleus tridentatus* that have turned over, the higher preference for the water waves for them to turn over.

**C. 3.1.5 Variables:**

**The following table shows the variables of this experiment.**

| Independent variables | Dependent variables | Controlled variables |
|---|---|---|
| √ the presence of water waves | √ the number of Juvenile *Tachypleus tridentatus* which had successfully turned back | √ volume of water in tank<br>√ Salinity of the water<br>√ Temperature of the water |

**C. 3.1.6 Results:**

Table 3.1 shows the number of Juvenile *Tachypleus tridentatus* successfully turning over with water waves

| Order of trial | 1st trial | 2nd trial | Average rate |
|---|---|---|---|
| Number of crabs: | 6 | 6 | 75% |

Table 3.2 shows the number of Juvenile *Tachypleus tridentatus* successfully turning over without water waves

| Order of trial | 1st trial | 2nd trial | Average rate |
|---|---|---|---|
| Number of crabs: | 3 | 3 | 37.5% |

**C.3.1.7 Discussion:**

The experiment allowed us to conclude the existence of water waves enhanced the success rate of turning over of Juvenile *Tachypleus tridentatus*. Over 75 percent of the Juvenile *Tachypleus tridentatus* turned over successfully with the help of water waves. This indicated that if they were stranded in nature, they could turn over by themselves so that they would not die because of desiccation and attack by other organisms. One reason that Juvenile *Tachypleus tridentatus* were in danger was that if they were not quick enough to turn upside down, they would be eaten by predator such as gulls. This reduced their chance of survival.

We should also be more careful when we pick up Juvenile *Tachypleus tridentatus*. We should not pick them up by its tail as this would damage the ball and socket joint which was very similar to a human hip joint. Since the telson was connected to the abdomen by a ball and socket joint, which allowed the tail to move in multiple planes. If their tails were damaged, they would be more susceptible to desiccation or predation as they had difficulty in turning over.

**C.3.1.8 Limitation:**

1. There were some limitations in this experiment. First, we could not damage the tail of Juvenile *Tachypleus tridentatus* to investigate how they used their tails to turn over as breaking their tail would nearly kill them. Then we only studied them by observing how they turned over.

2. Water waves created by us may not be consistent because we created this manually. Each time the waves created may be with different degree of force which will affect our observation.

**C. 3.1.9 Conclusion:**

The existence of water waves enhanced the successful rate of the turning over of Juvenile *Tachypleus tridentatu*

## Reflection

To conduct a simple investigation using organisms in nature is not simple and easy. Firstly, horseshoe crabs were not easily to identify in nature. As we went out to seek them, we often could not find them and this also indicated that their number is decreasing and they are in danger of becoming extinct compared to their abundant number in the past.

Through this investigation, we learnt to have more patience and perservance when we tried different sets of experiments. Actually, we had done more than that we presented in this report but most of those were failed or had no significant meaning or findings. But we enjoyed the processes as we could have more chances to carry out experiments and observation using horseshoe crabs like what scientists would do.

We also learnt that reading scientific literacy is important for us to understand science, its methodology, observations, and theories. It helps us plan and carry out our investigation based on previous knowledge.

We should pay special attention about the variables in each experiment especially the controlled variables. We ought to concern more on how we can provide a fair condition to study the factor concerned so that we can obtain a more accurate result.

## Conclusion

After these investigations, we have a basic understanding of Juvenile *Tachypleus tridentatus*. They showed a preference toward light regardless of the hungry or full conditions and be more active under light condtion. They preferred food in order of clams, followed by the squids, A*rtemia* and blood worms respectively. They also preferred fresh clams than that of frozen clams as they showed a higher activity when they were fed with fresh clams. Moreover, it was also found out that the success rate of turning over of Juvenile *Tachypleus tridentatus* was enhanced by the water waves. Most of them turned themselves in the presence of water waves with the help of telson in the experiment.

## Further investigation suggested

1. Will different levels of light intensity affect the activity of horseshoe crabs?

2. How do horseshoe crabs obtain fresh from the clams with shells?

3. Is the size of the horseshoe crabs related to their success rate of turnover?

4. Will the temperature of the surrounding affect the activity of horseshoe crabs?

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