

OBSERVATIONS ON NUDIBRANCH BEHAVIOUR PATTERNS UNDER LABORATORY CONDITIONS

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Graphical abstract



Abstract

Phyllidiid Nudibranchs *Phyllidia varicosa* and *Phyllidiella pustulosa* were observed under laboratory conditions and abnormal behaviours were recorded. This study described the manoeuvring, mating behaviour, egg laying and abnormal activities. Some abnormal behaviours were recorded while retaining the sample in the aquarium such as emitting noxious chemicals, lifting up the mantle edge to expose the gills and crawling towards the water surface and lie there. Maneuvering behaviour was distinguished when two individuals were interacting with each other before the mating. Mating behaviour started by extending and joining the reproductive organs followed by gamete exchange for several minutes. During the mating behaviour, there was a limited movement like contracting of the body and hiding the rhinophores into the rhino-tube. Egg deposition was observed in the aquarium as natural behavior after mating and as a stress response after placing the sample in the captivity.

Keywords: *Phyllidia varicosa*, *Phyllidiella pustulosa*, nudibranch behavior, egg deposition, mating behavior

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1.0 INTRODUCTION

The dorid nudibranchs *Phyllidia varicosa* and *Phyllidiella pustulosa* are common tropical Indo-Pacific nudibranchs species [1], and they belong to the Phyllidiidae family that is a distinctive group of various species. These species are also quite abundant in coastal water of Balok - Pahang- Malaysia (personal observation). The two collected species are the ideal choice that can be used for the observations because they exist in the same habitat. To the best of our knowledge, no more information is available about the behaviour of this family. In contrast, they have been widely studied for their secondary metabolites, defense mechanisms, description of new species and their phylogenetic relatedness [1-6].

All of the phyllidiid species are hermaphroditic, and they reproduce by internal fertilization like other

nudibranchs. Mating behaviour of the phyllidiidae is poorly investigated due to the difficulty of accessibility to the natural habitat. Some mating behaviors and reproduction were studied in the laboratory such as simultaneous hermaphrodite molluscs nudibranch *Aeolidiella glauca* due to the difference between *Aeolidiella glauca* and other nudibranchs [7]. Some behaviors of other species like *Tritonia diomedea* have been analyzed such as mating, swimming, feeding, escape swims, and egg-laying in field behavior [8, 9]. In general, some information can be found in forums that provide useful knowledge about the sea slug such as (www.seaslugsforum.net) by sharing information with the worldwide audience.

Eggs are deposited in various forms of gelatinous masses on diverse substrates. These forms are divided into three types in nudibranchs, type A is a flat form with gelatinous ribbons attached to the substratum,

and this form is the most common in dorid nudibranchs. Type B egg masses are gelatinous globules with an extended end attached to the sand, and this is more common in cephalaspideans. Finally, Type C egg masses are gelatinous cords attached to the substratum by a gelatinous sheet, and this form is most common in Aeolid, Dendronotid, and ascoglossans [10]. Several species of nudibranchs were observed for their feeding behaviour due to the ability to feed them in aquarium such as *Aplysia californica* [11], *Pleurobranchaea californica* [12] and *Melibe leonine* [13]. The studied species cannot be fed in the aquarium because they are specialist feeders on sponges, which can emit noxious chemicals when they are disturbed [1].

The behaviour of the phyllidiid nudibranchs is poorly investigated. This study focused on the mating behaviour, egg deposition and other behaviours that can be found under laboratory conditions.

2.0 METHODOLOGY

Individuals from two species *P. varicosa* and *P. pustulosa* were collected from the coastal water of Balok and placed in a plastic container equipped with an aeration pump and live rock as a substrate (Figure 1). They were Then immediately transported to International Islamic University Malaysia and kept alive in a continuous water flow glass aquarium with well-maintained filtration system (Figure 2 and 3). Various parameters for the aquarium were set up with ideal range for marine husbandry. The ideal range is shown in Table 1. Nudibranchs species collected in this study were identified through the coloration pattern of their mantle and external morphology.



Figure 1 Nudibranchs species **A.** *P. varicosa* and **B.** *P. pustulosa*

Table 1 Various parameter ranges for the seawater and ideal ranges for marine husbandry

Parameter	Seawater range	Acceptable range	Ideal range
Temperature (°C)	21-30	24-28	26-28
Salinity (ppt)	23-40	33-38	34-36
pH	7.4-8.4	7.8-8.8	8.2-8.6

All parameters above were maintained and equipped with own individual filtration system (Figure 2).

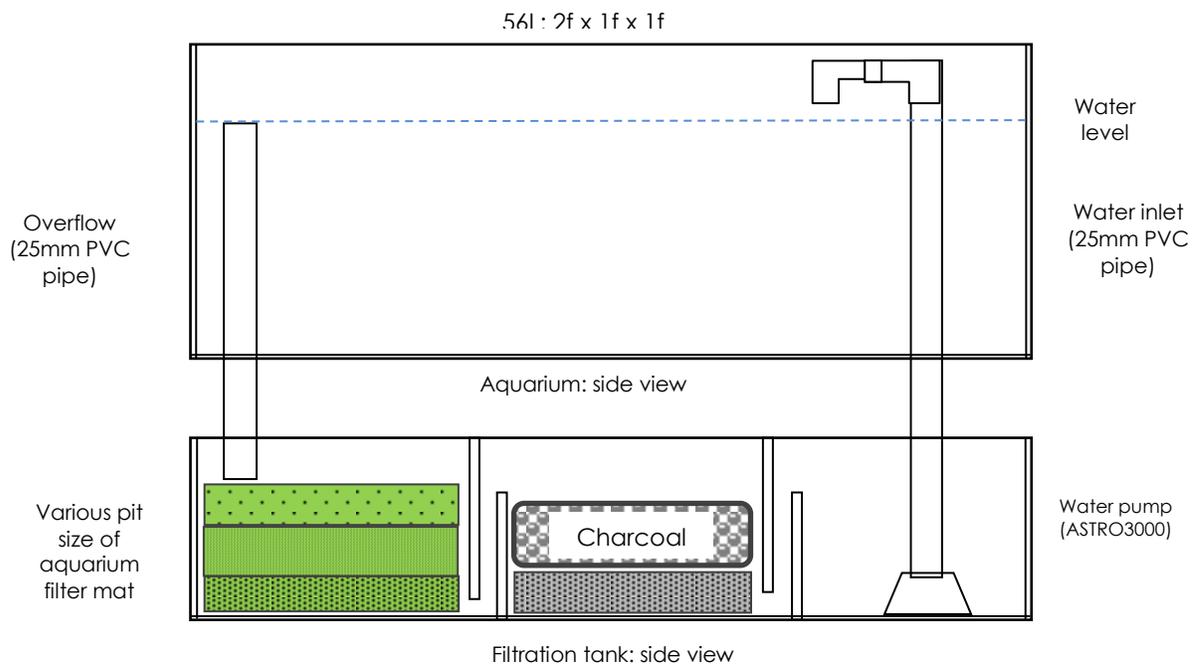


Figure 2 Side view of aquarium setting

The time in captivity was different due to the frequent sampling trips and the observations period in the aquarium was not more than 14 days due to the lack of food source and to avoid any adverse behaviour. All the observations were conducted at 10.30 am and finished at 5 pm. The order of the observation was based on the behaviour displayed by the individual.



Figure 3 Glass aquarium used for observing the behaviour of phyllidiid species: A. The outlet; B. The inlet of filtered sea water; C. Filtration system

3.0 RESULTS AND DISCUSSION

3.1 Abnormal Behaviors

The conspicuous behaviour of Nudibranch phyllidiid species was recorded inside the glass aquarium. They were seen crawling and resting either on the wall of aquarium or the artificial live rock. Some activities were observed, that included when the nudibranch species raise up their mantle edge to expose their gills (Figure 4), and when they crawl on the glass and lied upside down close to the water surface (Figure 4). Collected phyllidiid species were tended to secrete milky fluid when they were handled and transferred to new habitat as a response to stress conditions. These behaviours can be considered as unusual response, and it shows how hard it is to maintain the nudibranchs naturally in an aquarium. Despite the fact that the circulation of the water in the aquarium was well maintained, there were anoxic actions recorded on phyllidiid species. The observed behaviours may not be considered as a normal pattern like in situ but stimulated by stress conditions caused by captivity. There were observed responses in the studied species such as naked gills to acquire sufficient amount of Oxygen and to crawl towards the water surface to search for a more convenient place for settlement.

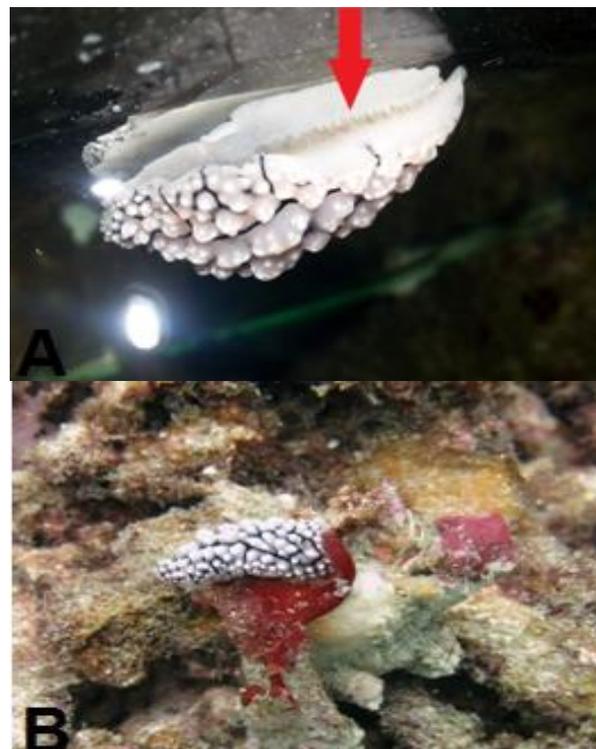


Figure 4 A. *P. varicosa* species lift up their mantle edge to expose their gills in the aquarium. B. *P. varicosa* in the field



Figure 4 The unusual behavior of nudibranch species in an aquarium: *Phyllidia varicosa* started to rest upside down on the water surface

3.2 Normal Behaviours

3.2.1 Mating Behaviour

In Figure 6, the blackish tube joining the two nudibranchs display that they are mating. Like other nudibranchs species, they are hermaphrodites with completely functional male and female reproductive organs. Mating behaviour between the same species was also recorded in *P. varicosa* and showed the reproductive organs (Figure 6). This behaviour was observed when two members from the same species were facing each other for several minutes and the

reproductive organs were coupled. After manoeuvring around in opposite circles, the two individuals become close to each other then they decided they were appropriate and the mating started with the joining of the sexual organs where the tubular organ extended from the right side of each mantle (Figure 6). Confined movements were observed during the mating such as contractions near to the gonopore.



Figure 6 Mating between two *P. varicosa* species, red circle indicate to the reproductive organ (blackish tube) and shows the interchange of semens and copulation of both individuals

3.2.2 Egg Deposition

It was noticed that the *P. varicosa* laid eggs in a spiral shape on the glass surface of the aquarium (Figure 7). The eggs of the phyllidiid species are stuck together and appear in a ribbon. They are always laid out in a circle or spiral shape. This behaviour was also distinguished when the individual started to produce a mucous matrix in a spiral form. This process lasted up to four hours in the collected species. As a response to the stress conditions the collected species started laying a ribbon without fertilized eggs in it or ribbon without egg just an irregular string of yolk (www.seaslugsforum.net). This was clearly observed while placing the samples in the aquarium after few days of sampling (Figure 7). The shape of the egg ribbon is spiral and it is laid counterclockwise, and this allows the organism to perform a better spiral shape and to anchor the egg ribbon with its foot. The colour of the ribbon is creamish white and it is laid flat without edges as shown in Figure 8 and 9. Laying egg ribbon after a while of putting the samples in the aquarium is observed as a stress response. The deposited ribbon might include non-fertilized egg or just a yolk material. Several studies have been conducted to show the behaviour of marine organisms in captivity. Changes in behaviour patterns caused by stress, in

many cases these patterns induced as an adaptive response to increase the probability of survival. Fish showed maladaptive behaviour patterns under stress conditions [14, 15]. Marine invertebrates also displayed stress responses to unfavourable conditions. For example, catechol-amines were detected in the haemolymph of stressed oysters *Crassostrea gigas* and that led to abnormal behaviour [16]. Therefore, the observed behaviours could be the unnatural response for these species.

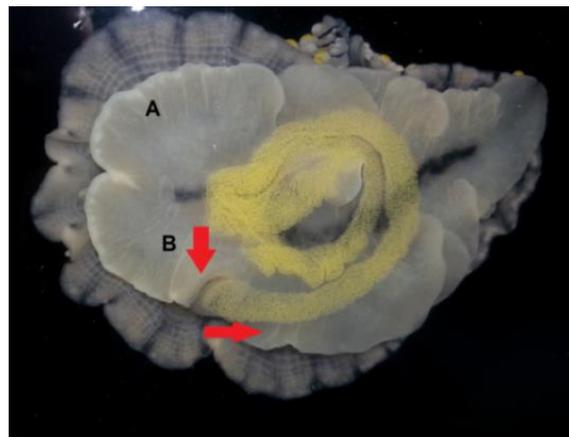


Figure 7 Egg deposition by *P. varicosa* form a counterclockwise spiral, as viewed from above and starting from the centre and working out. A. Foot; B. Gonopore



Figure 8 Egg ribbon of *P. pustulosa*



Figure 9 Spiral egg formation of *P. varicosa*, its creamish white and laid flat

The difficulty of keeping nudibranchs in the aquarium has been examined by Brunckhorst (1993), and he referred that to the feeding preferences of nudibranchs, and some nudibranchs species secrete poisonous mucous capable of affecting other captive marine organisms. Studying the behaviour pattern of these creatures could be a valuable approach to understanding higher organisms. Wyeth and Willows (2006) have studied *Tritonia diomedea* and concluded that *Tritonia diomedea* could be used as a useful model for future neuro-ethological experimentation. Much more analysis should be conducted to understand the behaviour of nudibranch that cannot be sustained in the aquarium.

4.0 CONCLUSION

The observed behavioural patterns in this study have provided an insight into the various behaviour patterns displayed by the phyllidiid nudibranch species under laboratory conditions and we classified them into normal and abnormal behaviour.

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