The Comparative Skeletal Structure of *Blenniella bilitonensis* (Skippers) and *Bathygobius fuscus* (Remainers)

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**Abstract.** Intertidal Zone is an area with high biological diversity including vertebrates. In order to survive in area with rapid change of environmental condition, animals need to be adapted accordingly. One type of adaptations that mostly employed is structural adaptation specifically ones that related to locomotor performances. Differences on how to navigate during high tides or when the water level subsided as well as types of locomotion between skippers and remainers suggested that there might also be structural differences in their skeleton. This research is aimed to study the skeletal structure of two species that use different strategy in their locomotion. Two species of fish from two main groups of intertidal fish (skippers and remainers) were caught and preserved in 96% Ethanol. The skeletal preparation was made using Inouye’s Alizarin Red Alcyan Blue Method. Data were collected by comparing skeletal structure between two species particularly the axial (vertebrae) and appendicular (pelvic, pectoral, caudal fin) skeleton. Based on the observation, skippers and remainers skeletal features has been adapted to different function during locomotion. Modifications on pelvic, pectoral and caudal bones were found in skippers and remainers. In *B. bilitonensis*, there are fusion of bones, reduction of some structures and also smaller bone area to provide agility. In remainers, modification mostly found in pelvic and pectoral fin. Pelvic fins possess larger and more rigid basal lepidotrichia as it was used as an attachment apparatus while fish cling to the substrate during high tide. In *B. fuscus*, pectoral fins are used as main propellers during swimming instead of caudal fins, hence larger bones area to provide more muscle attachment are observed in this species.

**Keywords**: Remainers, Skipper, skeletal structure, intertidal zone

1. **Introduction**

Tropical intertidal zone is rich in species diversity including fish. This area is characterised by the fluctuation of sea level, in which causing extreme periodical changes of environmental factors during 24 hours. To be able to cope with rapid changes of abiotic factors, either behavioural, physiological, morphological adaptation (or combination of three) is needed, therefore only certain species can thrive and survive in this zone[1].

There are three groups of amphibious fish inhabiting tropical intertidal zone, which are: Remainers, Skippers, and Tidepool emerger[2]. Remainers are group of amphibious fish characterised by their small range of mobility during lowtide. Remainers tend to stay in the crevices of the rock, under the boulders or tidepools with only millimetres deep when sea level subsided. Tidepool emergers only emerge from water when physical condition of water becomes inhospitable during low tide. Tidepool emergers
crawled out from one tidepool to another to find more suitable place to hide particularly during low tide. On the other hand, skippers can actively move out of water to forage, defend territories or mate. Different strategy employed by remainers, tidepool emergers and skippers suggested that there are different morphological structures related to locomotor performances.

Locomotion in land and water requires distinct morphological features particularly in musculoskeletal system. According to Hsieh, morphological innovation in locomotor system enables aquatic organisms to find and invade new ecological niche, allowing them to thrive in new habitat. Gibb et al. distinguishes three types of fish locomotion when they are out of water, which are: Trashing, tail-flip jump and prone jump. Trashing is uncontrolled and uncoordinated movement of fish in land. Experimental observation by Gibb, et. al showed that in flat surface, trashing does not result in clear orientation. However, when fish were placed in a surface with 30° of slope, trashing can be effectively used to descent the terrain. Tail flip jump usually can be found in fish that occasionally move out of water particularly to escape from predator.

Difference between tail flip jump and trashing lies on the direction of the movement. In a flat surface, trashing does not result in a clear direction, however tail flip jump usually produces a movement with clear directionality, which is toward the posterior part of the body. Another type of fish locomotion in land called prone jump. Prone jump is an active movement from water to land and can easily be observed in fish of family Gobiidae and Blenniidae. This type of movement requires good level of coordination and effectively results in clear direction of movement.

To perform coordinated movements, well adapted locomotor system is needed. One part of the locomotor systems that might undergone evolutionary changes to enable fish moving in land is skeletal system. According to, group of fish with ability to navigate themselves on land are differs from fully aquatic fish by having dorsoventral body form, with ratio between height to total length is more than 4.5. This means that amphibious fish possess more vertebrae compared to fully aquatic fish with the same size.

Aside from vertebrae, fins, particularly pectoral and caudal fins play important roles during fish locomotion on land. Harris (1960) mentioned that there is enlargement of cleithrum bone in Periophthalmus koelreuteri ‘s pectoral fin. This modification allows more place for muscle to attach hence more agility and power to navigate on land.

Other type of modification in fish is Pelvic Sucker Organ (PSO). Pelvic sucker organ, a modification of pectoral fins, used by fish to attach themselves on substrate. PSO can be found in fish that lives in the area with high turbulence such as waterfall and intertidal zone.

Remainers are known to stay inside tidepools during low tide while skippers tend to jump on to the land. Therefore, how are the structure of skeleton differing in Remainers and skippers? What kind of skeletal structure modification can be found in these two group of fish? This research is aimed to study the skeletal structure of two groups of fish namely remainers and skippers that use different strategy in their locomotion.

2. Research Method

2.1 Specimen Sampling
Fish specimen were collected from Siung Beach, one of the beaches with rocky intertidal zone in the southern part of Yogyakarta. Two species of amphibious fish namely Bathygobius fuscus and Blenniella bilitonensis, were collected as representation of remainers and skippers, respectively. They were collected by hands or small nets. Fish then sacrificed and fixed in Ethanol 96% for 1 week.
2.2 Skeletal Staining

After a week, fish skin and visceral organs were removed. Skeletal preparation was made using Alizarin Red Acyan Blue skeletal staining method by Inouye [12] with modification. Coloured skeletal specimen were analysed descriptively. Description were made particularly in the structure and number of vertebrae and also in appendicular skeleton namely: pectoral, pelvic and caudal fins.

3. Results and Discussion

3.1. Axial Skeleton

Based on observation, most of fish species that lives intertidal zone possess elongated body form. Fish with this type of body rely on the movement of the posterior part and tail to swim. Force were generated by the contraction of the muscles in caudal part of the body, which also causing the movements of tail. Other fins like pelvic and pectoral fins are used for manoeuvring such as changing direction, making turn or stopping. Since posterior part of the body are used in generating force for swimming, more numbers of vertebrae are needed for muscle attachments. These pictures represent the full body skeleton of two species used in this research:

Figure 1a. Skeleton of *Blenniella bilitonensis*, species belongs to skippers group

![Figure 1a](image1.png)

Figure 1b. Skeleton of *Bathygobius fuscus*, species belongs to remainers group

Axial skeleton comprises three parts, which are: cranium, vertebrae and ribs. In fish, vertebrae play pivotal role in locomotion, particularly caudal vertebrae. The main functions of the vertebrae are
to provide attachment for muscles fibres also to support fish’s body. Based on the observation, the number of caudal vertebrae of *B. bilitorosis* is 21 while *B. fuscus*’s body supported by 15 vertebrae. The difference in the number of the vertebrae suggested different number of muscles fibres attached to them. In fish, body muscles are arranged in rows or segments called Myomers. Myomer as a contractile unit of the muscle generate power during locomotion. With more numbers of vertebrae, more segments or rows of muscle can be attached to them therefore more power can be produced.

Figure 2 depicted the detailed features of vertebra structure on both species. Each vertebra consists of structures such as centrum, neural spine, haemal spine and hypapophyses. However, the difference between species observed can be point out in the angle and direction of the spine. In *B. bilitorosis*, direction of the spines is toward the posterior part of the body with 60° angle between centrum and spine. Meanwhile in *B. fuscus*, angle formed by each centrum and spine is 90° towards dorsal and ventral side of the body. Yet, the impact of this arrangement toward the contraction of the muscles fibres isn’t fully understood and more research needs to be done.

**Figure 2. Lateral view of *B. bilitorosis* (a) and *B. fuscus* (b) vertebrae**

### 3.2. Appendicular Skeleton

Appendicular skeleton in fish comprises of bones that construct fins on the specific regions of the body. Fins are named based on their locations and can easily be distinguished from one to another. In this research we focused on three fins that differ in function namely Pectoral, Pelvic and Caudal Fins.
Table 1. Comparison of appendicular bones structures in *B. bilitonensis* and *B. fuscus*

| Appendixal Skeleton | *B. bilitonensis* | *B. fuscus* |
|---------------------|------------------|-------------|
| **Pectoral Fins**   |                  |             |
| Os Supracleithrum   | Enlargement on the dorsomedial part of the bone, blunt end | No enlargement on dorsomedial parts of the bone, sharp end |
| Os Cleithrum        | Large *christa cleithralis* | Narrow *christa cleithralis* |
| Os Coracoideum      | Oval shaped, attached to Os Radalia III dan IV | Triangle shaped, attached to Os Radalia IV |
| Os Radialia         | 4, shape is varied between each bone; cleft between each radial bone | 4, uniform in shaped, no cleft between each bone |
| Lepidotrichia       | 14, no branch at the end, segmented, all parts consist of osteon | 18, branched at the end (2-6 branch), unsegmented, medial parts consist of osteon while distal end consist of cartilage |
| **Pelvic Fins**     |                  |             |
| Basypterygium       | Consists of three bones with triangle shape | Consist of two bones with rectangular shape |
| Lepidotrichia       | Reduction on the number lepidotrichia → 3 pairs, consists of osteon, unbranched, segmented basal lepidotrichia is modified into a ball and socket joint → more flexible movement | 5 pairs of lepidotrichia, branched (2-6 branches), unsegmented, medial parts consist of osteon while distal part are made of cartilage Basal lepidotrichia attached to lepidotrichia by hinge joint movement of lepidotrichia is restricted |
| **Caudal Fins**     |                  |             |
| Os Pleural Centrum  | 5 bones, posterior part modified into urostyle | 2 bones, posterior part modified into urostyle |
| Os Hyural           | 4, fusion between Hypural I and II (Hypural 1+2), and also between Hypural III and IV (Hypural 3+4) | 5, fusion between Hypural I and II (Hypural 1+2), and also between Hypural III and IV (Hypural 3+4). Hypural V located between Hypural 3+4 and Os Epural |
| Os Epural           | 2, separated | 2 → fused into one disc-shaped bone |
| Lepidotrichia       | Number of principal rays 14, segmented, each posterior end branched into two branches consist of cartilage. Segmented (anterior parts of the fin) are made of osteon | Number of principal rays 14, unsegmented, each posterior end branched into 6 branch (hair like – branch), basal lepidotrichia are made of osteon |
|                     | - 6 Proccurent rays, made of osteon, unbranched, unsegmented | - 4 Proccurent rays, made of osteon, unbranched, unsegmented |

3.2.1 Pectoral Fins

Pectoral Fins are one of two paired fins found in lateral side of the body. In both species these fins are required during swimming mainly in manoeuvring, changing direction or slowing down and stopping.
However, other functions of the fins can be observed in *B. bilitonensis*. In *B. bilitonensis* pectoral fins can be utilized as hook and stabilizer when fish clings to rocks. According to [13], pectoral fins of fish from Family Blennioidea consists of two regions called Fanfield and Hookfield. During swimming, fanfield and hookfield on both sides can be expanded to create larger surface area. This method is applied when fish needs to slow down and finally stop. However, in *B. bilitonensis*, hookfield can also be used as “hook” when fish attach themselves on the surface of the rocks. Hookfield (fig 3) can be distinguished from fanfield by having sharp end and dorsally oriented lepidotrichia. Branched end of lepidotrichia is not observed in *B. bilitonensis*, but present in *B. fuscus*, which main function of pectoral fins is as mentioned above. During manoeuvre, surface area of the fins is regulated by expanding or contracting the lepidotrichia. Branched end of lepidotrichia allows fish to maintain the expanding state of the fins for as long as it needed.

Aside from lepidotrichia, differences in pectoral fins structures can also be found in other bones such as Cleithrum, Radialia and condyles of pelvic and pectoral girdle. List of the detailed comparison between *B. bilitonensis* and *B. fuscus* bone structures provided in table 1.

![Figure 3: Lateral View of Pectoral fins in *B. bilitonensis* (a) and *B. fuscus* (b)](image)

### 3.2.2 Pelvic Fins

Pelvic fins in *B. bilitonensis* are used mainly in land, as a structure to supports fish body as well as “walk” in solid surfaces such as sand and rocks. However, different function of these paired fins is observed in *B. fuscus*. In *B. fuscus*, pelvic fins have been modified into Pelvic Sucker Organ (PSO) (fig 4), which used to attach fish body onto the substrate when there’s high turbulence. PSO allows fish to maintain its position even during hightide. In rare occasions, PSO can also be used by fish to cling on a vertical substrate. Since Pelvic fins in both species were operated in different circumstances, different structures were observed. In *B. bilitonensis*, there are fusions of lepidotrichia and also flexible joint connecting lepidotrichia and basal leidotrichia. Combination between the rigid structure of lepidotrichia (because of the fusions) and flexible joint allow fish to stand on its pelvic fins as well as walk on land.

In *B. fuscus*, lepidotrichia were separated into 5 rays on each side and each lepidotrichium was branched and segmented. The joint between lepidotrichia and basal leidotrichia were rigid, allowing less movement of lepidotrichia. Nonetheless, this modification allowing *B. fuscus* to create a vacuum system that can be utilized as a sucker organ, which help this fish to survive in a highly turbulent environment. Vacuum system was created by the assistance of membrane between each lepidotrichia (fig. 4).
3.2.3 Caudal Fin

The function of caudal fin in general are to maintain direction also to generate force so fish can move forward during swimming. In *B. fuscus*, tail mostly utilized to generate power for swimming while in *B. bilitonensis*, aside of swimming, tail can also be used when fish moves out of water as well as navigate on land. According to [8] for fish that able to move in land, additional function of tail has been observed. These additional functions including maintaining body’s balance and also acting as the propeller when the fish jump \[4\]. Based on the observation, there are slight differences in caudal fin structure between *B. bilitonensis* and *B. fuscus*, mostly related to the number of the bones. Comparison between caudal fin’s bones can be seen in table 1 and figure 5. Since bones provide place for muscle attachments, additional number of bones can be used as the place of attachment of more muscle fibres. *Blenniella bilitonensis* possess more caudal vertebrae as well as bones that construct caudal fins. It can be suggested that more type of muscle can be found in *B. bilitonensis* as part of its adaptation to navigate on land. Tail serves its function not only for swimming but also jumping, therefore agility, flexibility and robustness of the tail is required.

Figure 4. Dorsal view of Pelvic girdle in *B. bilitonensis* (a) and *B. fuscus* (b)

Figure 5. Lateral view of Complex Caudal Vertebrae - Caudal Fin of *B. bilitonensis* (a) and *B. fuscus* (b)
4. Conclusion

Based on our observation, it can be concluded that there are differences between axial and appendicular skeleton of *Blenniella bilitonensis* and *Bathygobius fuscus*. *Blenniella bilitonensis* was adapted to navigate on land in which modification can be observed in the number of vertebrae, fusions of lepidotrichial bones in pelvic fin and enlargement of some bones, mainly to provide more muscle attachments. In *B. fuscus* notable adaptation can be found in pelvic fins which modification is Pelvic Sucker Organ, allowing this species to attach to its substrate in a turbulent environment.

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