CHAPTER 1

1.1 CEREBELLUM

The cerebellum which means 'little brain' is present in all vertebrate brains. Located underneath the posterior region of the skull and rostrocaudal to the occipital lobe, it is attached to the brainstem by a pair of cerebellar peduncles (Seeley et al., 2000). Generally, the cerebellum is an obvious brain structure although smaller than the other brain parts, such as forebrain of higher vertebrates and midbrain of birds. In higher vertebrates, the weight of cerebellum is about ten percent (10%) of the total weight of the cerebral hemispheres. A series of fissures subdivide the surface of cerebellum into many folia and lobes, results in the existence of various subdivisions of cerebellum. Because of that, it is believed that the surface area of the cerebellar cortex is roughly fifty percent (50%) of the area of cerebral cortex (Singh, 2006).

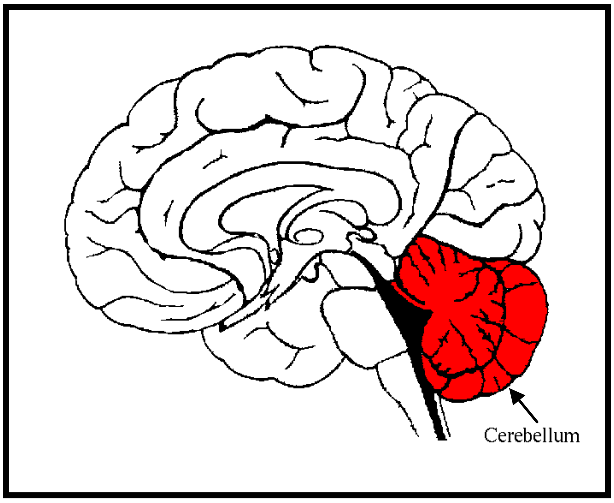

Figure 1.1: Human cerebellum (Adapted from http://allaboutthebrain.tripod.com/id13.html)

1.1.1 Functions of cerebellum

The general function of the cerebellum is related to the maintenance of posture and balance, and to coordinate movements of various body parts. The cerebellum acts as a monitor to the other centers of the brain and is accountable for muscle synergy throughout the body. It monitors the centers that are involved in muscle contraction or movement in general, but not the initiator of such motion. Voluntary movements can proceed without its aid, but such movements are clumsy and disorganized (Sasaki and Gemba, 1992).

Generally, the cerebellum is believed to function as a type of computer that is concerned with the accurate and effective control of movement. Basically, the cerebellum is responsible in integrating and coordinating all information that is flowing into it directly from various neural pathways. The subsequent cerebellar output will then either go down the spinal cord to innervate muscle groups or return back to the cerebral cortex for necessary adjustments to achieve the coordinated movement intended (Eccles et al., 1967).

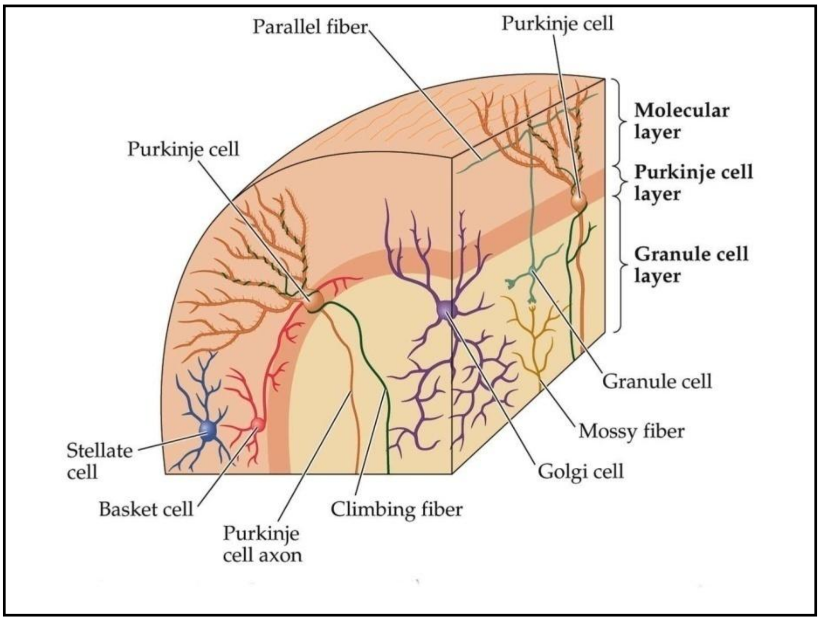

Figure 1.2: The neuronal organization in cerebellum (Purves, 2011)

Figure 1.2 shows the neural circuitries in cerebellum of human that are composed of many neural pathways in order to ensure the effectiveness of cerebellum functions. The cerebellum is thought to organize the flow of a particular motor pattern arising elsewhere in the nervous system that should be expressed (Bone and Moore, 2008).

Apart from that, cerebellum serves as the comparator. To initiate the voluntary movement, impulses from the cerebral motor cortex will go down to the spinal cord before activating muscle contractions. Simultaneously, impulses from the proprioceptive neurons that innervate the joint, tendon and muscle arrived at the cerebellum to provide information about the position of the body. Then, cerebellum compares information about the target movement from motor cortex with the sensory information from the structures that perform the movements. If any difference is detected, the cerebellum sends impulses to motor neurons in the motor cortex and also spinal cord to correct the discrepancy. For example, if we close our eyes, we could touch our nose smoothly and easily by using our fingers, assisted by the cerebellar comparator. If this function does not work accordingly, our fingers would not be sure the whereabouts of the target (For more information, refer to Prosser, 1973). Thus, the traditional view that the cerebellum is an organ of motor control might be only partially correct. The cerebellum is better viewed as a sensory processor that analyzes and processes sensory information for the motor system (Sarnat and Netsky, 1981).

Some anatomical, behavioral, and neurophysiological studies that were done in the beginning of mid-1980s began to suggest that the role of the cerebellum extends beyond a simple motor domain. Finally, the neurophysiological evidence that implicated the cerebellum in some aspects of cognition was provided by development of neuro-imaging techniques (Fiez, 1996).

In our daily experiences, learning of specific skills is achieved by repeated training of relatively similar movements. Hence, the training gradually changes awkward unsmooth movements into voluntary movements which appeared to be unconsciously and automatically executed. This may largely involve the participation of the cerebro-cerebellar interaction (cerebellarization) (Sasaki and Gemba, 1992).

The cerebellum functions can be hindered by alcohol consumption. Volkow et al. (1988), reported that alcohol consumption will decrease the blood flow to the cerebellum, which leading to the shrinkage of the cerebellum. As a result, the balance and pace will be impaired and may cause collapse.

Cerebellar ataxia is caused by cerebellar damage and characterized by postural deficiencies and disintegration of movements. It is manifested in many ways; i.e. dysmetrias (Yanagihara, 2010). This is in agreement with Kandel et al. (2000), who proposed that a class of disorders called dysmetrias is a disruption of the ability to measure the precise trajectory of movements. In addition, it has been shown in animal studies that cerebellar lesions can disrupt simple learned motor actions.

1.1.2 Morphology of cerebellum

On gross inspection, the three lobes which could be distinguished in the human cerebellum are the flocculonodular lobe (Archicerebellum), the posterior lobe (Neocerebellum) and the anterior lobe (Paleocerebellum). Several research groups have found that different lobes of the cerebellum play different important roles in the learning of motor behavior and coordination, such as locomotion, arm movement, posture control and typical conditioning of eye-blink responses (Kawato and Gomi, 1992). Morphologically, human cerebellum is comprised of two cerebellar hemispheres and a central midline vermis. On the cerebellar surface, there are numerous folia and folium. The corpus cerebelli consisted of anterior and posterior lobes (Pansky and Allen, 1980).

At the cellular level, the mammalian cerebellum is made up of the cortical layer and the medullary layer. Three cellular layers of the cerebellar cortex from its dorsal surface are molecular, Purkinje and granular layers (Figure 1.2).

1.1.3 Cerebellum of fishes

The teleosts, have evolved a more highly complex brain than urodeles. The advanced development of the cerebellum in fishes and relative lack of the cerebellum in urodeles could be associated with the complexity of the anterior part of the brain. The fishes are also capable of being involved in more complex activities than salamanders (Sarnat and Netsky, 1981). The existence of a cerebellum is controversial in hagfish, with some anatomists saying it is present while others saying that it is not (Bell, 2002). Although most anatomists conclude that a cerebellum is present in lampreys, this cerebellum is small and difficult to distinguish.

Preliminary studies in some of teleost fishes have shown interesting findings on the cerebellum. Instead of only caudally/posteriorly oriented cerebellum as commonly seen in other vertebrates, the cerebellum of fish exist in either two opposite directions. From gross morphological aspect, different orientations of cerebellum observed were; rostrally directed for *keli* (*Clarias* sp.) (Tiong, 2005; Shamiza, 2008) and *baung* (*Mystus nemurus*) (Ngui, 2006), and caudally directed for *tilapia merah* (*Oreochromis* sp.) (Adela, 2004) and *jelawat* (*Leptobarbus hoeveni*) (Chia, 2006). This research will characterize the morphology and histology of cerebellum which represents these two types of orientation.

The significance of the cerebellar orientation is still unknown and no detailed reports of these cerebellums could be found in established literature. Table 1.1 shows the orientation of the cerebellum of selected freshwater fishes for this research:

Table 1.1: Orientation of cerebellum of selected local freshwater fishes for study.

Types of fish	**Orientation of cerebellum**
Baung (*Mystus nemurus*)	Rostrally/Anteriorly directed
Keli (*Clarias* sp.)	Rostrally/Anteriorly directed
Jelawat (*Leptobarbus hoeveni*)	Caudally/Posteriorly directed
Tilapia merah (*Oreochromis* sp.)	Caudally/Posteriorly directed

The existence of two opposing orientations of the cerebellums of these fishes could possibly reflect some differences in the hodology and architectonic of the cerebellum. Neurohistological information of the cerebellar tissues could give indication on how the cerebellum is involved in the behavior and movement control of the fishes.

The cerebellar contribution to processes besides the motor domain in non-mammalian vertebrates is only modestly known compared to the mammalian counterparts. Interestingly, some early lesion studies already related the fish cerebellum to the process of learning and memory, such as motor conditioning and avoidance learning (Duran et al., 2005).

Recent studies showed that in the teleost fish, like in mammals, the cerebellum plays an important role in the classical conditioning of simple motor reflexes, in emotional learning and in spatial cognition. Cerebellar lesions completely and permanently abolish eyeblink conditioning and impaired autonomic emotional responses (e.g. heart rate classical conditioning) in goldfish. Furthermore, goldfish with cerebellum lesions present a severe impairment in spatial cognition (Duran et al., 2005)

Extensive work has been done to demonstrate the effect of cerebellar lesion on behavior. There was various range of impairment after the partial or extensive removal of the cerebellum; from no discrepancy in equilibrium, spawning, swimming or parental behavior to severe impairment in swimming movements and sexual behavior (William and David, 1970). This is in agreement with Duran et al., (2005) who reported that cerebellum lesions do not produce any noticeable motor discrepancy as indicated by the swimming activity.

Brown (1957) reported that very early experiments in which parts of the cerebellum were removed gave similar results. This included works by Corso in 1895 who removed both the entire cerebellum and portions of it from various fishes and found no changes in locomotion or muscular power; Bethe in 1899, who removed the entire cerebellum or one-half of it from dogfish (*Scyliorhinus*) also did not observe any disturbances of movement (Brown, 1957).

In contrast, Karamyan (1956), quoted by William and David (1970), stated that the complete removal of the cerebellum in the teleosts resulted in disturbance of muscle tonus followed by a spontaneous recovery of function. However, this usually resulted in death.

Some of the previous works that had been done on cerebellum was tabulated in Table 1.2. Most of the works were done regarding cerebellar lesion, mechanisms of cerebellum and cognitive function by cerebellum.

Table 1.2: List of some researches on cerebellum of different classes of vertebrates.

No	Researchers	Findings	Sample
1	Brown (1957)	No disturbances of movement after removal of cerebellum	Dogfish (*Scyliorhinus*)
2	William and David (1970)	Removal of cerebellum resulted in various range of impairment.	Dogfish, goldfish and carp
3	Kawato and Gomi (1993)	Feedback-error-learning model of cerebellar motor control	Monkey
4	Fiez (1996)	Cerebellar contributions to cognition	Human
5	Bell (2002)	Evolution of cerebellum-like structures	Hagfish and lampreys
6	Duran et al. (2005)	Cognitive and emotional functions of the teleost fish cerebellum	Goldfish
7	Yanagihara (2010)	Mechanisms of locomotor control in the cerebellum	Rat

Many studies done on cerebellum did not involve species similar to those Malaysian local freshwater fishes. This study undertaken detailed out the cerebellum of four selected juvenile local freshwater fish species. It was conducted to characterize the differences in histology and anatomy of cerebellum of different fishes.

Since the cerebellum is an established brain structure related to movements, it would be expected that the histology and gross morphology of cerebellum would reflect its effects on swimming pattern. The shape or form of fish body would also be taken into account because it plays important role in determining the swimming pattern. Thus, the correlation between histology and gross morphology of cerebellum, fish body shape and swimming pattern will be discussed in this study. The cytoarchitectonic structure of fish cerebellar cortex would also be ascertained so as to identify whether it has similarities with cerebellar cortex in higher vertebrate, especially mammals.

The four selected juvenile Malaysian local freshwater fishes were from the class Actinopterygii. Development of trade and marketing of these freshwater fishes, together with the development of aquaculture industry is increasingly taken into consideration because the fisheries industry nowadays is more dependent on the fish harvested from farm rather than the sea-caught fish (Froese and Pauly, 2012).

1.2 FRESHWATER FISHES WITH ROSTRALLY ORIENTED CEREBELLUM

Peninsular Malaysia has the high diversity of freshwater fishes in the world (Zakaria-Ismail, 1990). According to FishBase, the number of freshwater fishes species recorded in Malaysia is 614. The freshwater fishes inhabit a variety of habitats, ranging from small torrential streams to estuaries, highly acidic ecosystems and alkaline stream (Ahmad and Khairul-Adha, 2007). In this study, two types of juvenile Malaysian local freshwater fishes were used as representative of rostrally oriented cerebellum. They were *keli* (*Clarias* sp.) and *baung* (*Mystus nemurus*). Mokhsin and Ambak (1983) and Froese and Pauly (2012) have described the details on these fishes that come from the class of Actinopterygii.

1.2.1 *Keli* (*Clarias* sp.)

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Actinopterygii
Order :	Siluriformes
Family:	Clariidae
Genus:	*Clarias* (Gunther, 1864)
Local/common name:	*Keli* / freshwater catfish

Figure 1.3: Taxonomy of *keli*

Catfish has more than 2,700 species representing approximately one third of all freshwater fish. It is one of the most economically important groups of freshwater fishes and brackish water fishes in the world (Burgess, 1989).

This fish has an elongated body shape and it has no scales. The skin which is covered by mucus is used in cutaneous respiration; the fish breathes through its skin. Whitish dorsally, and silvery on its ventral and lateral sides are also distinctive freshwater catfish features. The freshwater catfish has two pairs of short rounded barbels on lower chin, maxillary barbels nearly as long as the head and absent on nostrils (Mokhsin and Ambak, 1983 and Teugels et al., 1999). This fish is unable to protrude its mouth as other fishes such as carp. It has a broad sub terminal mouth, with rows of villiform teeth aligned on the upper and lower jaws (Zakaria-Ismail, 2003). Serrated spines located at the front of the dorsal and pectoral fins, adipose fins, and a forked caudal fin are freshwater catfish features. All catfish, except members of Malapteruridae (electric catfish), possess a strong, hollow, bonified leading spine-like ray on their dorsal and pectoral fins. Its dorsal and pectoral fins do not have elongates first rays (Mokhsin and Ambak, 1983 and Teugels et al., 1999).

As a defense mechanism, these spines may be locked into place so they stick outwards, hence can cause severe wounds. In several species, these fin rays could be used to deliver a stinging protein in response to irritation. This venom is produced by the glandular cells in the epidermal tissue covering the spines (Zakaria-Ismail, 2003).

Clarias sp. is widely distributed throughout Malaysia. They are very common in ponds, rivers, paddy fields, swamps and canals. It is capable of lying buried in mud (Figure 1.4) for lengthy period if water in ponds and lakes evaporate during dry seasons. Its pores are full with mucous in order to protect itself from dehydration. On top of the hole, there is an empty portion containing air, to allow it to breath. It can move out of the water using its extended fins. During food deprived situation, the freshwater catfish's activities would be slowed.

Figure 1.4: Catfish in a man-made muddy hole. (Adapted from http://agrolink.moa.my/pqnet/kwln/pelihara_ikan_keli.htm)

This fish is also used as biological agent in pest control in paddy field. It eats larvae, insects and worms in the paddy field; thus, reducing the cost for crops by reducing the usage of pesticides (Teugels et al., 1999). In addition, catfish is also an important food fish. It is cultivated on a big scale nowadays and has high demand in the market.

1.2.2 *Baung* (*Mystus nemurus*)

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Actinopterygii
Order:	Siluriformes
Family:	Bagridae
Genus:	Mystus
Species:	*Mystus nemurus* (Valenciennes, 1840)
Local/common name:	*Baung /* River catfish

Figure 1.5: Taxonomy of *baung*

Mystus nemurus is a brown large Asian Catfish which can grow up to two feet long. The distribution of this species extends from many regions in Asia, namely Mekong, Chao Phraya, Xe Bangfai basins, Peninsula Malaysia, Sumatra, Java, and Borneo. It can be found in a variety of habitats, from tidal zone to the upstream of rivers, but it is most frequently found in large muddy rivers, with slow current and soft bottom. It is also nocturnal and likes hidden places. It eats a variety of food including prawns, insects, crabs and fish larvae (Mokhsin and Ambak, 1983 and Rainboth, 1996).

This fish has an elongated body shape. It has four pairs of barbels on the head, dorsal and pectoral spines. Its head is flattened rather than conical; the head is broader than high. It is territorial and best kept with only a couple other large species in big tanks. It also is a definite tankbuster that needs a tight-fitting canopy (Mokhsin and Ambak, 1983). *Baung* is one of the dominant species landed by fishers and has big potential in aquaculture field.

1.3 FRESHWATER FISHES WITH CAUDALLY ORIENTED CEREBELLUM

As fishes with rostrally directed cerebellum, two types of juvenile Malaysian local freshwater fishes were also chosen as representative of caudally oriented cerebellum. They were *jelawat* (*Leptobarbus hoeveni*) and *tilapia* (*Oreochromis* sp.). Mokhsin and Ambak (1983) and Froese and Pauly (2012) have described the details on these fishes also. They are both also from the class of Actinopterygii, which is known as ray-finned fishes.

1.3.1 *Jelawat (Leptobarbus hoeveni)*

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Vertebrata
Class:	Actinopterygii
Order:	Cypriniformes (carps)
Family:	Cyprinidae
Genus:	*Leptobarbus*
Species:	*Leptobarbus hoeveni* (Bleeker, 1851)
Local/common name:	*Jelawat /* River carp

Figure 1.6: Taxonomy of *jelawat*

Leptobarbus hoeveni can be found in big rivers and small streams connected to the river. Although reported to be non-migratory, it involves in local tropic migrations to and from inundated forests. The distribution of this fish in Asia covers Thailand to Sumatra and Borneo (Mokhsin and Ambak, 1983 and Roberts, 1989).

This fish has an elongated body shape (Kottelat, 2001). The head is broad and flattened above. The scale color is greenish silver and the fins are reddish. The juveniles have black longitudinal stripe on the sides of the body. They have barbels which are small with rostral barbels smaller than the maxillary barbels (Mokhsin and Ambak, 1983).

Jelawat is one of the pond-cultured species native to northern Malaysia, Thailand and Borneo. It is occasionally seen in some rural fish ponds and was one of the costliest freshwater fishes in Malaysia, due to its taste and difficulty in sourcing it.

1.3.2 *Tilapia merah* (*Oreochromis* sp.)

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Vertebratae
Class:	Actinopterygii
Order:	Perciformes
Family:	Cichlidae
Genus:	*Oreochromis*
Species:	*Oreochromis* sp. (Peters, 1852)
Local/common name:	*Tilapia merah /* Freshwater snapper

Figure 1.7: Taxonomy of *tilapia merah*

Tilapia belongs to a family of fish known as cichlids which is a member of the mouth brooders. The body of this fish is whitish red in color. There are no enlargements of the jaws in mature fish. In the breeding season, the throat and all the fins become deep red. However, the females are less intensely colored compared to males (Mokhsin and Ambak, 1983). *Tilapia* can be found throughout Malaysia, China and South East Asian countries and usually inhabits freshwater habitats; in ponds, mining pools and rivers. However, some species are known as highly euryhaline (able to live and survive in different concentrations of salt water). *Tilapia* exhibits maximum growth rates at temperatures between 25 and 30°C (Meyer, 2002), which makes them more likely to become established and invasive in tropical climates. However, both tolerances for water temperature and for salinity differ massively between species (Gupta and Acosta, 2004).

Nowadays, *tilapia* makes up about 3.5% of the global aquaculture output number. Tilapias are well adapted to artificial culture environments as they can gain weight quickly at optimum conditions and reproduce without special management or infrastructure (Meyer, 2002). *Tilapia* is usually produced in the pond and cage cultured in open water bodies, where this is the most common culturing system.

1.4 HISTOLOGICAL PROCEDURES

Histology is the study of microanatomy of tissue. Various histological procedures are used in order to display general structural features. Histology is important in order to understand the morphology of tissue and to correlate structure with function (Young, 2006). The difficulty to histologically process non-mammalian neural tissue as compared to processing mammalian neural tissue has limited neurohistological work in fish. In this study, light microscope which is the main tool in histology was used to examine the cerebellar neural tissue components. Prior to tissue examination under the microscope, good histological procedures are very important in order to get the optimum results. The steps involved in processing were fixation, dehydration, clearing, infiltration, embedding, sectioning, staining and coverslipping of the tissue. Formalin 10% was chosen as a fixative in this study. For staining of the tissue, Haematoxylin and Eosin (H&E), Nissl and Thionin stainings were used to make tissue visible under the microscope.

1.5 SWIMMING MODE OF FISH

The various swimming movements of fishes normally involved locomotion. The term locomotion conveys the meaning of the forces that originate from the contraction of body musculature and give rise to forward progress. On the other hand, sinusoidal undulation is the term that is used to signify the movement of the body into a series of successive S-shaped curves. The control of both forward progress and undulation are very important to determine the movement direction of a fish. In this matter, the cerebellum might play important role for the smoothness of fish movements. Three types of swimming modes were focused on this study. The locomotions observed were anguilliform, subcarangiform and carangiform.

Anguilliform locomotion is sideways undulation that involves the entire body length and produces successive waves of muscular contraction being passed backward along the sides of body (Figure 1.8). This locomotion could be considered as a relatively inefficient mode of locomotion because it is impossible to achieve high speed. Nevertheless, this kind of locomotion is an efficient way to get around in water (William and David, 1978). This is proven by the finding in this study in which *keli* displayed this kind of movement and had a fine efficiency in moving around the viscous surrounding medium, e.g. muddy area.

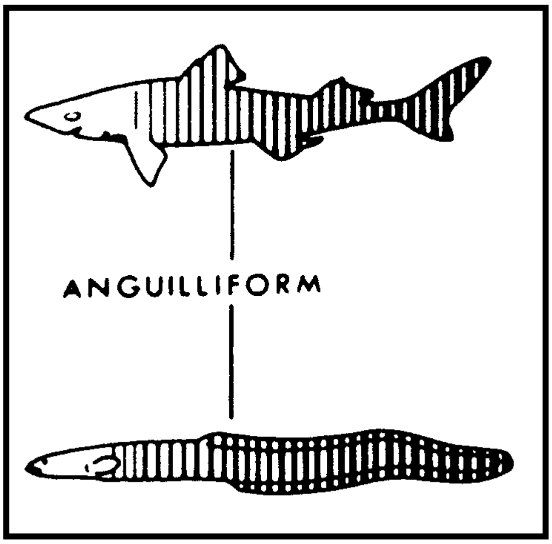

Figure 1.8: The musculature involvement in anguilliform locomotion (Pietsch, 2010).

As for subcarangiform locomotion, it is a swimming style which involves the movements of body and caudal fin (Figure 1.9). The trunk of the fish is undulated approximately two-thirds to one-half of the body in order to produce propulsive wave. Caudal fin is not the major propulsive force during normal forward swimming. In fact, the caudal fin is used for rapid acceleration, fast turning and high speed maneuverability (William and David, 1978), as being showed by *baung* and *jelawat* studied.

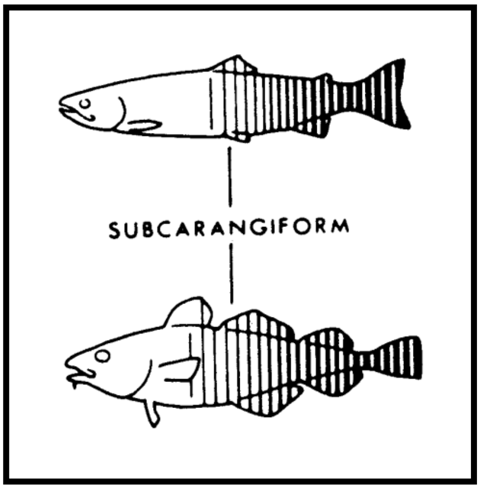

Figure 1.9: The musculature involvement in subcarangiform locomotion (Pietsch, 2010).

Carangiform locomotion as demonstrated by *tilapia* studied only involved the posterior part of the fish that is capable of large flexure (Figure 1.10). Side to side undulations is confined to the last third of the length of the body, while caudal fin provides the propulsive force (William and David, 1978).

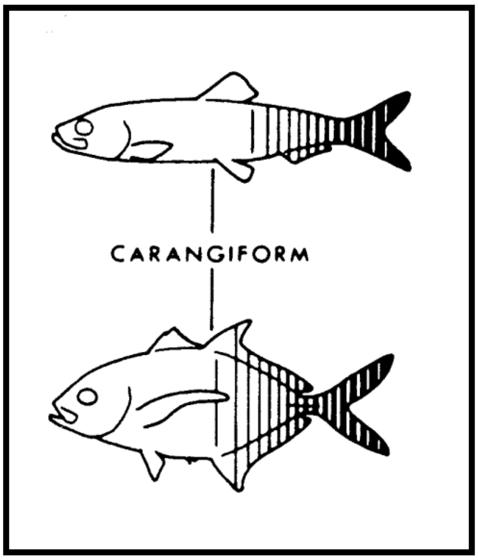

Figure 1.10: The musculature involvement in carangiform locomotion (Pietsch, 2010).

The coupling between body curvatures with muscle activity relies on the characteristic of the fish body form, as well as the swimming mode displayed by the fish. In addition, the interaction between the fish body and water would determine the body curvature wave, and the outcome of this interaction (swimming mode) is influenced by their body form (Wardle et al., 1995).

Lighthill, (1971) quoted by Wardle et al., (1995), reported that the fish body does not interact with the water. In fact, the power generated by the muscle will be passed down along the fish length in order to produce thrust at the tail blade. This model is in agreement with established models of carangiform swimming, and in this study as displayed by *tilapia*. More representatives of fishes with these different cerebellar orientations need to be looked at in the future studies. This is to validate the findings seen.

1.6 OBJECTIVES OF RESEARCH

General objectives of the study would be to conduct a neuroscience based study on local freshwater fishes, as well as to set up or establish neurohistological protocols for the study of non-mammalian neural tissue in local laboratory. Hence, this will spur more neurobiology based research which will use findings of details elucidated as basis for more advanced studies. The data from the neurohistological characterization of the neural regions could be the basis for systematic documentation and record-keeping, e.g. database development. The findings from this study would also be compared to mammalian neural system, so as to understand the relationship between the less complex neural systems of non-mammalian with the more complex ones.

The focus in this study was on cerebellum, which is part of the central nervous system of fish. Specific objectives outlined for the study were:

1. **Orientation of cerebellum and specific features of fishes (Chapter 2)**
   - To correlate different orientation of the cerebellum with specific gross morphological features of selected local freshwater fishes.
2. **Gross morphology of cerebellum (Chapter 3)**
   - To characterize the gross morphology of rostrally/anteriorly and caudally/posteriorly oriented cerebellum of selected local freshwater fishes.
3. **Neurohistology of cerebellum (Chapter 4)**
   - To characterize the neurohistology of rostrally/anteriorly and caudally/posteriorly oriented cerebellum of selected local freshwater fishes.