APPENDIX A: PREPARATION OF CHEMICAL SOLUTIONS

General

1. **Preparation of 10% neutral buffered formalin (1litre)**

   Formalin……100ml

   Distilled water…….……...900ml

   Sodium phosphate, monobasic, monohydrate (NaH_2PO_4)……4g

   Sodium phosphate, dibasic, anhydrous (Na_2HPO_4)……..6.5g

2. **Preparation of 70% alcohol solution**

   95% Ethyl alcohol……...…..700ml

   Distilled water……...250ml

3. **Preparation of 85% alcohol solution**

   95% Ethyl alcohol…….....……...…..850ml

   Distilled water……...100ml

4. **Preparation of 20% sucrose solution (100ml)**

   Sucrose…….....20g

   Distilled water……...100ml

APPENDIX B: PERSONAL OBSERVATIONS ON FISH SWIMMING BEHAVIOUR

Personal observations were made on the swimming behaviour of selected fishes used in this study. Emphasis was placed on the function of fins in fish locomotion. It should be noted that the observation made was limited only on the behavior of fish in the aquarium. Scientific conclusion on the fish swimming behaviour can only be drawn by complete behavioral experiments.

(a) PERSONAL OBSERVATIONS ON FISH SWIMMING BEHAVIOUR

Table 1 below summarized general usage of the fins. Detail information is described for each fin.

Table 1: Summary of fin functions

	Fish with long & continuous dorsal fin		Fish with short & non-continuous dorsal fin	
	Channa micropeltes	*Clarias* sp.	*Mystus nemurus*	*Pangasius* sp.
Dorsal fin	Direction maneuver, stabilizer, smoothen turning movement, propulsor	Direction maneuver, stabilizer, smoothen turning movement, propulsor	Direction maneuver, stabilizer, smoothen turning movement	Direction maneuver, stabilizer, smoothen turning movement
Adipose fin	-	-	Stabilizer	Stabilizer
Anal fin	Propulsor, Direction maneuver	Propulsor, Direction maneuver	Propulsor	Propulsor
Caudal fin	Propulsor, remain still	Propulsor	Propulsor	Propulsor
Pectoral fins	Direction maneuver, stabilizer, halt	Direction maneuver, stabilizer, halt	Direction maneuver, stabilizer, halt	Direction maneuver, stabilizer, halt
Pelvic fins	Control pitching	Control pitching	Control pitching	Control pitching

(i) FISH WITH LONG AND CONTINUOUS DORSAL FIN

Channa micropeltes

When swimming straight forward, the fish approximately two thirds of the body musculature to produce forward thrust. However, once it was disturbed, it moved by passing muscular contraction along the body to make turns or aggressive movement.

Dorsal and anal fins both acted to help generating forward propulsion. They also had a role to play in direction changing. Part of the dorsal fin or the whole of it, and also the anal fin could be pressed closely to the body in order to move up or down. Two types of movement could be performed by the caudal tail. It expanded to its maximum and then narrowed to the minimum next. This could be observed when it was trying to remain at a fixed position in moving water. Besides that, the caudal fin could also perform wave-like motion, especially while trying to generate forward propulsion. The pectoral fins were very flexible and the fish kept flipping them forward and backward almost all the time. They were used to change direction and also to remain a stand-still position. Pectoral fins were also used as stabilizers. Pelvic fins, on the other hand, acted as “rudders”. It helped to fish to lift, or to descend to the bottom.

The fish liked to stay still, i.e. to make no movement at all sometimes. It would move to the surface to breathe in air from time to time. Once disturbed, it moved rapidly. Besides that, the fish could perform a little backward motion.

***Clarias* sp.**

The fish moved by successive curving of body trunk into “S” shape. Caudal fin aided in the movement to produce even more powerful thrust. When only small movement was involved, for example, moving forward a little, only undulation at the caudal end of the trunk was observed. However, if the fish swam around the aquarium, body undulation could be observed at the whole body trunk.

Dorsal and anal fin helped to control the direction by initiating wave-like movement originating from the anterior to the posterior part. As it moved by undulation, dorsal fin helped to make the movement smooth. It also played important role for the fish to make a smooth turning. Besides that, dorsal fin also acted as stabilizer which prevented the fish from rolling and at the same time allowed it to lean on one side. Pectoral fins acted to decide the direction of where the fish was heading. The fish halted by flipping open the pectoral fins widely to make a sudden stop. At resting position, the head was slightly tilted and it gently flipped its pectoral fins up and down, two at a time, probably to maintain its balance. Pelvic fins acted in conjunction with pectoral fins to generate forward movement. When the fish was not moving, the pelvic fins could be seen opened up as if to steady the fish in resting position.

The fish could perform swimming with very high flexibility, unlike some fishes which can only swim straight forward; they can go any direction they want. Besides, they could swim in great flexibility at very high speed, but only for a short period. It was also important to note that the flexibility of the body movement achieved by this fish enabled it to leap up from the aquarium. However, sometimes they remained still, usually at the bottom of the aquarium, with the head lifting. Since the fish had labyrinthic organ for air breathing function, it came to the water surface frequently to breathe.

(ii) FISH WITH SHORT AND NON-CONTINUOUS DORSAL FIN

Mystus nemurus

The form of swimming for this fish was carangiform locomotion. It used mainly the caudal fin to generate forward propulsion. The caudal tail was able to produce powerful stroke which generated great forward thrust. Hence, the forward movement of the fish mainly depended on the caudal tail.

Dorsal fin helped to control the direction it was heading. Besides that, helped the fish to swerve either to the left or right in the direction it was heading. Adipose fin, which was located between the dorsal and caudal fins, stabilize the fish. The anal fin, which was located near to the caudal fin, helped in generating forwards thrust. The pair of pectoral fin assisted in direction control and body balancing. Besides that, they also functioned to halt the movement. Pelvic fins, on the other hand, acted mainly as "rudders". The fish descended from its original position when the pelvic fins were pressed closely to the body, and vice versa.

***Pangasius* sp.**

Pangasius sp. swam in the form of carangiform locomotion, which was caudal type of propulsion. Dorsal fin acted as a stabilizer, to keep the fish from rolling over, and to allow the fish to lean its body on a certain side. It, too, allowed a smooth turning while the fish was changing direction, for example when performing a "U-turn". Adipose fin did not move in both swimming and resting mode. It probably acted as stabilizer which maintained the balancing of the fish body. Anal fin helped in forward propulsion by having wave-like movement originating from the anterior to the posterior part. Caudal fin, on the other hand, was the main propulsion generator. Pectoral fins involved in controlling the direction and able to halt the movement. For instance, when the fish turned right, it moved only the right pectoral fin. Besides that, the widely open pectoral fins stabilized the fish in the water. They could also control the position of fish in the aquarium. When they were lifted upwards, the fish descended from its original position, and vice versa. Pelvic fins had similar functions and acted in conjunction with pectoral fins.

This fish usually swam straight. They were able to turn left and right, move up and down, but the movement was not as flexible as what observed in *Clarias* sp. was not seen in them. Side to side movement of the head (yawing) was observed when it swims. They were constantly moving and came to the surface to breathe from time to time. They liked to hover in the aquarium. However, it was interesting to note that a stressed fish would lie on one side or dash madly at the sides of the aquarium. In a worse case scenario, the fish swam with the head kept to the bottom and the tails uplifted.

(b) PERSONAL OBSERVATIONS ON DORSAL FIN AMPUTATION

To further investigate the function of dorsal fin in swimming, the dorsal fin was amputated to study its effects on swimming locomotion. The fish was first anaesthetized using MS222 into the state of surgical anaesthetization before the fin was cut away. It was important to note that the dorsal fin was trimmed to just above the base of fin rays. After that, the fish was placed in water (without MS222) in order for it to regain consciousness. Its swimming behaviour after dorsal fin amputation was observed.

For future study, the quality of work might be improved by using better instruments. Visual observation on the fish swimming movement, both normal and after fin amputation could be made perfect by permanent records over long period of time. Besides, two cameras can be used to record the fish movement from above and from the side simultaneously.

All the fishes were affected by the dorsal fin amputation. The swimming movement was not as stable. Details of the effects are described below.

Table 2: Summary of effects of dorsal fin amputation on the selected fishes

	Fish with long and continuous dorsal fin		Fish with short and non-continuous dorsal fin	
	Channa micropeltes	*Clarias* sp.	*Mystus nemurus*	*Pangasius* sp.
Effect of amputation	• Awkward body undulation and turning • Loss of balance • **Less propulsion**	• Awkward body undulation and turning • Loss of balance • **Less propulsion**	• Awkward turning • Loss of balance	• Awkward turning • Loss of balance

(i) FISH WITH LONG AND CONTINUOUS DORSAL FIN

Channa micropeltes

When the fish regained consciousness, it tried to leap out of the aquarium which might be caused by the stress and pain it experienced. However, without the dorsal fin, its leaping became less powerful compared to the time before amputation.

The fish lost its balance without the dorsal fin. Some of the time, it could not control the steadiness of its body, and ended up leaning to its left or right side, or rolling over.

Besides that, the fish had some problem in raising its body. This motion became slower compared to normal fish.

Other noticeable changes included awkward body undulation and direction changing. Additionally, without the dorsal fin, less forward propulsion thrust was generated as the movement became slow and sluggish.

However, it was interesting to note that after the dorsal fin amputation, the fish was still able to make backward movement.

***Clarias* sp.**

Amputation of the dorsal fin affected the swimming behaviour of the fish. The loss of dorsal fin caused the undulation movement to be awkward compared to the time before where it swam smoothly with undulation movement.

Besides that, the undulation movement performed by the fish also produced less propulsive thrust. The movement was observed to be clumsy.

The fish had the ability to leap out from the aquarium easily. With the dorsal fin amputated, less propulsion was generated and thus the leaping was unsuccessful.

Without the dorsal fin, the fish encountered with some problem when swerving either to the left or right in the direction it was heading. The movement was awakward.

Besides that, it was difficult for the fish to gain its balance without the dorsal fin. When they performed undulation movement, it easily rolled to one side of the body.

(ii) FISH WITH SHORT AND NON-CONTINUOUS DORSAL FIN

Mystus nemurus

The fish, with the dorsal fin amputated, had slightly lost its balance. It struggled to gain its balance in order not to roll over. It could be observed when the fish was swimming and also when it tried to remain still in the aquarium.

It could not perform a smooth "U-turn" after its dorsal fin was amputated. The fish performed awkward turnings. It was also important to note that it had to repeatedly adjust its body in order to make a turn.

***Pangasius* sp.**

As soon as the fish regained consciousness, it performed abnormal swimming behaviour. It glided in the aquarium with the head at the bottom but the tail lifted upwards, flipping vigorously. However, when the time progressed, the fish did not perform this swimming behavior again.

The fish seemed to have a hard time to gain balance without its dorsal fin. The fish had to adjust itself from time to time in order not to fall on the left or right side.

Another noticeable effect of fin amputation was the difficulty to make turns. With the dorsal fin, the fish could perform a smooth turn. After the dorsal fin was amputated, the fish could not make a turn in one shot. Instead, it had to repeatedly adjust its body in order to make a turn.

APPENDIX C: HISTOLOGICAL AND STAINING PROCEDURES

(a) H&E Staining

STEP	DURATION
Deparaffinization	
Xylol I	3 min
Xylol II	3 min
Hydration	
95% Alcohol I	3 min
95% Alcohol II	3 min
70% Alcohol	3 min
Distilled water	Rinse
Distilled water	Rinse
Staining	
Harris' Alum Haematoxylin	15 seconds
Under running tap water	Rinse
HCl 0.2%	2-3 seconds
$NaHCO_3$	1 min
Under running tap water	Rinse
Eosin	40-60 seconds
Dehydration	
95% Alcohol III	1-2 seconds
95% Alcohol IV	1-2 seconds
100% Alcohol I	3 min
100% Alcohol II	3 min
Xylol III	3 min
Xylol IV	3 min
Mounting	
Mount in Canada Balsam	-
Store in oven	24 hours

Preparation of chemicals:

1. **Eosin stain**

   Eosin Y solution……..…….....0.2ml

   95% alcohol……...100ml

2. **Harris' alum haematoxylin stain**

   Haematoxylin crystals……...1g

   Absolute alcohol……10ml

   Aqueous aluminium ammonium sulphate……...1ml

(b) Nissl Staining using Cresyl Violet as Dye

Deparaffinization	
Xylol I	3 min
Xylol II	3 min
Hydration	
95% Alcohol I	3 min
95% Alcohol II	3 min
70% Alcohol	3 min
Distilled water	rinse
Distilled water	rinse
Staining	
Cresyl violet stain	20 min
Dehydration	
70% Alcohol	3 min
TBA I	3 min
TBA II	3 min
TBA III	3 min
Xylol III	3 min
Xylol IV	3 min
Mounting	
Mount in DPX	-

Preparation of Chemicals:

1. **Cresyl Violet**

   Cresyl violet acetate ……..0.2g

   Distilled water……150ml

2. **Buffer Solution pH 3.5**

   0.1M acetic acid (6ml/1000ml water)……...……94.0ml

   0.1M sodium acetate (13.6g/1000ml water)…….……..…...6.0ml

3. **Working Solution**

   Buffer solution ……..100ml

   Cresyl violet…….....6-12ml

(c) Myelin staining - Lillie's Varient and Weil-Weigert Method

1. After fixation in 10% formalin, the tissue was soaked in 2.5% potassium dichromate for four days.
2. Tissue was dehydrated in a series of alcohol with gradually ascending concentrations, from 70% alcohol, 85% alcohol, 95% alcohol I, 95% alcohol II, absolute alcohol I to absolute alcohol II. The tissue was then soaked in two changes of wax and ended in toluene wax. After infiltration in three changes of wax, the wax embedded as paraffin block.
3. After sectioning, the slides were stained in the following process.

Xylol I
↓
Xylol II
↓
95% alcohol
↓
85% alcohol
↓
70% alcohol
↓
50ml 4% iron alum + 50ml 1% alcoholic haematoxylin (1-5 days old)
(55-60^{o}C, 40 minutes)
↓
Wash in distilled water
↓
Decolorize in 0.5% iron alum (30-40 minutes), control under microscope
↓
Wash in distilled water
↓
Blue in 1% borax + 2.5% potassium ferriacyanide solution (10 minutes)
↓
Wash in distilled water
↓
Counterstain in safranin O (5 minutes)
↓
Acetone
↓
Acetone + xylene
↓
Xylene
↓
Mount in clearmount

Preparation of chemicals:

1. **10% alcoholic haematoxylin**

Haematoxylin……..……..10g

Absolute alcohol……..…….100ml

2. **0.5% iron alum**

Iron alum……..0.5g

Distilled water……..100ml

3. **2.5% potassium ferricyanide**

Potassium ferricyanide……..……2.5g

Distilled water……..100ml

4. **Safranin O**

Safranin……0.1g

1% acetic acid……...……...100ml

5. 4% iron alum

Ferric ammonium sulphate……..4g
Distilled water……..…….……..100ml

6. 1% acetic acid

Acetic acid……...1ml
Distilled water……...……..99ml

(d) Wholemount

1. After fixation in 10% formalin, the tissue was passed dehydrated by passing through 30% alcohol, 50% alc0hol, 70% alchol, 85% alcohol, 95% alcohol (I and II) to abosolute alcohol (I and II).
2. The tissue was then cleared in methyl salicylate.
3. Immediately after that, it was mounted in a pool of Canada Balsam.

APPENDIX D: DATA AND TABLES

Table 1: Average number of motor neurons innervating caudal fin of *Clarias* sp.

Specimen	**1**	**2**	**3**	**4**	**5**	**6**	**Average**	**SD**
Length (cm)	13.5	13	13	14	14.5	13	13.5	0.58
Weight (g)	15.95	16.15	12.96	15.15	22.3	16.07	16.43	2.85
Labeled neurons	133	135	143	137	139	167	142.33	11.47

Table 2: Average number of motor neurons innervating caudal fin of *Pangasius* sp.

Specimen	**1**	**2**	**3**	**4**	**5**	**6**	**Average**	**SD**
Length (cm)	13.5	13.5	14	13.15	14.5	13.5	13.69	0.44
Weight (g)	18.8	20.3	19.7	20.15	18	20.01	19.49	0.83
Labeled neurons	183	185	168	192	168	163	176.5	10.66

Table 3: Distribution of *Clarias* sp. caudal fin motor neurons at different spinal segments

Segment	IL	DM	VM	C	VL	DL	RDL	Sum
V-6	3	1	2	1	3	0	0	10
V-5	4	3	4	2	3	0	0	16
V-4	6	7	9	3	3	0	0	28
V-3	5	5	7	2	4	0	0	23
V-2	7	9	11	5	4	0	0	36
V-1	6	7	8	4	2	0	0	27

Table 4: Distribution of *Pangasius* sp. caudal fin motor neurons at different spinal segments

Segment	IL	DM	VM	C	VL	DL	RDL	Sum
V-6	0	0	0	0	0	0	0	0
V-5	2	0	3	1	0	0	0	6
V-4	5	6	11	5	7	0	0	34
V-3	7	9	21	5	20	2	0	64
V-2	6	5	15	7	14	1	0	48
V-1	1	2	13	3	7	0	0	26

Table 5: Percentage of motor neuronal shapes (motor neurons innervating *Clarias sp.* caudal fin)

Shape	Percentage (%)							
	IL	DM	VM	C	VL	DL	VDL	Sum
tear	14.96	16.15	21.98	9.5	10.69	0.12	0	73.4
oval	1.43	2.14	2.14	0.48	0.24	0	0	6.43
cone	0.95	1.9	2.95	0.83	0.95	0.12	0	7.7
spindle	0.36	0.36	0.24	0.24	0.48	0	0	1.68
irregular	2.02	2.5	3.09	1.78	1.54	0	0	10.93

Table 6: Percentage of motor neuronal shapes (motor neurons innervating *Pangasius* sp. caudal fin)

Shape	Percentage (%)							
	IL	DM	VM	C	VL	DL	VDL	Sum
tear	11.96	8	27.57	8.8	17.1	1	0.17	74.6
oval	1	0.67	1.16	0	1.16	0	0	3.99
cone	0.67	1.83	2.82	0.17	0.83	0.17	0	6.49
spindle	0.5	0.67	0.83	0.5	2.16	0.17	0	4.83
irregular	1.5	1.16	2.99	1.16	2.99	0.33	0	10.13

APPENDIX E: SELECTED PUBLICATIONS (PROCEEDING AND ABSTRACT)

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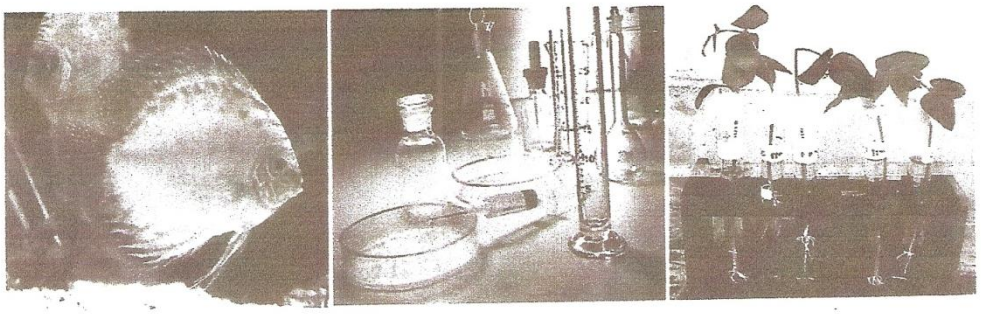

12-14 November 2008
Pusat Asasi Sains Universiti Malaya, Kuala Lumpur

ANATOMY OF SPINAL CORD OF *Clarias Macrocephalus* (IKAN KELI) AND *Pangasius Pangasius* (IKAN PATIN)

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Abstract

The spinal cord has ascending and descending tracts enabling communication of the body with the brain. It also acts as reflex center. This anatomical study investigated the spinal cord of Clarias macrocephalus and Pangasius pangasius; from gross morphological and histological aspects. General external morphology and histology of spinal cord sections stained with H&E and Nissl for both Keli and Patin were similar. However, differences were observed in terms of motor neuronal distribution in the ventral horn grey matter. They were not distributed randomly but seemed to be clustered. This could reflect the different distribution of fins, which help in the movement of fish; Keli has long and continuous fins along the body length but Patin has short and non-continuous fins.

1 INTRODUCTION

Clarias macrocephalus (Keli) and *Pangasius* pangasius (Patin) are two different local freshwater fishes. Clarias *macrocephalus* or broadhead catfish has a labyrinthic organ which enables it to live in shallow and open water or even lying buried in the mud for a lengthy period [1]. *C. macrocephalus* is known as carnivorous and they feed on almost anything that comes to them. *Pangasius pangasius* or yellowtail catfish has a shark-like body shape which makes them a rapid swimmer in large rivers and estuaries. It feeds on snails, other mollusca and plants [2].

Movement of fish involving its fins is orchestrated by the brain and spinal cord [3]. The spinal cord, through the ascending and descending tracts in it, transmit impulses between body and the brain. A very distinctive characteristic for fish is usage of fins in movement [4]. Each fin is designed for a specific function. Fins can be paired or median fins. Median fins include dorsal fin, caudal fin, anal fin, and adipose fin which are located along the centerline of the fish. Paired fins, on the other hand, are pectoral fins and pelvic fins [4]. Keli has long and continuous median fins [Figure 1(a)] while Patin has short and non-continuous median fins [Figure 1(b)].

The objective of this study is to investigate the morphology and histology of spinal cord of Keli and Patin which have differently distributed fins.

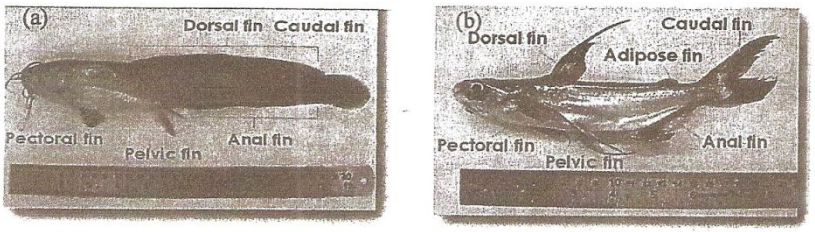

Figure 1: Lateral view of (a) *Clarias macrocephalus* (Keli) and (b) *Pangasius pangasius* (Patin).

Harnessing The Potential Of Biodiversity

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Diversity of a Central Nervous System Structure: Spinal Cord of a Terrestrial Frog in Comparison to an Aquatic Fish

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Abstract

Besides the brain, spinal cord is another central nervous system (CNS) structure that plays a significant role in a vertebrate's daily activities. The control of voluntary or reflex movements of the body is directly related to the spinal cord. The nature of the nervous tissue, in this case the spinal cord, reflects the possible movements which formed the animals' behaviour. Thus, this study was conducted to highlight the anatomical differences of the spinal cord structures in two animal species with different living behaviour. The experimental models being studied were the common species of Grass Frog (*Fejervarya limnocharis*) and Catfish (*Clarias* sp.). The former being a terrestrial creature, inhabits cleared as well as disturbed land areas while the latter adopts the aquatic lifestyle, spending most of its life in shallow, open waters. Gross morphological analysis and histological works involving H&E and Nissl staining methods were employed. Comparatively, the features observed between the spinal tissues of both species differed in terms of shape, the presence of enlargements at particular levels along the spinal cord, as well as the distribution patterns of the grey and white matters. Some of these dissimilarities could be associated with their distinctive mode of movements in correspondence to the different natural habitats they live in, for instance, the execution of saltatory movement by the frog on firm grounds and body undulation of the catfish with the help of fins across the water. The overall organization of spinal cord between both species nevertheless resembled those of higher vertebrates.

Introduction

The spinal cord is no less essential than the major controlling centre, i.e. the brain in any living vertebrates especially when it comes to mediating locomotory movement and reflex control of the body (Oksche and Ueck, 1976). Two experimental models with each demonstrating different modes of locomotion, *Fejervarya limnocharis* (Grass Frog or 'Katak Sawah') and *Clarias* sp. (the Catfish or 'Keli'), were put under scrutiny in this anatomical study of the spinal cord structure from both the morphological and histological aspects. The terrestrial frog spends most of its life on land as opposed to the catfish that adopts the aquatic lifestyle in shallow, open waters. Originating from such distinctive habitats, these two animals display differences in their own means of movement. With the presence of fore- and hind limbs, the frog is capable of executing saltatory movements on the firm ground, e.g. jumping, leaping and hopping. Conversely, the fish maneuvers its limbless body in the water to move by body undulation together with the help of fins (Bond, 1979).

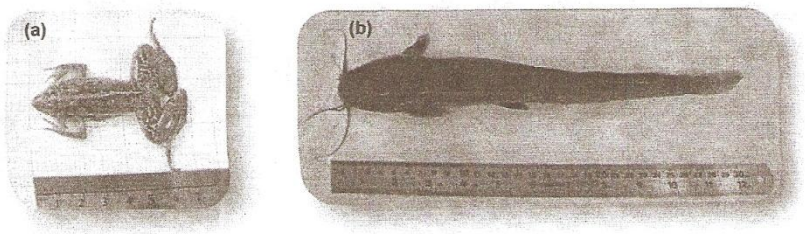

Figure 1: Dorsal view of (a) *Fejervarya limnocharis* (b) *Clarias*

Materials and Methods

Hee et al. (eds.). Harnessing the Potential of Biodiversity, page 300-303

Dannyyahr!

Proceedings

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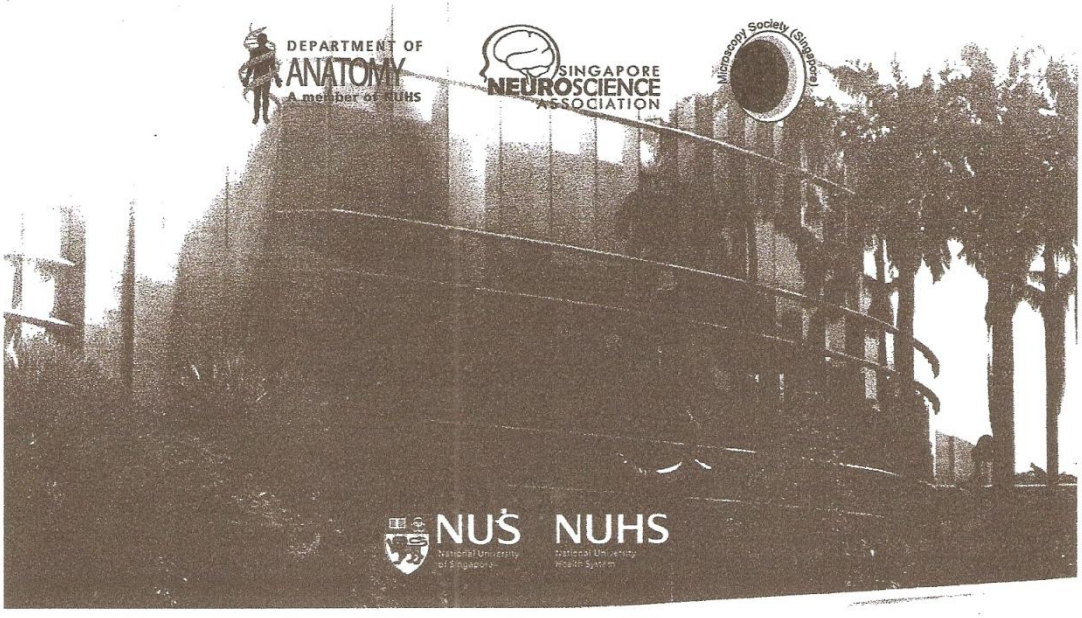

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NUHS National University Health System

28-010

Presentation slot: 28 May, 13:00-13:30

SPINAL CORD CYTOARCHITECTURE OF TWO FISHES WITH LONG AND CONTINUOUS DORSAL FINS: JUVENILE *CHANNA MICROPELTES* (TOMAN) AND *CLARIAS SP.* (KELI) WITH EMPHASIS ON MOTOR NEURONAL ORGANIZATION

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The spinal cord facilitates conduction of electrical impulses between the brain and body and controls effector organs in response to the internal and/or external environment. It houses millions of nerve cells that serve as its structural and functional units. Juvenile *Channa micropeltes* (Toman) and *Clarias sp.* (Keli) with average length of 17.5cm and 15cm, respectively, were used as the experimental subjects. Each intracardiacally perfused fish had its spinal cord extracted for gross morphological examination and three specific sites chosen for histological investigation. The cytoarchitecture of the spinal cord was delineated by H&E, Nissl and myelin stains. The spinal cord diameter of both fishes gradually decreased towards the caudal end. A distinctive spinal feature of these species was the lack of posterior funiculus, thus, the appearance of inseparable dorsal horns. Both fishes exhibited similarity in terms of motor neuronal organization. Somas of motor neurons located at the grey matter occupied five cell columns, namely intermediolateral, dorsomedial, ventromedial, central, and ventrolateral columns. However, additional groups of motor neuronal somas could also be found at dorsolateral and retrodorsolateral cell columns in mammals. The lack of these motor neuronal groups might be credited to less complexity of fin movements compared to the limb movements of mammals. It was observed that motor neurons at the medial column were usually big and of tear and oval shaped, while those at lateral column were smaller in size and could be found in tear, cone, spindle or irregular shaped. In summary, the motor neuronal organization of the spinal cord reflected control over external morphology (including appendages) of an organism.

28-011

Presentation slot: 28 May, 13:30-14:00

COLLAGEN FIBRIL DIAMETER VARIATION CORRELATES WITH BIOMECHANICAL HETEROGENEITY IN PERIPHERAL NERVES

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INTRODUCTION: Previous research has identified that the stiffness of rat peripheral nerves varies longitudinally according to where they traverse joints. Fibrillar collagen is the predominant matrix protein in the peripheral nervous system and is known to play an important role in the mechanical properties of many tissues during normal physiological movement. This study explores how the ultrastructural features of fibrillar collagen vary in specific regions of rat peripheral nerves.
OBJECTIVES: To measure the diameter of collagen fibrils and the unit cross sectional area of collagen in joint and non joint regions of rat median and sciatic nerves.
PROCEDURES: Joint and non joint regions of rat median and sciatic nerves were resected post mortem from 250-350g rats, maintained at their *in situ* tension and processed for transmission electron microscopy. Collagen fibril diameter and unit area of collagen per region of interest was measured and analysed.
RESULTS: In the median nerve, collagen fibrils were significantly thinner at joint regions compared to non joint regions. This phenomenon was evident in both the epineurium and the endoneurium of the median nerve (42.1nm ± 2.86 vs 47.1nm ± 2.87 and 66.1nm ± 5.74 vs 77.9nm ± 6.61 respectively), but not the sciatic nerve. Unit cross sectional area of collagen was comparable in the joint and non joint regions in the endoneurium and epineurium of the median nerve and sciatic nerves.
CONCLUSIONS: These results suggest that localised variation in nerve stiffness may be due to the size of collagen fibrils, with an increased number of thinner fibrils at joints and no difference in the overall collagen cross sectional area between regions.

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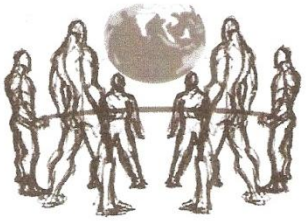

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PT1- 10

Spinal Cord Cytoarchitecture of Two Fishes with Short and Non Continuous Median Fins: Juvenile *Mystus nemurus* (Baung) and *Pangasius pangasius* (Patin) with Emphasis on Motor Neuronal Organization

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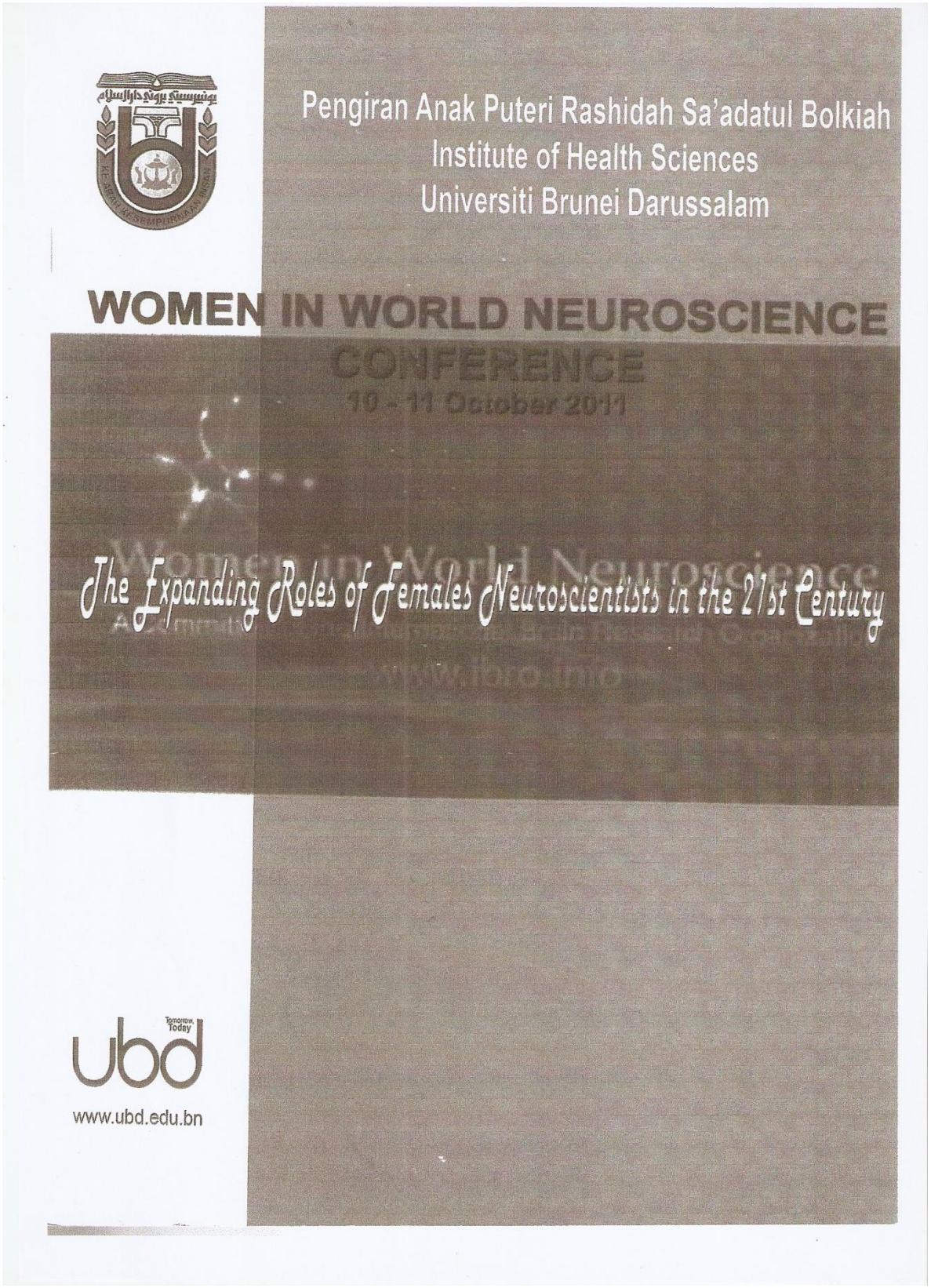
Spinal cord serves as a conduit connecting brain and peripheral nervous system, hence permitting neural transmission of both sensory and motor information. Lower motor neurons within spinal ventral horn have their axons projecting towards effector organs such as fin muscles. This study aimed to delineate motor neuron organization in juvenile *Mystus nemurus (Baung) and Pangasius pangasius (Patin)* with average length of 14cm and 16cm, respectively. Spinal cord was extracted from the vertebral column of each intracardiacally perfused fish for gross morphological inspection and histological investigation. The cytoarchitecture of the spinal cord was outlined by H&E, Nissl and myelin stains. The spinal cord diameter and grey matter area gradually decreased towards caudal end. Both fishes exhibited similarity in terms of motor neuronal organization. In contrary to mammals which have seven cell columns within ventral horn, only six cell columns, namely intermediolateral, dorsomedial, ventromedial, central, ventrolateral and dorsolateral columns were found in the fishes. An additional group of motor neuronal somas could be found as retrodorsolateral cell column in mammals, probably credited to the complexity of limb movement. At any given section, neurons in mammals are clustered as neuronal groups, whereas in the fish they were randomly distributed along the spinal length of the columns, usually one to three at each section. In this study, motor neurons were classified into tear, oval, cone, spindle and irregular shapes with tear-shaped somas having highest distribution. In summary, the motor neuronal organization of spinal cord reflected control over external morphology (including appendages) of an organism.


Pengiran Anak Puteri Rashidah Sa'adatul Bolkiah
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Spinal Motor Innervations of Caudal Fins with Different Morphology: Rounded Caudal Fin of Keli (Clarias macrocephalus) and Forked Caudal Fin of Patin (Pangasius pangasius)

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This study sought to understand the correlation of motor neuronal organization with caudal fin morphology. Juvenile fishes representing two caudal fin types were utilized; *keli* (average length=13.5cm) which exhibited rounded caudal fin and *patin* (average length=13.69cm) which displayed forked caudal fin. Forked caudal fin of *patin* enabled it to be a better swimmer than *keli*. The caudal fin muscles were located longitudinally anterior to the fin. When checked under the stereomicroscope, it was revealed that there were two muscle layers of the caudal fin; superficial and deep muscle layers. These muscle layers for both fishes demonstrated significant dissimilarities, with the caudal fin of *patin* expressing a more complicated organization of musculature at both layers. Six main nerve trunks in *keli* and four nerve trunks in *patin* supplying the caudal fins were identified. To retrogradely label the motor neuronal somas, the nerves were crushed prior to immersion in 50% horseradish peroxidase (HRP) solution. After 48 hours of survival, the specimens were sacrificed and the abstracted spinal cords were freeze-sectioned. The HRP deposited in the ipsilateral neuronal somas was reacted with chromogen 3,3'-diaminobenzidine/DAB) to form brownish HRP-DAB reaction product. Motor neurons were organized according to cell column classification consisting of intermediolateral (IL), dorsomedial (DM), ventromedial VM), central (C), ventrolateral (VL), dorsoventral (DV) and retrodorsolateral (RDL) cell columns. Most of the motor neurons innervating *keli* rounded caudal fin were located medially (IL, DM and VM cell columns). In contrast, those innervating *patin* forked caudal fin were mostly at the ventral areas (VM and VL cell columns). The differences of the motor neuronal organization might be credited to further muscle differentiation in deep muscle layer of *patin* which exhibited a more complicated organization than *keli*. Further studies are needed to ascertain topographical relation of motor neurons to the control of fin specific areas.