

**POPULATION PARAMETERS, FEEDING AND
REPRODUCTIVE BIOLOGY OF *Rasbora* spp.
FROM THE STREAMS OF MUDA RESERVOIR,
KEDAH, MALAYSIA**

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FROM THE STREAMS OF MUDA RESERVOIR,
KEDAH, MALAYSIA**

by

NURUL SHAFIKAH BINTI MOHD NOOR

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LIST OF SYMBOLS AND ABBREVIATION

<i>b</i>	Growth coefficient
<i>B'/R</i>	Relative biomass per recruit
BW	Body weight
C.flow	Current flow
cm	Centimeter
Cond	Conductivity
DO	Dissolved Oxygen
DOE	Department of Environment
DID	Department of Irrigation and Drainage
<i>E</i>	Exploitation rate
ELEFAN I	Electronic Length-Frequency Analysis I
<i>E_{max}</i>	Maximum allowable limit of exploitation
<i>F</i>	Fishing mortality
FiSAT II	FAO-ICLARM Stock Assessment Tools II
GSI	Gonadosomatic index
g	gram
<i>K</i>	Growth constant
<i>Kn</i>	Relative Condition Factor
L	Litre
LWR	Length-Weight Relationship
<i>L_∞</i>	Asymptotic length
<i>M</i>	Natural mortality
m	Meter
mg	Milligram
mm	Millimeter
NH ₄ ⁺	Ammonia
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
PAST	Paleontological Statistics Software Package for education and data analysis
PCA	Principal Component Analysis
PO ₄ ³⁻	Phosphate
<i>r</i> ²	Regression coefficient

RGL	Relative Gut Length
s	Second
sd	Standard deviation
SL	Standard Length
SPSS	Statistical Package for Social Science
ST	Station
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
TL	Total Length
t_{\max}	Potential longevity
vBGF	von Bertalanffy growth function
W	Weight
Y'/R	Relative yield per recruit
Z	Total mortality
\emptyset'	Growth performance index
μS	Micro Siemens

PARAMETER POPULASI, BIOLOGI PEMAKANAN DAN PEMBIAKAN
***Rasbora* spp. DARI ANAK - ANAK SUNGAI DI EMPANGAN MUDA, KEDAH,**
MALAYSIA

ABSTRAK

Satu kajian mengenai aspek biologi ikan *Rasbora* spp. telah dijalankan di lima anak sungai di Empangan Muda yang dipilih selama 12 bulan dari November 2014 hingga Oktober 2015. Kajian ini merangkumi penentuan parameter kualiti air dan komposisi ikan, hubungan berat badan, faktor keadaan relatif, parameter populasi dan biologi pemakanan *R. dusonensis* dan *R. vulgaris*, dan biologi pembiakan *R. dusonensis*. Untuk penilaian kualiti air, tiada perbezaan signifikan ($P > 0.05$) diperhatikan di antara stesen persampelan pada suhu air, kekonduksian, kedalaman air, jumlah pepejal terlarut, oksigen terlarut, aliran air, nitrat dan fosfat. Nilai analisis kualiti air dari anak-anak sungai di Empangan Muda berada di tahap selamat di Kelas I dan IIA. Dua puluh tujuh spesies ikan dari 12 keluarga telah direkodkan menggunakan jala dengan saiz lubang jaring 2.5 cm dan indeks kepelbagaian spesis kelompok ikan dari anak sungai di Empangan Muda berada dalam tahap sederhana 2.174. Analisis hubungan panjang-berat menunjukkan *R. dusonensis* dan *R. vulgaris* mengikuti pertumbuhan isometrik. Selain itu, faktor keadaan relatif (Kn) yang diperolehi oleh kedua jantina *R. dusonensis* adalah 1.00, sedangkan nilai faktor keadaan relatif yang diperolehi oleh *R. vulgaris* adalah 0.93 untuk betina dan 0.92 untuk jantan, menunjukkan keadaan yang baik untuk kedua spesies ini di anak sungai Empangan Muda. Daripada kajian parameter populasi, *R. dusonensis* dan *R. vulgaris* dianggarkan mencapai panjang infiniti (L_{∞}) pada 19.5 cm dan 17 cm, dengan kadar pertumbuhan sederhana, $K = 0.57 \text{ tahun}^{-1}$ (*R. dusonensis*) dan kadar pertumbuhan perlahan, $K = 0.23 \text{ tahun}^{-1}$ (*R. vulgaris*). Panjang pada penangkapan pertama (L_c) untuk *R. dusonensis* dan *R. vulgaris* dianggarkan pada 8.69 cm dan 7.86

cm. Jumlah kematian (Z) *R. dusonensis* adalah 4.93 tahun⁻¹ dengan kematian semasa penangkapan (F) = 3.51 tahun⁻¹ lebih tinggi dari kematian semulajadi (M) = 1.42 tahun⁻¹. Sedangkan jumlah kematian (Z) *R. vulgaris* adalah 1.13 tahun⁻¹ dengan kematian semasa penangkapan (F) = 0.32 tahun⁻¹ adalah lebih rendah daripada kematian semula jadi (M) = 0.81 tahun⁻¹. Kadar eksploitasi (E) untuk *R. dusonensis* adalah 0.71 tahun⁻¹ dan sedikit tereksploitasi daripada tahap eksploitasi optimum ($E_{0.1}$) iaitu 0.66 tahun⁻¹. Bagaimanapun, kadar eksploitasi (E) untuk *R. vulgaris* adalah 0.28 tahun⁻¹ dan berada bawah eksploitasi berbanding tahap eksploitasi optimum ($E_{0.1}$) iaitu 1.00 tahun⁻¹. Pengeksploitasian kedua-dua spesies ini perlu diuruskan dengan betul dengan cara yang mampan untuk kesinambungan sumber ikan sebelum ia mencapai anggaran maksimum eksploitasi (E_{max}) yang dianggarkan iaitu sebanyak 0.78 tahun⁻¹ dan 1.00 tahun⁻¹. Dari kajian biologi pemakanan, didapati usus oesophagiogastric untuk kedua-dua spesies ini mengandungi makanan campuran haiwan dan makanan berasaskan tumbuhan, yang menjadikan spesies ini sebagai omnivor-eurifagus. Kandungan makanan utama adalah tumbuhan, Insecta, Bacillariophyta dan cacing (Annelida dan Nematoda). Dari kajian biologi pembiakan, nisbah seks betina kepada jantan untuk *R. dusonensis* adalah 1: 1.5. Peringkat kematangan betina dan jantan *R. dusonensis* dikelaskan kepada lima peringkat kematangan. *R. dusonensis* adalah pembiak berganda berdasarkan analisis histologi dan pecahan oosit dilepaskan pada musim peneluran dengan tindakan peneluran berulang. Betina *R. dusonensis* mencapai kematangan jantina pada saiz lebih besar (9.6 cm) daripada jantan *R. dusonensis* (9.2 cm). Fekunditi *R. dusonensis* adalah tinggi dengan fekunditi relatif antara 1226 hingga 7902 telur per gonad dan ia mempunyai hubungkait yang lebih tinggi dengan berat ovari daripada berat badan dan panjang keseluruhan. Penilaian parameter populasi, biologi pemakanan dan reproduktif kedua-dua spesies membuktikan bahawa terdapat interaksi yang baik antara persekitaran dan *Rasbora spp.* di anak sungai Empangan Muda.

**POPULATION PARAMETERS, FEEDING AND REPRODUCTIVE BIOLOGY
OF *Rasbora* spp. FROM THE STREAMS OF MUDA RESERVOIR, KEDAH,
MALAYSIA**

ABSTRACT

A study on the biological aspects of the *Rasbora* spp. was carried out in five chosen streams of Muda Reservoir for duration of 12 months from November 2014 until October 2015. This study includes the determination of water quality parameters and fish composition, length-weight relationship, relative condition factor, population parameters and feeding biology of *R. dusonensis* and *R. vulgaris*, and reproductive biology of *R. dusonensis*. For water quality assessment, there were no significant differences ($P > 0.05$) were observed among sampling stations in temperature, conductivity, water depth, total dissolved solids, dissolved oxygen, current flows, nitrate and phosphate. The values of the water quality analysis from the streams of Muda Reservoir were in safe level in Class I and IIA. Twenty-seven fish species from 12 families were recorded using experimental cast nets with 2.5 cm mesh sizes and the species diversity index of fish communities from the streams of Muda Reservoir is in moderate levels of 2.174. Analysis of the length-weight relationship shows that *R. dusonensis* and *R. vulgaris* followed the isometric growth. Besides, the relative condition factor (K_n) obtained for both sexes of *R. dusonensis* was 1.00, while the value of relative condition factor obtained for *R. vulgaris* was 0.93 for females and 0.92 for males, showing the better condition of these two species from the streams of Muda Reservoir. From the population parameters study, *R. dusonensis* and *R. vulgaris* were estimated to reach length infinity (L_∞) at 19.5 cm and 17 cm, with moderate growth rate, $K = 0.57 \text{ year}^{-1}$ (*R. dusonensis*) and slow growth rate, $K = 0.23 \text{ year}^{-1}$ (*R. vulgaris*). The length at first capture (L_c) of *R. dusonensis* and *R. vulgaris* were estimated at 8.69 cm and 7.86 cm. The total mortality

(Z) of *R. dussonensis* was 4.93 year^{-1} with the fishing mortality (F) = 3.51 year^{-1} was higher than the natural mortality (M) = 1.42 year^{-1} . While, the total mortality (Z) of *R. vulgaris* was 1.13 year^{-1} with the fishing mortality (F) = 0.32 year^{-1} was lower than the natural mortality (M) = 0.81 year^{-1} . The exploitation rate (E) for *R. dussonensis* was 0.71 year^{-1} and was slightly exploited than the optimum exploitation level ($E_{0.1}$) of 0.66 year^{-1} . However, the exploitation rate (E) for *R. vulgaris* was 0.28 year^{-1} and under exploited than the optimum exploitation level ($E_{0.1}$) of 1.00 year^{-1} . The exploitation of both species should be managed properly in sustainable way for fish resources continuity before it reaches the estimated maximum allowable limit of exploitation (E_{max}) of 0.78 year^{-1} and 1.00 year^{-1} . From the feeding biology study, the oesophagiogastric guts for both species contains a mixed diet of animal and plant-based food, which make the species to be considered as euryphagous-omnivores. The main food contents were plant matters, Insecta, Bacillariophyta and worms (Annelida and Nematoda). From the study of reproductive biology, the sex ratio of female to male for *R. dussonensis* was 1: 1.5. The maturity stages of female and male of *R. dussonensis* were classified into five maturity stages. *R. dussonensis* is a multiple spawner according to histological analysis and oocyte size frequency distribution with fraction of the oocytes was released during the spawning seasons with repeated spawning acts. Female *R. dussonensis* reach sexual maturity at bigger size (9.6 cm) than the male of *R. dussonensis* (9.2 cm). The fecundity of *R. dussonensis* was high with the relative fecundity was ranged from 1226 to 7902 oocytes per females and it is more related to ovary weight rather than body weight and total length. The assessment of the population parameters, feeding and reproductive biology of both species proves that there is good interaction between the environments and *Rasbora spp.* in the streams of Muda Reservoir.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Lakes or reservoirs in Peninsular Malaysia

Most of lakes and reservoir in Malaysia are man-made lakes with only a few were identified as natural lakes. The most common example of natural lakes is Lake Bera and Chini. There are about more than 73 man-made lakes and reservoirs that is created for the purpose of hydroelectric generation, flood mitigation, irrigation, water supply for domestic and also for industrial use (Zati and Salmah, 2008). Until mids 90's there are about 46 reservoirs located in Peninsular Malaysia and a later study by Khoo *et al.* (2004) reported about 63 reservoirs in Malaysia. The increased number of reservoirs over the years is due to the industrialization factor and also the country's swift development (Ho, 1994; Zakaria-Ismail, 1996; Shah and Ali, 2002). The common identified differences of natural lake and reservoir is their drainage basin, compared to the natural lakes, reservoir drainage basin is more elongated, narrower and much larger (Kalff, 2002). As such, lakes, rivers and its catchment area are the main component of natural heritage that has to be preserved. However, studies of whole streams or rivers systems, or even specific stretches of streams that confluent to reservoir are still lacking. Forest in the catchment area should not be disturbed and need to be preserved as they are the repository of our natural heritage besides their association with rivers which are important water resources (Uzzell, 1989).

1.2 The important of Muda Reservoir and its fisheries activities

Muda Reservoir is one of the main reservoirs in the northwestern coast of Peninsular Malaysia other than Pedu, Ahning, Timah Tasoh and Beris Reservoir. The Muda Reservoir has a large catchment area (984 km²) with the storage capacity of 160 million m³ and covering an area of 25.6 km² (MADA, 2015). Flowing out of the Muda Reservoir

is Muda River. The Muda River being developed to become one of the important water resources for states like Kedah and Penang for agriculture and water supply, both states have the right to use the resources from Muda River (Lee, 2009). Muda River originates from the convergence of Bahoi River and Lasor River in the UMFR (Ulu Muda Forest Reserve) and drains into Muda Reservoir (Zalina, 2014). The important tributaries of the Muda River are Lasor River, Teliang River, Bahoi River, Kawi River and Kalir River (DID and JICA, 1995). These rivers play crucial roles in maintaining the number of fish population in this reservoir by providing natural food sources to the fishes, providing nursery ground for fingerling, spawning and breeding habitats for fishes and acting as fish refuge areas.

Muda Reservoir is a suitable area for recreational anglers, researchers, wildlife conservation and nature tourism. The forests around Muda Reservoir provide values include the values for ecotourism with a potential value of RM13 million annually; non-timber forest products; educational and scientific purpose; medicinal and pharmaceutical values; cultural and heritage values; and the existence values of the diverse flora and fauna (Lee, 2009). Apart from its faunal diversity, the abundance of natural saltlicks from streams of Muda Reservoir was added to the conservation value of the Ulu Muda Forest Reserve (Stevens, 1968) because saltlicks are important feature of Ulu Muda's forest that contribute to the wellbeing of its wildlife populations (Zalina, 2014), particularly for herbivorous mammals and birds in tropical rainforests it serve as natural mineral supplements (Matsubayashi *et al.*, 2007).

The common activities in Muda Reservoir and its streams were fisheries. The fisheries activity in the Muda Reservoir is relatively small with an estimated number of 30 fishermen or less. Most of these fishermen were doing this fishery as fulltime job as they

engaged in fishing for their main source of income and fished for at least three times a week (Lee *et al.*, 2013). Previous study in Muda Reservoir by Samat and Mazlan (2003), Samat *et al.* (2005), Shah *et al.* (2006), Shah *et al.* (2012) and Lee *et al.* (2013) recorded 11, 17, 21, 25 and 36 fish species respectively.

Moreover, Lee *et al.* (2013) recorded two types of fisheries at Muda Reservoir, which are commercial inland fishery and recreational fishery. Fishing is the main economic activity in Muda Reservoir so the inland fishery mainly engaged in by locals living in nearby areas. The recreational fishery at Muda Reservoir mainly consists of recreational anglers. Fishermen around Muda Reservoir were divided into two specialized group based on their sampling gears. First group usually target cyprinids and tilapia as their target species and most of them used gillnet as their fishing gears and some used cast nets (jala) to supplement their catch. Second group utilized longlines (rawai) to target predatory fishes such as baung and haruan.

Mostly, the cyprinid family dominates the Muda Reservoir and more prominent at riverine sites compared to lacustrine sites. The four most dominant fish in the riverine environment are *Mystacoleucus obtusirostris*, *Rasbora dusonensis*, *Osteochillus vittatus* and *Barbodes binotatus* (Lee *et al.*, 2013). The estimated annual fish production of Muda Reservoir is less than 21kg ha⁻¹ and there a potential annual yield of 50 tones for Muda Reservoir's fisheries (Khoo *et al.*, 2004; Lee *et al.*, 2013). Reservoirs fisheries provided important economic activities especially for the rural communities. It is becoming a source of income to local fisherman and a cheap source of protein to riverine communities. The average daily catch is 10.5kg per fisherman, providing an average income of RM800 per month. Their income could get up to RM1000 per month

especially during rainy season or known as good month. However, during bad month their income was between RM300-RM500 per month (Lee *et al.*, 2013).

1.3 Problem statements

About four million people in the three northern states in Malaysia (Kedah, Perlis and Penang) are dependent on Muda Reservoir as a regional water catchment area. Present study was conducted in the two sub-catchment areas in Muda River (Labua River, Sira Jawa River, Nyeh River and Debu River) and Teliang River. This ecosystem can be considered as vulnerable to human alterations. Based on Malaysiakini (2018), legal and illegal potential deforestation and unsustainable logging activities, affecting water quality around Muda Reservoir and its streams thus threaten its role as a water catchment area. Moreover, the clearing of forest patches increases the run-off of soil into Muda Reservoir and streams, making raw water supply murky and more expensive to treat. Any changes to catchment areas will lead to the fish habitat degradation and becoming a treat to the existence freshwater fishes and plankton (Chong *et al.*, 2010).

Increasing of severe incidents of floods and droughts affected by these activities disrupt the forest's ability to absorb and retain rainwater. Due to climate change from these events, the water quality in Muda Reservoir (WWF-Malaysia, 2018) is affected. A recent report by Lee *et al.* (2013), concluded that there are slightly increment in temperature, pH and total suspended solid (TSS) in streams of Muda Reservoir. The sustainable of water quality status are crucial not only for freshwater fish community but also the other wildlife species around Muda Reservoir's forest.

The practice of unsustainable fishing from local and outsider (fishermen as far as Baling) such as the deployment of smaller mesh-size gillnets, trammel nets, cyanide and dynamite in fishing led to the declination of fish stocks around Muda Reservoir (Lee *et*

al., 2013). This practice removed the juvenile fishes from the population which resulting in a less resilient fish population that have more difficulty to recover. The declination of the fish catches around Muda Reservoir noticeable by locals after year 2008. According to Chong *et al.* (2010), often uses of unsustainable fishing lead to overexploitation of the fish resources within, and the wide-scale destruction or degradation of the habitats. Most of the freshwater fishes in Malaysia are being overfished (Chong *et al.*, 2010). This overfishing coupled with the degradation of the riverine environment by pollution and siltation has led to the demise of the riverine fisheries. Thus, led to the disruption of freshwater fishes communities as an important cheap source of protein to the riverine rural communities and inland.

Apparently, to compensate for the lower catch, fishermen in Muda Reservoir begin to increase their catch by using more nets or longer nets and fishing for longer hours. Beside that, the uses of small size gill nets for example 3'' to 2 ½'' gill nets to catch more low value of mixed fishes (*ikan putih*) for 'pekasam' (fermented fish) industry to supplements their income. The abundant of vegetation such as weeds and aquatic plant in shallow coves make it as a fabulous place for juvenile's fishes to gather for sheltering and feeding. However, unhealthy fishing of 'merambat' is set across the cove and the net is deployed over the entire water column. This scares the fishes and drive them toward the net and get entangled with gillnets. This practice will reduce recruitment of juvenile fishes in to the reservoir (Lee *et al.*, 2013).

Besides that, there is lacking of reliable data regarding to the fisheries in the Muda Reservoir. Less attention of the Muda Reservoir received from outsider and its fisheries only supports a small-scale inland artisanal fishery until the WWF-Malaysia making an effort in promoting the protection and conservation of Ulu Muda Forest Reserve.

1.4 Rational of the study

Some of previous study focused on the freshwater fishes in the upstream of Muda River especially in Lasor River by Samat *et al.* (2005), while Shah *et al.* (2012) investigated fish checklist distribution in Sira Batu River, Surat River, Air Hangat River, Bahui River and Jawa River. Then, Lee *et al.* (2013) explored the freshwater fish in riverine environment (Muda River, Teliang River, Charok Tera and Che-Song River) and lacustrine environment. Although a number of documentations on the water quality and species composition and diversity at Muda Reservoir have been recorded, the assessment with regards to some biological aspects of fishes there has received little attention. The knowledge of the fish condition (growth performance and relative condition factor), population parameters, reproductive biology and feeding habits of fish species in this area is very limited and has not been properly documented and disseminated.

Besides that, there is limited information about fish diversity in streams of forest reserves in Peninsular Malaysia. Most species have not been studied, although they are utilized by local or indigenous people as food (Othman *et al.*, 2002). Current research done in two sub-catchment areas, which is Muda River (Labua River, Sira Jawa River, Nyeh River and Debu River) and Teliang River is an effort to do detail study on the streams mentioned above regarding its fish species composition. *Rasbora* spp. is chosen for detailed studies regarding its condition, population parameters, feeding and reproductive biology study because its abundance and dominance. This genus are one of the most dominant species found it this area. Therefore, the present study attempted to provide valuable information and knowledge on some aspect on the population biology of *Rasbora* spp. thoroughly for the management of the Muda Reservoir, which is vital for sustainable development of the capture fisheries particularly in this water body. Hence, through this study it is hoped that insights towards the significance of fish

biological studies from streams of Muda Reservoir could be obtained. Moreover, the findings could be used as baseline data for the purpose of sustainable fisheries resources. Moreover, as fish is the major source of dietary protein for the national sector, the growth of fisheries industry should be importance for a positive effect on the microeconomic of local communities in this area.

1.5 Objectives of the study

The main objectives of this study are as follows:

1. To evaluate the diversity of fish from the streams of Muda Reservoir (Labua River, Sira Jawa River, Nyeh River, Debu River and Teliang River).
2. To determine the length-weight relationships, relative condition factor and the population parameters of *R. dusonensis* and *R. vulgaris* through the estimation of growth parameters, mortality coefficients and recruitment patterns using FiSAT II software.
3. To investigate the feeding aspects and the diet of *R. dusonensis* and *R. vulgaris* regarding gut fullness index, relative gut length and the diet of both species between male and female, length size group, sampling stations, monthly and during dry and wet months.
4. To estimate some aspects on reproductive biology of *R. dusonensis* regarding to sex ratio, gonad maturity stages, length at first maturity, gonadosomatic index, relative fecundity and oocyte diameter.

1.6 Limitation of study

The main limitation of this study is insufficient of fish biodiversity data due to lack of sampling gears used. This research only used cast net as main sampling gear to collect fish samples aimed for the streams of Muda Reservoir as the streams chosen consist of shallow habitat. Cast netting is one of active fishing method other than gill netting used by fisherman in Muda Reservoir (Lee *et al.*, 2013). Besides, it is involved only single fishing operation in the streams of Muda Reservoir. Amongst the limitations faced by this study were the limitation of published fisheries statistics on inland fish landing and recreational fishery data for the Muda Reservoir. Hence, the data gathered during this study only provided a glimpse of the current state of the Muda Reservoir fishery. Therefore, the results from this study do not represent a realistic conclusion about fish species composition, fish species diversity, and population parameters of *Rasbora* spp. in Muda Reservoir. Thus, the results such as fish species composition, fish species diversity, population parameters, reproductive biology and feeding of *Rasbora* spp. are based on the cast net catches and the productivity of this gear from the streams of Muda Reservoir.

1.7 Hypothesis of the study

Rivers play crucial roles in maintaining the number of fish population in the reservoir by providing natural food sources to the fishes, providing nursery ground for fingerling, spawning and breeding habitats for fishes and acting as fish refuge areas. Therefore, the rivers with clean water quality and habitat that rich with food resources will provide better growth condition, feeding, breeding and nursery ground for most of the fishes.

CHAPTER 2

LITERATURE REVIEW

2.1 Freshwater fishes in Malaysia

Freshwater is a finite resource that is important for human, industry and agriculture. Freshwater also sustains the terrestrial and aquatic habitats needed for the growth of animal species and a myriad of indigenous plant (Zati and Juhaimi, 2010). Freshwater fishes in Malaysia are important as fish trading for live hood and they become the main sources of protein for rural population. Malaysia is recognized as hot mega diversity country because of its richness in biodiversity. Within 3500 freshwater fish tantamount to 35-40% of the world freshwater ichthyofauna in Asia, at least 1000 species are present in the freshwater system of Southeast Asian trophic. Moreover, species composition of freshwater fish in Peninsular Malaysia has a lot similarity with Siamese and Indonesian freshwater fishes (Zakaria-Ismail, 1994; Kottelat and Whiten, 1996, Chong *et al.*, 2010; Hashim *et al.*, 2012).

Malaysian region is considered as the southern center for distribution of primary freshwater fish (Mohsin and Ambak, 1983). Freshwater fishes in Malaysia can be divided into two zoogeographic regions, Peninsular Malaysia and West Malaysia (Sabah and Sarawak) (Mohsin and Ambak, 1991; Yap, 2002; Zakaria-Ismail, 1994). Various foreign researchers started the studies on the diversity of freshwater fishes in Malaysia on the mid 19th century. These studies trigger numerous studies by local Malaysian researchers in focus to record the fish biodiversity in Malaysia. The exact number of ichthyofauna in Peninsular Malaysia is still unknown. Cantor (1849) is the first person that recorded and listed the 15 freshwater fishes species from Pulau Langkawi, Pulau Pinang and Melaka in Malaysia (Zakaria-Ismail, 1996).

A study by Kottelat and Whitten (1996) noted that the upper reaches of rivers are the least studied riverine habitats, and likely contain undiscovered endemic species. Several studies on freshwater fish species have been recorded in Malaysia's reservoir such as the study by Shah and Ali (2002) in Ahning Reservoir, Mansor *et al.* (2010) on freshwater fish species in Pedu Reservoir; Kamaruddin *et al.* (2012) in Kenyir Reservoir; Muzzalifah *et al.* (2015) in Bersia and Temengor Reservoir; Mohd-Shafiq (2016) and Mohd-Syaiful *et al.* (2018) in Bukit Merah Reservoir, preliminary study of fishes of Beris Reservoir by Shah *et al.* (2016) in Kedah and fish checklist of Pergau Reservoir in Kelantan (Shah *et al.* 2017). Moreover, there are several studies on freshwater fish distribution in various tributaries of Muda Reservoir had been previously report such as Samat and Mazlan (2003) studied the fish distribution in Weng River, Samat *et al.* (2005) in Lasor River, Shah *et al.* (2012) in Sira Batu River, Surat River, Air Hangat River, Bahui River and Jawa River and Lee *et al.* (2013) in riverine (Muda River, Teliang River and Charok Tera River) and lacustrine environment and the latest was the study conducted by Nor-Aziella (2018) focused on fishes in the tributaries of Muda Reservoir (Labua River, Sira Jawa River, Nyeh River, Debu River and Teliang River).

2.2 The fishes of the genus *Rasbora*

According to Liao *et al.* (2010), there are at least 120 species of fishes in genus *Rasbora* have been discovered in the last 20 years. This genus includes small-to-moderate-sized with a streamlined body and have been adjudged as a group of great economic importance from aesthetic, medical and fishery (Jain and Tilak, 2010). This genus is known as surface feeder - feeding forms with a symphyseal knob in lower jaw that fits into the emargination in the upper jaw and mouth opening directed obliquely upwards. These special characteristics render these fishes most efficient and useful as larvivorous fishes (Jain, 1987). This genus is a freshwater fish that inhabits lowland and food-hill

streams, large rivers, ditches, rice paddy fields, ponds, lakes and swamps and was found throughout South and Southeast Asia (Kottelat and Tan, 2012). Besides, it is one of the most successful and widely distributed genus in some of the countries in Asia such as in Sri Lanka, Bangladesh, Burma, India, Pakistan, Indo-China, Thailand, Phillipine, Borneo, Indonesia and Malay Peninsula (Brittan, 1998).

The increase of numerous new species such as *R. patrickyapi* in Central Kalimantan, Borneo (Tan, 2009); four new species of the *Rasbora* of the Trifasciata group and *Rasbora* of the Sumatrana group from Northwestern Sumatra, Indonesia (Lumbantobing, 2010; 2014) make the exact numbers of this genus known species underestimated. The similarity in size, morphological and high variations in colourization among species in this genus bring a problem to the taxonomic and systematic identification. Brittan (1954a and 1954b) recognized three subgenera in the genus of *Rasbora*, which are *Rasbora*, *Rasboroides*, and *Megarasbora*. Then, this species in this genus were categorized into eight groups referred as species complexes, which are the *lateristriata*, the *sumatrana-elegans*, the *caudimaculata*, the *trifasciata*, the *argyrotaenia*, the *daniconius*, the *einthovenii*, and the *pauciperforata* complexes and are recognized as a ‘catch-all’ polyphyletic grouping because no synapomorphy has been proposed to diagnose it. Many author use the Brittan’s species complexes widely as a practical system for the classification of the group (Kottelat and Vidthayanon, 1993; Kottelat, 2005; Liao *et al.*, 2010). But, Kottelat and Vidthayanon (1993) replaced the category of species complex with ‘species group’. After Brittan (1954a), some authors have created several new genera for example *Amblypharyngodon*, *Boraras*, *Brevibora*, *Horadandia*, *Kottelatia*, *Pectenocypris*, *Rasboroides*, *Rasbosoma*, *Trigonopoma*, and *Trigonostigma* for some lineages within *Rasbora* (Kottelat and Witte, 1996; Liao *et al.*, 2010; Milton and

Jonathan, 2018). Currently, *Rasbora* constitutes the most species-rich genus with total of 97 species (Eschmeyer, 2013; Froese and Pauly, 2013; Kottelat, 2013).

Most of previous study on genus *Rasbora* had been done by researcher from Indonesia such as reproductive biology and food habits study on *R. lateristriata* in Ngrancah river, Kulon Progo regency by Djumanto *et al.* (2008); Djumanto and Setyawan (2009). Prior to work by Muchlisin (2010), he studied about *R. tawarensis* known as Depik in Lake Laut Tawar, Aceh, Indonesia. He reported that *R. tawarensis* is widespread in the lake but most abundant in shallow water and near shore however, the size is small on average for this area. Whereas, the bigger fish are found in deep water of the lake, but with lower abundance. Lumbantobing (2010) studied about four new cyprinid species of the *Rasbora trifasciata*-group, which are *R. api*, *R. nodulosa*, *R. kluetensis*, and *R. truncata*, that are described from northwestern Sumatra, Indonesia. Then again Lumbantobing (2014), found four new species of the minnow genus *Rasbora* of the Sumatrana group, which are *R. arundinata*, *R. haru*, *R. maninjau*, and *R. bindumatoga* from northern Sumatra, Indonesia. The most recent study is by Aprilian *et al.* (2016) and Evron *et al.* (2017). Aprilian *et al.* (2016) study are about morphological variation of Bada fish (*R. maninjau*, Lumbantobing) from Maninjau Lake and Evron *et al.* (2017) studied about the aspects of fisheries biology on *R. lateristriata* from Central Lombok Indonesia.

In Peninsular Malaysia, the fish is known as “ikan seluang” among the Malays. There are scarce detailed study on the biology of this genus been done in Malaysia. However, the presence of this genus from the species of *R. paviana* had been recorded in the study by Martin-Smith (1996) in Sabah; Shukor *et al.* (2008) in different geographical area of Peninsular Malaysia; Mansor *et al.* (2010) in Pedu Reservoir; Mohd-Shafiq *et al.* (2012)

in Kerian River and *R. vulgaris* by Amiruddin and Zakaria (2014) in Temenggong Reservoir and Mohd-Shafiq (2016) in Bukit Merah Reservoir.

Rasbora spp. also known one as 'ikan putih' and sold as mixed fishes. Then, it provided source of income to local fisherman in that area and as source of protein for local people. This genus had been chosen in this study because there are no detailed about biological studies of freshwater species especially *Rasbora* spp. from streams of Muda Reservoir were reported before and two species in this genus which are *R. dusonensis* and *R. vulgaris* are common, very abundant, most dominant and can be found in all sampling sites during sampling period. The domination of this species provided enough and sufficient data needed for biological aspect of fish research in all study sites. Thus, this present study is an effort to study and record a more detailed on the biological aspects of this genus by focusing on two species in this genus, which are *R. dusonensis* and *R. vulgaris* from the streams of Muda Reservoir.

2.2.1 *Rasbora dusonensis* (Bleeker 1850)

Morphologically, *R. dusonensis* (Figure 2.1) is distinguished from congeners by having a broad, dark-brown sharply defined midlateral stripe on body extending from opercula to caudal fin base and separated from the dark-brown supralateral stripe and dorsum by a highly contrasting pale stripe (longitudinal area). *R. dusonensis* have total of dorsal rays between 8-9, anal soft rays are 9–10 and total of vertebrae are 29-30. This species more intense posteriorly and not extends onto the caudal fin. *R. dusonensis* have a bright yellow caudal fin and usually with black posterior margin (carmine red without or with a very narrow black posterior margin) with dorsal-fin origin is closer to the eye rather than to the caudal-fin base. Besides, they have more slender caudal peduncle and 3 scale rows

between the lateral line and the midventral row (Rainboth, 1996; Kottelat, 1998; Ng and Kottelat, 2013).

In Asia, this species distributed mainly in Mekong and Chao Phraya basins, Malay Peninsula (from the Bernam River drainage eastwards and southwards to the Endau River drainage), Borneo (from the Baram River drainage southwards to the Barito River drainage) and Sumatra (from the Indragiri River drainage southwards to the Musi River drainage). It is present in a variety of habitats, but seems to prefer large rivers with slow current and murky water and commonly found along shores in large rivers (Kottelat, 1998; Kottelat and Widjanarti, 2005; Ng and Kottelat, 2013). Moreover, this species occurs at or near the surface in clear waters of rivers and streams with depth of about 5 m. Mainly, this species feeds on exogenous insects, some small crustaceans and algae (Rainboth, 1996).

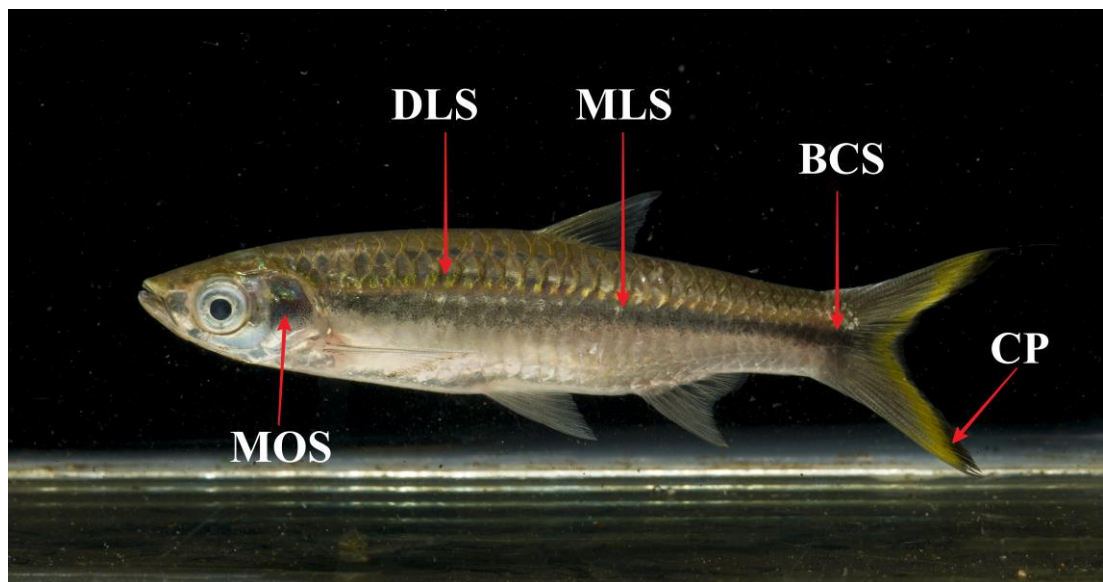


Figure 2.1: Photographs of the *R.dusonensis* (Photo credited to Muhammad Rasul Abdullah Halim). BCS = Basicaudal Spot; CP = Caudal Pigmentation; DLS = Dorsolateral stripe; MLS = Midlateral Stripe; MOS = Midopercular stripe.

2.2.2 *Rasbora vulgaris* Duncker 1904

Morphologically, *R. vulgaris* (Figure 2.2) is easily recognized by a narrow, black midlateral stripe along the sides starting at the gill opening and ending shortly in front of the caudal peduncle. It is widening into a diamond-shaped blotch under the dorsal fin than anteriorly and posteriorly. The midlateral stripe of *R. vulgaris* runs below and usually separated from the mid-axial streak (Kottelat and Widjanarti, 2005). Sometimes, it is recognized by the present of black tips on caudal lobes (Rainboth, 1996). Previously, *R. vulgaris* in Peninsular Malaysia has been treated as synonym of *Rasbora sumatrana* or *Rasbora paviana* (Brittan, 1954c) then, Lim and Tan (2002) on their study has treated this species as a valid species.

In Asia, this species widely distributed in the Peninsular Malaysia, from southernmost Thailand and west Peninsular Malaysia (Liao *et al.*, 2010). Inhabits lowland to mountain streams with muddy-sand to sandy-gravel bottoms, mostly forest covered. It is adaptive to still water bodies. Dominantly presence during dry season and exhibit small peak in beginning of rainy season (Baran *et al.*, 2005). There is very small fishery interest in this species and occasionally it is imported for aquarium trade but not much seen in the markets. The habitat degradation is a potential threat for this species (IUCN, 2017).

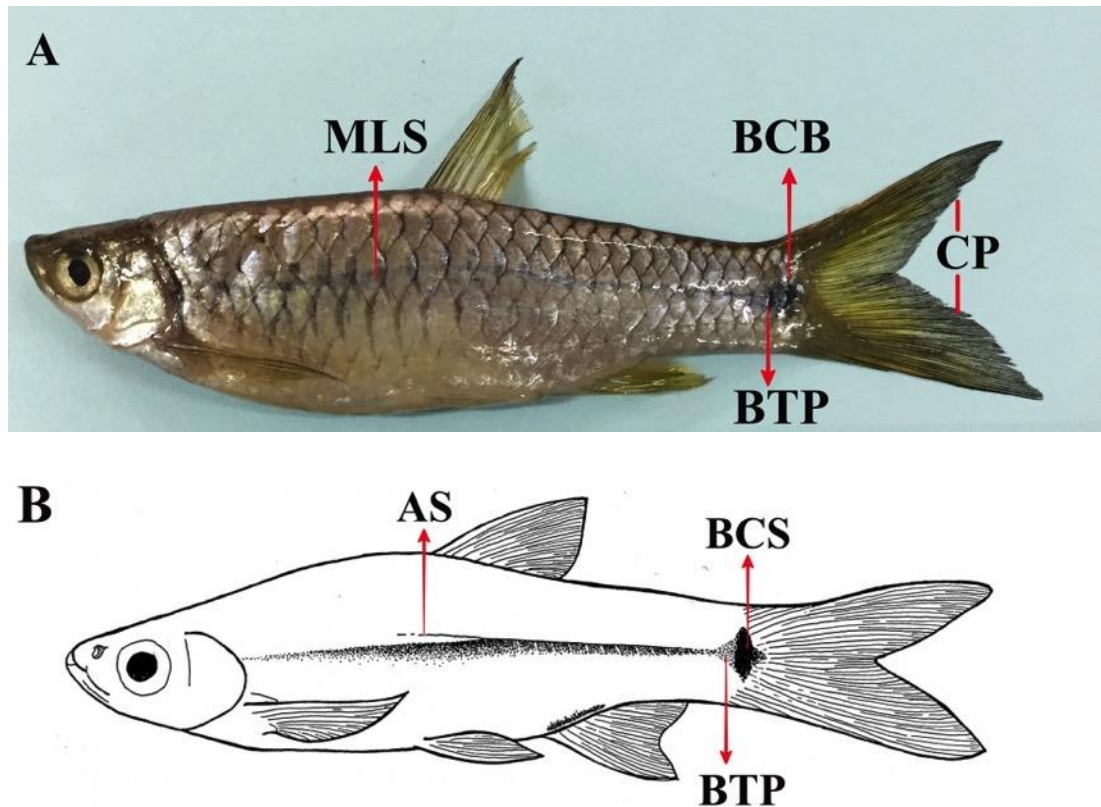


Figure 2.2: Lateral view body of the *R.vulgaris*: (A) Photographs of *R.vulgaris* (B) Schematic drawing of lateral body pigmentation (Source: Lumbantobing, 2014). AS = Axial Streak; BCB = Basicaudal Blotch; BCS = Basicaudal Spot; BTP = Basicaudal Triangular Patch; CP = Caudal Pigmentation; MLS = Midlateral Stripe.

2.3 Physico-chemical water quality

Water quality is a fundamental aspect in determining the healthiness of aquatic ecosystem. Healthy aquatic ecosystem and good water quality are vital in order to sustain fish species and other aquatic organisms (Arimoro *et al.*, 2008). The deterioration on water quality may cause higher mortality rates of fishes, changes the fish assemblage of certain area and changes the physical behavior of fishes (Borja *et al.*, 2012). In Malaysia, the water quality assessment in lakes and rivers commonly use the Interim National Water Quality Standard for Malaysia (INWQS) and water quality index (WQI) as suggested by the Department of Environment (DOE). The classification is based on annual basis by categorizing rivers in Class I, II, III, IV and V based on the INWQS. The

classification guidelines of the INWQS are show in Appendix 4.2. According to Yisa and Jimoh (2010), the INWQS is applicable for both rivers and lakes environment, its standardisation measurement is important and most helpful in assessing and monitoring surface water. Therefore, in this study, only INWQS was used to estimate the water quality status in the streams of Muda Reservoir. Previous studies of water quality status in various streams of Muda Reservoir were conducted by Mohd-Kamil *et al.* (2005) and Lee *et al.* (2013). Latest research on water quality in tributaries of Muda Reservoir was conducted by Nor-Aziella (2018). This water quality status regarding the physico-chemical parameters is expected to be changing over time in the streams of Muda Reservoir. This study was to access if there are differences in the physico-chemical parameters found from the streams of Muda Reservoir with the previous studies conducted there.

2.4 Length-weight relationship (LWR)

Frequently, length-weight relationship has been used extensively for analysis of fisheries data worldwide (Mendes *et al.*, 2004). Samat *et al.* (2008) stated that the LWR data are being used as a standard to predict fish growth parameter as well as mortality rate in their fish-sampling program. Besides, it is being used to estimate coefficient of condition factor (Schneider *et al.*, 2000). According to (Lawson, 2011), the study of LWR is also important for the conservation and management of fishes in aquatic system including freshwater system. According to Mohamad-Radhi *et al.* (2018), Cyprinidae family constituted 46% of the fish species family that are most often studied for length-weight relationship in Malaysia. Geographically about 92% of the length-weight relationship studies have been conducted in West Malaysia compared to East Malaysia, where only eight (8%) studies have been reported. While, Northern states (Perak and Kedah) have contributed to 35% of the studies. Moreover, according to Mohamad-Radhi the mean of

b value for 64 length-weight relationship studies of 49 freshwater fishes in Malaysia was 3.0139, which indicated that the growth is still in the normal range ($2.5 < b < 3.5$) suggested by Froese (2006) for freshwater fish.

Previously, there are several length-weight relationship of freshwater fishes studies conducted in Peninsular Malaysia's Reservoir such as Mansor *et al.* (2010) scrutinize length-weight relationship for 12 freshwater fish species in Kerian River basin and Pedu Reservoir, Kedah; Kamaruddin *et al.* (2012) study the relative growth coefficient (b) values for *Barbonymus schwanefeldii*, *Notopterus* sp. and *Hampala macrolepidota* from the Kenyir Reservoir, Terengganu; whereas, Muzzalifah *et al.* (2015) reported length-weight relationship of seven major species *Pristolepis fasciata*, *Cyclocheilichthys apogon*, *Hampala macrolepidota*, *Labiobarbus leptocheilus*, *Mystacoleucus obtusirostris*, *Osteochilus vittatus* and *Oxygaster anomalura* in Temengor Reservoir. However, to date, there has been no evaluation on these important fish parameters featuring the streams of Muda Reservoir and there is very lacking of length-weight relationships studies have been reported in streams of Muda Reservoir except the length-weight relationship study of *C. apogon* reported by Nor-Aziella (2018).

2.5 Relative condition factor (Kn)

According to Le Cren (1951), the condition of a fish reflects biological circumstances, physical and fluctuates by interaction among feeding conditions, parasitic infections and physiological factors. The first condition measure is a Fulton's condition factor (Bolger and Connolly, 1989) then, the condition measurement has evolved with several new approaches to measure the condition such as relative condition factor (Kn), relative weight (Wr), length-weight regression and residual analysis. There are several condition factor of freshwater fishes studies conducted in Peninsular Malaysia such as Mohd Shafiq *et al.* (2012) establish condition factors for six freshwater species in Kerian River,

Malaysia and Mansor *et al.* (2010) scrutinize relative condition factor (Kn) for 12 freshwater fish species in Kerian River basin and Pedu Reservoir, Kedah; whereas, Muzzalifah *et al.* (2015) reported condition factor of seven major freshwater species in Temengor Reservoir. However, the studies on the condition factor of freshwater fish from the streams of Muda Reservoir were limited. Previously, Nor-Aziella (2018) reported the study on relative condition factor of *C. apogon* in tributaries of Muda Reservoir. She reported the relative condition factor values (Kn) in the tributaries of Muda Reservoir did provided a favourable environment and suitable habitat that trigger the growth of *C. apogon*. Hence, this study was performed to evaluate the relative condition factor (Kn) of freshwater fishes from the streams of Muda Reservoir by focused on *R. dusonensis* and *R. vulgaris* as sample research.

2.6 Population parameters

Growth studies contribute in estimation of production, stock size, recruitment and mortality of fish populations that becoming an essential instrument in the management of fisheries resources (Jobling, 2002). As such, information about age and growth is extremely important in almost every aspect of fisheries (Giuseppe, 2007). The estimation of population parameters of freshwater fishes in Peninsular Malaysia's reservoir is still lacking. There were only two reported studies on population parameters in lacustrine environment such as the population parameters study of *Barbonymus schwanefeldii* in Pedu Reservoir, Kedah (Mansor *et al.*, 2012) and six selected species of *C. apogon*, *O. anomalura*, *B. gonionotus*, *B. schwanefeldii* and *O. vittatus* from family Cyprinidae and *Notopterus notopterus* from family Notopteridae by Mohd-Shafiq (2016) in Bukit Merah Reservoir. While, most recent study on population parameters was conducted by Nor-Aziella (2018) on *C. apogon* in tributaries of Muda Reservoir (riverine environment). All previous study mention above had estimated the growth parameters of L_{∞} (length

infinity), K (growth rate), growth performance index (ϕ'), mortality coefficient namely total mortality (Z), natural mortality (M) and fishing mortality (F), length at first capture (L_c), yield per recruit and biomass per recruit and annual recruitment pattern of fishes because these population parameters are important to describe the growth of species and also are inputs in several fishery production models (Sparre and Venema, 1992; Hilborn and Walters, 1992). Normally, the estimation of growth parameters is based on the length-frequency data. This data analyzed using special program package for length-based stock assessment called FiSAT II (FAO-ICLARM Stock Assessment Tools - Version 1.2.2).

2.7 Stomach content analysis of fish

Analysis of stomach content is a fundamental practice in animal ecology and fisheries research and this method is a widely used to ascertain the food and feeding habits of fish species (Sivadas and Bhaskaran, 2009). The study of feeding ecology is an essential tool to understand fish roles within their ecosystems (Hajisamaea *et al.*, 2003). Moreover, the pattern of interspecific competition of fishes can be assessed through the food and feeding habits studies (Shillewar *et al.*, 2009). Information of feeding habit of fish in their natural habitat is important to develop feeding practices and breeding technologies to support the aquaculture industry and domestication process (Muchlisin *et al.*, 2015).

Many researchers around the world have studied about food and feeding habit of various different fishes and the feeding biology of several tropical freshwater fishes have been well documented in different geographical habitat in Malaysia such as *N. soroides* in Gombak River (Khaironizam, 2010); *B. schwanefeldii*, *Notopterus* spp., *H. macrolepidota*, *H. nemurus*, *C. micropeltes* and *P. fasciatus* in Pulau Gawi, Kenyir Reservoir by Mustafa-Kamal *et al.* (2012); *C. apogon* in Temengor Reservoir

(Muzzalifah *et al.*, 2013); six selected species, *C. apogon*, *O. anomalura*, *B. gonionotus*, *B. schwanefeldii*, *O. vittatus* and *N. notopterus* by Mohd-Shafiq (2016) in Bukit Merah Reservoir; *Devario regina* in Kerian River (Mohd-Shafiq *et al.*, 2017) and recent study by Nor-Aziella (2018) on *C. apogon* in tributaries of Muda Reservoir. However the diet of *Rasbora* spp. has never been investigated in Malaysia. In other countries such as Indonesia, the food habit of *Rasbora* spp. had been thoroughly studied such as feeding habits study on *R. lateristriata* in Ngrancah River, Kulon Progo regency (Djumanto and Setyawan, 2009) and *R. tawarensis* by Muchlisin (2010). Moreover, in India, Weliange and Amarasinghe (2007); Gaikwad *et al.* (2009) studied on the food and feeding habit of *R. daniconius*. However, there is no published data available on the food and feeding habits of *R. dusonensis* and *R. vulgaris* in Malaysia. So, the frequency of occurrence and numerical method were chosen in this study in order to analyze the oesophagiogastric gut content of this two species from streams of Muda Reservoir.

2.8 Reproductive biology

Studies of teleost reproduction often favor valuable or commercial native species (Smith and Walker, 2004). Besides, knowledge of the reproductive cycle and the factors affecting it becomes important issues in fish and fisheries biology (Tomkiewicz *et al.*, 2003). Although Peninsular Malaysia has considerable numbers of native fishes, there is limited information available about their reproductive biology (Chong *et al.*, 2010). There are several studies on the reproductive biology of freshwater fishes was conducted in Peninsular Malaysia such as previous study conducted by McAdam (1994) on aspects of reproductive biology of *Barbonymus schwanefeldii* in Perak River; Khaironizam and Zakaria (2013) on spawning period and fecundity of *N. soroides* in Gombak River and reproductive biology of the introduced sailfin cat fish (*Pterygoplichthys pardalis*) in Langat River by Samat *et al.* (2016).

Then, there are several studies on reproductive biology of freshwater fishes conducted in Peninsular Malaysia's reservoir such as reproductive biology of a tropical catfish (*Hemibagrus capitulum*) in Chenderoh Reservoir by Khan *et al.* (1990); research on reproductive biology and breeding cycle of tropical cyprinid (*Thynnichthys thynnoides*) from Chenderoh Reservoir by Ahyudin and Kamaruzaman (1996) and recently, research on reproductive biology studies by focusing on sex ratio, gonad maturity stages, length at first maturity (L_{50}), spawning season, fecundity and egg diameter of *C. apagon* in tributaries of Muda Reservoir by Nor-Aziella (2018).

However, the reproductive biology of *Rasbora* spp. has never been investigated and evaluated in Malaysia. Therefore, *R. dusonensis* are chosen to do the reproductive biology because it was dominance species found from streams of Muda Reservoir and at present, no assessment of the status of the *R. dusonensis* population and its reproductive biology has been made in Malaysia. The data presented here is restricted to the availability of the fish species during the course of the study period, the present work constitutes vital information on the aspects of the fish reproductive biology, particularly the sex ratio, gonad maturity stages, length at first maturity (L_{50}), spawning season, fecundity and egg diameter. Owing to the fact that little is known about these aspects, the data gathered in this study provide benchmark information for the management, propagation and conservation of *R. dusonensis* from streams of Muda Reservoir.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Study area and sampling stations

Muda Reservoir is located in the Ulu Muda Forest Complex, district of Sik, Kedah Malaysia. It lies at the latitude of: 6°14' 18.71" N and longitude: 100° 45' 59.71" E. Muda Reservoir construction started in 1966 and impoundment started in 1969. This impoundment created new lentic habitats and served as important role in the provision of freshwater fishes in northern west-coast state of Kedah, Penang and Perlis (Suksuwan, 2008). It developed important and active inland fisheries. This reservoir fishery is important economic activities for the rural communities (Lee *et al.*, 2013). In addition, they are used for fishing (small scale fishing and sport fishing) and aquaculture (Lee, 2009). It is known as a popular site for recreational anglers from within the state, as well as from Penang (Suksuwan, 2008). Beside that, they are built as important water resources for agriculture to irrigate the vast and important paddy fields of the states of Kedah and Perlis and are also crucial for domestic and industrial water supply for three states, which are Perlis, Penang and Kedah; including the tourism island of Langkawi.

Generally, Muda Reservoir can be divided to three main sub-catchment areas; Muda River that flows from south to northwest, Teliang River flowing from northeast to southwest; and Weng River that flows from north to southeast (Lee *et al.*, 2013). This study was conducted in these two main sub-catchments; Muda River (Labua River - ST 1, Sira Jawa River - ST 2, Nyeh River - ST 3 and Debu River - ST 4) and Teliang River - ST 5. These sampling stations were in riverine or moving water environment (lotic). Riverine site always become main production of fisheries that provided higher number

of native species. Map of the sampling stations are illustrated in Figure 3.1. All the descriptions and locations were described in Table 3.1.

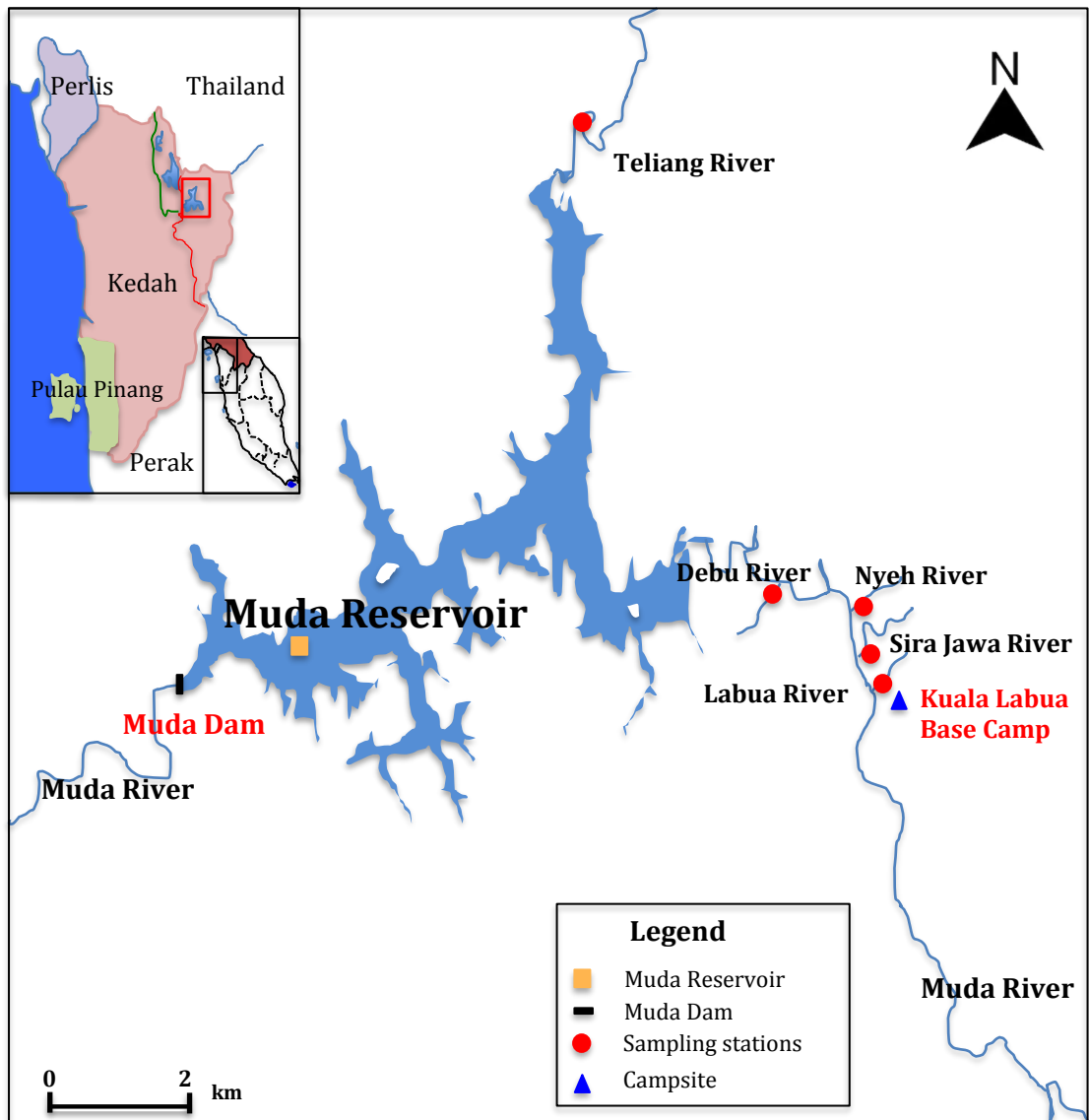


Figure 3.1: Location of sampling stations at five chosen streams of Muda Reservoir (ST 1: Labua River, ST 2: Sira Jawa River, ST 3: Nyeh River, ST 4: Debu River and ST 5: Teliang River).