DIVERSITY OF FRESHWATER FISHES IN ACEH PROVINCE WITH EMPHASIS ON SEVERAL BIOLOGICAL ASPECTS OF THE DEPIK (Rasbora tawarensis) AN ENDEMIC SPECIES IN LAKE LAUT TAWAR

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by

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KEPELBAGAIAN IKAN AIR TAWAR DI PROVINSI ACEH DENGAN PENEKANAN PADA BEBERAPA ASPEK BIOLOGI IKAN DEPIK (*Rasbora tawarensis*) SPESIES ENDEMIK DI TASIK LAUT TAWAR

ABSTRAK

Kajian intensif keatas kepelbagian spesies ikan air tawar telah dijalankan selama 6 bulan pada 17 lokasi yang merangkumi lima kawasan di Aceh bertujuan untuk membangunkan data asas komprehensif kepelbagian ikan air tawar di kawasan ini. Sejumlah 711 ikan yang terdiri daripada 114 spesies, 69 genera, 41 famili dan 12 order telah direkodkan dalam kajian ini. Cyprinidae didapati golongan ikan air tawar yang dominan di Provinsi Aceh. Sungai Lembang memiliki indeks kekayaan dan kepelbagian spesies yang paling tinggi di dalam Provinsi ini.

Berdasarkan kawasan pula, indeks kepelbagian spesies lebih tinggi di kawasan utara. Namun begitu kekayaan spesies yang paling tinggi terdapat di kawasan barat Aceh. Enam species dikenalpasti memiliki taburan yang luas ialah; *Puntius brevis, Channa striata, Anguilla bicolor, Anabas testudineus, Clarias teijsmani,* dan *Hampala macrolepidota.* Penilaian terhadap status pemuliharaan menunjukkan bahawa 10 spesies tergolong sebagai ikan yang terancam dan daripada jumlah ini, dua adalah endemik (*Poropuntius tawarensis* and *Rasbora tawarensis*), sementara 9 spesies ikan pendatang. Berdasarkan hasil kajian penentuan keutamaan, spesies *R. tawarensis* khasnya, dan genus *Rasbora* amnya telah dipilih sebagai spesies/genus sasaran dan menjadi tumpuan dalam penyelidikan lanjutan.

Berdasarkan pendekatan morfometrik (morfometrik tradisional, morfometrik jaringan truss dan data meristik) yang disokong oleh kajian genetik (berdasarkan gen barkod COI) adalah disimpulkan hanya terdapat dua spesies *Rasbora*, Eas dan Depik merupakan spesies yang sama, iaitu *R. tawarensis*, manakala Relo berkemungkinan spesies kriptik di dalam tasik Laut Tawar. Gen COI juga secara amnya mengesahkan status taksonomi 14 spesies ikan di tasik ini, bertepatan dengan penkelasan morfologi. Sejumlah 31 jujukan haplotip telah dihasilkan dimana *Puntius brevis* dan *Poropuntius tawarensis* memiliki hubungan yang sangat rapat.

Kajian panjang-berat dan faktor kondisinya ke atas 1,159 ekor ikan *R. tawarensis* dan 626 ekor ikan kawan *P. tawarensis* telah menunjukkan bahawa secara am saiz (panjang dan berat) *P. tawarensis* adalah lebih besar dengan signifikan berbanding *R. tawarensis*. Namun begitu kedua-dua spesies menunjukkan pola pertumbuhan alometrik negatif. Faktor kondisi kedua spesies ialah pada tingkat sederhana.

Hasil tangkapan ikan Depik (CPUE) ditunjukkan menurun selama beberapa tahun kebelakangan ini; daripada 1,165.72 g/m^2 jaring pada 1970an kepada 14.99 g/m^2 sahaja pada 2009. Hasil tangkapan harian nelayan menunjukkan bahAwa hasil tangkapan ikan *R. tawarensis* lebih tinggi pada awal bulan mengikut sistem kalender Islam.

Pola taburan pula menunjukkan bahawa ikan ini tersebar secara meluas di dalam tasik ini. Secara amnya kelimpahan ikan akan berkurangan seiring dengan peningkatan jarak dari pantai, namun saiz ikan akan meningkat dengan jarak dari pantai. Tiada perbezaan pada saiz ikan yang ditangkap pada musim kemarau ataupun musim hujan, akan tetapi kelimpahan ikan lebih tinggi dijumpai pada musim hujan. Dengan itu, saiz *R. tawarensis* tidak bergantung pada musim akan tetapi lebih bergantung kepada tempat. Namun demikian, kelimpahan ikan bergantung kepada kedua-duanya.

Sejumlah 901 ekor ikan betina dan 341 ekor ikan jantan *R. tawarensis* telah digunakan untuk kajian biologi pembiakan. Penyampelan ikan dilakukan setiap bulan bermula April 2008 sehingga Mac 2009. Berdasarkan pengamatan secara makroskopik dan mikroskopik, perkembangan gonad ikan Depik telah dibahagikan kepada 5 peringkat, iaitu tidak matang, sedang matang, matang, sangat matang dan salin. Secara amnya kadar kematangan tertinggi pada bulan September.

Nilai indek somatik gonad (GSI) bulanan ikan betina bervariasi daripada 6.65 di bulan April hingga 18.16 di bulan September dan 4.94 pada bulan Julai hingga 8.56 di bulan Mac bagi jantan. GSI betina tinggi pada bulan Sebtember, Mac dan Disember sementara jantan pada bulan Mac, Mei, September dan Disember. Ini menunjukkan bahwa aktiviti pembiakan kedua-dua jantina tinggi pada bulan Mac, September dan Disember. Nisbah jantina menunjukkan ikan betina lebih banyak berbanding ikan jantan. Secara purata, saiz ikan betina lebih besar secara signifikan berbanding daripada jantan. Namun, ikan betina matang pada saiz yang lebih besar daripada jantan.

Kesuburan mutlak bulanan berjulat daripada 2,744 biji telur sehingga 4,327 biji telur dan kesuburan relatifnya ialah daripada 334 sehingga 631 telur/g berat badan. Kekerapan pembiakan (spawning frequency) bulanan dianggarkan selang setiap 2 hingga 11 hari. Sejumlah 13 kawasan pembiakan (spawning ground) *R. tawarensis* telah dikenalpasti di Danau Laut Tawar, namun hanya empat sahaja yang aktif semasa musim kemarau. Oleh itu projek ini telah menyumbang data penting untuk pemuliharaan spesies ikan air tawar di Provinsi Aceh secara am, dengan masing-masing *R. tawarensis* dan Danau Laut Tawar sebagai organisma dan habitat model.

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ABSTRACT

Intensive survey of fish biodiversity was conducted for six months in 17 locations covering the five regions of Aceh Province to build a comprehensive database in this area. A total of 711 fishes belonging to 114 species,o 69 genera, 41 families and 12 orders were recorded in this study. Cyprinidae was the predominant freshwater fish group in the Aceh Province. The Lembang River had the highest species richness and diversity index in the Province. Regionally, the diversity index was highest in the northern region. However, the highest species richness was found in the western region. Six species i.e. *Puntius brevis*, *Channa striata*, *Anguilla bicolor*, *Anabas testudineus*, *Clarias teijsmani*, and *Hampala macrolepidota* were the most widely distributed. The evaluation of conservation status showed that 10 species were threatened and of these, two species (*Poropuntius tawarensis* and *Rasbora tawarensis*) were endemic while 9 species were introduced. Based on priority evaluation, this study was focused in Lake Laut Tawar and *R. tawarensis* as a target species in particular and genus *Rasbora* in general.

Based on examination of the morphometric approaches (traditional morphometric, truss network morphometric and meristic data) and supported by genetic (based on the barcoding COI gene) data, it is concluded that there were only two valid species of *Rasbora* were present in the lake, where Eas and Depik

were shown to be the same species, *Rasbora tawarensis* and the other, Relo was probably a cryptic species (*Rasbora sp*.). The COI gene also generally validated the taxonomic status of the 14 species in the lake in agreement with morphological classification. A total of 31 haplotypes sequences were produced where *Puntius brevis* and *Poropuntius tawarensis* were found to be very closely related.

A length-weight relationships and condition factors study on 1,159 *R. tawarensis* and 626 *P. tawarensis* revealed that in general, the size (length and weight) of *P. tawarensis* was significantly higher than *R. tawarensis*. However, both species showed an allometric negative growth pattern. The condition factor values of both species were moderate.

In general the catch per unit efforts (CPUE) of *R. tawarensis* was found to have decreased over the years; from 1,165.72 g/m^2 of net in 1970s to only 14.99 g/m^2 in 2009. Based on fishermen daily production data the CPUE was found to be higher in the first week (new month) of Islamic calendar. The distribution pattern showed that this species was widespread in the lake. In general, fish abundance decreased with increasing distance from the shore; however, fish size increased with the distance from the shore. There was no significant difference in fish size caught in the dry and the wet seasons, but fish abundance was highest in the wet season. Therefore, size of *R. tawarensis* was not seasonally but more spatially dependent. However, fish abundance was both seasonally and spatially dependent.

A total of 901 females and 341 males of *R. tawarensis* were used for biological reproductive studies. Sampling was conducted monthly from April 2008 to March 2009 in Lake Laut Tawar. Based on macroscopic and microscopic evaluation, the gonadal development stages of *R. tawarensis* were divided into five classes i.e. immature, develop, mature, ripe and spent. In general higher proportions of mature

fish were present in September. The monthly GSIs of female *R. tawarensis* varied from 6.65 in April to 18.16 in September and 4.94 in July to 8.56 in March for male. Female GSI was high in September, March and December, while male GSI was high in March, May, September and December. This indicated that the reproductive activity of *R. tawarensis* was highest in March, September and December.

The sex ratio showed that females were dominant and bigger. In general, females were significantly larger than males. The female attained first maturity at a larger size than males. The monthly fecundity ranged from 2,744 to 4,327 eggs for absolute and 334 to 631 eggs/g body weight for relative fecundities. Monthly spawning frequencies were estimated to be every 2 to 11 days. A total of 13 spawning grounds of *R. tawarensis* were detected in Lake Laut Tawar. However, only four grounds remained active in the dry season. Thus this project has contributed very critical data for the conservation of freshwater species in the Aceh Province in general, with the *R. tawarensis* and Lake Laut Tawar as the model organism and habitat, respectively.

CHAPTER 1

GENERAL INTRODUCTION

1.1 General Description of Aceh Province

1.1.1 Geography and administration

The Aceh Province is situated in western Indonesia at the northern tip of Sumatera Island (Figure 1.1) with an area of 57,366 km^2, equivalent to 12.3% of the island. The population was estimated at 4.16 million in 2008 (Bappeda, 2008). In 1966 it was recognised as an independent province from the Northern Sumatera Province, with autonomy in religious, education and cultural legislations and in 2006 it was given special autonomy by the Central Government of Republic of Indonesia.

The province is subdivided into 18 districts or *kabupaten* and five cities or *kota* (Appendix A1). The capital and the largest city is Banda Aceh (*Koeta Radja*), located on the coast near the northern tip of Sumatra. Geographically, Aceh Province is bordered by the Malacca Straits in the east, the Indonesian Ocean in the west, and the Bengal Straits and Andaman Sea in the north and the Northern Sumatera Province in the south.

The province has an entire coastline of 2.310 km and the province controls 295.370 km^2 of territorial waters including an economic exclusive zone of 238.807 km^2 with at least 119 isles (Bappeda, 2008). Besides the territorial seas, Aceh Province has vast inland water resources i.e. 73 major rivers, marshes and at least two lakes. The important lakes are Lake Laut Tawar and Lake Aneak Laot situated in Aceh Tengah district and Weh Island respectively.

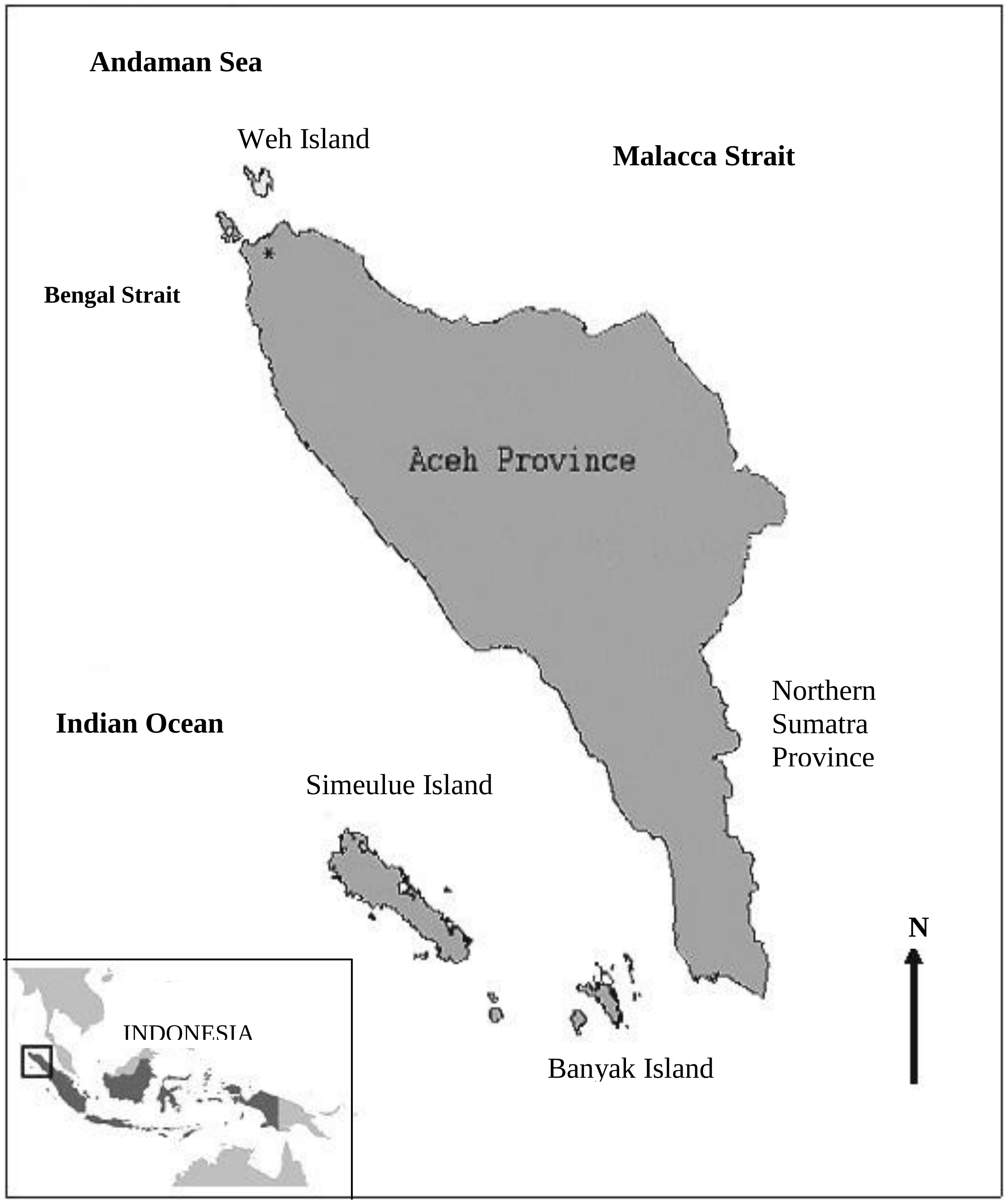

Figure 1.1. The map of the Aceh Province, Indonesia and borders

1.1.2 Socio-economic and cultural

The economy of the people of Aceh is extremely dependent on small scale agriculture, plantations and fishing with low investment and technology. Agriculture, including fisheries is the main source of income for approximately more than 50% of the population.

The livelihood of the people in the agricultural and fishery sectors was hardest hit with high losses of income in the 2004 tsunami catastrophe; this sector made up more than one third of the total losses. However, in the ensuing few years the economy of Aceh grew by 7.7% in 2006 (World Bank, 2008). This growth was primarily driven by the reconstruction efforts, with high growth in the construction sector. After the massive tsunami in 2004 and the ending of the long drawn political conflict in 2005, the reconstruction programs changed the economic structure significantly. The service sector took a more dominant role, whilst oil and gas production decreased. The poverty level increased slightly in 2005 after the tsunami (World Bank, 2008) (Appendix A2). However, the poverty level then fell in 2006 to below the pre-tsunami level, suggesting that the rise in tsunami-related poverty was short lived. The rebuilding activities and the end of the conflict most probably facilitated this decline.

The Aceh Province is home to at least seven indigenous ethnic groups each with different languages or dialects. The major ethnic groups are the Acehnese, distributed throughout Aceh; Gayonese, in the central region (Aceh Tengah, Gayo Lues and Bener Meriah); Alasnese, most living in the south-eastern region (Aceh Tenggara and Aceh Singkil); Tamiangnese in the eastern region (Aceh Tamiang district); Jammee, the descendants from Minang Kabau (West Sumatera) in southern and south-western region of Aceh; Kluet in South Aceh, and Simeulue in

Simeulue Island. In addition, there are migrant Chinese who play a vital role in business and trade. Besides that some Javanese and Batak ethnics form minority groups.

1.2 General Description of Aceh Tengah District and Lake Laut Tawar

This doctoral study gave special focus on the Depik, *Rasbora tawarensis* in the Lake Laut Tawar, Aceh Tengah district as an aquatic habitat model for the biodiversity and conservation aspect of the Aceh Province in general. The district is located at 4°10”- 4°58”N and 96°18” - 96°22”E with a total area of 4,138.39 km^2, a total population of 170,766 and population density estimated at approximately 39 per km^2. The district is divided into 14 sub districts and 271 villages and is bordered by the Bener Meriah district in the North, Gayo Lues district in the South, Aceh Timur district in the East, and Nagan Raya and Pidie districts in the West (Figure 1.2). Detailed description of the map and borders are provided in Appendix B. The primary livelihoods of the residents are agriculture and horticulture and the primary commodities of the district are coffee, paddy, cacao, sugarcane, and pecans (Bappeda, 2009).

The Lake Laut Tawar is the biggest lake in the Aceh Province, Indonesia. This lake is situated in the Gayonese Highland, approximately 1200 meters above sea level. The lake is an old volcanic caldera of 16 km in length, 5 km in width with an average depth of 35 meters and a maximum predicted depth of 80 to 115 meters. It is surrounded by mountains reaching over 2000 meters. Several short tributaries discharge into Lake Tawar, the main outflow being the Peusangan River. The watershed is covered by forests, which are increasingly affected by deforestation and agricultural land conversion.

The lake plays an important role in the livelihood of the local people by providing water resources for domestic needs, agricultural and aquaculture activities as well as industries. In relation to fishery, both capture fisheries and aquaculture are practised in Lake Laut Tawar.

Figure 1.2. The map of Aceh Province showing Aceh Tengah district and Lake Laut Tawar

1.3 Current Fisheries Activities in Lake Laut Tawar

1.3.1 Capture fisheries

Three sub-districts directly border Lake Laut Tawar and at least 27 fishing villages are present along the coastline of the lake. Currently, capture fisheries is the main activity in the Lake. The total fishermen number has decreased up to 62.2% during the last five years, for example the total number was 596 in 2004, but had decreased to 225 in 2008.

The Depik, *R. tawarensis* is the main target capture fish with approximately 46% of the fishermen focusing on this fish**.** However, recent statistics have shown that the overall fish production of Lake Laut Tawar has decreased up to 83.5% during the last two decades (Table 1.1). A parallel trend also occurs for the Depik, revealing decreases of catch-per-unit effort (CPUE) over the years. As a consequence, the fishermen have changed their fishing strategies from seasonal fishing with traditional gears for example *penyangkulan, bubu* and *dedeseun* to more efficient but destructive gears for example selective small mesh size gillnet and operating during all seasons (rainy and dry seasons).

A total of 48 fish landing sites at 18 locations are found in the lake, most are situated in the Bintang sub-district. In general, most of the fishermen are artisanal fishers with low technology practices and low average daily income of Rp51,000 or US$5 (personal communication, fishermen).

Table 1.1. Annual fishery production during the last two decades

Years	Total production (tons)
1988	455*
1994	223**
2006	79.1***
2007	9.7***
2008	74.5***

Sources:
*Fisheries Department of Aceh Province (1989). **Estimation annual production by Kartamihardja *et al*., (1995). ***Bappeda, (2009).

1.3.2 Aquaculture

Although, the fish landings from the Lake Laut Tawar have drastically reduced, market demand has increased over the years as human population in the region and awareness on the nutritional advantages of fish protein increased. Therefore, as fishermen seek for alternatives to meet the market demand, aquaculture has been identified as the most obvious solution to these problems. Presently, at least 150 fishermen are utilising the lake for aquaculture activities.

Aquaculture is a new fishery activity in the lake, beginning only after 2000. However, this activity is increasing rapidly. For example, the total cage floating nets in the lake increased from 16 cages in 2006 to 143 cages in 2008.

Unfortunately, most of the cultured fishes are introduced species. Furthermore, not much attention is given to educate the fishermen on sustainable aquaculture by the relevant fisheries agencies. Intensive aquaculture activities by irresponsible or ignorant fishermen may negatively affect the lake water quality with adverse consequences on the fish community. The introduction of exotic species to the lake whether incidentally or purposely via aquaculture activities will also pose a serious threat to native species.

1.3.3 Other utilization of the lake

In addition to fisheries activity, the lake is also utilized for domestic water supply by a government agency (PDAM Tirta Tawar). The water intake is estimated at 100 liter/second. Furthermore, the Aceh Provincial Government has established a master plan to develop a dam to produce power electricity for Aceh Province. Presently, electricity supply for the district comes from the North Sumatra Province but the capacity is lower than the demand.

The dam is also used for agricultural water supply. From a conservation perspective, these activities pose serious threats and have negative impact to the lake especially to fish communities and eventually to the human community. Other threats to the region include logging activities, forest fires, development of a ring road and urbanization activities around the lake.

1.4 Problem Statement

Currently, the fisheries resources in Aceh Province has neither been fully utilised nor being well managed. According to Razali (2007), more than 60% of the Aceh fisheries resources has not been optimally exploited. For example in 2002, fish production was 60,894 tons (25.7% from aquaculture), but decreased to 47,658 tons (31.4%) in 2005 (Fisheries and Marine Department of Aceh Province, 2006**)** (Figure 1.3). Possible reasons for this observation in addition to the unsustainable fisheries practices could be due to the security condition of the province as a result of the political turmoil during the last three decades until the year 2005 as well as the technological and aquaculture facility limitations. This situation hindered economic and social development in general and resulted in higher poverty levels. According to World Bank (2008) of the 975,374 families or 4.16 million people in Aceh, at least 510,633 (52.35%) families lived below the poverty line.

A monumental event occurred on August 15, 2005 when the Government of Indonesia and Aceh Independent Movement (GAM) signed the memorandum of understanding for peace. This agreement gave special autonomy to Aceh, which permitted the province to manage its own natural resources including fishery resources optimally for the prosperity of the local people. This has brought a new era and a turning point for future development of the Aceh Province especially in the fisheries sector.

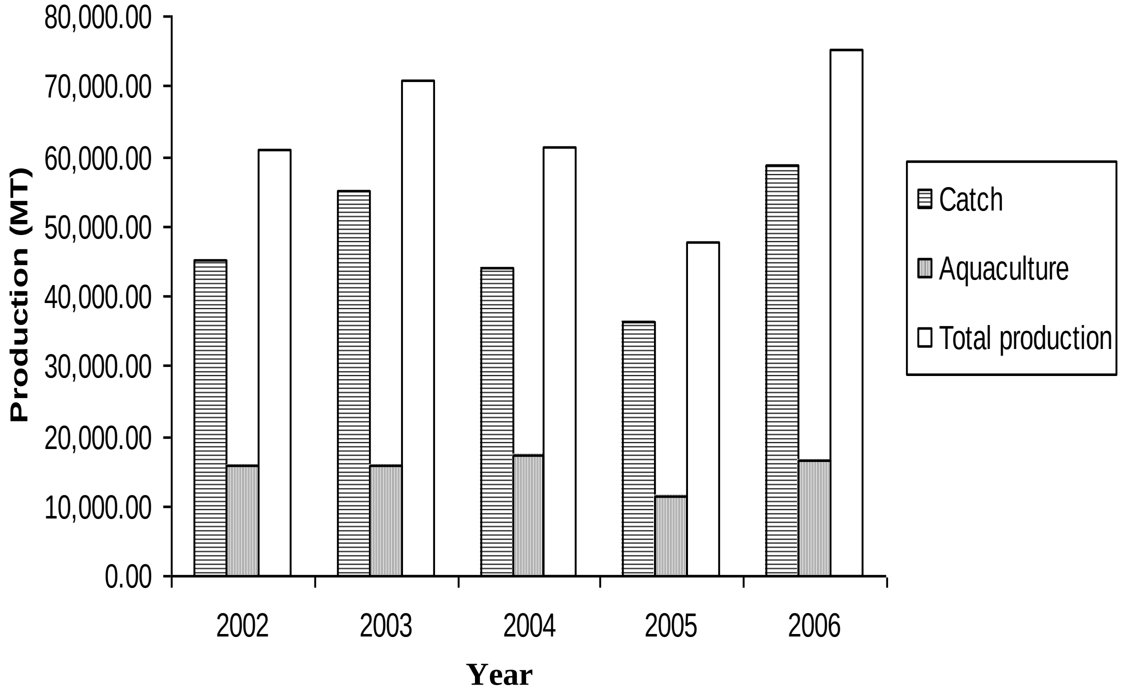

Figure 1.3. Fishery production trends of Aceh Province from 2002 to 2006. (Sources: Fisheries and Marine Department of Aceh Province, 2006).

The fishery industry is one of the most important sectors for the livelihood of the local people. According to Yusuf (2003), at least 55% of the Acehnese are dependent directly on the fisheries resources (both direct and indirect dependent was about 80%). The growth of the fisheries industry should be of high importance and a priority for a positive effect on the micro economy of this area. On the other hand, this is also associated with negative impacts on the environment such as over exploitation and pollution, ecological degradation and possible extinction of the native species. Therefore, future development of the fisheries sector in Aceh should be towards an ecologically friendly and sustainable programme.

Ecological database including aquatic resources and fish biodiversity is a critical prerequisite for sustainable development and this data can be used for monitoring and evaluating the impact of social economic development in the future. Presently, no database on the fish biodiversity in inland Aceh waters is available. The present study would be an important effort to overcome such a need especially in freshwater fish resources management and conservation. In addition to investigate the overall biodiversity and related aspects, special emphasis would be given on various biological aspects of the Depik, *Rasbora tawarensis* which is an endemic and a critically endangered fish in Lake Laut Tawar as a model for the sustainable management of the fish biota in this area.

1.5 Objectives of the Study

The main objectives of the present studies were:

a. To develop an inventory of freshwater fishes of the Aceh waters and evaluate their conservation status and economic value.
b. To barcode freshwater fishes of Aceh focusing on Lake Laut Tawar fishes.

c. To conduct a study on distribution and production trend of the Depik, *R. tawarensis* in Lake Laut Tawar.
d. To evaluate several biological aspects of the Depik, *R. tawarensis*; morphometric and genetic differences, length-weight relationships, condition factor and its reproductive biology for management and conservation programme.

1.6 Outline of the study

This study was divided into two stages:

a. In the first stage, an intensive fish biodiversity survey was done for six months throughout the Aceh Province and involved all five regions of Aceh. The objective of the survey was to develop an inventory of freshwater fishes in the Aceh waters and to evaluate their distribution, conservation status and initial economic values. This data was used to screen and determine the priority species for further studies in the second stage.
b. Based on the data above, the Depik, *R. tawarensis* was selected for detailed investigation in the second stage with Lake Laut Tawar as the study area. The approach of the investigation were (i) barcoding of freshwater fishes from Lake Laut Tawar, (ii) morphometric and genetic differences among *Rasbora* presumed taxa in Lake Laut Tawar, (iii) length-weight relationships and condition factors of two endemic fishes from Lake Laut Tawar, (iv) distribution and production trend of the Depik, *R. tawarensis,* and (v) its reproductive biology.

The thesis is presented in six working chapters. Each chapter is comprised of a brief introduction, objectives, materials and methods, results and discussion. Three additional chapters i.e. General Introduction, Literature Review, General Conclusion and Recommendations are included to give a comprehensive understanding of the overall study.

CHAPTER 2

LITERATURE REVIEW

2.1 Fish Diversity and Conservation

Biodiversity or biological diversity is the degree of variation of life at all levels of biological organization and is classified both by evolutionary (phylogenetic) and ecological (functional) criteria (Colwell, 2009). Therefore, fish biodiversity is the degree of variation of fish species in certain location.

Worldwide, Vida and Kotai (2006) have estimated a figure of approximately 50,000 fish species. Of these about 22,000-25,000 species have been named with valid description (Allen, 2000; Gilbert and Williams, 2002) and new species are being discovered or recognized at a rate of approximately 200 species per year of which 40% are freshwater fishes (Nelson, 1994). The family Cyprinidae makes up one of the largest numbers of the teleosts with over 1300 species on record (Moghraby and Rahman, 1984).

In Asia, there are at least 3500 freshwater fish tantamount to 35-40% of the world freshwater ichthyofauna (Kottelat and Whiten, 1996). Of these 1000 species are present in the freshwater system of Southeast Asia (Zakaria-Ismail, 1994). Cyprinid diversity in Southeast Asia is staggering in comparison to that in other parts of the family range and at the family level, only China has greater diversity than Southeast Asia (Rainboth, 1991). Furthermore, Rainboth (1991) pointed out that approximately 70 genera of cyprinids are endemic to Southeast Asia. By comparison, 95 genera are endemic to the East Asian region, while 15 are endemic to High Asia, the Tibetan Plateau. Cyprinids are the predominant fishes in Asia as well as in Southeast Asia (Rainboth, 1991; Kottelat and Whiten, 1996; De Silva *et al*., 2007). For example in Thailand, of the 549 freshwater fish species, 214 are cyprinids (39%) (Rainboth, 1991). Cyprinids are also predominant in several rivers in Peninsular Malaysia (Zakaria-Ismail, 1994) and Bario Kalabit Highland, Sarawak (Nyanti *et al*., 1999). Similar observation has been recorded in India, for example in north-eastern Gondavari basin (Heda, 2009) and southern Western Ghats (Johnson and Arunachalam, 2009).

Djajadiredja (1977) estimated that there are 4000 fish species in the Indonesian waters, and more than 964 freshwater or brackish water species can be found in western Indonesia and Sulawesi (Kottelat *et al*., 1993). Suwelo (2004) further reported that there are 1000 species of freshwater fishes in the Indonesian waters.

It is likely that the total number of freshwater and marine fishes in Indonesia is underestimated as many areas have not been explored. For example, Kottelat and Whitten, (1996) discovered a total of 79 new freshwater species within a span of only five years. In addition, approximately 40 new species of both freshwater and marine fishes were also reported from Papua, Indonesia during 1995 to 1999 (Allen and Renyaan, 2000). The Indonesian freshwater fishes are also predominated by Cyprinidae, for example in East Borneo (Rachmatika *et al*., 2005) and Sumatera (Siregar, 1993; Hamidah, 2004).

Asia has the world's highest population, currently estimated at 3.68 billion and expected to reach 4.78 billion by the year 2025 (United Nation Population Program, 2009) and the total inland fish consumption in Asia is the highest in the world (Delgado *et al*., 2003 cited in De Silva *et al*., 2007). Most of the needs (over

70%) have been supplied by inland captured fisheries (De Silva., 2003). In addition, Asia is the epicentre of the global aquaculture industry, accounting for over 85% of the global production, of which a significant quantum is based on alien species (De Silva *et al*., 2006). Therefore, if the inland water resources are indiscriminately exploited there is a high risk to be in conflict with sustaining biodiversity, particularly in water bodies that have a relatively high degree of endemicity (De Silva *at al*., 2007). Therefore, conservation of aquatic resources has become an important issue over the last decade. Recently, conservation has grown into an immense global issue, requiring continual financial and institutional support at all levels and utilising various approaches (Economidis, 2002).

Freshwater ecosystem is more sensitive and faces higher risks to threats by anthropogenic activities compared to marine ecosystem. Therefore, the conservation of freshwater fishes has been recognized as an important global issue since the number of species that are extinct, endangered or becoming rare is dramatically increasing as a result of numerous anthropogenic perturbations (Cowx and Collares-Pereira, 2003) such as pollution, changes in land use, river management and dam building (McCully, 1995), deforestation as well as effluent discharges (De Silva *et al*., 2007).

Deforestation has a profound influence on the rivers and streams that form an important feature of the forest ecosystem and their associated flora and fauna, in particular the fish fauna (Wright and Flecker, 2004). In addition, Pringle and Benstead (2001) reported that intensive deforestation can result in changes to invertebrate communities dominated by small burrowing forms, such as larvae chironomids which may be less available to foraging fishes. The siltation can also provide anchorage for vegetation that can block low order stream (Welcome, 1983). According to Craig (2000) the biodiversity and productivity of fish communities are reduced as the extent of land or water ecotones decreases resulting in declining terrestrial food supply and spawning and nursing habitats.

Introduction of exotic species is also a serious issue that contribute to depletion of native species (Lucas and Marmulla, 2000). This biological invasion is widely considered to be the second most important cause of species extinction after habitat destruction (Vitousek *et al*., 1997; Simberloff, 2003). Nuove *et al.*, (2005) stated that introduced species or non-indigenous species are species that are not native to a definite locality or ecosystem, although they may be found elsewhere in the same country or beyond the country's borders. Fausch (2007) stated that the process of biological invasion could be divided into four stages i.e. introduction, establishment, spread and impacts. However, unfortunately the impact of invasion has been rarely assessed.

According to the Economidis (2002), aquatic resource management must become a national and international priority. This is crucial to avert the loss of many important aquatic species. Conservation management programmes must be considered on a species-by-species, case-by-case basis, but within a broad ecosystem context (Thorpe *at al*., 1995). This is because the conservation of species is closely related to the conservation of the ecosystems and their constituent communities. Furthermore, habitat protection and rehabilitation are the principal long-term means through which successful fish conservation could be achieved (Economidis, 2002).

2.2. Quantitative Morphological Variation of Fishes – Morphometrics and Meristics

According to Matthews (1998), the morphological variation of a fish species is influenced by at least three factors: (1) phylogenetic heredity that may constrain morphological diversification within the group, (2) adaptation of the body and fins for the hydrodynamic conditions where their live and (3) adaptation of head, jaw, and propulsive musculature for obtaining food.

Quantitative morphology of fishes can be studied through morphometric and meristic techniques. These are the two main numerical techniques used in the process of scientific description of fishes (Turan 1999; Loy *et al*., 2000; Barriga-Sosa *et al*., 2004 and Pinheiro *et al*., 2005).

Meristic involves counts such as the number of fin rays, spines, scales, bones and cartilages in different anatomical parts of the fish. External meristic features which are commonly studied are fin-rays or spines, gill-rakers and scales while internal meristic features are vertebrae, pyloric caeca, pterygiophores and branchiotegal rays**.** Meristics has been often used to identify or differentiate between genera, species (Palma and Andrade, 2002; Barriga-Sosa *et al*., 2004), strains and crossbreeds and population or groups within species (Cabral *et al*., 2003; Pinheiro *et al*., 2005). However, variability in meristic characters is generally conservative particularly for small fishes such as *Betta* species groups (Lim, 2008).

In general, morphometrics can be defined as a technique for describing body form (Kapor and Khanna, 2004). It is a widely used tool in the study of ichthyological systematics or taxonomy which looks at measurable component (i.e. measuring the length or distance between physical features or landmarks) of fish anatomy such as the size of body parts and fins and its ratio of body length. This technique is very useful for testing and graphically displaying differences in shape when combined with multivariate statistical procedures (Loy *et al*., 1993). Observation of fish shape, size, colour, and other general description are also conducted during such procedure.

Since the 80s, the use of the truss pattern approach has become a popular technique in morphometric study. This pattern involves many external measurements between landmarks (Bookstein *et al*., 1985). The landmark-based technique of geometric morphometric or known as Truss Network Morphometric poses no restriction on the directions of variation and localization of shape change, and are much more effective in capturing information about the shape of an organism (Cadrin, 2000; Palma and Andrade, 2002; Jayasankar *et al*., 2004). This approach is considered a revolutionary tool in morphometrics since it overcomes inherent weakness of traditional characters sets which tend to be aligned to the same horizontal axes (Poulet *et al*., 2004).

2.3 Role of Genetic Study in Fisheries, Aquaculture and Conservation

Although modern taxonomic work regularly employs many other traits, including internal anatomy, physiology, behaviour, genes, isozymes and geography, morphological characters remain the cornerstone of taxonomic treatments (Ward *et al*., 2009). However, there are difficulties in relying primarily on morphology when attempting to identify fishes during various stages of their development for example larvae or when examining fragmentary, partial or processed remains. Even when intact adult specimens are available, the morphological characters used to discern species can be so subtle that identification is difficult, even for trained taxonomists (Ward *et al*., 2009).

It is now well recognised that DNA-based identification system, can aid the resolution of the vast diversity of life with its millions of species (Tautz *et al*. 2003). It has much to offer to fisheries managers, especially in the provision of tools enabling unequivocal specimen identification and assessment of stock structure (Ward, 2000). Genetic data have become increasingly important in assessing gene flow between populations, which is important for the maintenance of genetic diversity (Palumbi and Cipriano, 1998; Prioli *et al*., 2002). Genetic technologies have been recognised for their usefulness in species identification (Prioli *et al*., 2002), monitoring fisheries resources (Menezes *et al*., 2006), aquaculture program (Liu *et al*., 1998; Barriga-Sosa *et al*., 2004) especially for selective breeding. Generally, individuals with higher genetic variability have higher growth rates, development stability, viability, fecundity and resistance to environmental stress and diseases (Carvalho, 1993; Dinesh *et al*., 1996).

Genetic data has also been useful in developing conservation efforts (Dizon *et al*. 1992; Mesquita *et al*., 2001). In many situations, genetics is the best way to determine whether a species is worthy of special protection under the Endangered Species Act (ESA) or other form of conservation status. It allows calculated decision on the course of action to be taken for the protection and conservation (Leuzzi *et al.*, 2004; Matoso *et al*., 2004) as well as managing different stocks (Salini *et al*., 2006). For instance, genetic uniformity of a threatened species will provide the option of restocking by translocation (Teske *et al*., 2003). On the contrary highly differentiated population would mean that each population should be independently managed (Brown and Epifanio, 2003).

In recent years the combination of morphometric and genetic data have been commonly used by researchers, for example in herring (Hedgecock *et al*., 1988), sea bass *Decentrarchus labrax* (Erguden and Turan, 2005), snakehead *Channa* genus (Norainy, 2007), vandace, *Coregonus albula* (Kaupinis and Bukelskis, 2010) and Japanese threadfin bream, *Nemipterus japonicus (*Lim, 2010) to investigate species and population variation.

2.4 DNA Barcoding

The main objective of the barcode of life project is to provide a data base of genetic sequences which can be used as a tool for universal species identification (Hebert *et al*., 2004a). The method promises fast, accurate species identifications by focusing analysis on a short standardized segment of the genome (Hebert *et al*., 2003a). Hebert *et al*. (2003a) proposed a single gene sequence would be sufficient to differentiate all, or at least the vast majority of animal species specifically through the use of the mitochondrial DNA gene cytochrome oxidase subunit I (COX1) as a global bio-identification system for animal. The sequence was likened to a barcode, with species being delineated by a particular sequence or by a tight cluster of very similar sequences (Ward *et al.*, 2005). However, in several instances different sequences have been used to investigate different taxonomic groups and in different laboratories (Ward *et al*., 2005). The barcode can also include some systematic or taxonomic information and also yield data not only on type but also on attributes such as origin, major classification and date of sampling.

However, this approach is associated with several controversy and debate among scientists for example on what degree of genetic similarity can species be defined (Sites & Marshall 2003; Blaxter, 2004) to the performance of different molecular markers in various taxonomic groups (Zardoya and Meyer, 1996; Vences *et al*., 2005b; Steinke *et al*., 2005) and how to incorporate intraspecific variation (Moritz and Cicero, 2004). Some have even expressed scepticism against this approach. The first objection is the concern that DNA sequence differences among closely related species will often be too small to allow their discrimination (Mallet and Willmot, 2003), the second objection is the fact that present strategies and programs suffer from difficulties of aligning sequences of different lengths (Lipscomb *et al*., 2003), especially in automated large-scale analyses.

Furthermore, Rach *et al.* (2008) documented that the DNA barcodes have been found to identify entities below the species level that may constitute separate conservation units or even species units. Instead they suggested that character-based DNA barcoding can be a rapid and a more reliable means for (i) the assignment of unknown specimens to a taxonomic group, (ii) the exploration of conservation units, and (iii) complementing taxonomic identification systems.

Despite these issues, the DNA barcode have been obtained for over 4000 species of fish and the COI sequences deposited in the Barcode of Life Database (BOLD) (Ratnasingham and Hebert, 2007). Additional fish COI sequences are available in GenBank (Smith *et al*., 2008).

The barcode has already been applied in many animals such as birds (Hebert *et al*., 2004b), springtails invertebrates (Hogg and Hebert, 2004), skipper butterfly (Hebert *et al*., 2004a), blowfly (Whitworth *et al*., 2007), leaf beetles (Jurado-Rivera *et al*., 2009), nematodes (De Ley *et al*., 2005), amphibian (Vences *et al*., 2005a), ants (Smith *et al*., 2005), crustacean (Lefebure *et al*., 2006) and scuttle flies (Boehme *et al*., 2010).

Presently, the barcode analysis is already a cost-effective option for species identification in some situations and this will increasingly be the case as reference libraries are assembled and analytical protocols are simplified (Hajibabaei *et al*., 2005). Besides the common preservation techniques of tissue samples fresh, frozen or stored in suitable preservative e.g. RNALater, FTA cards, EDTA lysis buffer or 95% alcohol, cooked or processed fish can also be barcoded (Ward *et al*., 2009).

2.5 Reproductive Biology of Fishes

Reproductive biology envelops the study of reproductive strategy, reproductive system, fecundity, gonadal development, spawning frequency and some factors influencing the reproductive performance of fish.

2.5.1 Reproductive strategy

Natural challenge leads the fish to the maximization of the lifetime production of offspring and more importantly to maximization of survivorship of offspring until adulthood (Murua and Sabodiro-Rey, 2003). The reproductive strategies of fishes are often reflected in the anatomical differences between the sexes, male and female. The objective of a reproductive strategy is to maximize reproductively active offspring in relation to available energy and parental life expectancy (Roff, 1992; Pianka, 2000); strategies and tactics to achieve this objective differ among groups (Balon, 1984).

The reproductive strategy of a species is the overall pattern of reproduction common to individuals of a species, whereas the reproductive tactics are those variations in response to fluctuations in the environment (Wootton, 1990; Roff, 1996). Information on the reproductive biology is essential for the development of the commercial aquaculture of an aquatic species.

2.5.2 Reproductive system

The majority of female teleosts are oviparous and much more fecund than elasmobranchs. Those with a viviparous mode usually exhibit internal fertilization, for example in killifishes, Poeciliidae (Bone *et al*., 1996), elasmobranches and *Sebates* sp (Murua and Saborido-Rey, 2003). The ovaries of adult fish exist are paired structures attached to the body cavity on either side of the dorsal mesentery. Three patterns of ovarian development are generally observed in fishes, i.e. synchronous, group synchronous and asynchronous.

a. Synchronous pattern; in this category, all oocytes develop and ovulate in unison and there is no replenishment from the early stage. Fishes with synchronous pattern are known as total spawners. All eggs are shed within a short period of time. A single size distribution in the ovary is observed (West, 1990), example is in the common carp *(Cyprinus carpio*).
b. Group synchronous pattern; in this category, at least two populations of oocytes at different developmental stages are observed (Murua and Saborido-Ray, 2003). They are also known as multiple spawners. This pattern allows for multiple, distinct ovulatory events that typically follow seasonal, lunar, or diurnal cycles (Redding and Patino, 1993). This pattern has been documented in several fishes for example rainbow selebensis, *Telmatherina celebensis* (Nasution, 2005), white mullet, *Mugil curema* (Solomon and Ramnarine, 2007) and tucunare, *Cichla kelberi* (Normando *et al*., 2009).

c. Asynchronous pattern; in this category, the ovaries contain oocytes at all stages of maturity without any dominant population. A fish with asynchronous ovulator is also known as a batch spawner, where only a portion of the yolked oocytes is spawned in each batch, usually through the hydration process (Murua and Saborido-Rey, 2003). Batch spawning is a strategy to release eggs over a long period of time, allowing for protracted or continued ovulation (Redding and Patino, 1993; Murua and Saborido-Rey, 2003), thus increasing the survival probability of the offspring (Lambert and Ware, 1984). Examples are pounting *Trichopterus luscus* (Alonso-Fernandez *et al*., 2008), *Cichla kelberi* (Normando *et al*., 2009), and *Astyanax scabripinnis* (Veloso-Junior *et al*., 2009).

In order to maximize reproductive success, some fish produce eggs with attachment apparatus called villiform, especially in freshwater fish. The villiform is an adhesive filament functioning for attachment to substrates when the eggs are released into the water and protect them from being washed away in the water. Fish with this organ include *Lepadogaster lepadogaster*, *Diplecogaster bimaculatus*, *Apletodon dentatus* (Breining and Britz, 2000), *Polypterus ornatipinnis*, *P. senegalus*, *P. ornatipinnis* and *Erpetoichthys calabaricus*, (Britz and Bartsch, 1998)

Some species such as gouramis and grass carp produce buoyant eggs, not the floating type which may be best suited to freshwater, to counter excessive drift in rivers and streams. Marine fish eggs are usually smaller in diameter than freshwater fish and most are buoyant and liberated into the pelagic zone (Bone *et al*., 1996).

There is no distinguishing morphological character between freshwater and marine fish eggs and larvae although they have different osmotic problems (Bone *et al*., 1996), where the freshwater is hypertonic to environment and vice versa in marine fish egg.

In general, fish spermatozoa can be divided into two types i.e. aquasperm and introsperm. In the aquasperm or primitive spermatozoa, the spermatozoa are released into the water for external fertilization. Most external fertilizers have spermatozoa with spherical nuclei and short midpieces (Jamieson, 1991 quoted in Burn *et al*., 1998). In the introsperm or modified spermatozoa, where spermatozoa are introduced into the ovary of the female, the spermatozoa are characterized by an elongated nucleus, and enlarged midpiece or mitochondrial region (Mattei, 1991). Fish with this type of spermatozoa are described as internal fertilizers. Some species of male internal fertilizers have an apparatus called andropodium to facilitate transfer of spermatozoa into the female. This organ is formed by the anal fin (Brembach, 1976 quoted in Downing and Burn, 1995).

2.5.3 Fecundity

In general, fecundity is defined as the number of ripening eggs found in the female just prior to spawning (Bagenal, 1978). Fecundity data is essential for studies of population dynamics and life history of a fish (Kapoor and Khanna, 2004). There are at least six categories of fecundity descriptions, namely; potential annual fecundity, annual realized fecundity, total or absolute fecundity, relative fecundity, batch fecundity, and annual population fecundity**.**

Potential annual fecundity is the total number of advanced yolked oocytes matured per year (Hunter *et al*., 1992). Annual realized fecundity is the actual