



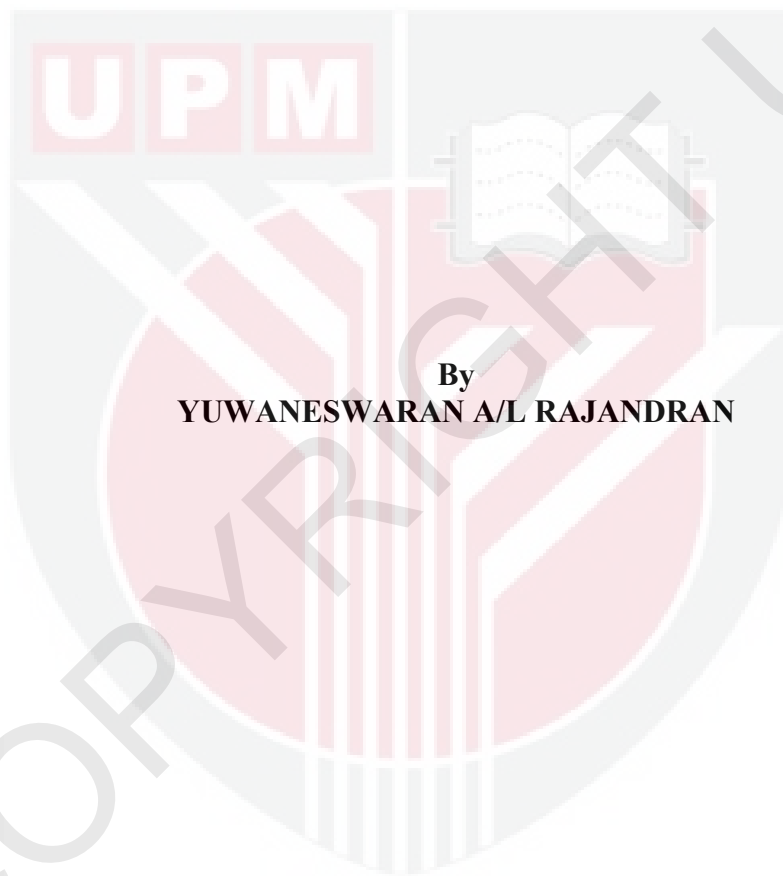
UNIVERSITI PUTRA MALAYSIA

***PIONEERING BREEDING STRATEGIES AND
BEHAVIOURAL STUDIES IN IBAN FIGHTING FISH
(*Betta ibanorum*) PRECEDING INDUCED METHODS***

YUWANESWARAN RAJANDRAN

**Ip
FSPH 2024 16**

**PIONEERING BREEDING STRATEGIES AND BEHAVIOURAL STUDIES
IN IBAN FIGHTING FISH (*Betta ibanorum*) PRECEDING INDUCED
METHODS**



By
YUWANESWARAN A/L RAJANDRAN

**A Project Report Submitted in Partial Fulfilment of the Requirement
for the Degree of Bachelor Science Aquaculture in the Faculty Of Agricultural
And Forestry Sciences, University Putra Malaysia Bintulu Sarawak
Campus**

2024

ABSTRACT

Betta ibanorum, also known as Iban fighting fish is a species of gourami endemic to southern Sarawak in Malaysia. The species name *ibanorum* was named after the Iban people, the largest group of people in the Sarawak. This species is a mouthbrooder, and grows to a length of 8.1 centimetres (3.2 inches). The lack of documented information on breeding strategies and behavioural studies makes it difficult for further studies. Therefore, this study investigated the breeding strategies and behavioural studies of *B. ibanorum* under laboratory conditions. Four pair of *B. ibanorum* were been injected using different types of hormones for gametes development for each trial. Observations on the embryonic development of *Betta ibanorum* was microscopically monitored and recorded. The broodstocks does produce plenty of eggs and less sperm for certain hormones. The behaviour of *B. ibanorum* were recorded such as the genital papilla and factors affecting embryogenesis and early development such as water temperature, water quality, and parental behaviour. However, there was fertilization failure and lack of progress in embryonic development for *Betta ibanorum* in captured environment. This study on breeding strategies and behavioural studies using induced methods in *B.ibanorum* has bridged a gap in the knowledge of developing eggs and morphology of the species in Malaysia. Although the attempted embryogenesis of *B.ibanorum*, the species of betta in East Malaysia; mostly found in Sarawak province has failed, further experimentation should be conducted to address the intrinsic and extrinsic variables that are known to impede embryogenesis experiments to optimise the process.

Keywords: Betta; *B.ibanorum*, East Malaysia

ABSTRAK

Betta ibanorum, juga dikenali sebagai ikan laga Iban adalah spesies gurami endemik di selatan Sarawak di Malaysia. Nama spesies *Betta ibanorum* dinamakan sempena orang Iban, kumpulan orang terbesar di Sarawak. Spesies ini akan melakukan persenyawaan dalam mulut, dan tumbuh sehingga 8.1 sentimeter (3.2 inci). Kekurangan maklumat yang didokumenkan tentang strategi pembiakan dan kajian tingkah laku menyukarkan kajian lanjutan. Oleh itu, kajian ini menyiasat strategi pembiakan dan kajian tingkah laku *B. ibanorum* di bawah keadaan makmal. Empat pasang *B. ibanorum* telah disuntik menggunakan jenis hormon yang berbeza untuk pembangunan gamet bagi setiap percubaan. Pemerhatian terhadap perkembangan embrio *Betta ibanorum* dipantau dan direkodkan secara mikroskopik.

Induk menghasilkan banyak telur dan kurang sperma untuk hormon tertentu. Tingkah laku *B. ibanorum* direkodkan seperti papila kemaluan dan faktor yang mempengaruhi embriogenesis dan perkembangan awal seperti suhu air, kualiti air, dan tingkah laku induknya. Walau bagaimanapun, terdapat kegagalan persenyawaan dan kekurangan kemajuan dalam perkembangan embrio untuk *Betta ibanorum* dalam persekitaran yang ditangkap. Kajian mengenai strategi pembiakan dan kajian tingkah laku menggunakan kaedah teraruh dalam *B.ibanorum* ini telah merapatkan jurang dalam pengetahuan membangunkan telur dan morfologi spesies di Malaysia. Walaupun percubaan embriogenesis *B.ibanorum*, spesies betta di Malaysia Timur; kebanyakannya ditemui di wilayah Sarawak telah gagal, eksperimen selanjutnya perlu dijalankan untuk menangani pembolehubah intrinsik dan ekstrinsik yang diketahui menghalang eksperimen embriogenesis untuk mengoptimumkan proses.

Kata kunci: Betta, *B. ibanorum*; Malaysia timur; Sarawak

ACKNOWLEDGEMENTS

I am humbled to express my gratitude and thanks to God, and I was able to conclude this report and express my sincere thankfulness. I would like to express indebted gratitude to our respected lecturer, Dr. Nora Faten Afifah Binti Mohamad for giving her valuable guidance and encouragement throughout this project. It was a great privilege and honor to work and study under her guidance. I would like to pay my special appreciation to the staff of the Department of Animal Science and Fishery for their technical support. I wish to express my deepest gratitude to my friends, Harith syazwan, Nabhan, Fiqman, Kavin raj, Karthigeswary, Sasikumar, Jaykumar and Mr. Lirong Yu Abit for their support along the way. Countless gratitude I sincerely express to everyone who directly or indirectly made it possible for me to widen my knowledge together and helped me finish my project on time. My warmest thanks are extended to my beloved family members for being the mediator of my success. I dedicated this writing as the mark of my hard work and the end of my study for a bachelor's degree. I hope this writing, continues to benefit everyone.

APPROVAL SHEET

I certify that this research project report entitled “Pioneering breeding strategies and behavioural studies in Iban fighting fish (*Betta ibanorum*) preceding induced methods.” has been examined and approved as partial completion of the requirement for the degree of Bachelor of Science in Aquaculture with Honors in the Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia Bintulu Sarawak Campus.

Dr. Nora Faten Afifah Binti Mohamad
Faculty of Agricultural and Forestry Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus (Supervisor)

Dr. Zamri Bin Rosli
Dean
Faculty of Agricultural and Forestry Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus

Date:

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	ii
ACKNOWLEDGEMENTS	iii
APPROVAL SHEET	iv
TABLE OF CONTENT	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF APPENDICES	xi
LIST OF ABBREVIATIONS	xii
CHAPTER	
1.0 INTRODUCTION	1
1.1 Research Background	1
1.2 Objective	3
2.0 LITERATURE REVIEW	4
2.1 Taxonomy	4
2.1.1 Family of Osphronemidae	4
2.2 Diversity and distribution of betta species	5
2.2.1 Southeast Asia	5
2.2.2 Malaysia	6
2.3 Morphology and general appearance	6
2.3.1 Morphology of group <i>Betta akarensis</i>	6
2.3.2 Morphology of <i>Betta ibanorum</i>	7
2.3.3 Coloration of <i>B. ibanorum</i>	8

2.4	Habitat of <i>Betta ibanorum</i>	9
2.5	Reproduction of <i>Betta ibanorum</i>	10
	2.5.1 Embryogenesis of <i>Betta.sp</i>	11
2.6	Behaviour of <i>Betta ibanorum</i>	12
2.7	Diet of <i>Betta ibanorum</i>	12
2.8	Maintenance of <i>Betta ibanorum</i>	12
2.9	Hormone	13
	2.9.1 Ovaprim	14
	2.9.2 Luteinizing hormone-releasing hormone (LHRH)	14
	2.9.3 Estradiol	15
	2.9.4 Human chorionic gonadotropin (hCG)	16
3.0	METHODOLOGY	17
3.1	Study area	17
3.2	Sample Collection and broodstock rearing	18
3.3	Hormone Preparation and administration	19
3.4	Gamete collection and <i>in -vitro</i> fertilization	20
3.5	Microscopic examination	21
3.6	Data analysis	21
4.0	RESULTS	22
4.1	Identification of genital papilla of <i>B.ibanorum</i>	22
4.2	Effects on eggs using different types of hormones	22
4.3	The collection of gametes	23

4.4	Characteristics of <i>B. ibanorum</i> eggs	24
4.5	The comparison of embryonic development of <i>B. ibanorum</i> and <i>B. splendens</i>	25
4.6	Comparison of pH level of sampling site with previous study	26
5.0	DISCUSSION	28
5.1	Housing and water parameters	28
5.2	pH and water salinity	28
5.3	Disease	29
	5.3.1 White Spot Disease (<i>Ichthyophthirius multifiliis</i>)	29
	5.3.2 Fin rot	30
	5.3.3 Fin clamp	30
5.4	Captured environment	31
6.0	CONCLUSION	33
	REFERENCE	34
	APPENDICES	38

LIST OF TABLES

Tables		Page
3.3	Dosage level of different types of hormones for male and female fish.	19
4.2	The effects of different types pf hormones on the production of eggs and fertility rate after 6 trials.	23



LIST OF FIGURES

Figure		Page
2.3.1	The general appearance of <i>B. akarensis</i> (Seriously fish n.d.).	7
2.3.2	The general appearance of <i>B. ibanorum</i> (Dunlop.c n.d.).	8
2.4	The blackwater habitat in the lower Batang River basin, Samarahan Division, Sarawak.	9
2.5.1	The embryonic development of <i>betta splendens</i> (Nivaldo, 2013).	11
3.1	The location of Nirvanna forestry and Borneo Base UPMKB.	17
3.3	The Syndel ovaprim (left), hCG, LHRH and the method of injecting hormone near the pectoral fin under the scales.	19
3.4	The incubator that was used during incubation process of the eggs.	20
4.1	The <i>B. ibanorum</i> genital papillae of female and male.	22
4.3	The result of the sperm and eggs during the stripping of Synthetic salmon gonadotrophin with estardiol.	24
4.3	The picture of unmaturred eggs using the hormone LHRH.	24

4.4	The weight of matured and unmatured eggs of <i>B. ibanorum</i> .	25
4.5	The failure of embryonic development of <i>B. ibanorum</i> .	26
4.6	The pH level of two different types of water where the samples were collected. Neutral pH level and acidic pH level.	27



LIST OF APPENDICES

Appendix		Page
6.1	Weight of broodstock (g)	38
6.2	Disease	39
6.3	Sampling site at UPM Bintulu Sarawak campus	40
6.4	Captive conditions	41
6.5	Microscopic examination	42



LIST OF ABBREVIATIONS

FAO Food and Agriculture Organization

B. ibanorum *Betta ibanorum*

sp Species

LHRH Luteinizing hormone-releasing hormone

hCG Human chorionic gonadotropin

& and

% Percentage

cm Centimeters

mg Miligram

g Gram

mm Milimeter

ppm Parts per million

h Hour

CHAPTER 1

1.0 INTRODUCTION

1.1 Background of the Study

The variety *Betta*, privately known as fighting fish, comprises of little freshwater battling fish that has a place with the Osphronemidae family. They are local to Southeast Asia and are found inside different sorts of water bodies, accordingly prompting their variety. There are presently 27 species of *Betta* fish recorded in Malaysia, with seven species beginning from Sarawak (Tan and Ng, 2005; Schindler and Schmidt, 2008; Tan, 2009; Tan and Ahmad, 2018; Kamal et al., 2020). *Betta* fish are possible fancy fish with monetary and natural significance (DoF, 2018).

The variety *Betta* species, prevalently known as fighting fishes, comprises of little fish (not bigger than 110 mm SL) adjusted to damp, stale water pool and slope stream natural surroundings. They have the capacity to inhale air, show specific egg and brood care (either oral agonizing or bubble-home structure) and show solid regional impulses (Rüber et al., 2004). The genus *Betta* currently contains some 70 species (Tan & Ng, 2005; Schindler & Schmidt, 2008; Tan, 2009; Tan & Ahmad, 2018; pers. obs.).

According to Tan, H. H. and Ng, P. K. L. (2004), *Betta ibanorum* are place under the family of Osphronemidae and class of Teleostei. *Betta ibanorum* are originate from the Malaysian province of Sarawak on the island of Borneo. It is generally circulated all through the south and west of the state and exists in a few variety structures relying upon territory. Type territory is Kuching, Bako Public Park, Bukit Gondol, Sarawak, Borneo, and it's known from different regions around the city

of Kuching, in addition to the areas of Matang, Serian, Gedong, Simunjan and Sri Aman with referred to run broadening eastwards to the extent that the gold-mining town of Bau (Tan and Ng, 2004).

They occupy clear streams with streaming water albeit most realized regions comprise of blackwater conditions in old peat swamps, however it is likewise been gathered from side of the road trenches and one populace has been found living in a little pool at the highest point of a cascade. The two sorts of living space are normally concealed from the sun with plentiful riparian vegetation. Blackwaters are naturally stained brown with humic acids and different synthetic substances delivered by rotting natural material. The breakdown mineral substance is quite often insignificant, pH as low as 3.0-4.0 and substrate made dominantly out of fallen leaves, branches and lowered tree roots. Clearwater streams typically take care of sandy or rough substrates with a layer of biofilm, and the fish will generally be found in peripheral zones where the stream is more fragile or oceanic plants developing (Tan and Ng, 2004).

Betta ibanorum is listed under the group of *Betta akarensis* gathering of intently related species inside the sort, of which individuals share the accompanying arrangement of characters: ownership of pre-orbital and post-orbital stripes (the post-orbital stripe is weak or hindered in certain species); jaw bar present; caudal-blade lanceolate with profoundly broadened middle beams in mature guys; caudal balance generally with cross over bars; greenish or pale blue brilliant scales on assortment of guys in certain species; opercle without luminous scales besides in adolescents (Tan and Kottelat, 1998).

1.2 Objectives

The lack of documented information on the embryogenesis and early development of *Betta ibanorum* makes it difficult for further studies. According to Tan and Ng (2004), this species has been discovered in the year of 2004. The recent founding of *Betta ibanorum* causes less information regarding this species. Therefore, the results of this study will be used in the future to provide significant knowledge regarding the embryogenesis and early development of *Betta ibanorum* for the purpose of breeding, hybrids or even can be sell as ornamental fishes in upcoming years.

- I. To identify and observe the morphology of the *B. ibanorum* species during embryogenesis development for further researches.
- II. To observe the characteristics of gametes and genital papillae of *B. ibanorum*
- III. To observe the best induced method for *B. ibanorum* using different types of hormones.
- IV. To identify factors affecting embryogenesis and early development such as water temperature, water quality, and parental behaviour.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Taxonomy

Betta Bleeker, 1850, is the most speciose genus in the family Osphronemidae. Britz (1994, 1995, 2001) and Britz et al. (1995) recently included in the Osphronemidae species previously placed in the families Luciocephalidae and Belontiidae. The large number of species of *Betta* is probably due to several factors. The warm and humid climate of Southeast Asia provides a stable environment with numerous niches and habitats (Whitmore, 1986: 15). The presence of many types of water-bodies; rivers, ox-bow lakes, forest streams, open country streams, hill streams, freshwater and peat swamps support a great diversity (see Kottelat & Ng, 1994). Many species are also adapted to survive in extreme environmental conditions, like in blackwaters with high acidity (pH between 3 and 5) in peat swamp forests. *Betta spp.* also tend to have ranges restricted to one or a few drainage systems, delimited by various obstacles like mountain ranges or large rivers (Kottelat & Ng, 1994).

2.1.1 Family of Osphronemidae

Osphronemidae is a type of fish group that has a labyrinth as a breathing apparatus. Its distribution is found in Asia, from Indonesia, India to China. This group of fish makes nests by making foam which is then used as a spawning ground for eggs (Sari, 2019). Fish belonging to the Osphronemidae family, namely twill fish (*Belontia hasselti*) (Fauziah et al., 2017) besides that there is also betta fish (*Betta edithae*), Siamese sepat (*Trichopodus pectoralis*), red-eyed sepat (*Trichopodus trichopterus*) (Iqbal et al., 2019). Fish of the Osphronemidae family are consumption fish and can also be used as ornamental fish (Ajai et al., 2020). In addition, in nature the fish group

Osphronemidae has an important ecological role for nature and humans. Siamese finches are fish that eat everything, be it aquatic plants, mosses and phytoplankton (Murjani, 2009); (Ramadhan et al., 2020). Furthermore, Diniya et al (2014) explained that in addition to eating small animals in the water, these fish also include grazer fish which eat the remains of organisms attached to the sidelines of aquatic plants such as weeds (Nursyamsiah et al, 2017).

2.2 Diversity and distribution of betta species

2.2.1 Southeast Asia

Concentrates on the variety and dissemination of Betta fish in the Southeast Asian district have likewise been accounted for from a few nations, for example, 12 species were accounted for from Thailand, specifically *B. simplex*, *B. prima*, *B. pugnax*, *B. pi* (Tan and Ng 2005; Tanpitayacoop and Na-Nakorn 2005), *B. smaragdina* (Tan and Ng 2005; Monvises et al. 2009), *B. mahachaiensis*, *B. siamorientalis* (Kowasupat et al. 2012, Citation2012), *B. imbellis*, *B. splendens* (Tan and Ng 2005; Kowasupat et al. 2014), *B. pallida*, *B. ferox* and *B. apollon* (Panijpan et al. 2014). Moreover, three species were additionally distinguished in Cambodian waters, to be specific *B. prima* (Tan and Ng 2005), *B. siamorientalis* and *B. stiktos* (Kowasupat et al. 2012, 2014), while two species were accounted for from Brunei (*B. macrostoma* and *B. akarensis*) and two species from Singapore (*B. pugnax* and *B. imbellis*) (Tan and Ng 2005). Moreover, 24 species were found in Malaysian waters specifically *B. coccina*, *B. pulchra*, *B. stigmosus*, *B. waseri*, *B. ibanorum*, *B. macrostoma*, *B. akarensis*, *B. chini*, *B. warrior*, *B. lehi*, *B. taeniata*, *B. brownorum*, *B. pi* (Tan and Ng 2005), *B. ocellata*, *B. persephone*, *B. tussyae*, *B. livida*, *B. hipposideros*, *B. pugnax* (Tan and Ng 2005;

Kowasupat et al. 014), *B. imbellis* (Tan and Ng 2005; Kowasupat et al. 2014), *B. kuehnei*, *B. stigmosa*, *B. waseri*, and *B. bellica* (Panijpan et al. 2014).

2.2.2 Malaysia

The latest modification, by Tan and Ng (2005), covered each of the 23 Malaysian species considered substantial around then: Peninsular Malaysia with 13 species; *B. bellica* Sauvage, *B. coccina* Vierke, *B. hipposideros* Ng and Kottelat, *B. imbellis* Ladiges, *B. livida* Ng and Kottelat, *B. Persephone* Schaller, *B. pi* Tan, *B. pulchra* Tan and Tan, *B. pugnax* (Cantor), *B. stigmosa* Tan and N, *B. tomi* Ng and Kottelat, *B. tussyae* Schaller, *B. waseri* Krummenacher; Sarawak with six species; *B. akarensis* Regan, *B. brownorum* Witte and Schmidt, *B. ibanorum* Tan and Ng, *B. lehi* Tan and Ng, *B. macrostoma* Regan, *B. taeniata* Regan; and Sabah with four species; *B. balunga* Herre, *B. chini* Ng, *B. warrior* Tan and Ng, *B. ocellata* de Beaufort. From that point forward, another three species have been portrayed and reported: *B. kuehnei* Schindler and Schmidt 2008 and *B. omega* Tan and Ahmad 2018 from Peninsular Malaysia, and *B. midas* Tan 2009 from Sarawak.

2.3 Morphology and general appearance

2.3.1 Morphology of group *Betta akarensis*

Individuals from the *B. akarensis* bunch are recognized by the following person suite (Tan and Kottelat, 1998): presence of preorbital and postorbital stripes (postorbital stripe might be interrupted or faint), and a jawline bar; caudal balance lanceolate, middle beams particularly extended in mature male, with expanding of caudal fin beams. 40 mm SL, generally with caudal-balance cross over bars (consistently particular in mature male); greenish to pale blue radiant body scales in certain species;

opercle without glowing scales (Witte and Schmidt, 1992; Ng, 1993). Grown-up examples may have greenish-gold opercle scales which will quite often vanish in imprisonment. Adolescent might have gold opercle scales, yet all the same these vanish with age. The faintly shaded greenish-gold opercle scales of the grown-ups could be because of stress from dealing with. All individuals are male oral brooders. The *B. akarensis* and *B. pugnax* gatherings can be promptly separated by two characters: the presence of faintly hued greenish-gold opercle scales in individuals from the *B. akarensis* bunch (versus the serious somewhat blue green opercle scales in individuals from the *B. pugnax* bunch) also, typically more butt-centric blade beams.



Figure 2.3.1 shows the general appearance of *B. akarensis* (Seriously fish n.d.)

2.3.2 Morphology of *Betta ibanorum*

Betta ibanorum is recognized from the other individuals from the *B. akarensis* bunch by the accompanying mix of characters: opercle without second postorbital stripe; eye variety yellow when alive; butt-centric balance beams 27-29 mm; subdorsal scales 5 mm; horizontal scales 30-32 mm; pre dorsal scales 21-23 mm; Post dorsal scales 10-11 mm; preanal length 45.8-49.4% SL; head length 31.7-36.4% SL; length of butt-centric blade base 50.0-55.8% SL. Body somewhat slim, caudal balance extensively lanceolate; dorsal furthermore, butt-centric blades pointed; pelvic balance filamentous. Enormous male with a sunken head profile. Beams of unpaired blades of guys prolonged, and may seem filamentous.



Figure 2.3.2 shows the general appearance of *B.ibanorum* (Dunlop.c n.d.)

2.3.3 Coloration of *Betta ibanorum*

According to Tan, H. H. and Ng, P. K. L. (2004), body dull brown dorsally, lighter ventrally. Mature male with glowing green to blue back half on body scales, dorsal and caudal cross over bars. Female with have less glow and without caudal cross over bars. Adolescent with a particular dark focal stripe on body and a dark caudal peduncle spot. A limited particular jaw bar in all examples. Opercle with little or no design.

2.4 Habitat of *Betta ibanorum*

According to Ruhana.H (2022), *B. ibanorum* lives in streaming water (lotic) and stale waters (lentic). These waters were found to go from somewhat acidic to unbiased. Amphibian vegetation was likewise in abundance, added with the inclusion from the timberland shelter. *B. ibanorum* and *B. brownorum* were both tracked down inside a similar living space, correspondingly to *B. midas* and *B. rutilans*. This is interestingly, with a concentrate by Nur et al. (2022), where the Betta species displayed regional conduct prompting no two species residing in a similar living space.



Figure 2.4 shows the blackwater habitat in the lower Batang River basin, Samarahan Division, Sarawak.

2.5 Reproduction of *Betta ibanorum*

Mouthbrooding, one of the most particular types of parental consideration is just tracked down in nine teleost families (Blumer 1982). The development from a "watching" type of care, such as substrate bringing forth or air pocket settling, to "mouthbrooding" is by and large thought to be connected with shifts in reproductive and life-history highlights as anticipated by Sparkle's "protected harbor" theory (Sparkle, 1978). Sparkle's protected harbor hypothesis states that propagule (i.e., egg) size is supposed to develop in light of relative survivorship rates during and after the propagule stage contingent upon the structure and quality of parental consideration. Regular determination is probably going to incline toward huge egg size when the egg stage can be viewed as a protected harbor because of parental consideration venture. The advances from no parental care to parental consideration, and furthermore changes in the structure and nature of parental consideration, for example, from "protecting" to "brooding," are subsequently expected to bring about shifts in conceptive what's more, life-history highlights. These movements incorporate, for instance, diminished fruitfulness, expanded egg size, and higher paces of adolescent endurance with higher parental speculation (Ruben, 2004). Mouthbrooders further show an overall reduction or loss of egg and larval connection frameworks (Peters what's more, Berns 1982). In any case, wide similar examinations with in hearty phylogenetic systems are as yet expected to distinguish the elements that molded the development of elective types of parental consideration in teleost fish. In generally 70% of the number of species inside the variety *Betta*, guys bring brought forth eggs into their mouths also, hatch them for as long as four weeks (Schmidt 1996). The staying 30% of the species inside the variety display bubble settling, the prevailing

regenerative style and plesiomorphic condition among osphronemid taxa (Britz and Cambray 2001), in which eggs and hatchlings are watched and guarded by the male.

2.5.1 Embryogenesis of *Betta.sp*

Main stages of the embryonic development in *Betta.sp* are first cleavage, morula, blastula begins, blastula ends, beginning of the gastrula, gastrula at the end of epiboly; beginning of the formation of embryo and yolk pigmentation, pre-larva, ready to hatch, showing the cephalic and tail regions and the presence of somites and hatching of the larva and eye pigmentation (Nivaldo, 2013).

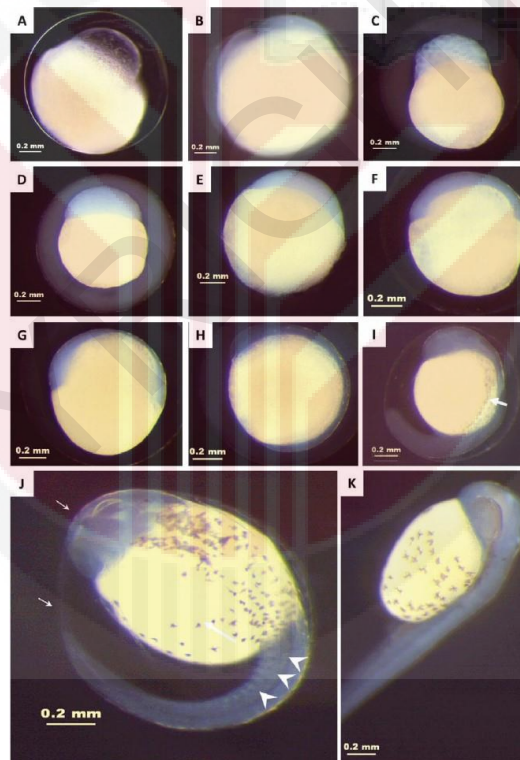


Figure 2.5.1 shows the embryonic development of *Betta splendens* (Nivaldo, 2013)

2.6 Behavior of *Betta ibanorum*

According to Water (1967) and Bronstein (1982), Betta fish is regional and forceful during the producing season and egg brooding. These regional and forceful propensities are connected with self-preservation and posterity assurance from hunters, rivalry for mates, and keeping up with social orders (Polgardani et al. 2017).

2.7 Diet of *Betta ibanorum*

Betta ibanorum likely to take care of generally on bugs and other little spineless creatures in nature. Hostage fish will typically acknowledge dried items whenever they're perceived as consumable, however ought to be offered a lot of little live or frozen food varieties, for example, Daphnia, Artemia or bloodworm consistently to guarantee improvement of ideal tone and condition. This species cannot be over feed as *Betta spp.* appear to be especially inclined to stoutness.

2.8 Maintenance of *Betta ibanorum*

According to Tan H.H (2004) *Betta ibanorum* can be kept up with in a completely embellished aquarium albeit numerous raisers don't really want to involve a substrate for simplicity of support. Driftwood roots and branches can be utilized and set such a way that a couple of obscure spots are framed while dirt plant pots or lengths of funneling can likewise be incorporated to give further haven. The option of dried leaf litter further stresses the normal feel and as well as offering extra cover for the fish carries with it the development of organism settlements as deterioration happens. These can give an important optional food hotspot for fry and the tannins and different synthetics delivered by the rotting leaves are likewise thought to be helpful for fishes from blackwater conditions.

There is compelling reason need to utilize regular peat, nonetheless, the assortment of which is both impractical and naturally horrendous. Like others in the variety this species appears to do best under genuinely faint lighting. You could add oceanic plant species that can get by under such circumstances, for example, *Microsorium*, *Taxiphyllum* or *Cryptocoryne spp.*, and a couple of patches of drifting vegetation would be helpful too. This species requires acidic circumstances with immaterial carbonate hardness and extremely low broad hardness so a converse assimilation unit or other strategy for getting delicate water might should be utilized, and this can be additionally fermented utilizing phosphoric corrosive or comparative if important. As it normally possesses languid waters filtration ought not be areas of strength for excessively, an air-controlled wipe channel set to turn over delicately sufficient. Keep the tank all around covered and don't fill it to the top as like all *Betta spp.* it requires periodic admittance to the layer of damp air that will frame over the water surface, and is a phenomenal jumper (Ng.P.K.L. 2004).

2.9 Hormone

Prompted rearing through chemical treatment and fake hatching of prepared egg enjoys benefits of better pace of treatment and incubating, better circumstances for development also, better assurance of hatchlings against ominous natural condition and predators (Olaniyi and Akinbola, 2013). Subsequently, concentrate on the utilization of hormonal measurement could assist with deciding the best grouping of chemical that could build the creation of hatchlings. There are different hormones that can be utilized in prompted rearing for seed creation counting pituitary concentrate or hypophysis from comparative or different fish, Deoxycorticosterone Acetic acid derivation (DOCA), Ovaprim, Ovulin, Ovatide, Human Chorionic Gonadotropin

(HCG), Ovopel, Dagin and Aquaspawn (Brzuska and Adamek, 1999; Cheah and Lee, 2000; Zohar and Mylonas, 2001; Hassan et al., 2018). The industrially accessible manufactured initiating chemicals in readymade containing GnRH and dopamine blocker receptor, for example, ovaprim, ovopel, dagin and water produce are turning out to be extremely well known and viewed as effective in effective generating of fishes (Hassan et al. 2018).

2.9.1 Ovaprim

Ovaprim is one of the most famous and viable hormones to animate the development of male and female grown-up fish. Ovaprim is utilized as a successful generating inducer for counterfeit reproducing of fishes (Chaube et al., 2014). Ovaprim is produced using Salmon Gonadotropin Delivering Chemical (SGnRGH) and Dopamine bad guy in a steady arrangement, ready in glycerine and liquor at specific amount (Sasmita, 2016). Ovaprim are utilized to enliven the ovulation cycle in female catfish and make sperm more fruitful in male fish. Ovaprim can speed up the last oocytes development and prompted bringing forth (Zadmajid et al., 2017). Fish treated with the Ovaprim showed expansion in gonadosomatic file, egg measurement, and wet weight comparative with controls. Fish that infused with high dose of chemicals produce huge number of egg creation (Zadmajid et al., 2017).

2.9.2 Luteinizing hormone-releasing hormone (LHRH)

Engineered analogs of luteinizing chemical delivering chemical (LHRH) are turning out to be broadly utilized for prompting ovulation and generating in various teleosts. For marine species, for example, milkfish, mullet, ocean bass, and rabbitfish, a solitary LHRH infusion or pellet embed gives off an impression of being viable. Various

spawnings of ocean bass have likewise been gotten following a solitary infusion or pellet embed of a high portion of LHRH. In various freshwater fishes, for example, the cyprinids, LHRH alone anyway has restricted adequacy. Normalized strategies utilizing LHRH along with the dopamine adversaries pimozone, domperidone and reserpine have been created for different types of carps. The method may likewise be relevant for generating marine teleosts that may not answer LHRH alone or where a high portion of the peptide is required (Marte C.L. 1986).

2.9.3 Estradiol

Estradiol is a type of estrogen, a hormone that is naturally produced in the bodies of various animals, including fish. In the context of fish, particularly in environmental science and biology, estradiol is sometimes studied due to concerns related to endocrine disruption. Endocrine disruptors are substances that can interfere with the endocrine system, which regulates hormones, and they can have various effects on the health and behavior of organisms. In the case of fish, exposure to exogenous (external) sources of estrogen-like compounds, such as estradiol, can lead to endocrine disruption. This exposure may result from the discharge of certain chemicals, including synthetic estrogens from pharmaceuticals, into water bodies. Such substances can affect the reproductive and developmental processes of fish, leading to issues like feminization of male fish or altered reproductive behaviours. Researchers and environmental agencies often study the presence and effects of estrogenic compounds, like estradiol, in aquatic environments to assess potential risks to fish populations and ecosystems. Monitoring and understanding these effects are crucial for managing and mitigating environmental impacts. It's important to note that the

specific research findings and regulations related to estradiol and other endocrine-disrupting compounds may vary by region and over time.

2.9.4 Human chorionic gonadotropin (hCG)

To regulate and promote spermatogenesis and spermiation in fish, gonadotropin-releasing hormone, gonadotropin, or steroid hormones can be injected or implanted. It is easier and less complicated to treat the testes than the ovaries. Fish spermiation is effectively stimulated by the hormone human chorionic gonadotropin (HCG). Spermiation in goldfish (*Carassius auratus*), rainbow trout (*Oncorhynchus mykiss*), seabream (*Sparus aurata*), milkfish (*Chanos chanos*), European eel (*Aguilla anguilla*), and Japanese eel (*A. japonica*) has been shown to be concentration-dependent when exposed to HCG at 100–10,000 IU/kg bodyweight (BW) (Donaldson & Hunter, 1983; Park et al., 1992). Carp pituitary extract (CPE) is a commonly used spawning inducer, yet its strength varies depending on the sex, maturity, and age of the fish.

CHAPTER 3

3.0 METHODOLOGY

3.1 Study area

The study was carried out in University Putra Malaysia Campus Bintulu, Sarawak (UPMKB). The exact location of sample collection selected at Borneo Base and Forest Nirvana inside UPMKB area. The coordinates of the areas are 3.209325, 113.093808 (Borneo Base forestry) and 3.207006, 113.074646 (Nirvana forestry). The average temperature of all these areas ranges from 25°C to 31°C and according to the average rainfall, the interannual variability ranges from 3618 mm to 4552 mm for these 5 years.



Figure 3.1 shows the location of Nirvana forestry UPMKB

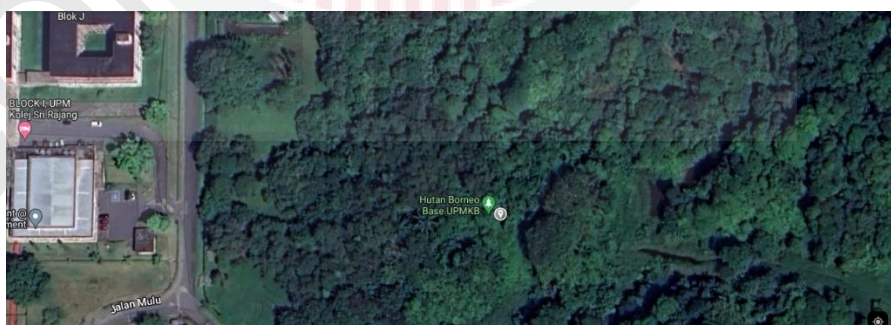


Figure 3.1 shows the location of Borneo Base forestry UPMKB

3.2 Sample Collection and broodstock rearing

A descriptive study was employed between June and September 2023 in Bintulu, Sarawak specifically at selected areas. Samples were collected throughout the area using a net. The collected samples were matured enough for breeding that was 10g and above for each. The samples were transported to Lab virology Complex Academy at the University Putra Malaysia campus Bintulu, Sarawak (UPMKB) and stock in a plastic container with continuous flow of aeration. All of the brood stocks were kept in a separate aquarium tank. The brood stocks were trained to feed with commercial pellet food. During the 14-day acclimatization period, the broodstock was housed in 1-liter containers for each and separate them according to their gender and species. The tanks were half filled with water and maintained at a Ph of 7.0, ammonia level of 0.0 ppm, 3 mg/l of dissolved oxygen, 0.1 mg/L of nitrite, 0-4ppm of nitrate, and a temperature of 25°C throughout the period. Fed with pellets twice a day (5% of body weight). Feeding was done twice daily using a floating commercial adult fish pellet (manufacturer) at a rate of 5% of body weight. The water was partially replaced every week, and chlorine was removed from the water by stock them in a 100-litre tank with aeration.

3.3 Hormone Preparation and administration

Spawning of wild brood stocks were injected using LHRH, hCG and Estradiol with Synthetic salmon gonadotrophin using 0.5mm syringe near the pectoral fin and the fishes were released in each tank where the ratio is 1 male for 1 female



Figure 3.3 shows the Syndel ovaprim (left), hCG (middle) and LHRH (right).

4 pairs of *Betta ibanorum* were tested for each hormone. The females were given full measurement of hormones while the males were given half of the dosages regulated to the females (Viveen et al., 1985). The females were injected estradiol 3 days before injecting Synthetic salmon gonadotrophin to make the eggs matured enough for stripping and the females were fed well with live feed such as rice prawns and worms.

Hormone	Male (ml/kg)	Female (ml/kg)
Synthetic salmon gonadotrophin	0.1	0.5
Estradiol (Female)	-	0.1
LHRH	0.1	0.2
hCG	0.1	0.2

Table 3.3 shows the dosage level of different types of hormones for male and female fish.



Figure 3.3 shows the method of injecting hormone near the pectoral fin under the scales

3.4 Gamete collection and *in -vitro* fertilization

After hormonal induction (7-12 h), the genital region was entirely cleaned with tissue to keep away from gamete tainting with excessive waste. Eggs were collected by applying slight strain to the mid-region and the sperm will be collected using a 2mm suction set. After assortment, the eggs will be dry treated in plastic dishes with pooled milt from hormonally actuated males. Then the sperm was gently mixed using chicken feather to fertilize the eggs. The fertilize eggs were transfer to an incubator that have slight aeration to keep the eggs flow up and down. Dead eggs were eliminated, and water was changed each 2 h. The eggs were monitored until hatching.



Figure 3.4 shows the incubator that was used during incubation process of the eggs.

3.5 Microscopic examination

Development stages were observed morphologically utilizing Leica Zoom 2000 Stereo microscope. Fertilized eggs were spread onto 9-cm Petri dishes. 10 embryos were inspected from treated eggs inside each Petri dish starting from the first 30 minutes after fertilizing the eggs. The eggs were observed and pictured every one hour until they hatch.

3.6 Data analysis

Photomicrographs of different stages of embryonic development were captured using a digital camera and presented descriptively. Comparisons between different types of hormones for *B. ibanorum* embryos was done by using morphological features of the gametes. The pH of water, genital papilla, collection of gametes, characteristics of eggs and the development of embryo.

CHAPTER 4

4.0 RESULTS

4.1 Identification of genital papilla of *B.ibanorum*

During the observation under Leica Zoom 2000 Stereo microscope, the matured female had a round shaped and reddish genital papillae, meanwhile the matured male had a triangle shaped and reddish genital papillae.



Figure 4.1 shows the *B.ibanorum* genital papillae of female (left) and male (right)

4.2 Effects on eggs using different types of hormones

In total 127 were captured in the surrounding of Borneo base and Nirvana forest that located in UPM Bintulu Sarawak Campus. For each hormone 3 pairs are selected according to their matured weight which is around 10 g. The fertility rate, average weight of samples and eggs were recorded after 6 trials of induced breeding method using three types of hormones (Table 4.2).

Hormones	Average weight of samples (g)	Average weight of eggs (g)	Fertility rate (%)
Synthetic salmon gonadotrophin with estradiol	12.785	28.67	0
LHRH	13.486	6.54	0
hCG	12.387	0	0

Table 4.2 shows the effects of different types of hormones on the production of eggs and fertility rate after 6 trials.

According to the results that have been shown in table 4.2, Synthetic salmon gonadotrophin with estradiol hormone is the best to use for induced breeding method for *B. ibanorum* species. Synthetic salmon gonadotrophin with estradiol produced high quality matured eggs compared to other hormones.

4.3 The collection of gametes

After receiving different types of hormone injection for 12 hours, the *betta ibanorum* gametes were extracted. Stripping method was used to extract the gametes by applying pressure on their belly and the sperm were extracted by using 1.3 mm catheter. A female had been produced a weight of 15g to 25 g of eggs. The most effective hormone is the Synthetic salmon gonadotrophin with estradiol because it produced matured eggs and good quality of sperm than hCG and LHRH. Hormone LHRH did produce some eggs but not matured enough for breeding: does not contain any sperm and hCG did not produce any eggs or sperm after 7 trials.



Figure 4.3 shows the result of the sperm (right) and eggs (left) during the stripping of Synthetic salmon gonadotrophin with estardiol.



Figure 4.3 shows the picture of unmatured eggs using the hormone LHRH

4.4 Characteristics of *B.ibanorum* eggs

The eggs were round in shape and bigger in size compare to other types of *Betta. Sp.* When the eggs are matured, the colour is light yellowish and when the eggs unmatured the colour is white. During stripping method applied, the female produced a white type of liquid similar like sperm that can be study in future researches. A female produced

approximately 20 to 30 eggs. The eggs are very sensitive because in this study some of the eggs got broke due to light pressure during handling.



Figure 4.4 shows the weight and the matured eggs of *B. ibanorum*



Figure 4.4 shows the unmaturing eggs of *B. ibanorum*

4.5 The comparison of embryonic development of *B. ibanorum* and *B. splendens*

Figure 10 summarises the observations on embryogenetic development of *Betta ibanorum* up to 24 hours post fertilization in the laboratory culture. It shows the failure of embryonic development during the first, second, third, fourth, and fifth attempts because of some factors. The expected embryogenesis development was shown in

figure 3.5.1 which is taken from other research study of same species which is *B.splendens*.

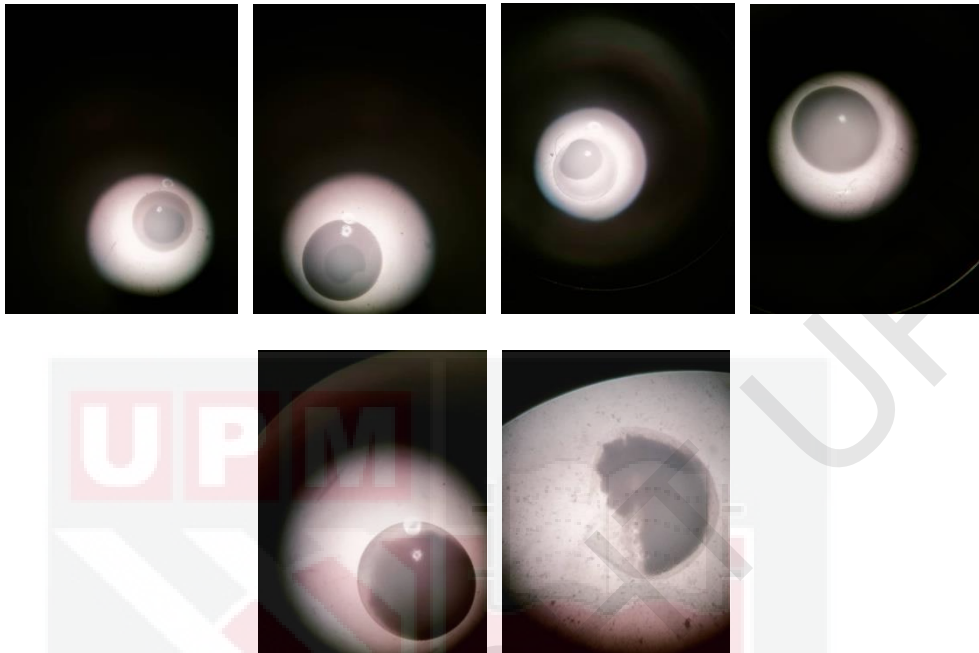


Figure 4.5 shows the failure of embryonic development of *B. ibanorum*

In the microscopic view of *B. ibanorum* eggs, the yolk and cytoplasm were visible. Some of the eggs were get ruptured because of pressure during stripping.

4.6 Comparison of pH level of sampling site with previous study

According to P. K. L. Ng, 1992, *B. ibanorum* had been found in stagnant ponds or peats that contains blackwater which had high acidity level. During this study, the sample collections were placed in two different locations that have stagnant and also flowing water. *B. ibanorum* had been found in both types of water that have different level of pH. The morphology of *B. ibanorum* had been the same eventhough there are from different types of water. The acidity was caused by the decompose of dead leaves from trees near the stagnant ponds.

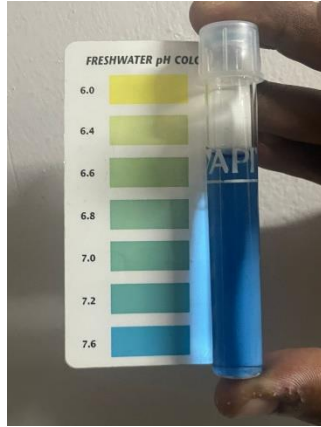


Figure 4.6 shows the pH level of two different types of water where the samples were collected. Neutral pH level (left) and acidic pH level (right)

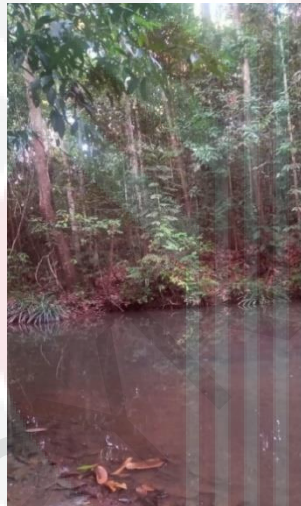


Figure 4.6 shows the two different types of location that have acid pH level of water (right) and neutral pH level of water (left)

CHAPTER 5

5.0 DISCUSSION

5.1 Housing and water parameters

It is essential to have high-quality water to maintain and grow healthy betta. When housed in low-quality water, betta are vulnerable to illness and disease, just like many other aquarium species. At concentrations above 0.25 ppm, ammonia, a byproduct of the decomposition of fish waste and uneaten food, has harmful effects on health (Madison, 2022).

5.2 pH and water salinity

Maintaining the health and well-being of *Betta splendens* depends critically on the ideal pH and salinity levels of their water. It's crucial to remember that betta fish are indigenous to Southeast Asian freshwater environments, especially those in Thailand, where they live in ponds, shallow streams, and rice paddies with slow-moving or stagnant water. Comprehending their native habitat is essential to emulating appropriate circumstances under confinement.

In general, *Betta sp.* do well in pH ranges from slightly acidic to neutral. Betta aquarium pH should be in the range of 6.5 to 7.5. The slightly acidic to neutral pH range of the water in their natural environments is matched by this range. *Betta sp.* fish immune system, metabolism, and general health all depend on maintaining the proper pH level (Hargreaves, 2006).

According to *Betta splendens* Care Guide. (n.d.), *Betta sp.* are freshwater animals, their ability to withstand salinity is restricted. The salt levels they face in their native habitats are minimal. Therefore, keeping Betta aquariums entirely freshwater is

often advised. It is generally not recommended to add salt to aquarium water because betta fish are not acclimated to brackish or salty environments.

5.3 Disease

In this study many problems had been faced because of disease. The disease that had been observed in this study were fungus attack, fin rot and fin clamp. Some of the *B. ibanorum* that were caught from wild were died after 3 days of acclimatization period because of fungal infection and fin clamp. Some of the broodstocks died because it cannot adopt to the new environment which is captive environment in lab. During the early stage of this study, half of the broodstocks were placed in a single plastic aquarium and another half in another plastic aquarium. When the breakout of fungal infection occurs it spreads fast enough to other broodstocks causes mortality because it is harder to cure eventhough by quarantine the broodstocks.

5.3.1 White Spot Disease (*Ichthyophthirius multifiliis*)

The symptoms of white spots disease are white cysts resembling salt grains on the skin, fins, and gills. Clamped fins, rapid gill movement and increased mucus production also another severe symptoms of white spot disease. The parasite has a three-stage life cycle, including a free-swimming stage (theronts) that infects fish, a trophont stage that forms cysts on the fish, and a tomont stage that produces daughter cells. This disease transmit through direct contact with infected fish or contaminated water. The management of this disease are increase water temperature gradually to speed up the life cycle of the parasite and use medications such as copper-based treatments, formalin, or malachite green. Isolate infected fish to prevent the spread of

the parasite. Improve water quality through regular water changes also helps to prevent this disease (Colorni, 1985).

5.3.2 Fin rot

When kept in inadequately clean water, betta fish get fin rot. Ammonia can accumulate in dirty water and burn a betta's fins. The fish's immune system may be weakened as a result of this stress. The weaker betta can then become infected by bacteria that grows in contaminated water. Fin rot is more likely to be caused by an abundance of aquarium bacteria than by a single dangerous species of bacteria. As long as your aquarium is cleaned on a regular basis, microorganisms are usually harmless. But, if betta has an injury of any kind perhaps he tore his tail off an aquarium ornament, for instance this can increase the fish's vulnerability to bacterial infections and fin rot.

If the fish is kept in good condition and given proper care, there is very little likelihood of a small tear or wound developing into fin rot. The fish's immune system will not deteriorate and make it more vulnerable until it experiences stress, which can happen as a result of bad care or unfavorable tank settings (Colorni, 1985).

5.3.3 Fin clamp

Poor water quality is the main contributing factor to betta fish having clamped fins. Bad water quality can cause betta fish to have a variety of additional issues, some of which can result in betta clamping its fins. The oxygen content of betta fish can be reduced, ammonia, nitrite, nitrate, and phosphate levels can rise, and bacterial and parasite illnesses can spread when they are overfed or housed in unclean water.

Betta fish prefer a water pH that's neutral or slightly acidic, with a range between 6.5 to 7 and a hardness of between 5-20 dGH. A betta that is unhappy with the chemistry of its water may exhibit distress by clamping its fins and becoming less active. Both of these disorders have the potential to become fairly harmful. Water becomes too hard and alkaline betta's skin will become irritated and likely see them itching themselves on the aquarium rocks and gravel. Both of these conditions can become quite serious, and a betta that is unhappy with its water chemistry may show distress by clamping its fins and becoming less active (Colorni, 1985).

5.4 Captived environment

The shape and size of the tank possess a major effect on the reproduction behaviour of mouthbrooding fish, as *Betta Ibanorum*, which uses a distinctive reproductive strategy in where one parent, generally the male, develops eggs that are fertilised in their mouths until the eggs develop. For this kind of fish, having a sufficient-sized aquarium is crucial for the reason that it promotes the development of domains and attracting practices, thereby minimising aggressiveness throughout the breeding process. Territorial aspects that mimic ecosystems in nature while offering secure areas for mating pairings to hide, including caves, stones, or other places to hide, are essential elements of the aquarium setting. In addition, the dimensions of the tank influence the way *Betta Ibanorum* get along with their tank mates; bigger aquariums are more capable to handle multiple breeding couples.

A well-organized tank enables the preservation of consistent water quality, the monitoring of breeding behaviours, and the capacity to take action when required, may enhance the breeding process as well as the well-being of fishes. However, (Craig Watson et al., 2019) mentioned that, as long as the quality of the water, eating habits,

along with other health-related parameters are effectively handled, male bettas do perform well in tiny containers, although inconsistent recommendations in the scientific and enthusiast books.



CHAPTER 6

CONCLUSION

This research contributes significantly to our knowledge of the *Betta ibanorum* breeding strategy and induced breeding behaviour. Given the failure of breeding attempts on *Betta ibanorum*, a common betta species in Malaysia, further investigation is needed to address factors inhibiting embryogenesis in studies. Even though the the eggs did not fertilize, Synthetic salmon gonadotrophin with estradiol succeed in producing high number of matured gametes compared to the other two tpes of hormones. By optimising the breeding process for *Betta.sp*, researchers in the future may be able to enhance indicators of performance including hatching rate, larvae viability, and overall growth and survival rates.

REFERENCES

- Betta splendens (Siamese Fighting Fish) - Care Guide. (n.d.). Retrieved from Practical Fishkeeping: <https://www.practicalfishkeeping.co.uk/fishkeepinganswers/freshwater/siamese-fighting-fish-care-guide/>
- Bronstein PM. 1982. Breeding, paternal behavior, and their interruption in *Betta splendens*. *Animal Learning & Behavior* 10:145–151. DOI: 10.3758/BF03212262.
- Colomi, A. (1985). Fish diseases caused by Protozoa. *CRC Critical Reviews in Microbiology*, 12(2), 87-118.
- Craig Watson, Matthew DiMaggio, Jeffrey Hill, Quenton Tuckett, and Roy Yanong. (2019, April 1). *FA212/FA212: Evolution, Culture, and Care for Betta splendens*. Ask IFAS –Powered by EDIS. Retrieved January 7, 2024, from <https://edis.ifas.ufl.edu/publication/FA212>
- Fernanda Nogueira Valentin, Ferreira, N., Cristina, Fernandes, J. B., Luiz Gustavo Giannecchini, & Satiko, L. (2015). *Early development of Betta splendens under stereomicroscopy and scanning electron microscopy*. 23(2), 247–256. <https://doi.org/10.1017/s0967199413000488>
- Forsatkar MN, Nematollahi MA, Amiri BM, Huang W-B. 2014. Fluoxetine inhibits aggressive behaviour during parental care in male fighting fish (*Betta splendens*, Regan). *Ecotoxicology* 23:1794–1802. DOI: 10.1007/s10646-014-1345-0.
- Ghosh, A. K., Biswas, S., Sarder, L., Sabbir, W., & Rahaman, S. M. B. (2022). Induced breeding, embryonic and larval development of Koi carp (*Cyprinus carpio*) in

Khulna, Bangladesh. *Mesopotamian Journal of Marine Sciences*, 27(1), 1–14.

<https://doi.org/10.58629/mjms.v27i1.167>

Giam, X., Koh, L. P., Tan, H. H., Miettinen, J., Tan, H. T., & Ng, P. K. (2012). Global extinctions of freshwater fishes follow peatland conversion in Sundaland. *Frontiers in Ecology and the Environment*, 10(9), 465–470.

<https://doi.org/10.1890/110182>

Goldstein, R. J. (2015). *The Betta Handbook*. Sourcebooks, Inc.

Grinang, J. (1970). Fishes and Macroinvertebrates of Padawan Limestone, Sarawak, Malaysia. *Borneo Journal of Resource Science and Technology*, 3(2), 1–14.

<https://doi.org/10.33736/bjrst.246.2013>

Hargreaves, J. A. (2006). Chemistry and fish toxicology. In: *Chemistry and Biology of Water, Air and Soil* (pp. 189-213). CRC Press.

Hui, T., & Grinang, J. (2020). *Parosphromenus barbarae*, a new species of *Licorice Gourami* from Sarawak, Borneo (Teleostei: Osphronemidae).

<https://doi.org/10.26049/VZ70-3-2020-07>

IUCN. 2020. The IUCN Red List of threatened species. Version 2020-1. Available: <https://www.iucnredlist.org>.

Radha Chaube, Singh, R. P., & Joy, K. P. (2014). *Effects of ovaprim, a commercial spawning inducer, on vasotocin and steroid hormone profiles in the catfish *Heteropneustes fossilis*: In vivo and in vitro studies*. 195, 190–200.

<https://doi.org/10.1016/j.ygcen.2013.11.009>

KAMAL, N. S. S., TAN, H. H., & NG, C. K. C. (2020). *Betta nuluhon*, a new species of fighting fish from western Sabah, Malaysia (Teleostei:

Osphronemidae). *Zootaxa*, 4819(1),187–194.

<https://doi.org/10.11646/zootaxa.4819.1.11>

Lim, S. H., Koh, Y. K., Chang, B. S., & Kim, D. H. (2014). Spermatogenesis of Siamese Fighting Fish, *Betta splendens*, Osphronemidae, Teleostei. *Applied Microscopy*, 44(1), 1–7. <https://doi.org/10.9729/am.2014.44.1.1>

Linke, H., 1991. Labyrinth fish. The bubble-nest-builders. Their identification, care and breeding. Tetra-Press (English edition), Melle, 174 pp.

Marte, C. L. (1989). *Hormone-induced spawning of cultured tropical finfishes*. 519–539.

Morisson, P., Ismail, Z., Asilah Abdul Mutalib, Ismail, F., Man, & Zahidah Ab Latif. (2020). *Induction Of Ovulation Using Ovaprim And Its Impact On Reproductive Parameter Of Clarias gariepinus*. <https://doi.org/10.12928/joves.v3i2.2931>

Nur, F. M., Batubara, A. S., Fadli, N., Rizal, S., Siti-Azizah, M. N., & Muchlisin, Z. A. (2022). Diversity, distribution, and conservation status of Betta fish (Teleostei: Osphronemidae) in Aceh waters, Indonesia. *The European Zoological Journal*, 89(1), 135–144. <https://doi.org/10.1080/24750263.2022.2029587>

Panijpan, B., Kowasupat, C., Laosinchai, P., Ruenwongsa, P., Phongdara, A., Senapin, S., Wanna, W., Phiwsaiya, K., Kühne, J., & Fasquel, F. (2014). Southeast Asian mouth-brooding Betta fighting fish (Teleostei: Perciformes) species and their phylogenetic relationships based on mitochondrial COI and nuclear ITS1 DNA sequences and analyses. *Meta Gene*, 2, 862–879. <https://doi.org/10.1016/j.mgene.2014.10.007>

- Rüber, L., Britz, R., Tan, H. H., Ng, P. K. L., & Zardoya, R. (2004). EVOLUTION OF MOUTHBROODING AND LIFE-HISTORY CORRELATES IN THE FIGHTING FISH GENUS BETTA. *Evolution*, 58(4), 799–813. <https://doi.org/10.1111/j.0014-3820.2004.tb00413.x>
- Tan HH, Ng PK. 2005. The fighting fishes (Teleostei: Osphronemidae: Genus Betta) of Singapore, Malaysia and Brunei. *Raffles Bulletin of Zoology* 13:43–99.
- Vahid Zadmajid, Sune Riis Sørensen, & Ian A.E. Butts. (2019). *Embryogenesis and early larval development in wild-caught Levantine scraper, Capoeta damascina (Valenciennes, 1842)*. 280(1), 133–148. <https://doi.org/10.1002/jmor.20926>
- WoRMS - World Register of Marine Species - *Betta ibanorum* Tan & Ng, 2004. (n.d). www.marinespecies.org. Retrieved June 12, 2023, from <https://www.marinespecies.org/aphia.php?p=taxdetails&id=1009453>
- Yayuk Astriani, & Khairul Khairul. (2022). Biodiversity Of Fish Family Osphronemidae In Swamp Waters In Bagan Bilah Village, District Of Panai Tengah Labuhanbatu Regency. 20(1), 8–8. <https://doi.org/10.19184/bioedu.v20i1.28344>

APPENDICES



(A) *Betta ibanorum*



(B) *Betta ibanorum*



(C) *Betta ibanorum*



(D) *Betta ibanorum*



(E) *Betta ibanorum*

Appendix 6.1. Weight of broodstock (g)



(F) Fin rot



(G) White spot disease



(H) Fin clamp

Appendix 6.2 Disease in *Betta ibanorum*



(I) Sampling site



(J) Sampling site

Appendix 6.3 sampling site at UPM Bintulu Sarawak campus



Appendix 6.4 Captive conditions



Appendix 6.5 Microscopic examination