UNIVERSITI KEBANGSAAN MALAYSIA *The National University of Malaysia*	UKM-UKMAEC-BO08	No. Semakan: 03	Tarikh Kuatkuasa: 09/12/2021
	BORANG PERMOHONAN KATEGORI AKUATIK (AQUATIC *CATEGORY APPLICATION FORM*)		

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1. PEMOHON (PENYELIDIK UTAMA)
APPLICANT (LEAD RESEARCHER)

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2. PROJEK PENYELIDIKAN
RESEARCH PROJECT

A.	TAJUK *TITLE*	Comparison of communal and separate rearing of families in a Catla catla genetic improvement population		
B	NO GERAN *GRANT NO.*	https://www.fishinnovationlab.msstate.edu/research/projects/advancing-aquaculture-systems-productivity-through-carp-genetic-improvement		
C.	INSTITUSI PEMBIAYA DANA *FUNDING INSTITUTION*	Fish Innovation Lab through WorldFish (Penang, Malaysia).		
D.	TEMPOH KAJIAN *DURATION OF STUDY*	MULA *START*	(Bulan/*Month*) FEB	(Tahun/*Year*) 2023
		TAMAT *END*	(Bulan/*Month*) JAN	(Tahun/*Year*) 2024

3. PENYELIDIK BERSAMA DAN KAKITANGAN YANG TERLIBAT (termasuk kakitangan makmal)
CO-RESEARCHERS AND STAFFS INVOLVED (including laboratory staffs)

Bil. *No.*	**Nama dan Alamat** *Name and Address*	**Kelayakan** *Qualification*	**Jawatan** *Position*	**Telefon & Emel** *Telephone &Email*
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4. PELAJAR
STUDENTS

Bil. *No.*	**Nama dan Alamat** *Name and Address*	**Program** *Program*	**Telefon & Emel** *Telephone &Email*
1.		☐ Prasiswasah *Undergraduate* ☐ Pascasiswazah *Postgraduate*	
2.		☐ Prasiswasah *Undergraduate* ☐ Pascasiswazah *Postgraduate*	
3.		☐ Prasiswasah *Undergraduate* ☐ Pascasiswazah *Postgraduate*	

4.		☐ Prasiswasah *Undergraduate* ☐ Pascasiswazah *Postgraduate*	
5.		☐ Prasiswasah *Undergraduate* ☐ Pascasiswazah *Postgraduate*	

BAHAGIAN 1: GAMBARAN MENYELURUH KAJIAN
SECTION 1: OVERVIEW OF STUDY

1.1 Latar belakang ringkas dan kewajaran projek (Tidak melebihi 300 perkataan)
Brief background and justification of study (Not more than 300 words)

WorldFish aims to increase aquaculture productivity and human wellbeing in Bangladesh through its rohu, catla and silver carp genetic improvement programs. These programs use a family-based selective breeding approach, which allows rapid genetic improvement, while controlling inbreeding and maintaining genetic variation in its breeding population.

Currently WorldFish nurses – from spawning until fish are large enough to be tagged with passive integrated transponders – each carp family in a separate un-replicated hapa (i.e.net). Common environmental effects resulting from separate nursing of full-sibling families are difficult to quantify, are difficult to account for in genetic analysis and potentially result in suboptimal responses to selection (i.e. genetic gains). An alternative, and potentially superior approach to nursing families in hapas, is to communally rear fish, obtain tissue samples at the time of tagging and reconstruct the pedigree (i.e. assign parentage) of each fish using molecular genetic markers. WorldFish is currently developing Single Nucleotide Polymorphism (SNP) panels for this purpose. Communal early rearing (CER) in combination with pedigree reconstruction allows families to be kept together from very early in life, avoiding or reducing common environmental effects associated with separate early rearing (SER). Furthermore, it can allow the number of families (including half-sibling families) to be increased without a need for additional facilities, thus increasing the precision of genetic parameter estimates (e.g. additive and non-additive genetic variances, heritabilities and genetic correlations), selection accuracy and ultimately response to selection (Ninh et al., 2011).

1.2 Adakah kajian yang serupa sedang/pernah dijalankan oleh penyelidik atau penyelidik lain? (Jika ya, sila berikan butiran dengan kata kunci (maksimum 3 penerbitan penuh) perlu dikemukakan bersama-sama borang permohonan)
Have similar studies been undertaken by you or others currently /previously? (If yes, give details with key references (maximum of 3 full publications) must be submitted together with the application form)

Results from this study will be specific to WorldFish Genetically Improved Catla and the SNP panel used for parentage assignment. However, similar studies have been conducted in other species and breeding populations (Ninh et al., 2011).

1.3 Matlamat kajian (Senaraikan matlamat umum dan khusus)
Study objectives (List general and specific objectives)

The objective of the study is to validate the use of a catla SNP panel for parentage assignment and compare progeny test results using communal early rearing (CER) and separate early rearing (SER) methods on Generation 1 WorldFish Catla Genetic Improvement Program families.

Specific objectives are to determine if:

- individuals can accurately and unambiguously be assigned using SNP markers
- pools of two samples from different catla individuals allow accurate and unambiguous parentage assignment
- equal numbers of individuals per family are present at tagging under CER
- the genetic correlation between CER harvest weight and SER harvest weight equals one

1.4 Hipotesis kajian
Study hypothesis

The null hypotheses to be tested are:

- Parentage of catla individuals can accurately and unambiguously be assigned using SNP markers (maximum likelihood method of assignment applied to SER fish) (Hamilton, 2021),
- Parentage of catla pools of two individuals can accurately and unambiguously be assigned using SNP markers (maximum likelihood method of assignment applied to SER fish) (Hamilton, 2021),
- There are equal numbers of individuals per family at tagging (within spawn runs) under CER (chi square test), and
- The genetic correlation between CER harvest weight and SER harvest weight equals one (likelihood ratio test) (Gilmour et al., 2014; Hamilton et al., 2022).

1.5 Berikan ringkasan rekabentuk kajian menggunakan CARTA ALIR (Sila masukkan kumpulan, spesies, bilangan dan umur haiwan yang digunakan, kaedah, tempoh kajian dan parameter-parameter kajian).

Provide a summary of the study design using a FLOWCHART. (Please include the, study parameters) groupings, species, number and age of animal used, methodology, duration of experiments

All fish used in this trial form part of the WorldFish Catla Genetic Improvement Program population and will be progeny tested and have their data analyzed as part of routine genetic improvement activities.

The 2022 catla spawning was difficult. Few candidate parents were in ready-to-spawn condition at time of spawning and some of those that were yielded very few, if any, viable gametes. Ultimately 81 catla full-sib families (of the targeted 120) were produced across two spawn runs in May-June 2022. These families were made and maintained in separate upwelling hatching jars with mesh at the top to prevent the loss of eggs (i.e. one family per jar). Approximately 3 days post hatching, hatchlings were be transferred from jars to be nursed. Forty of these families were nursed using two methods: separate early rearing (SER) in hapas (i.e. nets) and communal early rearing (CER) in small earthen ponds.

Separate early rearing represents the current nursing method adopted by WorldFish, whereby 1.5 g of hatchlings from each family is stocked into a single hapa – this represents approximately 1200 individuals per hapa (5.2 x 1.8 x 0.9 m) at 142 / m3 density. For the SER treatment, initially each hapa was constructed of saree cloth (~300 µm mesh) and mesh size was progressively increased during nursing – initially to mosquito netting (~1.4 mm) and ultimately to polyfilament netting (~5 mm). Hapas were cleaned at least every two weeks to ensure the movement of water and plankton into and out of hapas.

Once fish reached a size (~15 g) suitable for tagging with passive integrated transponder (PIT) tags, 50 individuals from each hapa were targeted for tagging, as per WorldFish Genetic Improvement Program standard operating procedures. In total, 2920 fish from 75 families were tagged and placed in grow out ponds between 24 Oct and 1 Nov 2022. Fish were tagged according to WorldFish standard operating procedures (WorldFish, 2019a) – fish were anesthetized with clove oil, a small incision made, a PIT tag inserted into the body cavity and fish monitored during recovery.

For the purpose of this study, communal early rearing involved the pooling of equal quantities of hatchlings from 32 families from each spawn run (64 total). Each of the two pools of were initially reared in small earthen ponds (160 and 200 m^2). Equal quantities of fry from each of these ponds were subsequently transferred to a single pond for the extent of the nursing period. 1213 of these fish were ultimately tagged on 7-8 Nov 2022 using the same procedure as above.

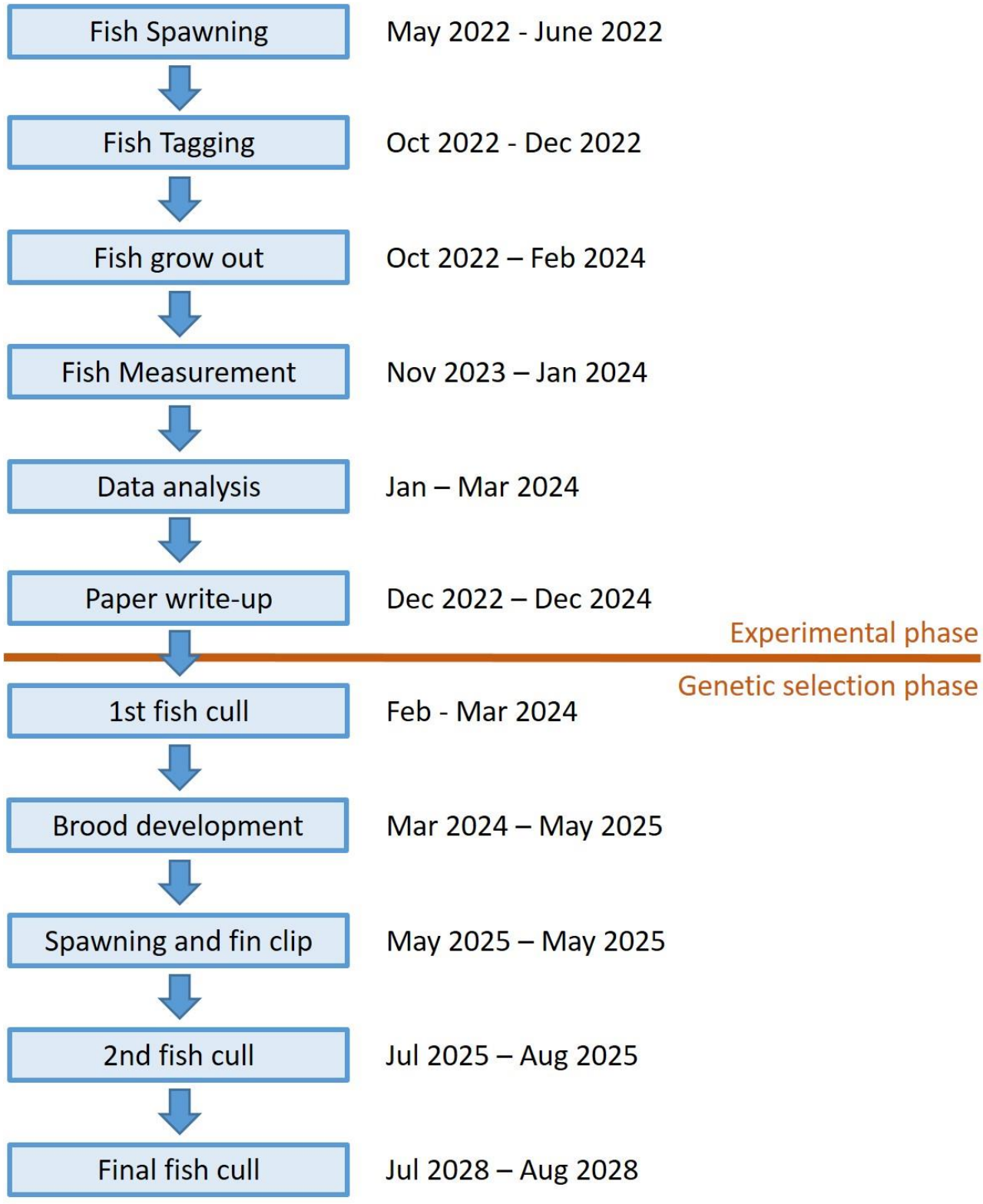
At the time of tagging, tissue samples (i.e. fin clips) were obtained according to WorldFish standard operating procedures (WorldFish, 2019b), with fin clips taken using a 2-mm-diameter hole punch. Fin clips were obtained using a hole punch rather than scissors as past experience revealed that staff sampling fish with scissors were prone taking a larger-than-required sample. A hole punch provide a consistently sized sample that is sufficient for DNA extraction and unlikely to be spoiled with long-term storage in our standard sample tubes. One fin clip was taken from all fish in the CER treatment and two fin clips was taken from 5 individuals per family in the SER treatment. Tissue samples from the SER treatment are necessary to validate methods of parentage assignment (Hamilton, 2021). Fin clips will be genotyped and Single Nucleotide Polymorphism (SNP) data used to assign parentage to individual and pooled samples.

Tagged fish are currently being out in ponds under biculture with silver carp (4 ponds) as part of routine WorldFish Carp Genetic Improvement Program progeny testing. Silver carp in grow out ponds were nursed and tagged according the same SER method adopted for catla. Tagged silver carp are also being grown under monoculture (4 ponds), as part of routine WorldFish Carp Genetic Improvement Program progeny testing. At ~18 months of age, all catla and silver carp will be assessed for harvest-age traits using waterproof digital scales (body weight) and a length board (standard length, body depth and body width) (WorldFish, 2019c). Using harvest-age measurement data, known pedigrees and parentage assignment data, analyses will be undertaken test hypotheses and to select candidate parents (Section 1.4).

Post harvest-age assessment and data analysis, approximately 20% of fish will be retained as candidate parents of the next generation of catla. Other fish will be humanely euthanized, according to the WorldFish Euthanasia Standard Operating Procedure (acceptable methods detailed in this SOP are anesthetic overdose, pithing, and percussive stunning) (WorldFish, 2019d). PIT tags will be retrieved post-euthanasia and, where possible, euthanized fish will be sold for human consumption.

This trial is being conducted at the WorldFish Carp Genetic Improvement Facility and WorldFish staff monitor fish behavior for indicators of stress (e.g. gaping, moving at the surface, jumping) daily. If handling of fish is deemed necessary to monitor health status and or growth, this will be undertaken in accordance with the WorldFish 'Handling of

Finfish', 'Sedation and Anaesthesia' and 'Weight and Length Measurement' Standard Operating Procedures (WorldFish, 2019c; d; e).



1.6 Kewajaran penggunaan haiwan.
Justification of using animals

The use of the catla for this experiment is justifiable in accordance with The Principles of Humane Experimental Techniques (Flecknell, 2002):

Replacement

- No non-sentient alternative can be used to replace the catla and still achieve the study objectives (see section 1.3).

Reduction

- All fish used in this trial will form part of the WorldFish Catla Genetic Improvement Program population and will be progeny tested and have their data analyzed as part of routine genetic improvement activities. To achieve genetic gain in such programs – requiring the estimation of breeding values to identify superior individuals (i.e. candidate parents of the next generation) – it is necessary to test a large number of individuals. The greater the number of individuals tested, the greater the selection intensity applied in the selection of new parents and the greater the genetic gains achieved in the next generation (Falconer and Mackay, 1996; Gjedrem and Baranski, 2009). However, the 'law of diminishing returns' applies – doubling the number of individuals tested does not result in double the genetic gains. Accordingly, WorldFish has adopted 50 individuals per family as its standard for progeny testing to achieve a high level of genetic gain without requiring the use of an excessive number of animals.

Refinement

- All staff involved in spawning, overseeing the on-farm grow out and sampling the catla at harvest are trained and competent in fish husbandry and handling.
- The number of individuals per family tagged for from the CER has been limited to 1213 fish from 64 families (~19 per family *c.f.* the targetted 50 per family for the standard SER treatment). This will allow estimation of genetic correlations to determine if CER can be adopted in place of SER.
- Fish will be humanly euthanized and, where possible (i.e. of sufficient size and quality, after tag removal), sold for human consumption.

1.7 Potensi manfaat kajian.
***Potential benefits of study*.**

☐ Meningkatkan kefahaman mengenai kesihatan haiwan/manusia
Increasing understanding of animal / human health

☐ *Pengekalan/penambahbaikan kesihatan haiwan/manusia*
Maintaining / improving animal / human health

☒ Penambahbaikan pengurusan haiwan
Improving animal management

☒ Lain-lain (sila nyatakan): *Others (please specify):*	Routine adoption of CER and parentage assignment using molecular markers has the potential to improve the precision of genetic parameter estimates and response to selection (i.e. genetic gains) (Ninh et al., 2011) in the WorldFish Catla Genetic Improvement program.

BAHAGIAN 2: MAKLUMAT HAIWAN KAJIAN
SECTION 2: INFORMATION ON EXPERIMENTAL ANIMALS

2.1 Jenis haiwan akuatik.
Types of aquatic animals.

	Jenis haiwan/*Animal type*	Nyatakan nama saintifik/*State the scientific name*
☒	Pisin *Piscine*	e.g. Tilapia *(Oreochromis niloticus)* *Catla catla* (grown out in biculture with *Hypophthalmichthys molitrix*)
☐	Krustasia *Crustacean*	e.g. Prawn (*Macrobrachium rosenbergii*)
☐	Amfibia *Amphibian*	e.g. Frog (*Rana temporaria*)
☐	Celonia *Chelonian*	e.g. Terrapin (*Malaclemys terrapin*)
☐	Setasia *Cetaceans*	e.g. Dolphin (*Sousa chinensis*)
☐	Lain-lain/*Others*	

2.2 Adakah sebarang permit diperlukan untuk menangkap, mengguna, memusnah atau melepaskan haiwan yang dilindungi atau haiwan transgenik? Jika ya, sila sertakan dokumen berkenaan.
Is there any permit that must be obtained for the capture, used, destruction or release of protected or transgenic animals?
If yes, please provide the necessary document(s).

☐ PERIKANAN/*FISHERY*
☐ UKM-IBC/*UKM-IBC*

2.3	**Peringkat hidup/umur** ***Life stage/Age***	WorldFish Genetically Improved catla and silver carp
2.4	**Berat/ Ukuran panjang badan** ***Weight/Body length***	10 g to ~18 months of age, weighing up to 2 kg

2.5 Bilangan haiwan
Number of animals

	Jantan *Male*	Betina *Female*	Jumlah *Total*
Generation 1 WorldFish Genetically Improved Catla (SER)	~1460	~1460	2920
Generation 1 WorldFish Genetically Improved Catla (CER)	~605	~606	1213
Silver carp reared in biculture with catla and monoculture. Silver carp represent Generation 2 WorldFish Genetically	~5156	~5156	10312

	Improved fish (9351 from 227 families) and new introductions obtained from the Bangladesh Department of Fisheries (961).			
	Jumlah *Total*	2066 catla 5156 silver carp	2077 catla 5156 silver carp	4133 catla 10312 silver carp

2.6 Sumber haiwan
Source of animals

☒ Institusi tempatan lain (Sila nyatakan)
Other local institution (Please specify): WorldFish Carp Genetic Improvement Program – Jeshore, Bangladesh

☐ Diimport
Imported

Syarikat pengimport
Importing company: _____

Negara asal
Country of origin: _____

SILA SERTAKAN SALINAN SIJIL KESIHATAN DAN PERMIT IMPORT APABILA MENDAPAT KELULUSAN.
PLEASE SUBMIT A COPY OF HEALTH CERTIFICATE AND THE IMPORT PERMIT UPON APPROVAL.

2.7 Penyesuaian dan rawatan profilaksis haiwan sebelum eksperimen
(Peralihan haiwan dari sumber ke tempat baru/kajian)
Acclimatisation and prophylactic treatment of the animals before the experiment
(Transitioning of the animals from the source to new/experiment setting)

2.7.1 Bilangan hari/*Number of days*: None, fish are coming from the internal Worldfish Carp Genetic Improvment Program population.

2.7.2 Rawatan/*Treatment*: _____

☐ Metilena biru/*Methylene blue*
☐ Air garam/*Salt water*
☐ Malakit hijau/*Malachite green*
☐ Akriflavin/*Acriflavine*
☐ Povidone iodine
☒ Others/lain-lain____None_____

2.8 Penempatan haiwan
Housing of animals
2.8.1

☒ Kolam
Pond

Jenis *Type*: ☒ Tanah *Land* ☐ Simen *Cement* ☐ Lain-lain (Nyatakan): *Others (Specify):* Fresh water earthen ponds for both nursing and grow out.

☐ Tangki
Tank

	Jenis *Type*	☐ Gentian *Fiber* ☐ Akuarium *Acquarium* ☐ Lain-lain (Nyatakan): *Others (Specify):*		
	Pelitup dasar kolam/tangki *Pond/tank's bottom lining*	☐ Ya/*Yes* ☒ Tidak/*No*	Sila nyatakan jika ya *(Please indicate if yes)*	
	Saiz/Isipadu/Kapasiti (contoh m^3/10 ton) *Size/Volume/Capacity (e.g. m^3/10 ton)*	See section 2.8.4		
	Sistem pengaliran air *Water flow system*	☐ Edaran semula/*recirculating* ☒ statik/*static* ☐ penapisan dalaman/*internal filteration* ☐ aliran terus/*flow-through* ☐ lain-lain/*others:* _____		
	Sistem disinfeksi *Disinfection system*	☐ bahan kimia/*chemical agent*, nyatakan/*state*: _____ ☐ sistem filterasi/*filtration system* ☐ sistem ultralembayung/*UV system* ☐ ozon/*ozone*		

2.8.2	Kepadatan pelepasan *Stocking density*	~1.1 fish per m^2
2.8.3	Sumber air *Source of water*	☐ air sungai (*river water*) ☐ air laut (*sea water*) ☐ air paip (*tap water*) ☐ lain-lain/*other*, nyatakan/*state*: __Ground water

2.8.4 Lokasi kolam/tangki
Location of the pond/tank

Jashore, Bangladesh

Pond	Pond Area (m2)	Feeding Treatment	Catla (SER)	Catla (CER)	Silver	Total
TAL_07	2064	Low input	0	0	2026	2026
TAL_08	1295	High input	508	210	550	1268
TAL_14	1902	Low input	0	0	1866	1866
TAL_15	2347	Low input	920	382	996	2298
TAL_18	1942	Low input	0	0	1905	1905
TAL_19	1335	Low input	524	218	572	1314
TAL_20	1376	Low input	0	0	1350	1350
TAL_21	2468	High input	968	403	1047	2418
	14729		**2920**	**1213**	**10312**	**14445**

Low input = Mustard oil cake and fertiliser
High input = commercial feed, mustard oil cake and fertiliser

2.9 Makanan haiwan
Animal feed

2.9.1 Sumber makanan
Source of feed

Jenis
Type

☒ Komersial
Commercial

☐ Tempatan
Local

Nama pengeluar
Name of manufacturer

☐ Diimport
Imported

See Section 2.8.4

Two feeding treatments will be applied – low input and high input (see Section 2.8.4 for details). The low input treatment reflect historic and current feeding regimes applied by poor small-scale farmers in Bangladesh. The high input treatment reflect practices increasingly adopted in Bangladesh as aquaculture production is intensified.

Fermented mustard oil cake (MOC): fermented residue from commercial mustard oil or meal production. Up to 1.2 g / m2 / week (50 g / decimal / week) will be used.

Apply urea at a rate of 2 g / m2 / week (81 g / decimal / week) and triple superphosphate at a rate of 2 g / m2 / week (81 g / decimal / week). The rate of fertiliser application will be increased if Secchi disc visibility is greater than 30 cm.

MOC and fertiliser promote natural feed growth (e.g. plankton)

Commercial feed will be Mega Pre Starter

Adakah makanan mengandungi sumber porsin?
Does the feed contains porcine ingredients?
☒ Tidak/*No*
☐ Ya/*Yes*
☐ Tiada maklumat/*No information*

☐ Formulasi sendiri
Self-formulated

Senaraikan bahan-bahan utama dalam formulasi
List the major ingredients in the formulation

Mega Pre Starter
PROTEIN (%) 33 (min)
CARBOHYDRATE (%) 28 (max)
FAT (%) 7 (min)
FIBRE (%) 6 (max)
ASH (%) 18 (max)
CALCIUM % 2 (max)
PHOSPHORUS (%) 0.6 (min)
MOISTURE (%) 12 (max)

☐ Makanan hidup/*Live feed*, nyatakan/*state*:	Natural food production – phytoplankton, zooplankton etc.

2.9.2 Kekerapan pemberian makanan
Frequency of feeding

☒ Setiap hari / *Daily*
☐ Dua kali sehari / *Twice daily*
☐ Tiga kali sehari / *Three times daily*
☐ *Ad libitum* / *Ad libitum*

2.9.3 Nyatakan berat makanan berdasarkan peratus berat badan haiwan (%)
(Contoh 2 % daripada berat badan haiwan sehari)
State the weight of the food based on the percentage of body weight of the animals (%)
(E.g. 2 % of body weight of the animals per day)

1-3% of body weight per day depending on age/size

2.10 Kualiti air
Water quality

2.10.1 Kekerapan menguji kualiti air
Frequency of testing water quality

☐ Harian / *Daily*
☐ Mingguan / *Weekly*
☒ Lain-lain (Nyatakan): / *Others (Specify)* — *1-4 weeks for individual ponds. Parameters gauged by measurements in adjacent ponds.*

2.10.2 Parameter kualiti air yang diuji dan nyatakan ketetapan nilai
Water quality parameters tested and state the cut-off values

Parameter / *Parameter*	Ketetapan nilai / *Cut-off value*
☒ Oksigen terlarut / *Dissolved oxygen (ppm)*	<2.0 mg / L
☒ Suhu / *Temperature (°C)*	>30 °C

☒ pH *pH*	>9
☒ Ammonia (ppm) *Ammonia (ppm)*	0.8-1.0 TAN
☐ Nitrat (ppm) *Nitrate (ppm)*	Not routinely measured
☐ Nitrit (ppm) *Nitrite (ppm*)	Not routinely measured
☐ Saliniti *Salinity (ppt)*	Not routinely measured

Sila berikan sebab jika tidak perlu dilakukan
Please provide reasons if this is not necessary

WorldFish has managed fish at the site for a number of years and has not observed issues with respect to these parameters. Additional emergency water quality testing will be conducted in the case of an algal bloom or phytoplankton crash, a sudden weather change, unusual behaviour of fish, an outbreak of fish disease, or as deemed necessary by experienced staff.

2.11 Kitar pencahayaan
Lighting cycle

☒ Cahaya semula jadi
Natural light

☐ Cahaya terkawal (sila nyatakan dalam jam/hari): *Controlled light (please specify in hours/day):*	

BAHAGIAN 3: TATACARA DALAM HAIWAN
SECTION 3: PROCEDURES IN ANIMALS

Sila tandakan [√] dalam kotak yang bersesuaian. Biarkan kosong jika tidak berkaitan.
Please tick [√] in the necessary boxes. Leave blank if not applicable.

3.1 Tatacara bukan pembedahan
Non-surgical procedures

3.1.1 Tatacara (Nyatakan dengan terperinci)
Procedures (Specify in detail)

☐ Suntikan bahan ujian
Injection of test substance

Isipadu bahan ujian *Volume of test substance*	
Kekerapan suntikan *Frequency of injection*	
Cara suntikan *Route of injection*	

☐ Pensampelan darah
Bleeding of animal

Isipadu darah *Volume of blood*	
Kekerapan pensampelan darah *Frequency of bleeding*	
Cara pensampelan darah *Route of bleeding*	

☒ Biopsi
Biopsy

Jenis *Type*	Fin clips
Saiz sampel biopsi *Size of biopsy sample*	Refer to WorldFish Standard Operating Procedures
Kekerapan *Frequency*	Once after nursing

☐ Gavaj oral
Oral gavage

Isipadu bahan ujian *Volume of test substance*	
Saiz jarum gavaj *Size of gavage needle*	
Panjang jarum gavaj *Length of gavage needle*	
Kekerapan gavaj *Frequency of gavage*	

	☐ Ubahsuaian tingkahlaku *Modification of behaviour*	
	☐ Aruhan tumor *Induction of tumours*	
	Agen/Bahan ujian karsinogenik *Carcinogenic agent/test substance*	
	Dos *Dose*	
	Kekerapan *Frequency*	
	Tempoh *Duration*	
	Langkah keselamatan (penyelidik) *Precautions measures (researchers)*	
	☐ Lain-lain (Sila lihat Bahagian 3.4) *Others (Please see Section 3.4)*	
3.1.2	Kaedah pengekangan haiwan (Nyatakan) *Method of animal restraint (Specify)*	
	☒ Manual *Manual*	Refer to standard operating procedures for PIT tagging, fin clipping and handling procedures (WorldFish, 2019a; b; e).
	☐ Mekanikal *Mechanical*	
	☐ Kimia *Chemical*	
	☐ Dadah *Drug*	
	Dos *Dose*	
	Cara administrasi *Route of administration*	
	☐ Lain-lain (Nyatakan) *Others (Specify)*	
3.1.3	Kekerapan mengekang haiwan *Frequency of animal restraint*	
	☐ Setiap jam *Hourly*	☐ Setiap hari *Daily*
	☐ Setiap minggu *Weekly*	☐ Setiap bulan *Monthly*
	☒ Lain-lain (Nyatakan) *Others (Specify)*	Fish will be anethasied for tagging and fin-clipping. Animals will be netted, anethasied and restrained to obtain measurement data at harvest age. Fish selected as parents will be restrained during spawning and fin clipped.
3.1.4	Kekerapan pemantauan haiwan *Frequency of animal monitoring*	

	☒ Setiap hari *Daily*	☐ Selang hari *Ponds monitored daily*
	☒ Lain-lain (Nyatakan) *Others (Specify)*	Ponds will be monitored daily. All fish will be weighted and measured for standard lengh at tagging. All fish will be weighted and measured for standard lengh, body depth and width at harvest. If additional handling of fish is deemed necessary to monitor health status and or growth, this will be undertaken in accordance with the WorldFish 'Handling of Finfish', 'Sedation and Anaesthesia' and 'Weight and Length Measurement' Standard Operating Procedures (WorldFish, 2019c; d; e).
3.1.5	Kaedah pemantauan haiwan *Method of animal monitoring*	
	☐ Pemerhatian tampakan/klinikal *Visual/clinical observation*	
	☒ Menimbang berat *Weighing*	
	☐ Pemeriksaan fizikal *Physical examination*	
	☒ Lain-lain (Nyatakan) *Others (Specify)*	Standard length, body depth and width.
3.1.6	Kekerapan pemantauan pasca-tatacara (pembedahan, suntikan intravena dll) *Frequency of monitoring post-procedure (surgery, intravenous injection etc)*	
	☐ Setiap jam *Hourly*	☐ Setiap hari *Daily*
	☐ Lain-lain (Nyatakan) *Others (Specify)*	The respiratory rate and gill colour of fish will be regularly monitored while they are exposed to, and recovering from, anesthesia in accordance with Standard Operating Procedures (WorldFish, 2019c; d; e).
3.1.7	Pemantauan pasca-tatacara *Post-procedure monitoring*	
	☒ Pemerhatian tampakan/klinikal *Visual/clinical observation*	
	☐ Menimbang berat *Weighing*	
	☐ Suhu *Temperature*	
	☐ Pemeriksaan fizikal *Physical examination*	
	☐ Lain-lain (Nyatakan) *Others (Specify)*	
3.1.8	Nasib haiwan yang sakit *Fate of sick animal*	
	☒ Rawat *Treat*	☒ Mematikan *Sacrifice*
3.1.9	Jika anda memutuskan untuk merawat haiwan yang sakit, nyatakan kaedah rawatan yang diberikan.	

If you decide to treat a sick animal, describe the method of management/treatment.

Treat in accordance with '2.6 Fish health Interventions' of the 'WorldFish carp genetic improvement program pond and fish management manual' (Hamilton et al., 2020).

3.1.10 Jika anda memutuskan untuk mematikan haiwan yang sakit, pilih kaedah perikemanusian yang digunakan.
If you decide to sacrifice a sick animal, choose a method of humane killing.

☐ Dislokasi tulang servikal
Cervical dislocation

☐ Mandian ais
Cold stunning in ice water/Freezer/Liquid Nitrogen

☐ Penyahairan
Dewatering

☐ Infusi CO_2 dalam air
CO_2 infusion in water

☒ Dos lewah dadah (Nyatakan)
Drug overdose (Specify)

Dadah *Drug*	Eugenol
Dos *Dose*	Five times the normal heavy anaesthetic dosage (>90mg/L)
Cara administrasi *Route of administration*	Immersion
☒ Lain-lain (Nyatakan) *Others (Specify)*	In accordance with 'Euthanasia of Finfish' Standard Operating Procedure (WorldFish, 2019d)

3.2 Tatacara pembedahan
Surgical procedures

3.2.1 Kategori
Category

☐ Pembedahan berakhir dengan kematian; haiwan dieutanasia.
Non-survival surgery; animal euthanised.

☐ Pembedahan minor. Tiada penembusan pada kaviti utama badan.
Minor surgery. No penetration to the main body cavity.

☒ Pembedahan major. Terdapat penembusan pada kaviti badan.
Major surgery. Penetration to a major body cavity.

☐ Pembedahan major. Terdapat penembusan pada kaviti badan yang mengakibatkan kemerosotan fizikal dan fungsi.
Major surgery. Penetration to a major body cavity which results in permanent physical of functional impairment.

3.2.2 Terangkan tatacara pembedahan secara terperinci.
Describe the surgical procedure in detail.

Passive Integrated Transponder (PIT) tags are implanted into the peritoneal cavity of fish and used to individually identify fish. Refer to 'PIT tagging of finfish' Standard Operating Procedure (WorldFish, 2019a).

3.2.3 Lokasi kemudahan pembedahan.
Location of the surgery facilities.

Hatchery adjacent to fish ponds (See section 2.8.4)

3.2.4 Anestesia/Analgesia/Ubat penenang
Anaesthesia/Analgesia/Tranquilisers

Agen *Agent*	Dos *Dose*	Cara *Route*	Kekerapan *Frequency*
Eugenol	dosed to effect	Immersion	Once at tagging and once at harvest-age measurement.

3.2.5 Kriteria penilaian anestesia
Criteria of anaesthesia assessment

☒ Kadar respirasi *Respiratory rate*
☐ Kadar denyut jantung *Heart rate*
☐ ECG *ECG*
☐ Relaksasi otot *Muscular relaxation*
☐ Picitan ekor *Tail pinch*
☐ Refleks kornea *Corneal reflex*

☐ Lain-lain (oksimeter denyut, respirator) *Others (pulse oximeter, respirator)*	*See 'Sedation and anaesthesia of finfish' Standard Operating Procedure (WorldFish, 2019f)*

3.2.6 Penilaian kesakitan dan ketidakselesaan pasca-pembedahan.
Assessment of pain and discomfort post-surgical procedures.

☒ Hilang selera makan *Loss of appetite*	☐ Kemerosotan berat badan *Loss of weight*
☒ Kegelisahan *Restlessness*	☐ Postur rehat tak normal *Abnormal resting posture*
☒ Perubahan daya gerak *Change of mobility*	☐ Gagal menunjukkan sifat ingin tahu *Failure to show natural inquisitiveness*
☒ Tiada tindak balas *Unresponsiveness*	☒ Kesukaran bernafas *Laboured breathing*

☐ Perubahan pada tampakan (Sila nyatakan:)
Change in physical appearance
(*Please indicate:*)

Refer to the WorldFish Passive Integrated Transponder (PIT) Tagging of Finfish Standard Operating Procedure (WorldFish, 2019a)

3.2.7 Terangkan penjagaan pasca-pembedahan.
Describe post-surgical care.

The groups of animals that are recovering, will be observed on a daily basis to ensure they recover, and their feed rates return back to normal. Those that are euthanised will not require any further observations. If there are increased mortalities noted, depressed feed rates or secondary infections from handling, a fish health professional will be alerted. (WorldFish, 2019f)

3.2.8 Kaedah mematikan haiwan secara perikemanusiaan.
Method of humane killingof animals.

☐ Dislokasi tulang servikal
Cervical dislocation

☐ Mandian ais
Cold stunning in ice water/Freezer/Liquid Nitrogen

☐ Penyahairan
Dewatering

☐ Infusi CO_2 dalam air
CO_2 infusion in water

☒ Dos lewah dadah (Nyatakan)
Drug overdose (Specify)

Dadah *Drug*	Eugenol
Dos *Dose*	Five times the normal heavy anaesthetic dosage (>90mg/L)
Cara administrasi *Route of administration*	Immersion
☒ Lain-lain (Nyatakan) *Others (Specify)*	In accordance with 'Euthanasia of Finfish' Standard Operating Procedure (WorldFish, 2019d)

3.2.9 Terangkan akhiran kajian ini (sekiranya berbeza daripada perancangan awal). Contoh: Dibuang /dimakan selepas tamat masa luput bahan ubatan dalam badan akuatic...

Describe the end-point of the study (at what instance would you sacrifice the animal, if there is any difference from the original plan) E.g. Disposed / consumed after withdrawal period….

Approximately 80% of all stacked fish will be culled after the harvest-age measurement and the candidate parents have been indentified. Culling will be done according to WorldFish Standard Operation Procedures. Where fish are of market standard, they will be sold for human consumption.

3.3 Penggunaan Bahan Berbahaya (jika berkaitan).
Use of hazardous agents (if applicable).

3.3.1 Adakah bahan berbahaya digunakan? ☐ Ya ☒ Tidak
Is any of the hazardous agents used? *Yes* *No*

Jika Ya, teruskan dengan soalan berikutnya.
If yes, please proceed to the following questions.

3.3.2 Jenis bahan berbahaya.
Types of hazardous agents.

☐ Organisma patogen *Pathogenic organism*	
Nama patogen *Name of pathogen*	
☐ Karsinogen kimia *Chemical carcinogen*	
Nama karsinogen kimia *Name of chemical carcinogen*	
Dos *Dose*	
Cara administrasi *Route of administration*	
Kekerapan *Frequency*	
☐ RNA/DNA rekombinan *Recombinant RNA/DNA*	
☐ Bahan radioaktif *Radioactive material*	
Nama *Name*	
Dos *Dose*	
Hayat simpan *Shelf-life*	
Cara administrasi *Route of administration*	

Kekerapan *Frequency*	

3.3.3 Terangkan bagaimana bahan berbahaya digunakan (**Sila lampirkan dokumen yang berasingan**).
*Describe how the hazardous agents are utilized (**Please attach as a separate document**).*

3.3.4 Terangkan tatacara pembendungan biokeselamatan (**Sila lampirkan dokumen yang berasingan**).
*Describe the **biosecurity** containment procedures (**Please attach as a separate document**).*

3.3.5 Terangkan bagaimana pelupusan sisa air dan haiwan dilakukan (**Sila lampirkan dokumen yang berasingan**).
*Describe the disposal of the used animals and waste water (**Please attach as a separate document**).*

3.4 Untuk penggunaan bahan toksik atau organisma patogen surat kelulusan Jawatankuasa Keinstitusian Biokeselamatan UKM atau mana-mana pihak bertanggungjawab mesti dikemukakan.
On the use of toxic materials as well as pathogenic organism, an approval letter from the UKM Institutional Biosafety Committee or any authority must be provided.

☐ Dikemukakan *Attached* ☒ Tidak berkenaan *Not applicable*

3.5 Jenis lain eksperimen (jika berkaitan).
Other types of experiments (if applicable).

☒ Bahan/peranti implan
Implanted devices/material

Saiz implan *Size of implant*	Passive integrated transponder (PIT) tag
Lokasi implan peranti *Location of implanted devices*	Body cavity
Tempoh *Duration*	15 months from Oct 2022 to Dec 2023

☐ Paralisis saraf-otot
Neuromuscular paralysis

Lokasi *Location*	
Kaedah *Method*	
Tempoh *Duration*	

☐ Imobilisasi-elektro
Electro-immobilisation

Jenis instrumen *Type of instrument*	
Tempoh *Duration*	
Saiz voltan *Voltage size*	

☐ Ujikaji toksikologi
Toxicology experiment

Senaraikan sebatian dalam kertas yang berasingan sekiranya anda menggunakan lebih dari satu sebatian. Terangkan sumber, kepekatan, dll. secara terperinci. Sila penuhkan Bahagian 3.3.

List the compound in a separate sheet if the compound is more than one. Describe in detail, source, concentration and etc. Fill up Section 3.3I.

☐ Ujikaji menggunakan janin/embrio (Sila lampirkan dokumen yang berasingan).
Foetal/Embryo experimentation (Please attach as a separate document).

3.6 Pengalaman dan kompetensi dalam tatacara pengendalian haiwan akuatik.
Experience and competency in handling aquatic animal procedures.

3.6.1 Adakah anda berpengalaman dalam mengendalikan haiwan kajian **akuatik**?
*Have you had any experience in handling **aquatic** animals?*

☒ Ya (Sila berikan bukti) / *Yes (Please provide an evidence)* ☐ Tidak / *No*

3.6.2 Adakah anda mempunyai kakitangan yang kompeten dalam pengendalian haiwan **akuatik**?
*Do you have competent staff in handling **aquatic** animals?*

☒ Ya / *Yes* ☐ Tidak / *No*

3.7 Tatacara pilihan.
Alternative procedure.

3.7.1 Adakah tatacara pilihan telah dikenalpasti?
Has alternatives to the procedures to the animals been searched for?

☒ Ya / *Yes* ☐ Tidak / *No*

3.7.2 Jika ya, sila sertakan bukti yang menyokong pernyataan anda (laman sesawang, jurnal, dll.)
If yes, please provide evidence/reference to support your claim. (websites, journals etc)

No non-sentient alternative can not be used to replace the fish and still achieve the study objectives (see Section 1.3).

BAHAGIAN 4: PENGELASAN PROJEK DAN KELULUSAN
SECTION 4: PROJECT CLASSIFICATION AND APPROVAL

4.1 Pengelasan Projek (pilih yang berkenaan)
Project Classification (select where applicable)

☐ A Tatacara dijalankan tanpa penggunaan anestesia dan agen kimia.
Procedure carried out without the use of anaesthesia and chemical agents.

☐ B Tatacara dijalankan di bawah pengaruh anestesia dan haiwan dimatikan.
Those procedures carried out under anaesthesia and the animal killed without regaining consciousness.

☒ C Projek biakbaka.
Breeding project.

☐ D Projek yang memerlukan haiwan dimatikan untuk pengambilan seluruh karkas haiwan atau sebahagian tisu selepas ujikaji dijalankan.
Projects requiring animals to be killed for the preparation of whole animal carcass or tissue specimens with prior experimentation.

4.2 Pengesahan veterinawan bertugas
Verification of attending veterinarian

Tandatangan/*Signature*:
Cop/*Stamp*:
Nombor APC/*APC number*:

(Sangat digalakkan/*highly recommended*)

BAHAGIAN 5: PERAKUAN DAN KELULUSAN
SECTION 5: ENDORSEMENT AND APPROVAL

5.1 PERAKUAN PENYELIDIK/PEMOHON
RESEARCHER/APPLICANT ENDORSEMENT

.....
Tandatangan & Cop Ketua Penyelidik
Signature & Stamp of Lead Researcher

13 Oct 2022
.....
Tarikh
Date

5.2 PERAKUAN KETUA JABATAN
HEAD OF DEPARTMENT ENDORSEMENT

.....
Tandatangan & Cop Ketua Jabatan
Signature & Stamp of Head of Department

09 Dec 2022
.....
Tarikh
Date

5.3 KEPUTUSAN JAWATANKUASA ETIKA HAIWAN UKM (UKMAEC)
DECISION OF UKM ANIMAL ETHICS COMMITTEE (UKMAEC)

……

……

……

5.4 NO. KOD KELULUSAN
NO. APPROVAL CODE

.....	.....
Tandatangan & Cop Sekretariat UKMAEC	Tarikh
Signature & Stamp of UKMAEC Secretariat	*Date*

.....	.....
Tandatangan & Cop Pengerusi UKMAEC	Tarikh
Signature & Stamp of UKMAEC Chairperson	*Date*

Hamilton, M.G., 2021. Maximum likelihood parentage assignment using quantitative genotypes. Heredity. https://doi.org/10.1038/s41437-021-00421-0

Hamilton, M.G., Rajts, F., Alam, M.B., Yossa, R., Delamare-Deboutteville, J.r.m., Chadag, V.M., Collis, B., 2020. WorldFish carp genetic improvement program pond and fish management manual

Ninh, N.H., Ponzoni, R.W., Nguyen, N.H., Woolliams, J.A., Taggart, J.B., McAndrew, B.J., Penman, D.J., 2011. A comparison of communal and separate rearing of families in selective breeding of common carp (*Cyprinus carpio*): estimation of genetic parameters. Aquaculture. 322, 39-46. https://doi.org/10.1016/j.aquaculture.2011.09.031

WorldFish, 2019a. PIT tagging of finfish (Version 1.0.0). WorldFish, Penang, Malaysia

WorldFish, 2019b. Fin clipping of finfish (Version 1.0.0). WorldFish, Penang, Malaysia

WorldFish, 2019c. Weight and length measurement (Version 1.0.0). WorldFish, Penang, Malaysia

WorldFish, 2019d. Euthanasia of finfish (Version 1.0.0). WorldFish, Penang, Malaysia

WorldFish, 2019e. Handling of finfish (Version 1.0.0). WorldFish, Penang, Malaysia

WorldFish, 2019f. Sedation and anaesthesia of finfish (Version 1.0.0). WorldFish, Penang, Malaysia