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Research protocols for the Xayaburi Dam PIT tag retention experiments

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Executive summary

PIT tagging is tolerated by many fish species worldwide and has already been demonstrated to work for two tropical Mekong species (Grieve et al. 2018a; Grieve et al. 2018b). It is intended to use PIT tagging to assess the passage effectiveness of the new Xayaburi fish pass facility on the Mekong River. However, there are over 200 migratory species at the Xayaburi site, and at least 26 of these species are considered to be of key importance. To gain assurance that PIT tags can generate useful data on the passage effectiveness of the Xayaburi fish pass facility, the tagging technique needs to be tested and refined for these Mekong fish species.

This report describes the research protocols for undertaking PIT tag retention experiments in tropical species from the Mekong Basin, to determine the suitability of using PIT tags on these species.

The protocols can be broken down into the following steps:

1. Collect the fish from the Mekong River
2. Transport the fish to the experimental facility
3. Randomly allocate the fish to treatment and control groups within the experimental tanks
4. Apply the handling procedures to the treatment and control groups
5. Start the PIT tag retention experiment(s) and assess the fish regularly
6. Conclude the experiment(s) and undertake final assessments
7. Release the fish.

This report also outlines the basic fish husbandry requirements for conducting PIT tag retention experiments and some options for analysing the results from such experiments.

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Introduction

Background

The diverse and productive fisheries of many tropical river systems are being placed under immense pressure due to the widespread development of irrigation and hydropower infrastructure in these systems (Ziv et al. 2012; Winemiller et al. 2016). In addition to altering flows, such infrastructure blocks fish from accessing vital feeding, spawning and nursery habitat, and subsequently precludes them from completing their life cycles. The Lower Mekong Basin (LMB) is presently home to the world's most productive inland fishery, but the fishery is under threat from the planned construction of eleven main stem dams. The first of these dams, at Xayaburi, in Lao PDR, became operational in October 2019 (Orr et al. 2012). A record level of investment has been assigned to alleviating the effects of the dam on fish passage; but the effectiveness of the fish passage facility remains untested, and a structured research program is critical for addressing this knowledge gap. Specifically, this research program needs to: (1) develop monitoring techniques for evaluating the effectiveness of mainstem dam fishways in the LMB; (2) quantify the performance of the Xayaburi Dam fishway; and (3) provide a standard for monitoring and constructing other mainstem dam fishways in the LMB.

Objectives of this report

PIT tagging was identified early on during the scoping phase of the research program as a potentially effective monitoring technique for assessing the effectiveness of fishways in the LMB. It has been successfully used for assessing the movements of temperate freshwater (Ombredane et al. 1998) and marine (Parker and Rankin 2003) fish species, but its usefulness in tropical species remains largely untested. In particular, very little is known about the retention rates of PIT tags in tropical species (Grieve et al. 2018a; Grieve et al. 2018b). This report describes the research protocols for undertaking PIT tag retention experiments on migratory Mekong species to determine the retention rates of PIT tags within these species.

Research protocols for the PIT tag retention experiments

The protocols for undertaking PIT tag retention experiments can be summarised in seven steps (Figure 1). These steps are explained in more detail in the subsequent sections.

Figure 1. Summary of the steps involved in performing the PIT tag retention experiments.

STEP 1: Collect the fish from the Mekong River

The experimental fish are collected from the Mekong River adjacent to the Xayaburi fish passage structure.

A. The fish are collected using boat electrofishing, by Xayaburi Power Company Limited (XPCL) staff, in accordance with the best practice guidelines developed for the project.
B. Fish are attracted to the electrode and then immobilised so they can be caught in a dip-net. Only target species will be removed from the water and every effort will be made not to immobilise non-target species. Once target species are dip-netted from the water, they are placed in an on-board live well that is supplied with flow-through water from the surrounding water body. The fish recover from the procedure in about two minutes. Each electrofishing 'shot' lasts for approximately 90 seconds.

STEP 2: Transport the fish to the experimental facility

The experimental fish are then transported to the on-site Xayaburi experimental facility (Hatchery) in accordance with best practice (Barker et al. 2002).

A. Fish will be allocated to a holding tank for a minimum of one day to recover from collection prior to receiving the tagging treatment.
B. One holding tank is required per species.
C. The aerated tanks have pumps and can be rapidly filled with source water. Transport from the Mekong River to the experimental facility takes less than 30 minutes. On arrival at the experimental facility, the transport tanks containing fish will be drained to a depth of approximately 20–30 cm.
D. Prior to transport, AQUI-S (Isoeugenol) needs to be added to the water (5-10 ml/1000 L) to induce light sedation and lower the activity and stress levels of the fish; facilitating easier handling and transfer to the holding tanks.
E. Following introduction, each holding tank needs to have ammonia levels tested, twice per day, to ensure all are within tolerable ranges. Water quality parameters such as dissolved oxygen, pH, temperature, conductivity and turbidity will need to be monitored before and after fish transfer to ensure there is no major difference between the water in the Mekong River and that in the the hatchery tanks. If there is over 10% variation, fish will need to be acclimated by gradually adding tank water to the holding tanks.

STEP 3: Randomly allocate the fish to treatment and control groups within the experimental tanks

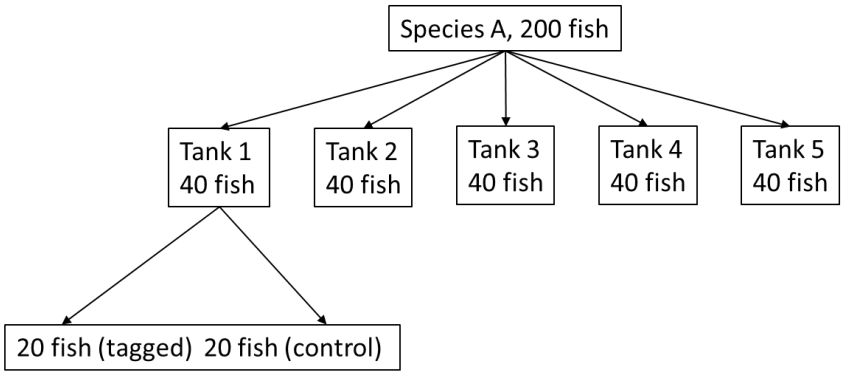
Once the fish have been acclimated for at least one day, they then need to be prepared for the tagging treatment.

A. Five replicate tanks will be required per species. Each tank needs to be labelled and prepared prior to fish allocations (Appendix II) .
B. For large species (>50 cm), there will be a maximum of 40 fish per tank (20 PIT tagged fish; 20 control (i.e. untagged) fish).
C. For small species (<50 cm), up to 3 species can be housed per tank (20 PIT tagged and 20 control individuals for each of the three species; so 120 fish total per tank). The tanks will be divided into smaller compartments using nylon mesh to separate each species.
D. On treatment day, the fish are dip-netted from the holding tank.
E. Each fish is assigned to being either a 'PIT tagged' or a 'control' fish, using a random treatment allocator like the one provided in Appendix III. For example, based on the 'START - random treatment allocator' table in Appendix III, the first fish randomly chosen for Tank 1 would be assigned to become a 'PIT tag' fish. The next fish

randomly chosen for Tank 1 would be assigned to become a 'control' fish, and so forth.

F. After being randomly assigned a treatment, each fish is then transferred to a holding tub containing aerated water with 25 mg/L AQUI-S anaesthetic solution (see the next step).
G. A different dipnet needs to be used for each tank, to minise the spread of disease between tanks, as this could inadvertently kill the fish.

a)



b)

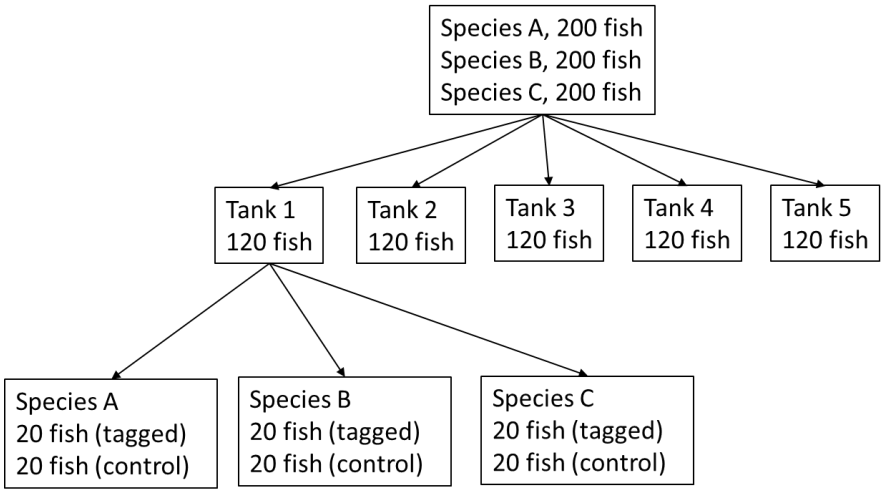


Figure 2. The experimental allocation of individuals for (a) a single large bodied species; and (b) three smaller-bodied species/cohorts per tank. In (b), the tank is divided into three discrete compartments (with buffers between compartments) using nylon mesh. The approach for smaller-bodied species/cohorts could be for just two species/cohorts depending on demand.

STEP 4: Apply the handling procedures to the treatment and control groups

After dipnetting, the fish then go through a handling procedure that involves being anaesthetised (see the previous step); PIT tagged (or not, in the case of control fish); and visual implant elastomer (VIE) tagged to provide a means of ascertaining their treatment allocation in the event of PIT tags being shed. The fish could potentially also be dart-tagged to allow for the identification of individuals if necessary for the study.

A. Fish are anaesthetised by immersing them in a 25 mg/L AQUI-S solution (see the previous step) (Barker et al. 2002).
B. Once the fish have been sufficiently anesthetised (as indicated by a loss of equilibrium and reduced opercular beat rate), they are weighed (g), measured (total length - cm), and PIT tagged, or handled and not PIT tagged (in the case of control fish). All fish are also VIE tagged (and dart tagged if this is considered necessary for the study). Mulitple operators should be used to increase working efficiency and minimise handling stress for the fish. The only practical way to allocate tagging treatments to individual fish is based on the order they are removed from the anaesthetising tank (see the previous step). Nevertheless, this may be lead to there being some relationship between this order and some other factor such as size (perhaps the big fish are unconsciously grabbed first). Consequently, the fish weights should be entered into the 'ONGOING species database' in Microsoft Excel (see Appendix IV) as they are tagged at the start of each experiment, so that adjustments can be made with the final fish tagged to balance the allocation of fish weights to tagging treatments (if necessary).
C. Species/cohorts > 175 mm (TL) receive 23 mm Biomark PIT tags, whereas species 60-175 mm (TL) receive 12 mm Biomark PIT tags.
D. Each PIT tag is inserted into the peritoneal cavity (i.e. gut) using a pre-loaded Biomark needle with the appropriate sized PIT tag. Gut-located PIT tags have been successfully applied worldwide with good retention, but can cause welfare issues if the tags are not inserted correctly (Bridger and Booth 2003). In particular, fish in spawning condition typically have well-developed gonads in the peritoneal cavity, and thus caution should be exercised to avoid inserting PIT tags into the gonad tissue and subsequently enhancing the risk of the PIT tags being expelled during ovulation. The best way of reducing the likelihood of this occurring is to place the fish on its dorsal region so that the gonads retreat, and then inserting the needle perpendicular to the body cavity (Bridger and Booth 2003).
E. The PIT tag for each fish is scanned and the tag number recorded in the 'ONGOING species database'.
F. For the VIE tagging, the elastomer is injected as a liquid and cures as a pliable solid. The tags are inserted beneath clear or translucent tissue so that they remain externally visible. The VIE tag colour for each fish is also recorded in the 'ONGOING species database' (next to its PIT tag number (if it has been allocated one)).
G. For the dart tagging (if incorporated into the study), the tags (HallPrint, Hindmarsh Valley, SA, Australia) should be inserted in the dorsal pterygiophores, ventrally adjacent to the dorsal fin (Lyon et al. 2019).
H. All fish descriptive data should be recorded in the 'ONGOING species database' at the start of each experiment (see Appendix IV). After recording the PIT tag number (if relevant) and VIE tag colour for each fish, record all of the other associated details (i.e. operator number and name, starting length and weight).
I. After fish have been anaesthetised and tagged (both PIT and VIE), they are placed into an aerated recovery tub, and then monitored until the effects of the anaesthetic have passed, before being placed into the nominated holding tank).
J. PIT tag detectability is tested at both the start and end of the study using a Biomark PIT reader (Biomark, Boise, Idaho, USA).

STEP 5: Start the experiment and assess the fish regularly

The experiment runs for 50 days. A 50-day duration is necessary to encompass the period when the probability of shedding or tagging-induced mortality will be highest (Dare 2003; Grieve et al. 2018a; Grieve et al. 2018b).

A. Throughout the experiment, every experimental tank is inspected twice daily to look for mortalities (and health issues).

B. Dead fish are removed and identified, inspected for the cause of death, and dissected for tag retrieval.
C. Also, EVERY DAY a high-powered magnet is used to sweep the bottom of each experimental tank to recover shed PIT tags.
D. Recovered PIT tags are scanned using the PIT reader, and the tag numbers are related back to their original fish.
E. The date of mortality, date of PIT tag shedding and/or any other relevant details are recorded for the relevant fish in the 'ONGOING species database' (Appendix IV), twice daily throughout the experiment following checks for mortalities and tag retention.
F. The water quality should be checked for each tank, daily (temperature, pH, conductivity, turbidity, salinity, dissolved oxygen, and ammonia (NH_3), nitrite (NO_2) and nitrate (NO_3) concentrations). The water quality data should be recorded EVERY DAY in the 'ONGOING WQ database' (Appendix V) throughout the experiment.

STEP 6: Conclude the experiment and undertake final assessments

The experiment is concluded after 50–60 days.

A. Fish are transferred into an anaesthetic tub containing a 25 mg/L AQUI-S solution (Barker et al. 2002).
B. Once the fish have been sufficiently anesthetised (as indicated by a loss of equilibrium and reduced opercular beat rate), they are identified, weighed (g), and measured (total length - cm).
C. They are then scanned for both PIT and the VIE tags.
D. All final fish descriptive data and water quality data should be recorded in the 'ONGOING species' and 'ONGOING water quality' databases (Appendix V), and then entered into the corresponding electronic databases.

STEP 7: Release the fish

Fish that survive PIT tagging will be released into the Mekong River adjacent to the Xayaburi fish passage structure.

Basic fish husbandry

Water quality monitoring

- The water quality (pH, turbidity, dissolved oxygen, temperature, conductivity, and ammonia, nitrite and nitrate concentrations) in each tank will be monitored twice weekly as part of basic fish husbandry (Barker et al. 2002). This will be done using a Horiba multi-probe water quality meter to check the flow-through conditions and inform the cleaning requirements.
- Each tank's nets and flow-through system pipes will be cleaned, and at least 25% water changed once per week OR whenever water quality parameters are outside tolerable ranges.
- If a disease outbreak occurs, tank water will be maintained in saline conditions (5 g/L) to control the outbreak of pathogens. This may be increased to 10 g/L and incorporate the addition of formalin (100–200 ppm) or oxytetracycline (100 mg/L) if pathogens are identified.
- Project staff will conduct regular checks to determine if prophylactic treatments are needed.

Fish feeding

- Fish will be fed manufactured pelleted feed at the rate of 2.5% of body weight per fish, twice per day (Nguyen 2013).

Euthanasing fish

- If any fish are found to be suffering and are beyond recovery, they will be euthanased using immersion in 100 mg/L Benzocaine (Ethyl-P-Aminobenzoate).

Data analysis

- PIT tag retention is evaluated by recording the number of tags shed per tank, while mortality is examined by recording the daily number of deaths in each tank (Grieve et al. 2018a).
- Growth is assessed from changes in weight between day 0 and day 50.
- All data analyses can be performed using IBM© SPSS © Version 19 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp) and the Generalised Linear Models procedure (GENLIN), as subsequently described.

Retention

- A Generalised Linear Model (GLM) with a binary response could be applied to assess whether PIT tag retention rate (shed = 1, retained = 0) is related to tank (random effect), treatment (fixed effect: treatment vs. control), starting weight of each fish (continuous covariate), and the interaction of tank and treatment.
- Tag survival analysis is performed to determine the proportion of tags lost (rejected or if fish died) at day 50.

Mortality

- A binary response model could be applied to assess whether mortality rate (died = 1, survived = 0) is related to tank (random effect), treatment (fixed effect: treatment vs. control), starting weight of each fish (covariate), and the interaction of tank and treatment.

Growth (as assessed by weight change)

- A normal response model could be used to assess whether the growth of each fish is related to tank (random effect), treatment (fixed effect: treatment vs. control), starting weight of each fish (covariate) and the interaction of tank and treatment.

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Appendix I. Equipment required for the PIT tag retention experiments.

Equipment	Use
Electrofishing boat and equipment	Collecting fish
AQUI-S anaesthetic	Anaesthetising fish during transport and tagging
Benzocaine	Euthanizing fish if required
Horiba water quality meter	Monitoring water quality
API ammonia, nitrite and nitrate water quality test kits	Monitoring water quality
Water quality photometer and test reagents	Monitoring ammonia, nitrite and nitrate
Pellet fish food	Feeding fish
Salt/formalin/oxytetracycline	Treating fish disease if required
Labels for the fish tanks	Labelling fish tanks
1 dip net per tank	Retrieving fish
Fish measuring board × 5	Measuring fish
Fish weighing scales	Weighing fish
Biomark PIT tag injector	Inserting PIT tags
VIE tag injector	Inserting VIE tags
Dart tag injector (optional)	Inserting dart tags (optional)
Disinfectant?	Disinfecting the tag injectors between fish
Hand held PIT tag readers	Scanning PIT tags
Magnet	Picking up PIT tags from the bottom of a tank
PIT tags (12 mm and 23mm Biomark tags)	Marking the fish
VIE tags	Providing a secondary mark to the fish
Dart tags (optional)	Giving fish individual identifications (optional)
Laptop with Excel databases (‘ONGOING species’ and ‘ONGOING water quality’)	Recording fish and water quality data
Processing tables	Processing fish (e.g. weighing fish, inserting PIT tags etc.)

Appendix II. Template for labelling each tank.

TANK:

SPECIES/LIFE STAGE:

PIT TAG SIZE:

DATE EXPERIMENT STARTED:

TREATMENT VIE COLOUR/LOCATION:
CONTROL VIE COLOUR/LOCATION:

OTHER COMMENTS:

Appendix III. 'STARTING – random treatment allocator' table.

Species/life stage: _____

PIT tag size (12 or 23 mm): _____

Tank	Treatment	Tank	Treatment	Tank	Treatment	Tank	Treatment	Tank	Treatment
1	PIT tag	2	Control	3	PIT tag	4	Control	5	PIT tag
1	Control	2	Control	3	PIT tag	4	PIT tag	5	PIT tag
1	Control	2	Control	3	PIT tag	4	Control	5	Control
1	PIT tag	2	PIT tag	3	Control	4	Control	5	PIT tag
1	PIT tag	2	PIT tag	3	Control	4	Control	5	Control
1	Control	2	Control	3	PIT tag	4	PIT tag	5	Control
1	Control	2	PIT tag	3	PIT tag	4	PIT tag	5	PIT tag
1	PIT tag	2	PIT tag	3	Control	4	Control	5	PIT tag
1	Control	2	Control	3	PIT tag	4	Control	5	PIT tag
1	Control	2	Control	3	Control	4	Control	5	Control
1	PIT tag	2	PIT tag	3	Control	4	Control	5	Control
1	Control	2	Control	3	PIT tag	4	PIT tag	5	PIT tag
1	PIT tag	2	PIT tag	3	PIT tag	4	PIT tag	5	PIT tag
1	Control	2	PIT tag	3	Control	4	PIT tag	5	Control
1	PIT tag	2	PIT tag	3	PIT tag	4	PIT tag	5	Control
1	Control	2	Control	3	Control	4	PIT tag	5	Control
1	PIT tag	2	Control	3	Control	4	PIT tag	5	PIT tag
1	PIT tag	2	Control	3	Control	4	PIT tag	5	Control
1	Control	2	PIT tag	3	Control	4	PIT tag	5	PIT tag
1	Control	2	Control	3	PIT tag	4	Control	5	PIT tag
1	Control	2	PIT tag	3	PIT tag	4	PIT tag	5	PIT tag
1	PIT tag	2	Control	3	Control	4	Control	5	PIT tag
1	Control	2	PIT tag	3	Control	4	PIT tag	5	PIT tag
1	Control	2	PIT tag	3	PIT tag	4	PIT tag	5	Control
1	PIT tag	2	Control	3	PIT tag	4	PIT tag	5	Control
1	Control	2	PIT tag	3	Control	4	Control	5	PIT tag
1	Control	2	Control	3	Control	4	PIT tag	5	Control
1	PIT tag	2	PIT tag	3	Control	4	PIT tag	5	Control
1	PIT tag	2	Control	3	Control	4	Control	5	Control
1	Control	2	Control	3	PIT tag	4	Control	5	PIT tag
1	PIT tag	2	PIT tag	3	PIT tag	4	Control	5	Control
1	Control	2	PIT tag	3	PIT tag	4	Control	5	Control
1	PIT tag	2	Control	3	PIT tag	4	Control	5	Control
1	Control	2	PIT tag	3	Control	4	PIT tag	5	PIT tag
1	PIT tag	2	PIT tag	3	PIT tag	4	Control	5	PIT tag
1	PIT tag	2	Control	3	PIT tag	4	Control	5	PIT tag
1	PIT tag	2	Control	3	Control	4	Control	5	Control
1	PIT tag	2	Control	3	Control	4	PIT tag	5	Control
1	PIT tag	2	PIT tag	3	PIT tag	4	PIT tag	5	Control
1	Control	2	PIT tag	3	Control	4	Control	5	PIT tag

Appendix IV. An example of the data to be recorded in the 'ONGOING species database' (for Tank 1 PIT tag fish).

Species/life stage	PIT_tag_size (12 or 23 mm)	Tank	Treatment	Operator number	Operator name	STARTING_PIT_tag_number	STARTING_VIE_colour	STARTING_dart_tag_number	STARTING_Fish number	STARTING_length_cm	STARTING_weight_g	Date_shed_PITtag	Date_of_mortality	ENDING_PIT_tag_number	ENDING_PIT_tag_orientation	ENDING_VIE_colour	ENDING_dart_tag_number	ENDING_OTHER_DETAILS?	ENDING_length_cm	ENDING_weight_g
		1	PITtag						1											
		1	PITtag						2											
		1	PITtag						3											
		1	PITtag						4											
		1	PITtag						5											
		1	PITtag						6											
		1	PITtag						7											
		1	PITtag						8											
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		1	PITtag						10											
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		1	PITtag						13											
		1	PITtag						14											
		1	PITtag						15											
		1	PITtag						16											
		1	PITtag						17											
		1	PITtag						18											
		1	PITtag						19											
		1	PITtag						20											

Appendix V. An example of the data to be recorded in the 'ONGOING water quality database'.

Species/life stage: _____

PIT_tag_size (12 or 23 mm): _____

Date	Time	Tank	Sampler	Temp (°C)	pH	SPC (mS/cm3)	Turbidity (NTU)	Salinity (ppt)	DO (mg/L)	DO (%)	NH3/NH4+ (mg/L)	NO2 (mg/L)	NO3 (mg/L)	Mortalities	Treatments	Comments

Appendix VI. Animal ethics approval certificate for the PIT tag experiments.

Charles Sturt University

ANIMAL CARE AND ETHICS
ETHICS AND COMPLIANCE UNIT
Locked Bag 588
Wagga Wagga NSW 2678
Australia
Tel: +61 2 6933 4322
Email: animalethics@csu.edu.au
https://www.csu.edu.au/division/deputy vc/rdi/ethics-and-compliance/animal

CHARLES STURT UNIVERSITY
AUTHORITY FOR THE USE OF ANIMALS FOR THE PURPOSE OF TEACHING AND RESEARCH

The Charles Sturt University Animal Care and Ethics Committee issue this authority to use animals for the purpose of teaching and research as detailed in accordance with the Australian code for the care and use of animals for scientific purposes.

Authority commencing **1 May 2019** and ending 30 **April 2020**

Protocol number: **A19040**

Project Name: *Assessing fisheries mitigation measures at Xayaburi Dam in Lao PDR*

Principal Investigator: A/Prof Lee Baumgartner

Contact Details: 0427 070 056

This authority covers the use of the following animals:

Animal to be studied (used): Fish

Number: 39800

Location: XPCL Holding Facility at Xayaburi hydropower dam site, Lao PDR

Purpose: A6 Research: animal management or production

Procedures: P4 Minor surgery with recovery

Should animals covered by this authority require urgent attention please contact the following:

Gary Thorncraft

Complaints or concerns regarding the treatment or use of animals cover by this authority should be directed in writing to: The Presiding Officer
Animal Care and Ethics Committee
animalethics@csu.edu.au

This authority is issued by the Presiding Office on behalf of the Vice Chancellor of Charles Sturt University, and remains in force (unless suspended, cancelled of surrendered)

Signed /Date: 12/4/19

Deputy Presiding Officer, Animal Care and Ethics Committee

CONTACT

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