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Suitability of tropical river fishes for PIT tagging: Results for four Lower Mekong species

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ABSTRACT

Tropical river systems support some of the most productive inland fisheries around the world, but their fisheries are being placed under growing pressure from disruptions to connectivity caused by river developments. Fish passage measures and complementary monitoring techniques are needed to mitigate the barrier impacts of river developments and validate the effectiveness of such measures, respectively. Passive integrated transponder (PIT) systems have been shown to be effective for monitoring the effectiveness of fish passage measures in temperate river systems but remain largely untested in tropical systems. The aim of this study was to investigate the suitability of four wild-caught tropical species from the Mekong River (*Hypsibarbus lagleri, Hemibagrus filamentus, Barbonymus schwanenfeldii, and Scaphognathops bandanensis*) to PIT tagging. This was achieved by undertaking PIT tag retention experiments in outdoor tanks onsite, to assess whether PIT tags can be retained in these species without impacting their survival and body condition. We found that there was no significant impact of PIT tagging on mortality; no fish lost condition from tagging; and the overall tag rejection rate was very low (4.5%) in all four species. The study findings indicate that *H. lagleri, H. filamentus, B. schwanenfeldii, and S. bandanensis* are all suitable for being PIT tagged in tropical river systems, and therefore could potentially be used to assess various fish passage metrics such as approach, attraction and passage efficiency.

1. Introduction

The extensive development of major tropical river systems for irrigation and hydropower generation is exerting pressure on the world's inland fisheries (Oldani & Baigun, 2002; Oliveira et al., 2018). The barrier impacts of dams and other structures can limit fish migration by blocking access to feeding, spawning and nursery habitats, and thus threaten the ability of fish to complete essential life-history stages (Jensen, 2001; Winemiller et al., 2016). Consequently, many inland fisheries face possible declines and even local extinctions as their habitats continue to be altered to meet rising irrigation and energy demands (Ziv et al., 2012).

Fish passage mitigation measures, such as fishways, are being increasingly used to alleviate the barrier impacts of river infrastructures (Brito-Santos et al., 2021; Wilkes et al., 2018). However, there is a general paucity of data on the effectiveness of fishways in restoring fish passage within tropical river systems in developing countries (Baumgartner et al., 2012; Oldani & Baigun, 2002). In addition, data on the efficacy of methods for assessing fish movement through fishways are also scant (Bao et al., 2019). Nonetheless, empirically proven methods for assessing fishway effectiveness are likely to be central to validate fishway design assumptions to optimize the sustainability of fisheries, especially in large tropical river systems like the Mekong, where the fisheries are invaluable for supporting livelihoods and food security in the region (Baumgartner et al., 2012).

The Mekong River Basin is currently home to the world's most productive inland fishery – with the inland fisheries productivity in the Lower Mekong River Basin (LMB) alone, ranging between 2 and 3 million tons per year (Lu & Siew, 2006; Phan et al., 2003). Its fish community is also highly diverse, comprising of at least 1200 species,

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representing a broad variety of families with a wide variety of morphologies and life histories (Coates et al., 2003; Poulsen et al., 2004). Despite the immense socio-economic and ecological value of the Mekong fishery, it is currently under serious threat from widespread river development. There are proposals to construct up to ten mainstream hydropower dams across the entire river channel: six in Lao PDR, two on the Lao PDR-Thailand reaches of the mainstream, and two in Cambodia (ICEM, 2010). Many organizations, such as the Mekong River Commission (MRC), and private sectors are increasingly investing significant amounts of time and money to mitigate the impacts of hydropower on the Mekong fishery, but there are little data available on reliable monitoring methods, and few technologies have been tested, under local conditions, for their ability to provide large, long-term, datasets. One promising method, Passive Integrated Transponder (PIT) tagging, has been proposed as an effective approach for monitoring the effects of hydropower development on fish in the LMB (Grieve et al., 2018), but little is known about the suitability of the majority of Mekong fish species for receiving tags.

Studies of temperate riverine fisheries have found PIT tagging to be a particularly effective mark-recapture technique for examining fishway effectiveness (Castro-Santos et al., 1996; Lundqvist et al., 2008). PIT tags comprise an antenna coil, capacitor, and circuit board encased in a small glass capsule, containing electronic circuitry that is individually coded with a 16-digit identification number (Roussel et al., 2000). The tags are powered electromagnetically when they pass within the vicinity of a suitable antenna, which can be set up at a location of interest within the fishway (Adams et al., 2006; Saboret et al., 2021).

PIT systems have been successfully used for assessing the movements of a range of migratory species (e.g. juvenile brown trout (*Salmo trutta L.*) (Ombredane et al., 1998); *Oncorhynchus tshawytscha* (Achord et al., 2012) in temperate river-floodplain systems around the world. Despite the wealth of knowledge regarding PIT tagging of such species in temperate rivers, the suitability of wild caught Mekong fish species for PIT tagging remains unvalidated (Baras et al., 1999) beyond two aquaculture Mekong fish species — *Pangasianodon Hypophthalmus* (Striped Catfish) and *Hypsibarbus Malcomi* (Goldfin Tinfoil Barbs) (Grieve et al., 2018). PIT tags were inserted into the shoulder, gut, or muscle of these two species without influencing their death or growth rates (Grieve et al., 2018). Nonetheless, it remains unknown as to whether the results for these two species can be effectively transferred to other wild Mekong fish species. Indeed, accurate data and its reliable interpretation hinges on the assumption that PIT tagged individuals can retain the tags without the tags affecting their survival or condition. One method of validating this assumption is by undertaking PIT tag retention trials.

The current study extended the work of Grieve et al. (2018) to assess the suitability of four wild-caught tropical species from the Mekong River (*Hypsibarbus lagleri, Hemibagrus filamentus, Barbonymus schwanenfeldii, and Scaphognathops bandanensis*) to PIT tagging. Specifically, it aimed to determine whether PIT tags can be retained in these species without impacting their survival or body condition, by undertaking PIT tag retention experiments in outdoor tanks onsite. Mortality and growth rates were assessed along with body condition. These results can be used to inform the suitability of wild-caught Mekong species for PIT tagging studies, and therefore, the potential for using such species to evaluate the effectiveness of fish passage mitigation measures in tropical river systems where they migrate.

2. Methods

2.1. Species selection

Hypsibarbus lagleri, Hemibagrus filamentus, Barbonymus schwanenfeldii, and Scaphognathops bandanensis were deemed as representative Mekong River species because of their 1) migratory behaviour, 2) economic importance, and 3) ecological significance. *Hypsibarbus lagleri* is widespread, inhabiting major rivers during the dry season and migrating to smaller rivers during the rainy season. It is classified as Vulnerable by the IUCN, because of overfishing and dam construction (IUCN). *Hemibagrus filamentus* is a commercially valuable catfish that inhabits slowly flowing or standing waters in all countries of the lower Mekong Basin (Ng, 1999). It was listed as Data Deficient by the IUCN in 2011. *Barbonymus schwanenfeldii* is listed as Least Concern and can be found in rivers of medium to large size in the Lower Mekong and Chao Phraya basins, the Malay peninsula, Borneo and Sumatra. It is a large freshwater fish that is commercially important for the aquarium trade and aquaculture. *Scaphognathops bandanensis* is listed as Vulnerable by the IUCN and is native to the LMB and used locally as food and in the aquarium trade. This species resides in the Mekong's mainstream during the dry season in slow, deep reaches, and then migrates to smaller streams or floodplains during the wet season (Rainboth, 1996). All the species (apart from *Barbonymus schwanenfeldii*) are classed as *'migratory main channel spawners'* and are likely to be impacted by hydropower development in the LMB (Commission, 2009).

2.2. Experimental setup

The Mekong River is 4900 km long and flows through six countries: China, Myanmar, Thailand, Laos, Cambodia, and Vietnam. It originates on the Tibetan Plateau and discharges into the South China Sea from Vietnam (Lu & Siew, 2006). The first of the ten mainstream hydropower facilities planned for the Mekong – Xayaburi Hydroelectric Power Plant (XHPP) in northern Lao PDR – has already been constructed and commissioned and includes a dual vertical slot fish pass and lock system (ICEM, 2010). All the wild Mekong fish for this study were caught in a trapping facility located near the exit of the XHPP fish pass (19°14'46.9"N 101°49'20.5"E). The trapping facility was activated at least four times per week. Typically, it required 45 min to lure the fish into the trap. Once fish entered the trap, the gate was closed, and the water was pumped out through a fine screen. Subsequently, captured fish were transported to the Fisheries Research Center at the XHPP, where they underwent a minimum acclimation period of three days in aerated tanks. The trial commenced once we had an adequate number of fish, ranging from 50 to 300 individuals. They were visually inspected daily and fed from the fourth day onwards with manufactured pelleted fish food (CP 9931) each morning (Nguyen, 2013). The PIT tag experiments were initiated after an acclimation period of at least 30 days.

The holding tanks were 12,000 L (5 m x 2 m x 1.2 m) in capacity, but water levels were maintained at 0.6 m (6000 L) for the duration of each trial. The tanks were set up on a flow-through system with a 10% volume of water changed per day, allowing fish to be held in filtered water drawn directly from the Mekong River. The temperature, DO, turbidity, and pH were assessed every morning using a HORIBA Model U-5000 multiparameter probe (Barker, 2017). Ammonia, nitrite, and nitrate concentrations were tested at the same time using test kits (Tetra Test NO_3 (nitrate), a Spectrum Brands Company, Germany) (Table 1).

2.3. Experimental design

The specific design of each trial depended on the number of fish, operators and housing tanks available. The general design principle was to randomly allocate fish to treatment (tag or control), operator, and tank for housing after handling. The proportion of control fish used varied between trials to maximize the number of tagged fish, with a minimum of 20% of fish always allocated to the controlled group (Table 2). In the first two trials, fish allocated for tagging were further randomly assigned to either scalpel (number 11 blades) or syringe (N206) methods for tag insertion. We tested scalpel and syringes as alternative PIT tag insertion techniques to determine if there are preferable ways to tag fish in future studies. Operator was treated as a random factor, where differences between operators could be partitioned before evaluating tagging. Nevertheless, feedback was given to

Table 1
Average water quality in each PIT tag trial at XHPP fish hatchery from June to November 2020. DO = dissolved oxygen concentration.

AVG±SD	Temp (°C)	pH	DO (mg/L)	Conductivity (μS/cm)	Ammonia (ppm)	Nitrate (ppm)	Nitrite (ppm)
Trial 1 *Hypsibarbus lagleri*	26.37 ± 0.43	8.27 ± 0.08	6.55 ± 0.35	349.47 ± 18.06	0.11 ± 0.14	0.00 ± 0.00	12.50 ± 0.00
Trial 2 *Hypsibarbus lagleri*	26.37 ± 0.44	8.27 ± 0.07	6.54 ± 0.35	349.22 ± 18.32	0.11 ± 0.14	0.00 ± 0.00	12.50 ± 0.00
Trial 3 *Hypsibarbus lagleri*	26.09 ± 0.62	8.35 ± 0.08	6.82 ± 0.20	231.87 ± 43.59	0.19 ± 0.27	0.08 ± 0.12	12.50 ± 0.00
Trial 4 *Hemibagrus filamentus*	25.95 ± 0.64	8.28 ± 0.08	6.81 ± 0.23	236.56 ± 40.38	0.22 ± 0.23	0.14 ± 0.22	14.77 ± 7.30
Trial 5 *Barbonymus schwanenfeldii*	26.70 ± 1.77	7.78 ± 0.31	7.98 ± 1.93	218.96 ± 19.06	0.00 ± 0.00	0.00 ± 0.00	12.50 ± 0.00
Trial 6 *Scaphognathops bandanensis*	26.39 ± 1.65	7.76 ± 0.18	7.44 ± 0.71	215.33 ± 16.08	0.17 ± 0.22	0.01 ± 0.03	12.50 ± 0.00

Table 2
Pretreatment descriptive statistics for the four species of fish used in six PIT tag trials.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
	Hypsibarbus lagleri			*Hemibagrus filamentus*	*Barbonymus schwanenfeldii*	*Scaphognathops bandanensis*
Tagging Date	22-06-20	23-06-20	05-08-20	07-08-20	07-10-20	08-10-20
Control	24	24	13	30	16	23
Syringe	18	48	57	59	68	79
Scalpel	25	48				
Total	67	120	70	89	84	102
Minimum length	199	135	126	191	102	140
Average length	290.46	182.42	200.11	285.71	206.29	191.34
Maximum length	445	224	304	400	265	282
Number of tanks	4	4	3	3	3	3
Number of operators	6	6	4	2	2	3

operators after each trial to aid in their performance. All operators had been trained and participated in two pilot trials prior to the current trials; however, it was expected that their tagging performance would improve throughout the study (Robinson et al., 2021).

Fish were housed in three or four separate tanks during each trial (Table 2), with a maximum of 50 fish per tank. Fish larger than 200 mm were chosen for *Hypsibarbus lagleri* trial 1, while fish smaller than 200 mm were chosen for *Hypsibarbus lagleri* trial 2. Fish from these two trials were tagged on consecutive days (trial 1 day 1, trial 2 day 2), but housed in shared tanks after treatment. *Hypsibarbus lagleri* (trial 3) and the other three species trials were completely independent and used individuals of various sizes housed in separate tanks.

2.4. Tagging methodology

The tanks were set up on a flow-through system with a 10% volume of water changed per day, allowing fish to be held in filtered water drawn directly from the Mekong River. All fish were anesthetized using Aqui-S® administered at a concentration of 60 mg/L to induce surgical anesthesia for fish tagging. Anesthesia was typically sufficiently induced (as evidenced by loss of equilibrium and reduced opercular beat rate) in less than 5 min (Lopes et al., 2016). Each fish was measured for length (mm), weight (g), and injected with visual implant elastomer (VIE) in their postocular eye tissue to identify treatment type (Ning et al., 2020). Tagged fish had a glass 23 mm PIT tag (ISO 11784/85, ICAR) inserted into the peritoneal cavity. All fish tagged and control were out of the water for less than 1 min. The peritoneal cavity was chosen as it has the lowest rate of mortality and shedding in other Mekong species (Grieve et al., 2018) and poses the least risk to fish consumers as surviving fish were to be released at the end of the trial. Control fish were subjected to the same handling procedures and time as treatment fish without the tag insertion. All fish were then placed in aerated 60 L recovery tubs, monitored until the anesthetic effects subsided, and then placed in the allocated housing tank which had the same flow system as the pre-tagging tanks. All tagged fish PIT tags were read using a handheld reader before and after insertion.

The trials began between June and October of 2020 and continued for 43–59 days. Mortality and shedding rates are greatest before 34 days (Grieve et al., 2018) and for the current experiments, a minimum of 43 days was chosen to accommodate both the early releasing date and the potential for delayed mortality (Barker, 2017). The fish were assessed daily for tag rejection, mortality and/or changes to their health. Any dead fish were measured in length and weighed; examined to determine the cause of death; and dissected for tag retrieval and placement assessment. In addition, a powerful magnet was utilized to look for and recover lost PIT tags from the bottom of each tank daily. On the final day of the experiment, the remaining fish were measured in length and weight, and then released back to the Mekong River.

2.5. Statistical methods

2.5.1. Mortality

A general linearized model with a binary response (mortality or not) was used to assess the mortality rates of fish in each trial. There were multiple potential factors affecting mortality that were investigated; namely:

Covariates – these included the size of the fish being tagged, weight, and body condition index, which is a combination of length and weight using Fulton's Index (Bolger and Connolly, 1989; Ricker, 1975).

Operator and housing tank – these were not of primary interest to the study but could affect the variability of the response and were considered random effect factors that were partitioned out before testing fixed factors.

In all trials, the comparison between tagged fish and untagged fish (control fish) was the primary fixed factor of interest. In *Hypsibarbus lagleri* trial's 1 and 2, the analyses also compared mortality rates between two types of tagging treatment (injecting a tag with a syringe or using a scalpel to prepare an incision for tag insertion).

The statistical model included all fixed and random factors, but as the covariates were correlated with each other, the analysis method included each covariate in the model independently. Results presented compare significance levels of each covariate to determine which, if any, of them added significant explanation to the final model. If none were significant, the final model included only the fixed and random factors.

2.5.2. Tag rejections

The tag rejection analyses considered the same covariates and random effects as the tag mortality model, but only included tagged fish. The primary factor(s) of interest were whether the likelihood of tag rejection was related to the size of the fish when tagged, in species that had a high tag rejection rate. This can inform suitable fish size ranges for future monitoring programs.

2.5.3. Body condition

As wild-caught fish were utilized in the trial, there were concerns regarding husbandry aspects such as feeding, disease, and tank environment. The Fulton Body Condition Index could provide a short-term measurement in this context. The Fulton Body condition index was compared between tagged and untagged fish (treatment; fixed factor), before and after the experiment (B/A; repeated measure), with housing tank included as a random factor and fish within tanks as random subjects in a repeated measures design. The analysis for body condition for trials 1 and 2 was pooled because of the shared tanks. That is, whilst tagged fish were uniquely identifiable, the control fish were shared between the two experiments. For the body condition analyses, the primary response of interest was the interaction of B/A and treatment (i.e. changes in condition that were independent of treatment were to be expected when all fish improve or decline in condition in response to husbandry or handling). However, if tagging affects fish condition or ability to change condition, then any difference in the condition in tagged and control fish after the trial would not be the same as any difference at the start of the trial.

The analyses to determine whether there had been a change in body condition associated with the tag was complex as the control fish did not have a unique identification number, and this was further confounded by tagged fish that had rejected their tag also not being uniquely identifiable. That is, they may have been identified as having been tagged and shed, but their measurements at the start of the study were not identifiable. Hence, the data formed a partially repeated measures experimental design, where changes in measurements of PIT tagged fish could be analyzed using say a paired t-test, but control or untagged fish would need to be analyzed using an independent samples t-test. To accommodate the partial repeated measures design, the analysis was undertaken using a linear mixed model with restricted maximum likelihood and general Satterthwaite approximation for the denominator degrees of freedom (Hrong-Tai Fai and Cornelius, 1996). The analyses did not include operator as an effect as the operator that handled a control fish at the start of the trial was unknown at the trial's conclusion. Significant effects were tested using follow-up pairwise comparisons of least squares means, adjusted for Type 1 error using Scheffe's correction.

Mortality and tag rejection analyses were performed using IBM SPSS Statistics 29.0.0.0, and body condition analyses were performed using SAS Institute. (2015) *SAS/STAT® 14.1 User's Guide*. Cary, NC: SAS Institute Inc.

Table 3
Results from statistical models to test the effects of fish body size parameters and experimental treatments on the tag related mortality and tag rejection rates (binomial models) and body condition index (mixed model) of the four species of fish assessed in the PIT tag trials. ^ Control fish were not included in the comparison between scalpel and syringe methods. Operators were not included in the body condition models.

MORTALITY RATES						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
	Hypsibarbus lagleri			*Hemibagrus filamentus*	*Barbonymus schwanenfeldii*	*Scaphognathops bandanensis*
Overall mortality rate	13.2%	0.8%	10.0%	16.9%	3.6%	36.3%
Covariates						
Starting weight	ns	ns	ns	$\chi^2 = 10.6$, df= 1, $p < 0.001$	ns	$\chi^2 = 5.8$, df= 1, $p < 0.03$
Starting length	ns	ns	ns	$\chi^2 = 10.4$, df= 1, $p < 0.001$	ns	$\chi^2 = 4.4$, df= 1, $p < 0.05$
Starting body condition	ns	ns	ns	ns	ns	ns
Random factors						
Operator	ns	ns	ns	ns	ns	ns
Tank	ns	ns	ns	ns	ns	$\chi^2 = 13.6$, df= 2, $p < 0.001$
Fixed factors						
Tagged vs Control	ns	ns	ns	ns	ns	ns
Scalpel vs Syringe^	ns	ns				
TAG REJECTION RATES						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
	Hypsibarbus lagleri			*Hemibagrus filamentus*	*Barbonymus schwanenfeldii*	*Scaphognathops bandanensis*
Overall rejection rate	5.9%	1.7%	15.7%	7.9%	0%	0%
Covariates						
Starting weight	ns	ns	ns	ns	ns	ns
Starting length	ns	ns	ns	ns	ns	ns
Starting body condition	ns	ns	ns	ns	ns	ns
Random factors						
Operator	ns	ns	ns	ns	ns	ns
Tank	ns	ns	ns	ns	ns	ns
Fixed factors						
Scalpel vs Syringe^	ns	ns				
BODY CONDITION						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
	Hypsibarbus lagleri			*Hemibagrus filamentus*	*Barbonymus schwanenfeldii*	*Scaphognathops bandanensis*
Random factors						
Tank	ns		ns	ns	F = 5.31. df = 2106, $p < 0.01$	F = 4.7. df = 2121, $p < 0.05$
Tank × Treatment	ns		ns	ns	ns	ns
Tank × B/A	F = 5.23. df = 2169, $p < 0.01$		ns	ns	ns	ns
Tank × B/A × Treatment	ns		ns	ns	ns	ns
Fixed factors						
Treatment	ns		ns	ns	ns	ns
B/A	ns		ns	F = 54.2. df = 1149, $p < 0.0001$	F = 4.55. df = 1125, $p < 0.05$	F = 21.67. df = 1146, $p < 0.0001$
Treatment × B/A	F = 5.25. df = 2164, $p < 0.01$		ns	ns	ns	ns

3. Results

3.1. Fish mortality

There was no significant effect (p > 0.05) of PIT tagging on the mortality of any of the four species across the six trials (Table 3). There was a relationship between the size of fish and mortality rate for two species (Table 3). *Scaphognathops bandanensis* mortality rate and size of fish were further confounded by husbandry issues associated with one of the housing tanks (Table 3). *Hemibagrus filamentus* mortality rates were similar in control (13%) and tagged (19%) individuals, suggesting that the mortalities were more likely caused by husbandry issues rather than tagging. Following the necropsy of the deceased *Hemibagrus filamentus* fish in trial 4, four control fish and 11 tagged fish had perished. Among the 11 tagged fish fatalities, only two were potentially attributed to injuries related to tagging (specifically, the tag was inserted too deep into the body cavity), but all mortalities occurred within 8 days after the tagging procedure. The remaining nine exhibited proper tag insertion without any internal injuries. The relationship between size and *Hemibagrus filamentus's* mortality was negative, and larger size fish were more likely to die (Fig. 1). For each 1 mm increase in the length of *Hemibagrus filamentus*, the probability of mortality of tagged fish increased by 2%; while the probability of mortality decreased by 2.4% for every 1 mm increase in the length of *Scaphognathops bandanensis* (Fig. 2). Smaller fish were more susceptible to disease outbreaks (Fig. 2).

3.2. Tag rejection

Two of the four species rejected PIT tags and had tag rejection rates of between 1.7% and 15.7% (Table 3). There was no significant relationship (p > 0.05) between tag rejection rates and the size of the fish or the tagging method.

3.3. Body condition

Body condition index was influenced by PIT tagging in *Hypsibarbus lagleri* trial's 1 and 2 (Table 3). Control fish had the same body condition before and after the trial, whereas syringe tagged fish marginally decreased in condition, but the decrease was not statistically significant (p = 0.973) (Fig. 3). Scalpel tagged fish in trial's 1 and 2 significantly increased (p = 0.015) in condition during the trial (Fig. 3). Nevertheless, body condition in *Hypsibarbus lagleri* was not affected by tagging during the next trial (Table 3). *Hemibagrus filamentus*, *Barbonymus schwanenfeldii, and Scaphognathops bandanensis* all had changing conditions in tagged and control fish during the trial (Table 3). The latter two species significantly improved in body condition, while *Hemibagrus filamentus* decreased in body condition in both control and tagged fish (Fig. 4).

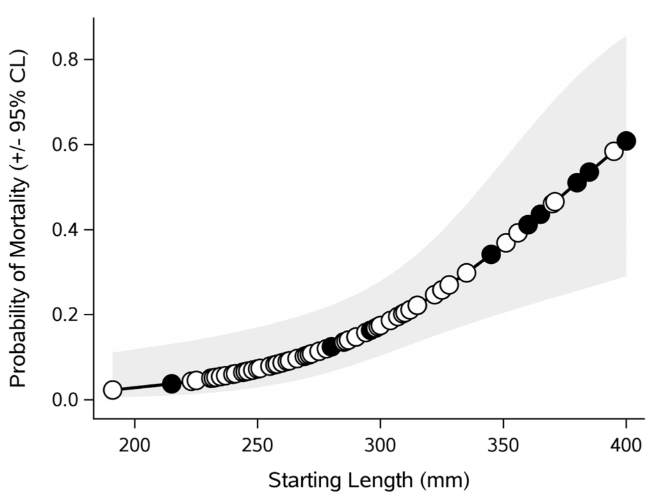

Fig. 1. Modelled relationship between size of fish and mortality rate of *Hemibagrus filamentus* during the PIT tag trial in August 2020 (both tagged and control fish were included). Blackline is modeled mortality, shaded area is 95% confidence interval, markers are mortality (solid) or survived (hollow).

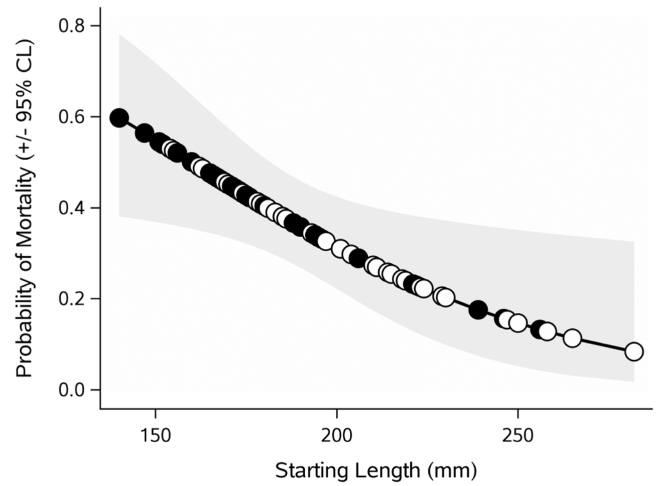

Fig. 2. Relationship between size of fish and disease related mortality rate of *Scaphognathops bandanensis* during the PIT tag trial in October 2020 (both tagged and control fish were affected). Blackline is modeled mortality, shaded area is 95% confidence interval, markers are mortality (solid) or survived (hollow).

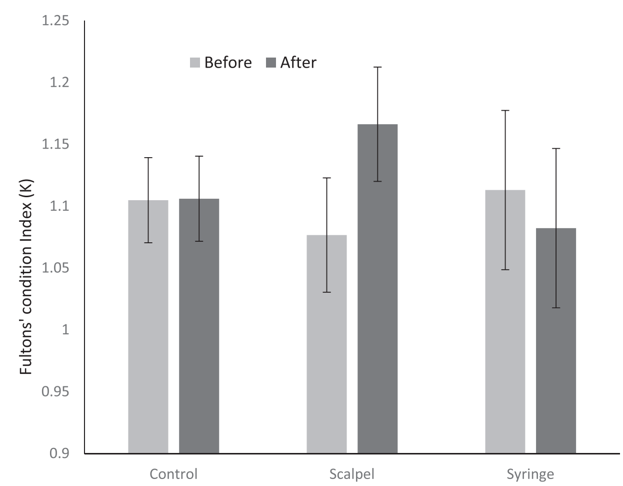

Fig. 3. Average (+/- STD err) Fulton Condition Index for *Hypsibarbus lagleri* for each treatment at the beginning and end of PIT tag trial's 1 and 2.

4. Discussion

This study expanded on (Grieve et al., 2018), by investigating the suitability of four wild-caught tropical species from the Mekong River (*Hypsibarbus lagleri, Hemibagrus filamentus, Barbonymus schwanenfeldii, and Scaphognathops bandanensis*) to PIT tagging. The four wild Mekong fish species used in this study were found to be suitable for tagging with 23 mm PIT tags. The slightly higher than desired mortality rates in *Scaphognathops bandanensis* and *Hemibagrus filamentus* were attributable to housing problems and not the tagging process. In the initial *Hypsibarbus lagleri* trial, the mortality rate for all operators was higher than in the subsequent trials and there was no difference in mortality between controlled and tagged fish. After accounting for confounding variables such as operator experience, retraining, and husbandry issues, rejection

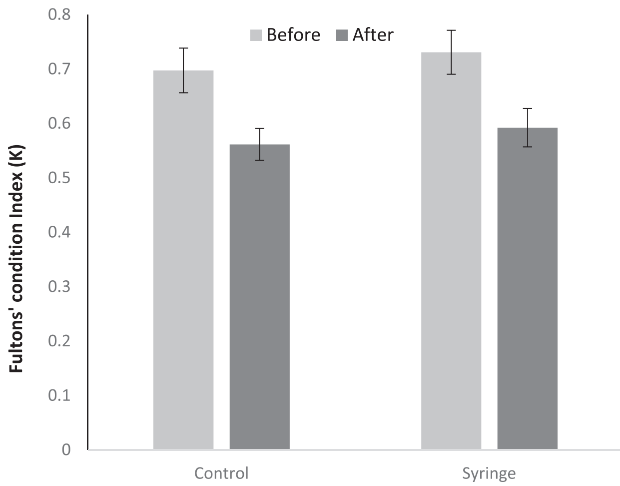

Fig. 4. Average (+/- STD err) Fulton Condition Index for surviving *Hemibagrus filamentous* at the beginning and end of PIT tag trial 4.

rates were low. During these trials, no species lost body condition from being tagged.

4.1. Mortality

There was no significant difference in mortality between the control fish and the tagged fish in these trials. These results are comparable to those found in hatchery-reared *Pangasianodon hypophthalmus* and *Hypsibarbus malcolmi* (Grieve et al., 2018). By adhering to best-practice tagging techniques, the risk of mortality was minimized (Grieve et al., 2018). Comparable mortality patterns were noted in the case of tagged fish, with the majority of deaths occurring in the initial two days following tagging (Brewer et al., 2016). However, *Hemibagrus filamentus* experienced size-related mortality in the tanks due to its morphology. This species has spikes that can damage the fish's body if it continuously makes sharp turns (Jawad, 2021; Noble et al., 2012). Due to the constant flow through system from the Mekong River, the individual fish that were used in our research were observed to constantly swim around the tanks. As the tanks were square-shaped, fish regularly swam into a corner and consequently spiked themselves when turning (Shadrack et al., 2021). This, in turn, frequently led to the gradual development of potentially fatal bacterial or fungal diseases and the observed high mortality rate, with larger fish being more susceptible. Notably, the mortality was observed in both control and tagged fish, as was the decline in body condition, which is consistent with a husbandry issue for this species (Kimball and Mace, 2020; Macaulay et al., 2021). In trial 6, one of the three tanks containing *Scaphognathops bandanensis* in the experiment experienced a disease outbreak, resulting in a high mortality rate in both tagged and control fish (Jawad, 2021; Noble et al., 2012). Despite this unfortunate event, it was possible to use the fish in the two remaining tanks to complete the experiment and affirm that this species is suitable for tagging, as tag-related mortality was low (Grieve et al., 2018; Ning et al., 2020). This demonstrates the value of well-designed experiments and housing appropriate to target species.

4.2. Tag rejection rates

The overall rejection rate was very low, and comparable to the rejection rates found for hatchery reared *Pangasianodon hypophthalmus* and *Hypsibarbus malcolmi* (Grieve et al., 2018). The overall rejection rate was comparable to the rejection rate in temperate species (e.g. Steelhead trout (1.2%): *Oncorhynchus mykiss* (Welch et al., 2007) and Chinook salmon (0.004%): *Oncorhynchus tshawytscha* (Dare, 2003). In trial 3, a higher rejection rate was the result of nine out of sixteen fish tagged by a single operator rejecting their tags.

PIT tag retention rates are believed to be affected by various factors, such as the tag insertion technique (Archdeacon et al., 2009; Gheorghiu et al., 2010), as well as the size of PIT tag (Larsen et al., 2013; Lopes et al., 2016) and the fish size (Baras et al., 2000; Gheorghiu et al., 2010). The analysis from this study revealed that the operator effect and fish size were not statistically significant, but tag retention rate improved with increased operator's experience (Cooke et al., 2003; Pennock et al., 2016; Robinson et al., 2021). In the last experiment, this operator's rate of rejection was improved to 1.7%. Experienced operators can work quickly and effectively, reducing the amount of time animals are removed from their natural habitat and minimizing their exposure to stress or injury (Cooke et al., 2003; Pennock et al., 2016).

4.3. Body condition

Due to the utilization of wild-caught fish in the trial, there were apprehensions regarding husbandry factors, including feeding, disease, and tank environment. The Fulton Body Condition Index could offer a short-term measurement in this regard Index (Bolger and Connolly, 1989; McCabe et al., 2019; Ricker, 1975). In the current study, PIT tagging affected the body condition index in trial's 1 and 2 only. The body condition of the control fish and the syringe-tagged fish remained the same before and after the experiment (Hahlbeck et al., 2023). The scalpel technique is potentially less susceptible to infection than the syringe technique, because it does not involve entering the body cavity (Archdeacon et al., 2009; Schiavon et al., 2023). However, the results of the body condition comparisons between trial's 1 and 2 should be interpreted with caution, because fish from two distinct size cohorts were housed together for logistical purposes. This increased the variability of the body condition index of the controlled fish, as the distribution of fish sizes in each tank became bimodal, with a group of smaller fish and a group of larger fish in each tank. Notably, the body condition of the control and syringe fish did not change significantly over the course of the experiment; only the body condition of the scalpel fish did. The random assignment of fish to treatments, tanks, and operators resulted in the scalpel treatment group beginning the experiment in poorer body condition, which may have meant that they were more likely to recover during the experiment (Kimball and Mace, 2020; Macaulay et al., 2021). Similarly, the syringe fish started in slightly higher condition on average and were therefore more likely to lose condition anyway.

Nonetheless, the potential influence of tagging on body condition in the first two trials was moderated as the operators gained experience (Cooke et al., 2003; Pennock et al., 2016). That is, *Hypsibarbus lagleri* is proposed to be suitable for tagging because there was no effect of tagging on body condition in the final trial, which also included small and large fish, tagged by more experienced operators.

5. Conclusion

Our results indicate that *H. lagleri, H. filamentus, B. schwanenfeldii, and S. bandanensis* are all suitable for being PIT tagged in tropical river systems. PIT tagging did not significantly impact mortality; no fish lost condition from tagging; and the overall tag rejection rate was very low in all four species. Nonetheless, due consideration must be given to husbandry and operator experience to minimize the potential for mortalities in PIT tagging studies.

CRediT authorship contribution statement

Huang Xiaodi: Formal analysis, Conceptualization. **Robinson Wayne:** Supervision, Methodology, Formal analysis. **Poomchaivej Thanasak:** Writing – original draft, Formal analysis, Data curation. **Baumgartner Lee J.:** Methodology, Conceptualization. **Ning Nathan:**

Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fishres.2023.106930.

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