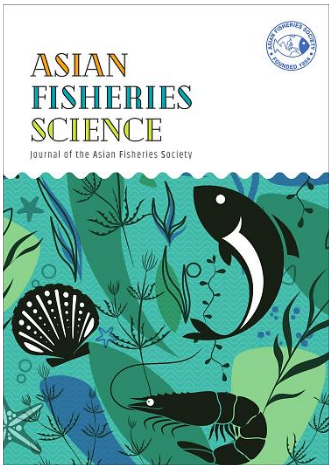

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Distribution of Suckermouth Armoured Catfishes (Siluriformes, Loricariidae) Across the Salinity Gradient of the Mekong Delta, Vietnam

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Abstract

It has been suggested that suckermouth armoured catfishes of the genus *Pterygoplichthys* Gill, 1858 (Siluriformes: Loricariidae) might use brackish water systems as a means to disperse between freshwater bodies. This study aimed to explore the dynamics of *Pterygoplichthys* spp. distribution in relation to salinity gradient and provide population characteristic data, namely length, weight, condition factors by sampling the Co Chien and Ham Luong rivers, Mekong Delta, Vietnam. Contrary to initial predictions, there was neither evidence for the presence of *Pterygoplichthys* spp. in brackish waters in the Mekong Delta nor for the fishes' colonisation of an area that fluctuates seasonally between freshwater and brackish water. Length-weight relationship and condition factor of the fishes demonstrated negative allometric growth and good physiological condition. The findings from this study could help fishery managers to map out the distribution of *Pterygoplichthys* spp. in the Mekong Delta as well as raise awareness on the perils of releasing aquarium pets.

Keywords: aquatic invasion, fisheries management, freshwater-saline transition, body condition, Southeast Asia

Introduction

The suckermouth armoured catfishes (Siluriformes: Loricariidae) comprise nearly 1,000 nominal species native to South and Central America (Armbruster and Page, 2006; Froese and Pauly, 2020). One genus in particular, *Pterygoplichthys*, contains popular aquarium animals that have subsequently invaded twenty-one countries on five continents (Orfinger and Goodding, 2018). At least four of the 16 described species exhibit established invasive populations, along with putative hybrids (Orfinger and Goodding, 2018; Anjos et al., 2020). Outside of its native Neotropics, this genus exhibits large populations, causing socioeconomic threats (e.g. damaging fisheries equipment, reducing fish catch) and disrupting ecological processes (e.g. altering food webs, competing with local fishes, causing bird mortality via asphyxiation, and introducing non-native parasites or diseases)(Hoover et al., 2004; Nico et al., 2009; Capps and Flecker, 2013; Orfinger et al., 2019).

As in other Southeast Asian countries, the suckermouth armoured catfishes first appeared in Vietnamese ornamental fish shops during the 1990s (Levin et al., 2008). Little is known when they successfully established in natural water in Vietnam, but it likely occurred before 2004 (Research Institute for Aquaculture No. 2, unpubl. report). Since then, two species, *Pterygoplichthys disjunctivus* (Weber, 1991) and *Pterygoplichthys pardalis* (Castelnau, 1855), have become established in natural waters in Vietnam including ponds, canals and rivers (Levin et al., 2008; Trần et al., 2013; Zworykin and Budaev, 2013; Gusakov et al., 2018). Consequently, the Vietnamese government first designated these introduced fishes as alien invasive species within the country under the circular 27/2013/TTLT-BTNMT- BNNPTNT, September 2013 and the status was retained in the new updated circular 35/2018/TT-BTNMT, December 2018.

The family Loricariidae is considered a strictly freshwater family of fishes in their native range throughout the Neotropics (Myers, 1949). However, scattered collection records of *Pterygoplichthys* spp. in saline waters (i.e. 5 - 8 g.L^{-1}) in their introduced ranges have raised questions regarding the breadth of their physiological tolerance to salinity (Capps et al., 2011; Brion et al., 2013). In addition, presumptive hybridisation among *Pterygoplichthys* species, e.g., between *P. disjunctivus* and *P. pardalis*, has been recorded in North American and Asia (Wu et al., 2011; Bijukumar et al., 2015; Orfinger and Goodding, 2018). Could such hybrids benefit from broader physiological tolerance than their non-hybrid counterparts? (Wu et al., 2011). Recent laboratory experiments suggest that these taxa can tolerate salinity up to 12 $g.L^{-1}$, raising concerns that they could use estuaries as salinity bridges to invade new waterways (Capps et al., 2011; Brion et al., 2013; Bijukumar et al., 2015). Therefore, the objective of this study was to evaluate the extent to which differences in salinity might compromise the dispersal of *Pterygoplichthys* spp. In addition, population characteristics in the form of length-weight data and condition factor were calculated to quantify the physical well-being of the Mekong Delta populations.

Materials and Methods

Study area

Co Chien and Ham Luong rivers are two tributaries of the Mekong River in Ben Tre Province, Vietnam. Hydrologic regimes in the rivers are regulated by the combination of flow from the Mekong River, seasonal effects (dry season: November to April; rainy season: May to October), semi-diurnal tidal influence from the South China Sea, and water runoff from surrounding agricultural areas (Tri, 2012). Water quality is still considered suitable for aquatic life (Kongmeng and Larsen, 2018). There are 114 fish species found in the rivers (Trần et al., 2013), however, fishing activity exerts intense pressure on these populations (Hortle, 2009).

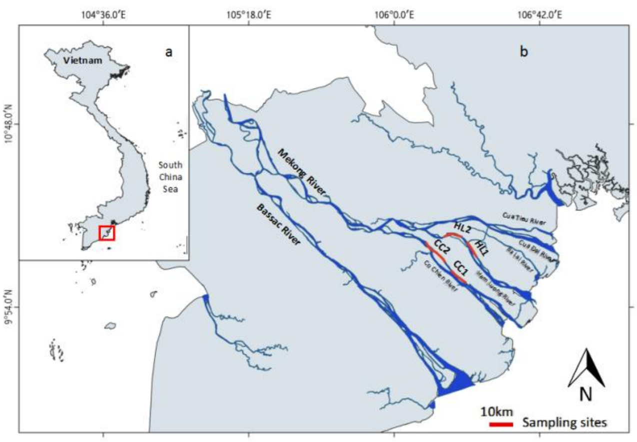

Fig. 1. (a) Ben Tre Province (square), Vietnam, the study site for the distribution of suckermouth armoured catfishes of the genus *Pterygoplichthys* spp. (b) Samplings sites in Co Chien (CC) and Ham Luong (HL) rivers in Ben Tre Province. CC1 and HL1 are downstream and characterised by freshwater in rainy season and saline water in dry season, while CC2 and HL2 are upstream and freshwater year-round.

Sampling regime

Samplings were carried out from March to July 2019 from two stretches in Co Chien River: stretch CC1 (10°02'17" – 10°04'32N; 106°20'50" – 106°16'43"E) and stretch CC2 (10°08'54" – 10°12'53"N; 106°13'25" – 106°09'19"E; Fig. 1). Stretch CC1 is 35 km from the coast and freshwater (hereafter referring to salinity <1 $g.L^{-1}$) in the rainy season and reaches salinity levels of 4-6 $g.L^{-1}$ in the dry season. Stretch CC2 is about 30 km upstream of stretch CC1 and freshwater year-round. Two additional stretches were sampled in Ham Luong River: stretch HL1 (10°09'14" – 10°13'12"N; 106°23'35" – 106°21'17"E) and stretch HL2 (10°15'26" – 10°14'48"N; 106°19'13" – 106°11'53"E). Stretch HL1 is about 40 km from the coast and freshwater in the rainy season and its salinity reaches up to 10 $g.L^{-1}$ in the dry season. Site HL2 is about 53 km from the coast and freshwater year-round.

Fish were collected using bottom trawl net (4 m wide, 7 m long, and 2 cm mesh size) in the morning. Time and sampling gear reflect the fact that members of *Pterygoplichthys* are gregarious benthic fishes. Each net was trawled along the river bank using a boat running at a speed of 3 $km.h^{-1}$ and hauled for every 20 min. An average of eight to ten hauls per sampling were conducted with a total sampling distance of 10 km per stretch. General physiochemical parameters of surface water including salinity ($g.L^{-1}$), dissolved oxygen (DO), pH, temperature (°C) and total dissolved solid (TDS, $mg.L^{-1}$) were measured *in situ* using a digital multimeter (CUSTOM, Japan).

Sampling frequency was determined for each site according to earlier information from local fishers and to meet research objectives. To evaluate *Pterygoplichthys* spp. ability to compromise difference salinity gradient, two samplings were carried out: one in the dry season (March) and another in the rainy season (July) in stretch CC1, where a temporal freshwater-saline shift was demonstrated between two seasons. In stretch HL1, where the salinity regime was similar to the CCI stretch, local fishers were interviewed for information on the presence of *Pterygoplichthys* spp., instead of the usual bottom trawl sampling. Two local fishers were interviewed at each stop spanning 3.5 km, and a total of six fishers were interviewed. For the interview, the fishers were asked three questions: 1) Have you caught *Pterygoplichthys* spp. in this area and, if so, when? 2) How many *Pterygoplichthys* spp. have you caught each time, on average? and 3) Do you see *Pterygoplichthys* spp. distributed in nearby water? Where the presence of *Pterygoplichthys* spp. were reported consistently, five independent samplings were conducted equally spaced throughout March to July 2019 in stretch CC2 and one sampling in June 2019 in stretch HL2, to evaluate the composition and physical well-being of *Pterygoplichthys* spp.

Local fishers were hired to report and collect the *Pterygoplichthys* spp. in the fishery bycatch in Co Chien River (CC1 and CC2). Collected fish were measured for total length (TL) to the nearest 0.1 cm and weighted for body mass (W) to the nearest 0.1 g. All fishes were tagged and labelled in the field, fixed in 10 % formalin solution, and brought back to the lab of the Institute of Tropical Biology and identified based on the number of dorsal fin rays and ventral patterning (Armbruster and Page, 2006; Page and Robins, 2006; Wu et al., 2011). A voucher specimen and DNA fin clip samples were deposited in the Aquatic Animal Collection of the Institute of Tropical Biology.

Data analysis

Specimens were identified and tabulated by month. Length-weight relationship was determined using the equation of Le Cren (1951):

$$W = a \times TL^b$$

where W is body mass (g), TL is total length (cm), *a* and *b* are parameters of the length-weight relationship equation.

Fulton's condition factor (K) was calculated using the equation of Bagenal and Tesch (1978):

$$K = 100 \times \frac{W}{TL^3}$$

and,

Relative condition factor (K_n) was calculated using the equation of Le Cren (1951):

$$K_n = \frac{W}{a \times TL^b}$$

To reduce the potential bias due to low sample size, bootstrapping with 1,000 replicates (Canty and Ripley, 2019) was performed to estimates 95 % confidence interval of K_n. ANOVA analysis was used to compare the difference of K_n between four size classes (TL: <20 cm, 20–30 cm, 30-40 cm and >40 cm, adapted from Samat et al., 2008). All analysis was performed using R (R Core Team, 2013).

Results

Environmental conditions

General physiochemical parameters of surface water measured during the field samplings are presented in Table 1. The highest salinity during our sampling regime in the dry season was 1.5 and 1.2 g.L^{-1} in CC1 and HL1 respectively, while the CC2 and HL2 were freshwater across all measurements (i.e., ~0.1 g.L^{-1}). Weekly measurement at the nearby monitoring stations in the dry season showed the salinity in CC1 ranged from 0.1 to 4.8 g.L^{-1} and in HL1 from 0.1 to 10 g.L^{-1}, depending on the year (Fig. 2a). Salinity intrusion has not reached the CC2 for the last 3 years, whereas the maximum salinity measured in the HL2 has reached ~ 2 g.L^{-1} more recently (Fig. 2b).

Table 1: River information (depth, width) and physiochemical parameters (salinity, temperature, pH, DO, TDS) of surface water at the sampling stretch.

Physiochemical parameters	CC1	CC2	HL1	HL2
Depth (m)	~ 11-12	~ 10-11	~ 12-13	~ 11-12
Width (m)	~ 2,100	~ 2,100	~ 1,000	~ 1,200
Salinity (g.L^{-1})	0.1-1.5	0.1	1.2	0.0-0.1
Temperature (°C)	28.3-32.1			
pH	6.28-7.43			
DO (mg.L^{-1})	5.1-6.5			
TDS (mg.L^{-1})	83-176	<30	100-190	<60

CC = Co Chien River, HL = Ham Luong River.

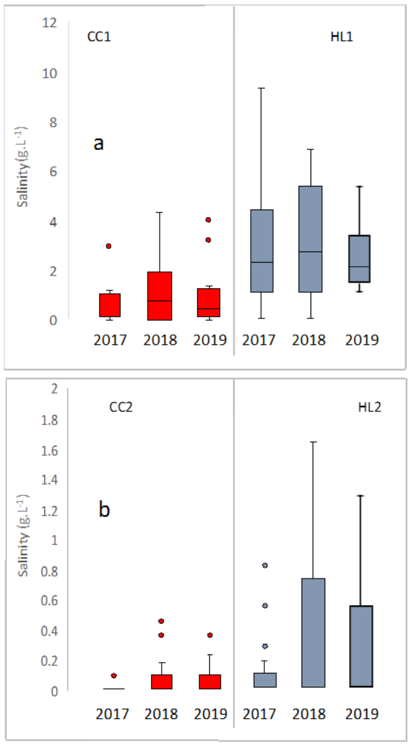

Fig. 2. Range of salinity (g.L^{-1}) measured in dry season (January to May) from 2017-2019 in Ben Tre by sampling site (BTEMC, 2019). CC1 and HL1 (a), and CC2 and HL2 (b) are samplings stretches downstream and upstream of Co Chien and Ham Luong rivers, respectively. The boxes show first quartile, median, and third quartile. The bars show minimum and maximum values. Circles represent outliers.

Sample composition

A total of 37 suckermouth armoured catfishes were caught throughout the sampling period. All were identified belonging to the genus *Pterygoplichthys* by possession of a single dorsal spine and twelve dorsal soft rays (Armbruster and Page, 2006). Sixteen samples were identified as *P. disjunctivus* by displaying solely coalesced spots on the abdomen (Fig. 3). Another 21 samples were identified as the putative hybrid of *P. disjunctivus* and *P. pardalis* (Wu et al., 2011) with the mixing of coalesced spots and discrete spots on the abdomen (Fig. 3). There were no *P. pardalis* samples recovered, which are identified by the presence of only discrete spots on the abdomen. The absence of *P. pardalis* suggests that likely hybridisation of *P. disjunctivus* and *P. pardalis* occurred in their native range or, more likely, in the aquarium trade before the fish were introduced to the area.

Pterygoplichthys distribution

All *Pterygoplichthys* were obtained from stretch CC2 and HL2 (Fig. 4). There were no *Pterygoplichthys* caught in stretches CC1 and HL01, where there are annual intrusions of salinity in the dry season. The absence of *Pterygoplichthys* in the CC1 and HL1 was corroborated by all six fishers interviewed during the samplings. Fishers also reported that *Pterygoplichthys* could be found in greater abundance in *Pangasius* (Siluriformes: Pangasiidae) aquaculture ponds along the rivers. It is possible that *Pterygoplichthys* are entering the ponds through daily water exchange practices. *Pterygoplichthys* seem to have colonised the ponds and cannot be eradicated due to the ponds' depths (~ 4 m), daily water exchange with surrounding waterways, and farmer's neutral conception toward the impact of these fish.

Length weight relationship

Total length (TL, cm) of fishes ranged from 14.5 to 45 cm while weight ranged (W, g) from 28 to 678 g. A strong relationship between length and weight of the *Pterygoplichthys* spp. was described as $W = 0.0252 \times TL^{2.681}$ ($a = 0.0252 \pm 0.0119$; $b = 2.681 \pm 0.198$, ± CI 95 %, $r^2 = 0.955$, $P < 2.2 \cdot 10^{-16}$; Fig. 5). Given that b = 2.681 ± 0.198, the population sampled demonstrated negative allometric growth.

Fulton's condition factor (K) and relative condition factor (Kn)

Fulton's condition factor (K) ranged between 0.61 and 1.22 with an average of 0.913, and K decreased with increasing length ($r^2 = 0.23$, $P = 0.0027$; Fig. 6). Relative condition factor (K_n) ranged from 0.68 to 1.31 with a mean of 1.01. The bootstrapping result of the K_n showed a very low bias value (~0.0011) of the mean K_n with the CI 95 % ranged from 0.965 - 1.062. ANOVA analysis of K_n did not show significant difference (P = 0.645) between any paired-size class (Fig. 7).

Discussion

This study documented the presence or absence of non-native *Pterygoplichthys* spp. in freshwater and saline transitional areas in two major tributaries of the Mekong River in Vietnam. Based on sampling efforts and interviews with local fishers on Co Chien and Ham Luong rivers, this study found no evidence of *Pterygoplichthys* spp. in brackish areas in the Mekong Delta. The result of the present findings contradicts a previous study conducted in southeastern Mexico (Capps et al., 2011), during which samples of *Pterygoplichthys* spp. were collected in an area with salinity of up to 8 g.L^{-1}. However, southeastern Mexico remains the only location from which *Pterygoplichthys* spp. are known to inhabit saline waters (Capps et al., 2011). To further confirm their distribution along salinity gradients, future work must be conducted to expand the scope to all main tributaries in Mekong Delta, Vietnam and other invaded waterways.

Despite the salinity tolerance of *Pterygoplichthys* spp. demonstrated in laboratory experiments (surviving up to 12 g.L^{-1}) (Capps et al., 2011; Brion et al., 2013; Kumar et al., 2018), the present field experiments suggest that salinity remains a limiting factor to the dispersal of the *Pterygoplichthys* spp. in the Mekong Delta. Like other catfish families with a less permeable integument (Kendall and Schwartz, 1968; Bringolf et al., 2005), the armoured plating of *Pterygoplichthys* may hinder osmoregulation. The impacts of salinity on the physiology of *Pterygoplichthys* remain unclear. For

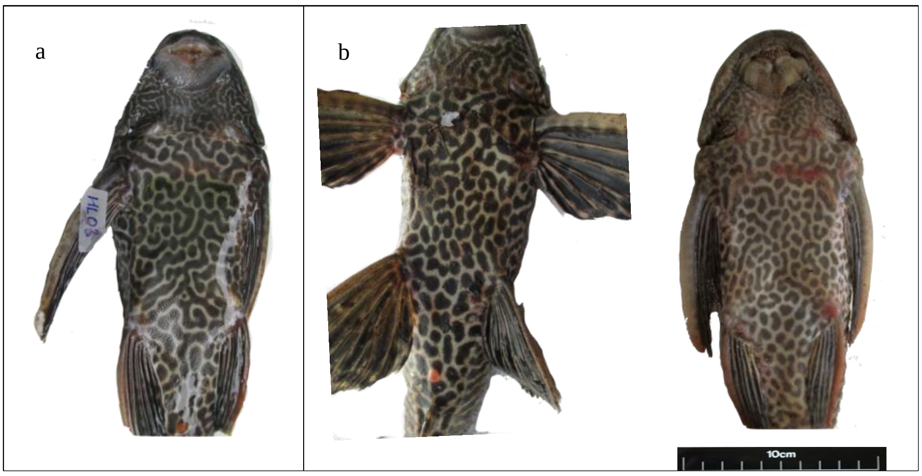

Fig. 3. Ventral view of representative samples. (a) *Pterygoplichthys disjunctivus*; (b) shows putative hybrids of *Pterygoplichthys disjunctivus* and *Pterygoplichthys pardalis*.

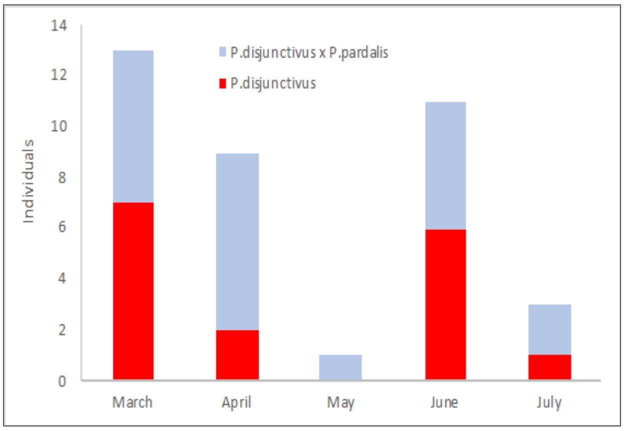

Fig. 4. Pterygoplichthys spp. sample composition by sampling month in Co Chien and Ham Luong rivers.

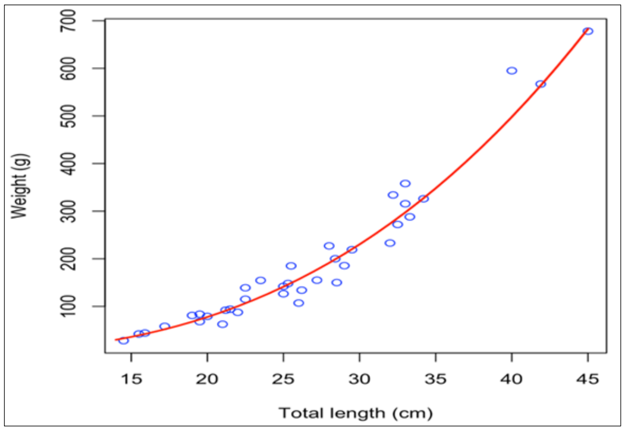

Fig. 5. Length weight relationship of *Pterygoplichthys* spp. in Co Chien and Ham Luong rivers.

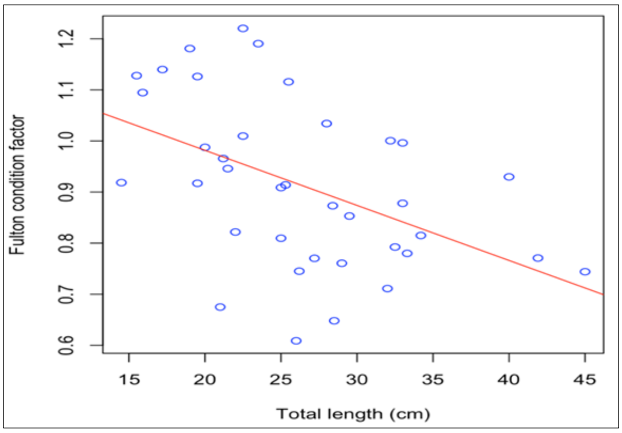

Fig. 6. Relationship between total length and Fulton's condition factor (K) of *Pterygoplichthys* spp. in Co Chien and Ham Luong rivers.

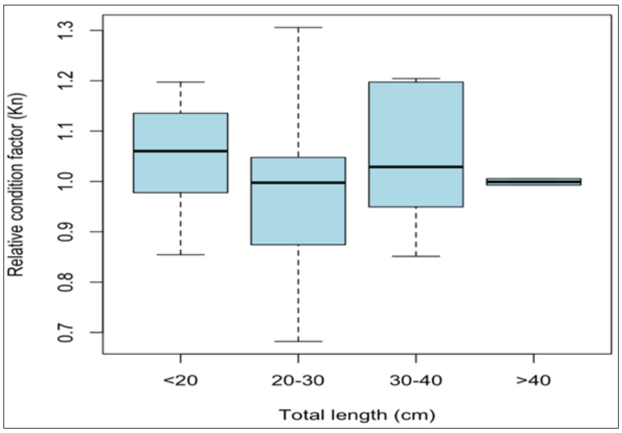

Fig. 7. Relative condition factor (K_n) per size class of *Pterygoplichthys* spp. The boxes show first quartile, median, and third quartile. The bars show minimum and maximum.

example, no spawning of ictalurid catfishes has been observed in salinity water greater than 2 $g.L^{-1}$ (Perry, 1973). Similarly, (Capps et al., 2011) did not find evidence of the *Pterygoplichthys* spp. reproducing in brackish-water habitats, perhaps lending support to the armour-osmoregulatory hypothesis. Alternatively, the observed lack of *Pterygoplichthys* in saline habitats could potentially be explained by indirect trophic effects. For example, restricted growth of preferred algal food sources and/or encouraging growth of sub-optimal food sources might occur at higher salinities, yielding an ecological barrier. Future field and laboratory studies should seek to address these physiological, bottom-up, and top-down hypotheses of dispersal limitation to determine the mechanistic processes governing the absence of the genus in saline areas of the Mekong Delta.

As is commonly seen throughout the non-native range of *Pterygoplichthys* spp., its allometry (b) is smaller than the cubic value of three (Jasso et al., 2013; Samat et al., 2016; Wei et al., 2017), demonstrating that the fishes grow faster in length than in weight. The growth parameters of *Pterygoplichthys* spp. also often fail to show impacts based on seasonality (Samat et al., 2016) or sex (Wei et al., 2017).

Condition factor (K) is a proxy of overall physical well-being. Relative condition factor (K_n) evaluates the physical condition while simultaneously compensating for changes in well-being with an increase in length (Samat et al., 2008). Even though Fulton's condition factor and relative condition factor (K_n) of suckermouth armoured catfishes in this study were both virtually equal to 1, it seems that *Pterygoplichthys* spp. in the Mekong Delta are in better physiological condition than in the nearby Malaysian Peninsula (Samat et al., 2008), inviting comparative studies about the taxonomy, physiology, and ecology of these fishes from their novel ranges.

Efforts have been taken to mitigate and reverse the negative impact of *Pterygoplichthys* spp. in their novel ranges. Some private firms in Mexico have marketed *Pterygoplichthys* spp. meat locally and internationally (Orfinger et al., 2019). Members of the genus *Pterygoplichthys* contain high protein and fatty-acid content (Mendoza et al., 2009) and have proven a viable component of commercial fish meal (Panase et al., 2018), and a source of antioxidants for nutrition supplements (Guo et al., 2019). Other potential uses of these fish are for biodiesel production (Anguebes-Franseschi et al., 2019) and even home and office décor (Hubilla et al., 2008). In Vietnam, these fishes were historically discarded by fishers partly due to its appearance, although it is now consumed by locals and is sometimes sold in local fish markets. Encouraging consumption of invasive species has been locally successful in controlling invasive apple snails (*Pomacea* spp.) in Vietnam (Do et al., 2018) and should be incentivised for the edible *Pterygoplichthys* spp. as well.

In Vietnam, the presence of *Pterygoplichthys* spp. in the wild has largely been ignored by the public. This neutral perspective is highlighted by the absence of scientific information about their dispersal and lack of biological agency to trade and release them into natural waters. Indeed, the work here provides preliminary information of established Vietnamese populations and a possible abiotic factor limiting their dispersal in two major tributaries in Mekong Delta. The data can be used to raise awareness and bolster media campaigns educating citizens on the perils of releasing aquarium pets into natural waters.

Conclusion

The findings from this study revealed no evidence of *Pterygoplichthys* spp. dispersal in brackish water of the Mekong Delta in Vietnam, nor their ability to colonise an area that demonstrates a temporal freshwater-saline shift, despite the evidence of their salinity tolerance in laboratory experiments. The *Pterygoplichthys* spp. in Co Chien and Ham Luong rivers are generally in good body condition signifying the conducive environmental condition for their growth. The Vietnamese government has designated *Pterygoplichthys* spp. as alien invasive species, although the presence of them in the wild in Vietnam has largely been ignored by the public. The work here provides preliminary information of a possible abiotic factor limiting *Pterygoplichthys* spp. to invade neighbouring water system. Continued research is needed to expand the search into other tributaries of the Mekong Delta to better characterise their dispersal ability in the entire river system.

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