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GRADUATE UNIVERSITY SCIENCE AND TECHNOLOGY

TRAN DUC DIEN

MORPHOLOGY, REPRODUCTION, FOOD SPECTRUM, DISTRIBUTION AND SPECIES COMPOSITION OF ALIEN SUCKERMOUTH ARMORED CATFISHES *Pterygoplichthys* IN VIETNAM

Major**: HYDROBIOLOGY**
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SUMMARY OF HYDROBIOLOGICAL DOCTORAL THESIS

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INTRODUCTION

1. The necessity of the thesis

Suckermouth armored catfish *Pterygoplichthy*s spp. originated from Southern America, have competed with native species for food and habitat with a wide food spectrum, high adaptability, high reproductive capacity and fast growth rate and they change the structure of species composition as well as reduce the quantity of freshwater fish resources. The suckermouth armored catfish is considered a serious threat to freshwater fisheries in Asia, Northern America and Mexico. They have invaded freshwater water habitats in 21 countries from Asia, Europe, and Africa to Northern America.

In Vietnam, the knowledge about alien fishes in particular and alien organisms, in general, is still very limited. While *Pterygoplichthys* spp. appeared in most of the main river basins and reservoirs in southern Vietnam. Some previous studies recorded the appearance of this fish in Vietnam only. The reproductive biology of *Pterygoplichthys* spp. was first examined in Dinh River (Khanh Hoa province). However, these results did not allow us to overview the morphology, species composition, food spectrum and reproductive cycle of *Pterygoplichthys* spp. and how they have evolved in different growth and reproductive strategies in various habitats.

According to Circular No. 35/2018/TT-BTNMT, 28th December 2018, The Ministry of Natural Resources and Environment has promulgated a list of 4 invasive species of suckermouth armored catfishes (among 6 invasive species), including *Pterygoplichthys pardalis, P. multiradiatus, P. disjunctivus, P. anisitsi.* In fact, the suckermouth armored catfishes has established its population in the wild, are encroaching on habitats, competing for food and causing harm to native organisms, with strong dispersal ability; tend or are causing an ecological imbalance in the water bodies they occur in Vietnam. This situation, continues to pose challenges for the management of invasive species of suckermouth armored catfishes and the protection of biodiversity in Vietnam. Therefore, the thesis topic: ***"Morphology, reproduction, food spectrum, distribution and species composition of alien suckermouth armored catfishes Pterygoplichthys in Vietnam"*** was conducted to clarify the biological characteristics of suckermouth armored catfishes when they enter and invade Vietnam's water bodies.

2. The research aims of the thesis

Improve understanding of some biological characteristics of the suckermouth armored catfishes *Pterygoplichthys*, as a scientific basis for impact assessment and management of invasive alien fish in Vietnam.

3. The main research content of the thesis

- Distribution of armored catfishes *Pterygoplichthys* spp. in Southern Vietnam;
- Morphological characteristics and species composition of suckermouth armored catfishes;
- Reproductive biology of suckermouth armored catfishes (species *P. disjunctivus*);
- Food spectrum of suckermouth armored catfishes.

4. New contributions to the thesis

Providing new data on the distribution of Pterygoplichthys spp. in Southern Vietnam. For the first time, data on the species composition of the alien suckermouth armored catfishes were identified based on both morphological and molecular methods.

For the first time, the reproductive biology of the alien suckermouth armored catfishes (species P. disjunctivus) has been studied fully and detail in two types of water bodies (lotic and limnic) in Southern Vietnam.

Provides important evidence on the composition of the food spectrum of the alien suckermouth armored catfishes containing groups of invertebrates.

Provide sufficient and highly reliable information for management and reduce the impact of the suckermouth armored catfishes.

CHAPTER 1. OVERVIEW

The group of fish of the family Loricariidae with 10 or more dorsal fin rays commonly known as "sailfin catfishes" or "suckermouth armored catfishes" has been studied and named *Pterygoplichthys*. However, because this variety has high phenotypic variability and the classification key is mainly based on the criteria of color and body ridges, it causes difficulties in the identification process. Genus *Pterygoplicthys* have invaded many freshwater bodies in 21 countries in Asia, Europe, Africa and North America. In Southeast Asia, suckermouth armored catfishes appeared about 30 years ago. First in the Philippines, then in Indonesia and Thailand. In Vietnam, this species was first recorded in 2003 (species *P. plecostomus*) in the Mekong Delta, Southern Vietnam. Up to now, this fish species has appeared in many water bodies from the delta to the mountains in Southern Vietnam.

Suckermouth armored catfishes are species with a mouth structure in the form of suckers that mainly feed on bottom sediments and detritus (in favour of plants). They grow fast (10 cm/year), have a relatively short lifespan (5 years) and after 2 years their size reaches over 30 cm. Suckermouth armored catfishes have a burrowing reproductive habit, a relatively large reproductive capacity (the highest is nearly 19,000 eggs) and the number of female fish is usually more than that of male fish... The breeding season is long and usually spawns in the dry season (some invasive areas have a rainy season like Malaysia). In Vietnam, a preliminary study on the reproductive characteristics of *Pterygoplichthys* in the Dinh River, Khanh Hoa province was carried out by Zworykin and Budaev (2013) when collecting samples in 2 batches (2 groups): Group 1 collected 34 samples from October to December 2010 and group 2 collected 10 samples in January 2012. The results showed that, in group 1, there were 5 females with gonads in stage IV and fertile from October to December. In group 2, there was also the ability to breed in January when there are 6 individuals in stages VI-III. Suckermouth armored catfishes had mass spawning in the 2nd group when 5 out of 6 individuals tested had ovaries at stage VI-III. The size of the oocyte in the ovary of mature individuals is 1-3 mm. The GSI index ranges from 6.59% to 15.77%. Rõ ràng, các nghiên cứu chưa cung cung cấp đầy đủ thông tin về đặc điểm sinh học của cá Tỳ bà giống *Pterygoplichthys*. Meanwhile, the scientific basis needs to be understood for the invasive management of this alien fish.

There have been many reports around the world about the impacts of this invasive alien fish species. They affect environmental ecology such as disrupting food chains in water bodies, competing for food and habitat with native species, and affecting ecological balance and biodiversity. They affect aquatic animals such as fish, aquatic plants, aquatic insects, aquatic mammals, and birds, impact on the bottom, cause landslides and transmission of diseases to native species. In addition, they also have socio-economic impacts such as reducing catches and native fish resources, damaging fishing gear, facilities and management, and affecting human health and life in society.

CHAPTER 2. MATERIALS, CONTENTS AND RESEARCH METHODS

2.1. Materials: suckermouth armored catfishes (*Pterygoplichthys*) were collected from 2017 to 2022 in the water bodies in Southern Vietnam on the main biological characteristics such as morphology and species composition, distribution, biological reproductive characteristics and food spectrum.

2.2. Methods: the methods are based on widely used guidelines in the world as well as support analysis from reputable experts from domestic and foreign research institutes.

Sampling populations: Used specialized sampling fishing such as gill nets, ignore fishing, fishing nets and fishing hooves with different methods. The fish is caught by local fishermen in the water bodies.

Sample processing: For each research content, there were different preservation methods such as transporting live to the laboratory, keeping alive or fixing it with 7% Formaldehyde.

Method of determining the parameters of the water body: Determined water parameters by multiparameter meter Hanna HI 9829 and Global Water.

The method of determining the distribution: Water bodies are confirmed to have a distribution when at least one sample of suckermouth armored catfishes is collected in the water body (fishes collected by at least 3 local fishermen). Geographical regions such as the South Central Coast, Central Highlands and Mekong Delta were surveyed to determine distribution in Southern Vietnam.

Morphology of suckermouth armored catfishes**:** According to Ambruster and Page (2006) and other references.

Methods to determine species composition: the species composition was determined with include morphology and molecular genetics.

Methods to determine size and length-weight relationship: The relationship between total length and the total weight was calculated for each site using power regression equation $W = aL^b e^{\varepsilon}, \varepsilon \sim N(0, \sigma^2)$.

Methods to determine some reproductive biological characteristics: sex ratio is the ratio of males and females obtained. Ovary development stages of fishes were determined using methods described by Nikolsky (1963) and King (1995). The spawning season was identified as a period with more than 50% of fish having ovaries at stages III, IV and V and greater GSI were observed. Other indices such as egg diameter, absolute fecundity, relative fecundity, and correlation between absolute fecundity and body size were also determined by widely used and reliable methods.

Methods to determine food spectrum: The food spectrum of fish was studied by qualitative and quantitative methods. Determine the species composition and mass of the major components present in the intestinal tract. The species composition and mass of the main components were analyzed by the research team of Dr. Gusakov V.A. (Papanin Institute, Russian Academy of Sciences).

2.3. Statistical analysis

Statistical algorithms are used on Statistica software (version 12.0) and R.studio software (versions 3.3.2 and 4.0.4) with data processing methods on comparison of distribution ratio and test the male: female ratio; test the distribution of data; compare values, distinguish groups of fish obtained, determine the size of the first sexual maturity of the population, estimate the regression parameters.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Distribution of suckermouth armored catfishes (*Pterygoplichthys*) in Southern Vietnam.

The results from a survey of 132 water bodies in Southern Vietnam. There were 88 water bodies recorded with the appearance of suckermouth armored catfishes (*Pterygoplichthys*) at the rate of 66.67% (Fig. 3.1).

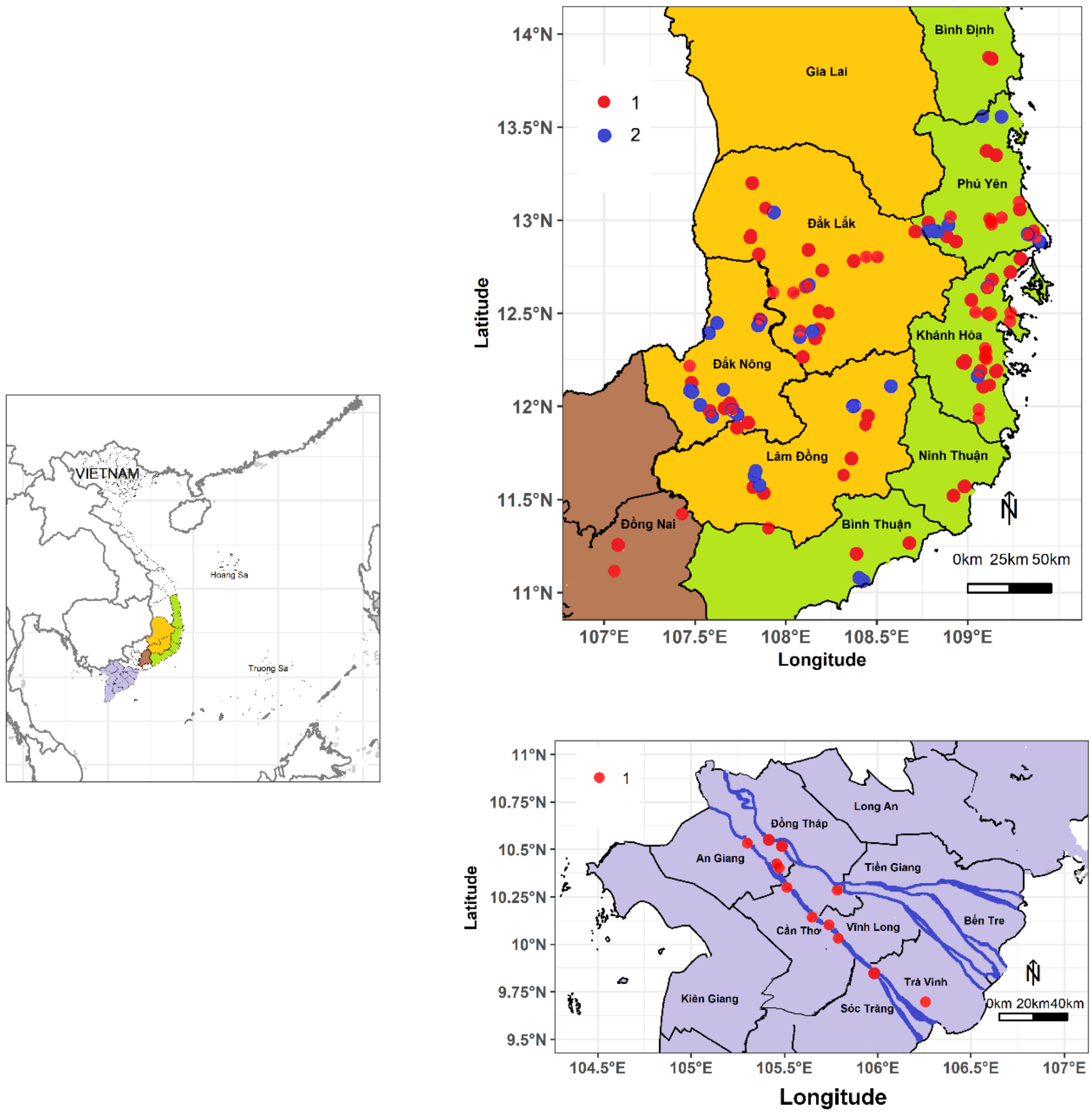

Figure 3.1: Map of the study area in Southern Vietnam and location of the studied water bodies: 1 – sites where catfish were found (red dots); 2 – catfish are absent (blue dots).

The results of the study have recorded the occurrence of suckermouth armored catfishes in all major river basins such as the Cai River system in Khanh Hoa, Ba and Da Rang Rivers in Phu Yen, Con River in Binh Dinh and Dong Nai Rivers in Dong Nai, Serepok River system in Dak Lak, Dak Nong and down Mekong River system (Tien River, Hau River).

The results of the study showed that there was a difference in the occurrence rate of suckermouth armored catfishes in the study areas ($p < 0.05$). In the Mekong River Delta, suckermouth armored catfishes appeared in all surveyed water bodies (100%), in the South Central Coast and Central Highlands, the occurrence rate in water bodies was also quite high, were 68.85% and 55.36%, respectively.

Suckermouth armored catfishes have been distributed in estuary areas where salinity ranges from 4 to 25‰. It was shown that the fish has a high adaptability to salinity and can live in brackish water. The fish can adapt to brackish water conditions in the estuary and can live in this water for a long time. Salinity adaptation of fish in natural conditions may be better than in experimental conditions, due to salinity stratification in the estuary and other ecological conditions. Obviously, the suckermouth armored catfishes were not only widely distributed in geographical areas but also in different salinity conditions. Concerns about the possibility of expanding the distribution of suckermouth armored catfishes to coastal aquaculture areas also need to be addressed.

3.2. Morphological characteristics and species composition of suckermouth armored catfishes *Pterygoplichthys* in Southern Vietnam.

3.2.1. Morphological characteristics of suckermouth armored catfishes *Pterygoplichthys.*

The *Pterygoplichthys* species obtained have 2 dorsal fins, namely, the first dorsal fin has a truncated anterior fin ray, followed by a spine fin ray and 10 - 13 soft rays; The second dorsal fin is a spine fin. The pectoral fins have 1 hard spine (spine fin) and 4 - 6 soft rays (usually 6 soft rays). The pelvic fin has 1 hard ray and 4 - 5 soft rays (usually 5 soft rays). The anal fin has 1 hard ray and 3 - 4 soft rays (usually 4 soft rays). The caudal fin has 2 hard rays and 14 soft rays. The number of lateral scales is 24 - 30 and there are 28 vertebrae. From the analysis results, it was shown that all the samples of the suckermouth armored catfishes belonged to the genus *Pterygoplichthys* (10-13 soft fin rays).

3.2.2. Species composition of suckermouth armored catfishes *Pterygoplichthys.*

According to species identification by external morphology using color patterns and taxonomic keys, two species of suckermouth armored catfish, *Pterygoplichthys pardalis* (Castelnau, 1855) and *P. disjunctivus* (Weber, 1991), as well as the intermediate group *P. pardalis* × *P. disjunctivus,* inhabit the studied water bodies and watercourses of Southern Vietnam (Fig. 3.2).

P. pardalis species has the main identifying feature as discrete (black) ventral spots, which can be combined, but as a rule of not more than 5 points (Figure 3.2a). Meanwhile, *P. disjunctivus* species has formed vermiculations on the ventral surface (Figure 3.2b). These form vermiculations can be bright fringes on a dark background or dark fringes on a light background. The results of determining the external morphology of these two species are consistent with the description of Ambruster and Page (2006).

However, given the wide phenotypic variation of *Pterygoptichthys* spp. species, the samples obtained showed a wide variety of ventral phenotypes that are intermediate between *P. pardalis* and *P. disjunctivus* and these species may be a hybrid between *P. pardalis* × *P. disjunctivus*. Specifically, on the ventral surface of the intermediate group, there are both vermiculations and distinct spots or spots with vermiculations made up of more than 5 spots combined (Figure 3.2c).

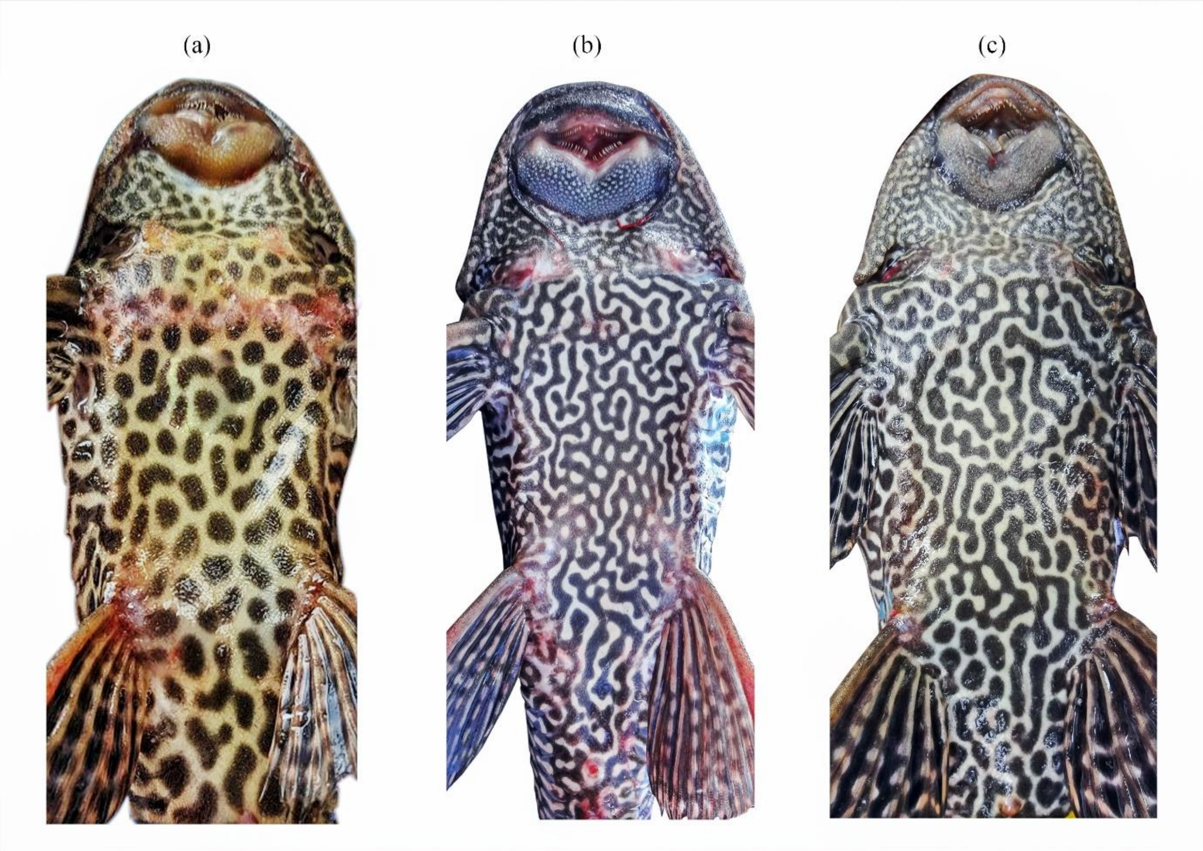

Figure 3.2: Characteristic patterns of coloration of the ventral side of the body of suckermouth armored catfish: (a) *Pterygoplichthys pardalis* (discrete black dots); (b) *P. disjunctivus* (vermiculations) và (c) *P. pardalis* × *P. disjunctivus?* (vermiculations and discrete black dots).

Stepwise discriminant analysis based on the measurement and counting criteria showed that the spatial distribution has a significant partition between the species of the genus *Pterygoplicthys* and the intermediate group between the two species (Figure 3.3). A stepwise discriminant analysis of morphological characters of different species and an interspecific hybrid of P*terygoplichthys* spp. made it possible to conclude that eight signs of 32 plastic and 6 meristic signs contributed the most for interspecific differences, namely, the length of the anal fin base, the length of the hard ray (spine) of the adipose fin, the distance between dorsal and adipose fin, the height of the caudal peduncle, the distance between dorsal and pelvic fin, the postorbital distance, the barbel length and the number of rays in the dorsal fin (Table 3.1)

The identification of different species of suckermouth armored catfish and their interspecific hybrid by morphological criteria was reliable (p < 0.001) according to the results of an analysis of the discriminant functions of the morphological characters of fish; the Wilks lambda was close to zero, 0.110 (Table 3.1). The proportion of correctly identified specimens of different species and hybrids of genus *Pterygoplichthys* by morphological characteristics reached ~ 98% (Table 3.2).

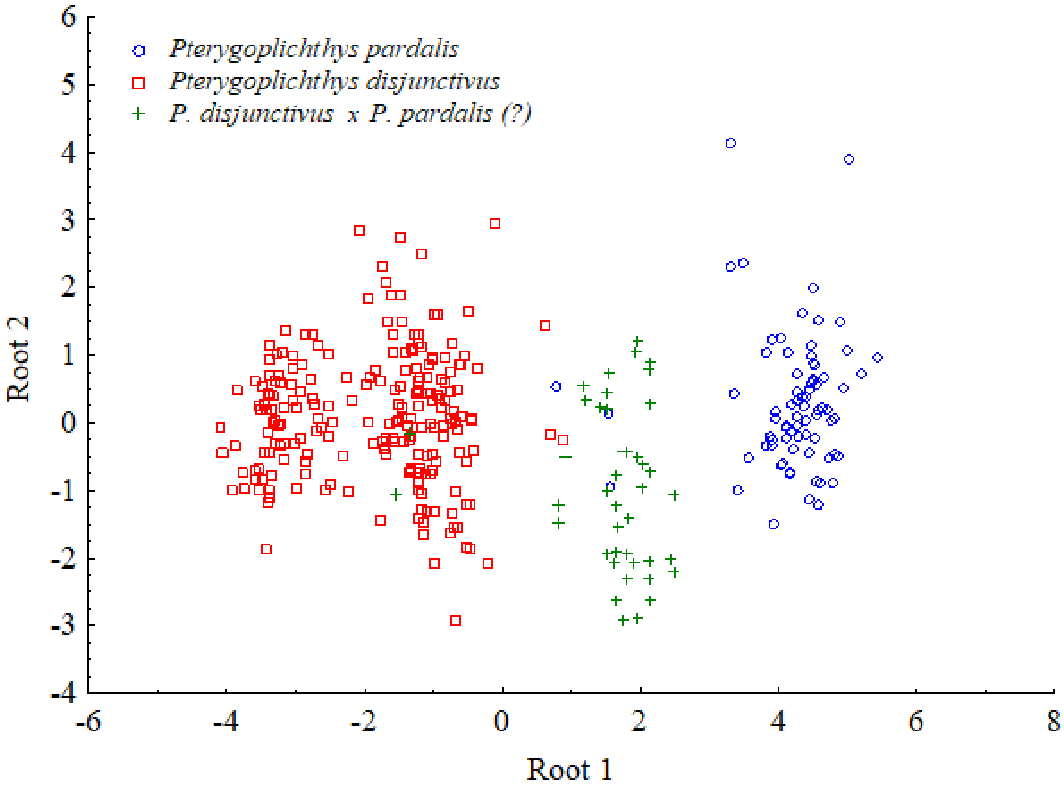

Figure 3.3: Distribution by plastic and meristic characters of different species and interspecific hybrid of suckermouth armored catfish in the space of the first and second discriminant functions. Root1 > 0 is the length of the anal fin base; Root1 axis below zero is the number of rays in the dorsal fin, barbell length; Root 2 axis above zero is the postorbital distance, dorsal adipose-caudal distance, the distance between the dorsal and pelvic fins; and Root 2 < 0 is the length of the adipose fin spine, the distance between the dorsal and pelvic fins.

Table 3.1: The result of the analysis of discriminant functions of different species and a hybrid of armoured catfish *Pterygoplichthys* spp.

Features	Wilks' Lambda: 0,110 approx, *F* (58,592) = 20,492, *p* < 0,0000					
	Wilks' Lambda	Partial Lambda	F-remove (2,296)	*p*	Tolerance ($1–R^2$)	R^2
SL	0,111	0,994	0,936	0,393	0,203	0,797
Predorsal L	0,111	0,999	0,105	0,900	0,176	0,824
Operculum-dorsal L	0,112	0,992	1,249	0,288	0,094	0,906
Operculum-pectoral L	0,111	1,000	0,078	0,925	0,103	0,897
Pectoral-spine L	0,111	0,998	0,290	0,749	0,828	0,172

Features	Wilks' Lambda: 0,110 approx, F (58,592) = 20,492, p < 0,0000					
	Wilks' Lambda	Partial Lambda	F-remove (2,296)	p	Tolerance ($1–R^2$)	R^2
Pelvic-spine L	0,112	0,989	1,695	0,185	0,553	0,447
Caudal peduncle L	0,111	0,999	0,122	0,885	0,611	0,389
Anal-spine L	0,112	0,991	1,386	0,252	0,656	0,344
Dorsal-pectoral D	0,113	0,981	2,823	0,061	0,288	0,712
Dorsal-spine L	0,111	0,996	0,631	0,533	0,763	0,237
Anal-fin base L	**0,113**	**0,979**	**3,137**	**0,045**	**0,610**	**0,390**
Dorsal-fin base L	0,111	0,993	1,092	0,337	0,307	0,693
Dorsal-adipose D	0,111	0,994	0,861	0,424	0,121	0,879
Adipose-spine L	**0,114**	**0,972**	**4,287**	**0,015**	**0,827**	**0,173**
Dorsal adipose-caudal D	**0,113**	**0,977**	**3,466**	**0,033**	**0,320**	**0,680**
Caudal peduncle Dp	**0,113**	**0,978**	**3,287**	**0,039**	**0,606**	**0,394**
Ventral adipose-caudal D	0,112	0,984	2,451	0,088	0,295	0,706
Dorsal-anal D	0,111	0,998	0,369	0,692	0,245	0,756
Dorsal-pelvic D	**0,114**	**0,969**	**4,745**	**0,009**	**0,378**	**0,622**
Head L	0,112	0,989	1,703	0,184	0,184	0,816
Postorbital D	**0,114**	**0,973**	**4,069**	**0,018**	**0,578**	**0,422**
Orbit Dia	0,111	1,000	0,013	0,987	0,665	0,335
Snout L	0,111	0,995	0,754	0,471	0,846	0,154
Barbel L	**0,114**	**0,974**	**4,004**	**0,019**	**0,651**	**0,349**
Head Dp	0,111	0,993	0,994	0,371	0,533	0,467
Internares W	0,111	0,992	1,191	0,305	0,851	0,149
Interorbital W	0,111	1,000	0,032	0,968	0,612	0,388
Cleithral W	0,111	0,998	0,305	0,737	0,221	0,779
Dorsal-fin rays	**0,536**	**0,180**	**7,031**	**0,001**	**0,769**	**0,231**

Ghi chú: *F remove – Criterion F concerns the exclusion of a character from the analysis; p is the significance level of the criterion F;* R^2 *is the explanatory coefficient. The values of the morphological indicators that contribute significantly to the discriminant analysis are in bold. CD – Length; KC – Distance.*

Table 3.2: Classification matrix for the common sample of the armoured catfish by morphological characteristics.

No	(*P. pardalis*)	(*P. disjunctivus*)	(*P. pardalis* x *P. disjunctivus?*)	Percentage (%)
1	72	0	3	96,0
2	0	209	1	99,5
3	1	2	39	92,9
Total	73	211	43	97,9

Note: The order numbers 1, 2, 3 in the column corresponding to the taxonomy matrix of P. pardalis, P. disjunctivus and possibly a hybrid P. pardalis × P. disjunctivus?

Molecular genetic analysis of suckermouth armored catfishes (*Pterygoplichthys*).

The results of molecular genetic analysis of suckermouth armored catfishes in Southern Vietnam have confirmed the accuracy of species classification by morphological method, including two species, *P. pardalis* and *P. disjunctivus*. Molecular genetic analysis demonstrates the presence of several phylogenetic lineages in the samples of the Vietnamese armored catfish.

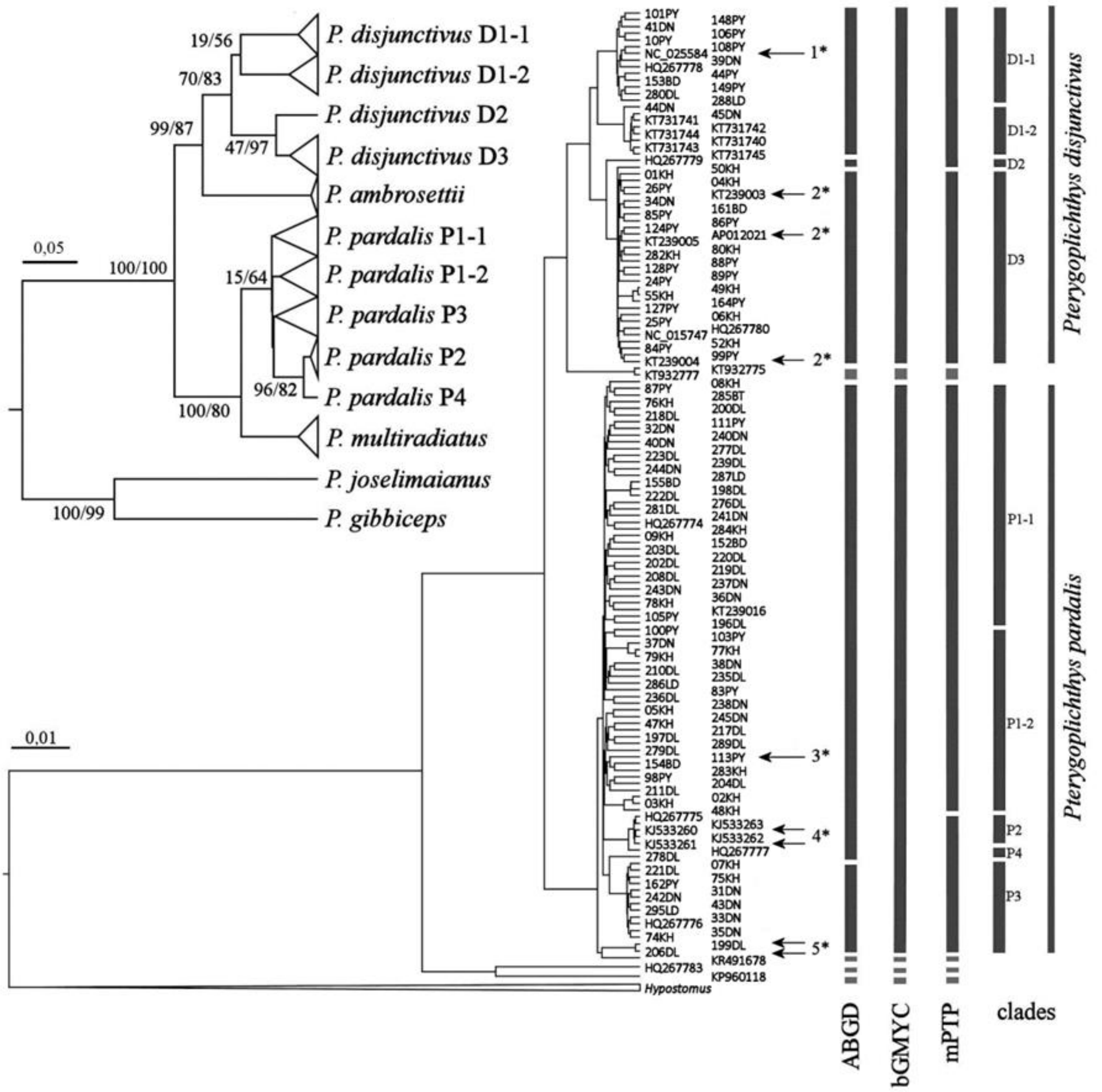

Figure 3.4: Rooted phylogenetic tree for the tRNA-Glu–cyt-b–tRNA-Thr locus. The collapsed tree with support for branching by key nodes (posterior probability values for BI (first digit) and bootstrap values for ML (second digit)) is presented in the inset. n=115.

Amazon sailfin catfish *Pterygoplichthys pardalis* in the water bodies and watercourses of Southern Vietnam is represented by clade P1 and P3 (according to the terminology of Wu et al., 2011), while clade P1 is subdivided into two slightly different subclades, P1-1 and P1-2 (Fig. 3.4). Representatives of the clade P2 and P4, previously described for the water bodies of Taiwan, were not found in our study. However, two specimens from the water bodies of Đak Lak Province (199DL and 206DL) clustered with the related species *P. multiradiatus*, although they were unambiguously identified as *P. pardalis* by morphology (Fig. 3.4: sequence no. 5*).

In the water bodies of Vietnam, *P. disjunctivus* itself is represented by clades D1 and D3 (according to the classification of Wu et al., 2011), while clade D1 is subdivided into two subclades. The D1-1 subclade belongs to the real D1 clade widespread in Southeast Asia, while the representatives of the D1-2 subclade were previously found only in India.

Morphological and genetic analysis has shown that there are different phenotypic and genotypic variations in some individuals obtained. The most probable hybrids include specimen no. 113PY, which has the diagnostic features of *P. disjunctivus* and the mitochondrial genome of *P. pardalis*, phylogenetic line P1 (Fig. 3.4: sequence no. 3*).

Morphological and molecular genetic data reliably indicate that two species of suckermouth armored catfish live in the water bodies and watercourses of Southern Vietnam, namely, *P. pardalis* and *P. disjunctivus*. Although, based on the external morphology, there is an intermediate morphological group between the two species *P. pardalis* × *P. disjunctivus* and the spatial distribution is divided between the two species *P. pardalis* and *P. disjunctivus*. This suggests that there is a hybrid phenomenon between the two species mentioned above in the water bodies of southern Vietnam.

3.3. Size, thelength–weight relationships and reproduction of suckermouth armored catfishes *P. disjunctivus* in Dinh River and Suoi Trau Reservoir.

3.3.1. Size of suckermouth armored catfishes in Dinh River and Suoi Trau Reservoir

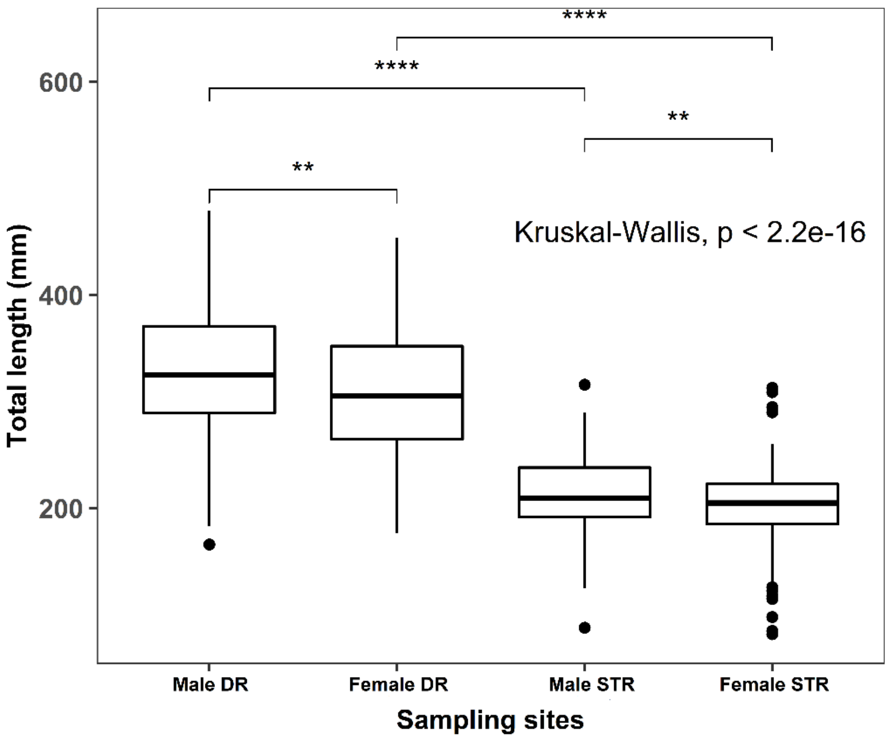

Figure. 3.5: Boxplot about Kruskal – Wallis test in the total length (Lt) of sexual *P. disjunctivus* in Dinh River - DR and Suoi Trau Reservoir - STR, Vietnam (**: P < 0.01; ***: P < 0,001).

There was a wide range in the total length of *P. disjunctivus* in both Dinh River (Lt = 177 - 451 mm, mean = 310.12 mm for females; and 183 - 479, mean = 327.33 mm for males) and Suoi Trau Reservoir (Lt = 82 - 313 mm, mean = 201.99 mm for females; and 88 - 316 mm, mean = 211.54 mm for males. The total length (L_t) frequency distribution of males and females of *P. disjunctivus* in Dinh River and Suoi Trau Reservoir was not normal distribution (Shapiro-Wilk test, $p < 0{,}05$).

There was a statistical difference in the mean total length of females and males and of fish between the two habitats (all P values < 0.01, Kruskal-Wallis). There was a difference in mean L_t between male and female fish in Dinh River ($p < 0.01$), in mean L_t between male and female fish in Suoi Trau Reservoir ($p < 0.01$), in mean L_t between the female of Dinh River and female of Suoi Trau Reservoir ($p < 0.001$) and in mean L_t between male of Dinh River and male of Suoi Trau Reservoir ($p < 0.001$). Total length tends to increase in both male and female fish living in rivers than in reservoirs (Fig. 3.5).

Overall, the study results showed that the growth of suckermouth armored catfishes (*P. disjunctivus*) in the lentic (Suoi Trau Reservoir) showed smaller values than in the lotic (Dinh River) habitat.

3.3.2. The length-weight relationships

Length-weight relationships were illustrated in Figure 3.6

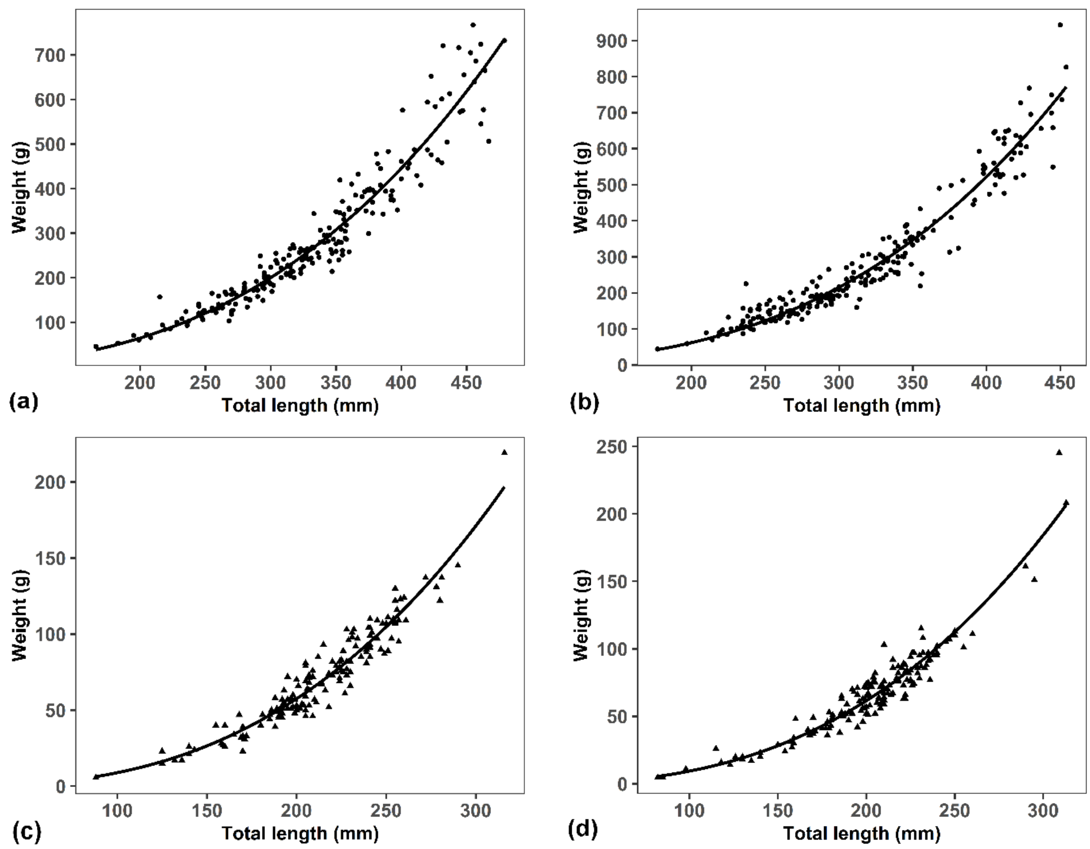

Figure 3.6: Total length-weight relationship of males and females of *P. disjunctivus* in Dinh River and Suoi Trau Reservoir, Vietnam: (a) – Males in Dinh River; (b) – Females in Dinh River; (c) – Males in Suoi Trau Reservoir; (d) – Females in Suoi Trau Reservoir.

Males in Dinh River: W = $0.00005L^{2.66}$, $R^2 = 0.94$, n = 190, $p \sim 0$

Females in Dinh River: W = $0.000023L^{2.82}$, $R^2 = 0.93$, n = 169, $p \sim 0$

Males in Suoi Trau Reservoir: W = $0.000035L^{2,70}$, $R^2 = 0.93$, n = 147, $p \sim 0$

Females in Suoi Trau Reservoir: W = $0.000039L^{2,70}$, $R^2 = 0.94$, n = 156, $p \sim 0$

All males and females had slope coefficients (b values) smaller than 3 regardless of the habitats (in Dinh River: t = -2.99; df = 167; P = 0.003 for females; and t = - 6.94; df = 188; P = 6.16 × 10-11 for males; and in Suoi Trau Reservoir, t = - 5.54, df = 154, P = 1,93 × 10-7 for females; t = - 4.76, df = 145, P = 4.6 × 10-6 for males), indicating a negative allometric growth pattern for all individuals from sampling populations. Both males and females of *P. disjunctivus* collected from the Dinh River (lotic habitat) and Suoi Trau Reservoir (lentic habitat) in this study had negative allometric growth (b = 2.66 – 2.82), these fish tend to grow in length more than weight as they live in water bodies.

3.3.3. Sex ratio

Morphology analysis of the gonad of these fish indicated that the sex of small fish (8 cm in length) could be determined using the naked eye. In both habitats, the ratio of armored catfish males and females was 1:1 (1.12:1 in Dinh River and 0.96:1 in Suôi Trau; *prop.test;* p > 0.05). There was no significant difference ratio of males and females between the two water bodies (0.53; 0.49; *prop.test;* p > 0.05).

3.3.4. Gonadal stages

Six maturity stages were identified based on the shape, size, and color of the ovaries and histological features in Southern Vietnam (Table 3.3, Fig. 3.7)

Table 3.3: Maturity stages of female *P. disjunctivus* introduced in Vietnam

Maturity stages	Characteristics of ovaries
I	The ovaries were pale pink to translucent in colour with inconspicuous vascularisation. Ovarian wall was very thin and no oocyte was visible with the naked eye. Histologically, oocytes, mainly PG types, were dense and closely distributed. (Fig. 3.7a).
II	Ovaries are light yellowish to opaque in color. Vascular supply increased and the blood capillaries became conspicuous. Oocyte was not visible through the ovarian wall with the naked eye. In histological sections, oocytes grew with a nucleus up to 50% of the whole oocyte area and limited vacuolation (Fig. 3.7b).
III	Ovaries increased in weight and volume and were in a light color. Oocytes (including stage II and III oocytes) can be seen by the naked eyes. Oocytes increased considerably in size and were pale pink in colour in H&E-stained sections (Fig. 3.7c).
IV	At close to spawning, the ovaries turned to deep yellow color (straw yellow or red-yellow). The blood supply increases considerably with a large blood vessel visible along the ovary. Oocytes reached a maximum in size and contained dense yolk particles. In H&E stained sections, the oocytes were dark purple in colour (Fig. 3.7d).
V	The ovaries were involved in reproduction. Ripened eggs were released directly into the abdominal cavity. Oocytes were separate and come out if pressed lightly on the abdomen.

VI	Ovaries appeared bruised; purple to dark pink in colour. The ovarian wall was thicker, ovaries are empty or left a few small eggs.

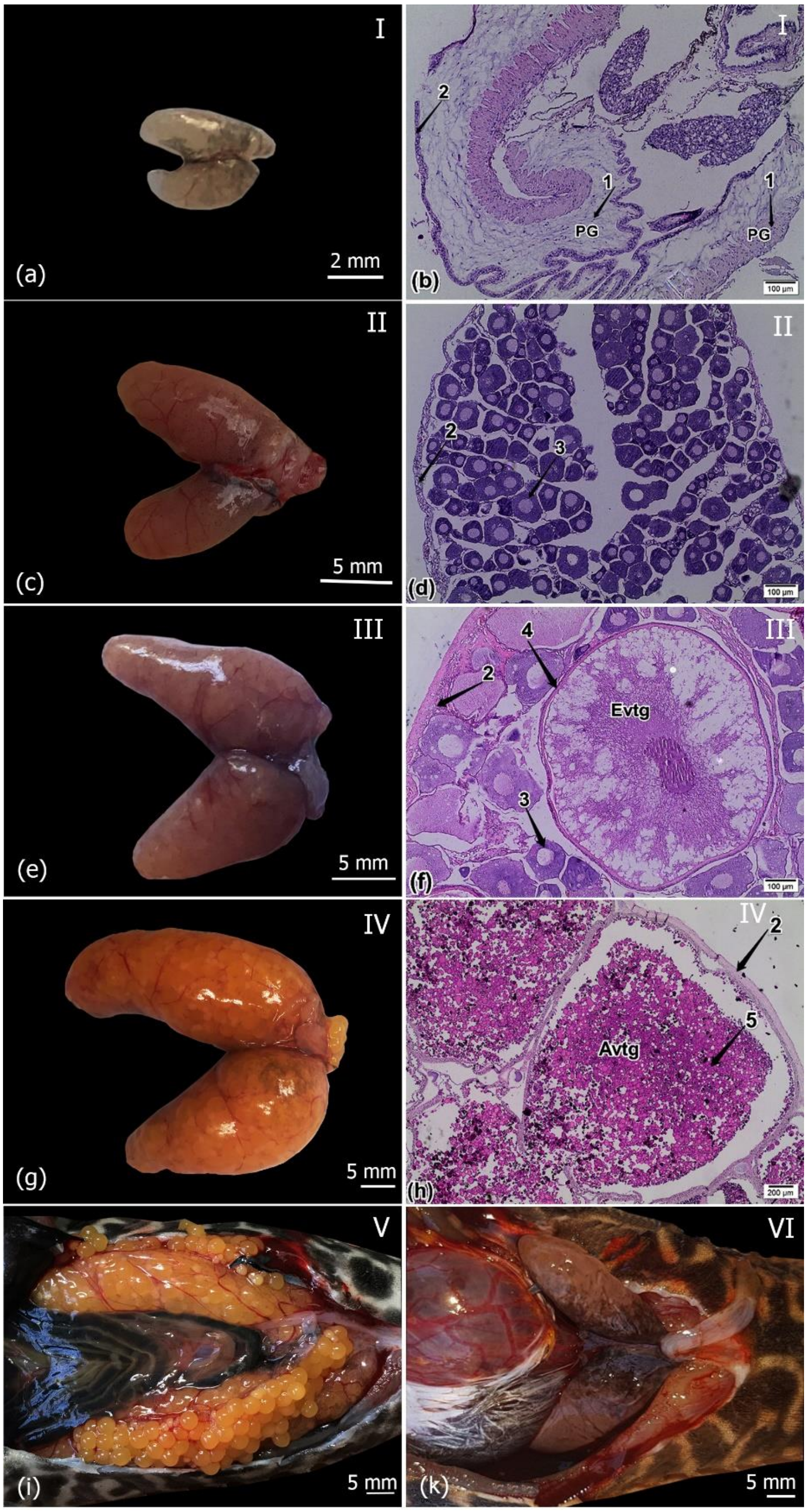

Figure 3.7. Macroscopic and microscopic appearance of the ovaries of the female *P. disjunctivus* introduced in Vietnam at various maturity stages.
Note: (a)*, (b): stage I; (c), (d): stage II; (e), (f): stage III; (g), (h): stage IV; (i): stage V and (k): stage VI; Evtg: early vitellogenic oocyte; Avtg: advanced vitellogenic oocyte; arrow 1--PG: primary growth oocyte; arrow 2 -- Ovarian wall; arrow 3 -- oocyte during previtellogenesis; arrow 4 -- oocyte during vitellogenesis; arrow 5 -- yolk granules in advanced vitellogenic oocyte*

3.3.5. Spawning season

The matured fish presented all year round but with a higher percentage during April-October compared to November-March in both water bodies. However, this rate varies between months of the year. The percentage of fish with matured ovaries peaked in August (100%) and was lowest in February (<10%). (Fig. 3.8a). The highest GSIs were 16.63 ± 1.25% in fish from Dinh River and 8.48 ± 1.12% in fish from Suoi Trau Reservoir, all in August. The lowest GSI was in February (0.46 ± 0.07%) for fish from Dinh River and (0.04 ± 0.03%) for fish from Suoi Trau in April (Fig. 3.8b).

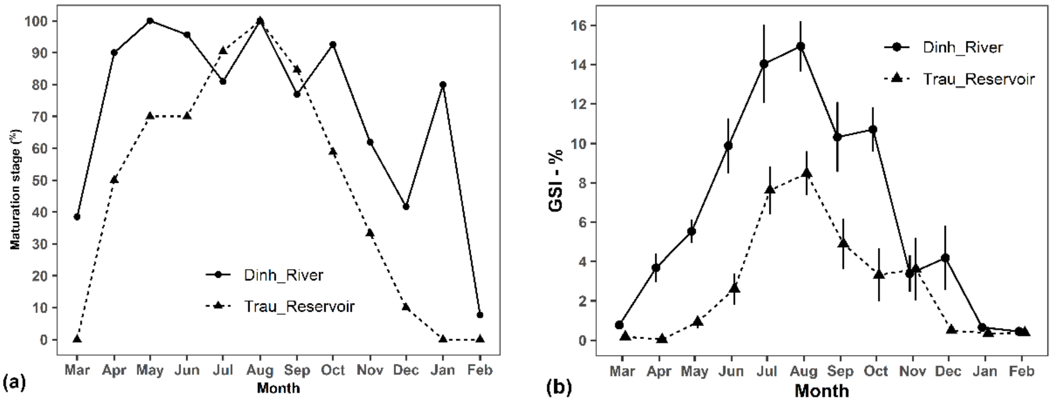

Figure 3.8. Monthly percentages of maturation stage (III-V) (Fig. a) and monthly changes of the Gonado-somatic index (GSI) of the non-native *P. disjunctivus* (Fig. b) in Vietnam.

So, the matured fish presented all year round, but with a higher percentage during April-October and peaked in June, and August (Fig. 3.8).

3.3.6. Length at first sexual maturity.

The total length at maturity of females was 179.7 mm (17.97 cm) for fish from Suoi Trau Reservoir and 234.3 mm (23.43 cm) for fish from Dinh River (Figure 3.9).

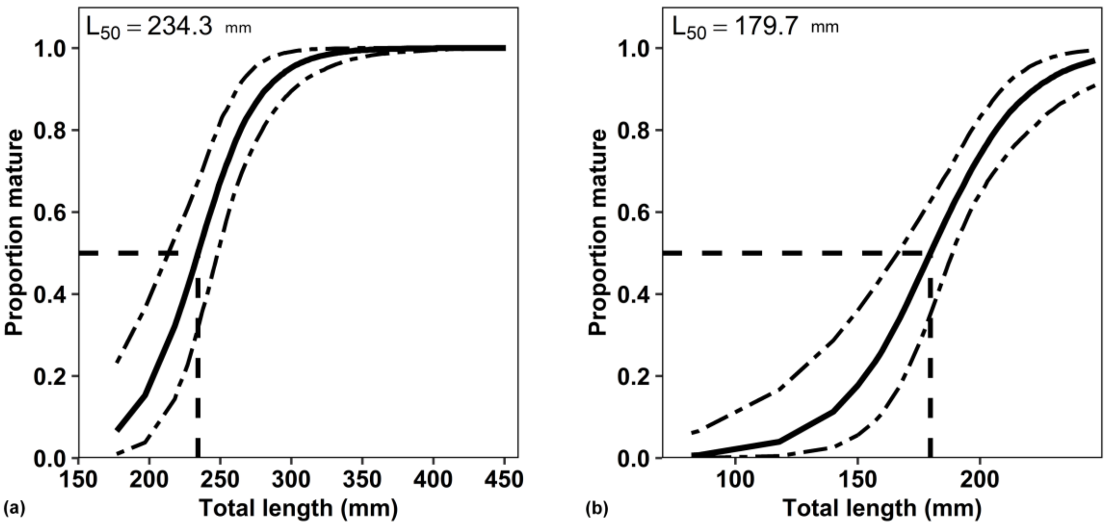

Figure 3.9. Length at the first sexual maturity 50% (L50) of the non-native *P. disjunctivus* in Vietnam: (a) – Dinh River, (b) – Suoi Trau Reservoir.

In our study, the size at first maturation of fish (in Dinh River and Suoi Trau Reservoir) is smaller than fish in native and other studies and the smallest matured fish was observed in Suoi Trau with a L_t of 16.7 cm and a body weight of 40 g,

3.3.7. Fecundity

The absolute first batch fecundity (F_b) of the *P. disjunctivus* in Dinh River and Suoi Trau Reservoir was 4812 ± 383 oocytes/ind. (ranged from 103 to 8208 oocytes/ind.) and 841 ± 91 oocytes/ind. (ranged from 150 to 1993 oocytes/ind.), respectively. The relative first batch fecundity (RF_b) of the *P. disjunctivus* in Dinh River and Suoi Trau Reservoir was 13.36 ± 0.81 oocytes/g (ranged from 0.69 to 21.32 oocytes/g) and 12.97 ± 1.19 oocytes/g (ranged from 3.26 to 25.23 oocytes/g), respectively. Specifically, the relative fecundity of the fish was not statistically different between the two habitats ($P > 0.05$) (Table 3.4).

The absolute total fecundity (F_t) in Dinh River and Suoi Trau Reservoir was 6000 ± 483 oocytes/ind. (ranged from 1303 to 10574 oocytes/ind.) and 995 ± 101 oocytes/ind. (ranged from 150 to 2255 oocytes/ind.), respectively. The relative fecundity (RF_t) in Dinh River and Suoi Trau Reservoir was 16.63 ± 0.92 oocytes/g (ranging from 7.05 to 26.00 oocytes/g) and 15.48 ± 1.35 oocytes/g (ranging from 3.26 to 28.55 oocytes/g). There was no difference in the mean relative fecundity of the fish among different sampling sites ($P > 0.05$) (Table 3.4).

One important finding of this study was that the absolute fecundity of *P. disjunctivus* was five times higher in Dinh River than in Suoi Trau Reservoir, although the distance between the two sampling sites is only 8 km, and with the same weather conditions. The difference in absolute fecundity of *P. disjunctivus* was associated with the larger body size of fish collected from these two habitats, as confirmed by the positive correlations between these two parameters. The index of Fb/Ft was 79.83 ± 2.73 % (ranging from 7.90 to 92.72 %) and 84.09 ± 2.00 % (ranging from 59.26 to 100%) for fish from Dinh River and Suoi Trau Reservoir, respectively ($p > 0.05$) (Table 3.4).

3.3.8. Oocyte diameter

The diameter of the Top Decile oocytes at stage IV-V ovaries of the *P. disjunctivus* in Dinh River and Suoi Trau Reservoir were 2.95 ± 0.04 mm (ranged from 2.50 to 3.41 mm) and 2.58 ± 0.01 mm (ranged from 2.42 to 2.80 mm), respectively. There was a significant difference in the mean diameter of the Top Decile oocytes among fish from lotic and lentic habitats (Table 3.4).

It seems that the size of the egg is smaller toward the lower latitudes which needs further investigation. In other studies in lotic habitats such as: in Volusia Blue Spring, Florida, U.S.A, the average diameter top decile class size of the period 2005-2007 was 3.44 mm, period 2012 - 2014 was 3.01 mm and the diameter of the top size class eggs was 3 - 4 mm for fish from Marikina River and 2.9 – 3,3 mm from East Kolkata, India. As for the water bodies in Vietnam, the smaller egg size in the reservoir allows the Pia to be well adapted to adverse environmental conditions such as higher water temperature, large changes in water levels during the year, and climate change. fluctuations of other environmental factors accompanied by a prolonged dry season in recent years.

Table 3.4: Reproductive parameters of *P. disjunctivus* from Dinh River and Suoi Trau Reservoir, Vietnam

Parameters	**Unit**	**Dinh River** **Mean ± SE (min-max)**	**Suoi Trau Reservoir** **Mean ± SE (min-max)**	***P***
F_b	oocytes/ind.	4812 ± 383 (103 – 8208)	841 ± 91 (150 – 1993)	< 0.05
F_t	oocytes/ind.	6000 ± 483 (1303 – 10574)	995 ± 101 (150 – 2255)	< 0.05
F_b/F_t	%	79.83 ± 2.73 (7.90 – 92.72)	84.09 ± 2.00 (59.26 – 100)	> 0.05
RF_b	oocytes/g	13.36 ± 0.81 (0.69 – 21.32)	12.97 ± 1.19 (3.26 – 25.23)	> 0.05
RF_t	oocytes/g	16.63 ± 0.92 (7.05 – 26.00)	15.48 ± 1.35 (3.26 – 28.55)	> 0.05
Diameter Top Decile of oocytes	mm	2.95 ± 0.04 (2.50 – 3.41)	2.58 ± 0.01 (2.42 – 2.80)	< 0.05

Note: *F_b – first batch fecundity; F_t – Total fecundity; RF_b – Relative first batch Fecundity; RF_t – Relative Total Fecundity; SE – Standard Error; Minimum (min) and Maximum (max); ind. – individual.*

3.3.9. The length–weight relationships and growth pattern

The results show that absolute fecundity (Ft) is positively correlated with body size (L_t and W_t) ($p < 0,05$). (Fig. 3.10)

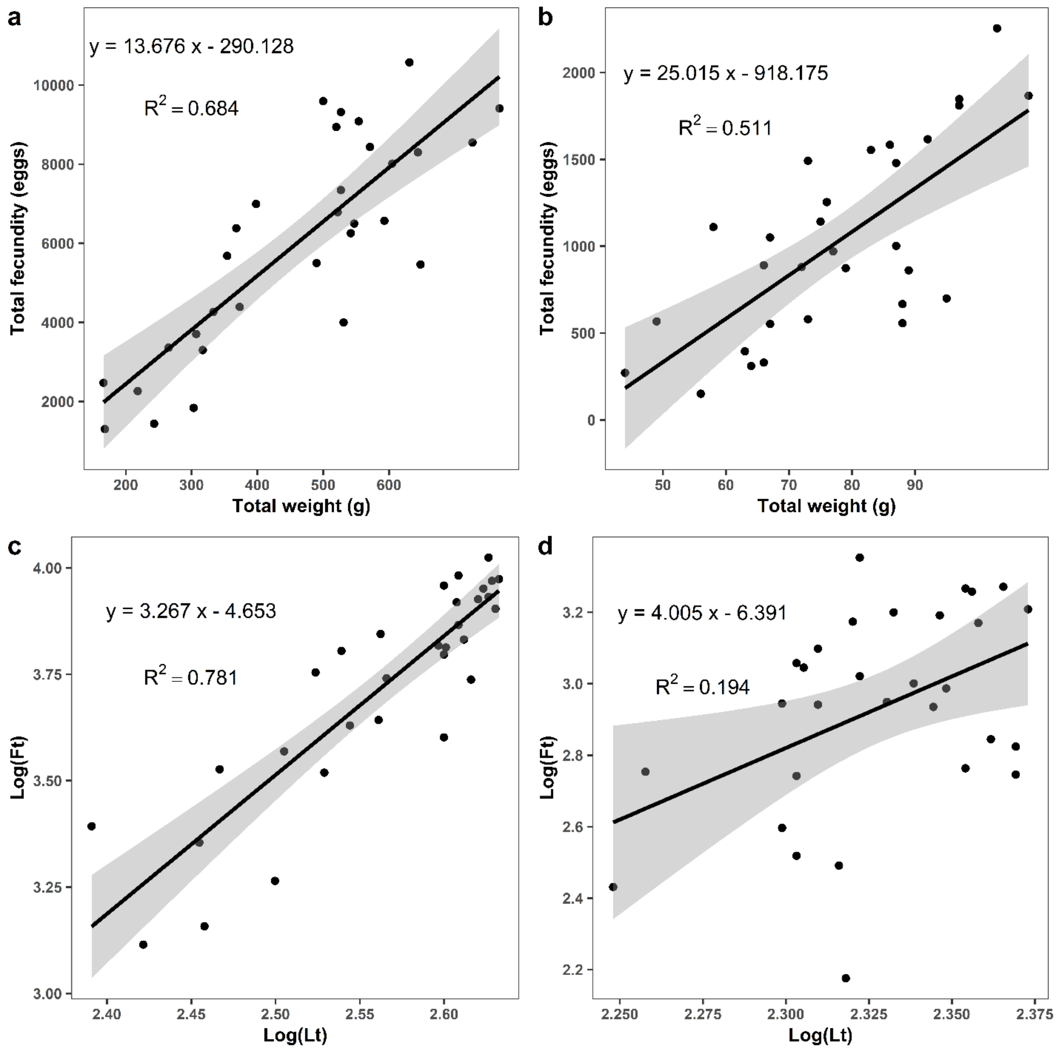

Figure 3.10: Correlation between total fecundity (Ft, eggs) and total weight (Wt, g) of the *P. disjunctivus* introduced in Vietnam. The grey area indicates a 95% confidence interval: (a) – Dinh River; (b) – Suoi Trau Reservoir. And correlation between total fecundity - Log(Ft) and total length - Log(Lt): (c) – Dinh River; (d) – Suoi Trau Reservoir

In general, the study results showed that the size, fecundity and egg diameter of suckermouth catfishes in limnic habitats were smaller than in lotic habitats.

3.4. Food spectrum

The fish analyzed for the food spectrum had an average total length of about 25 cm (ranging from 10 to 41 cm), and an average body weight of 148 g (ranging from 11 to 408 g). The fish that was obtained had a very long gastrointestinal tract, the average length of the gastrointestinal tract was 277 cm (from 112 cm to 606 cm). The length of the gastrointestinal tract of the studied armored catfishes of different sizes varied widely and exceeded the total length of fish 12 – 17 times (RLG = 7 – 12). There was a strong positive correlation between gastrointestinal tract length and total length (p < 0.01) as shown in Figure 3.11. Research results show that, the larger the fish size, the longer length of the gastrointestinal tract.

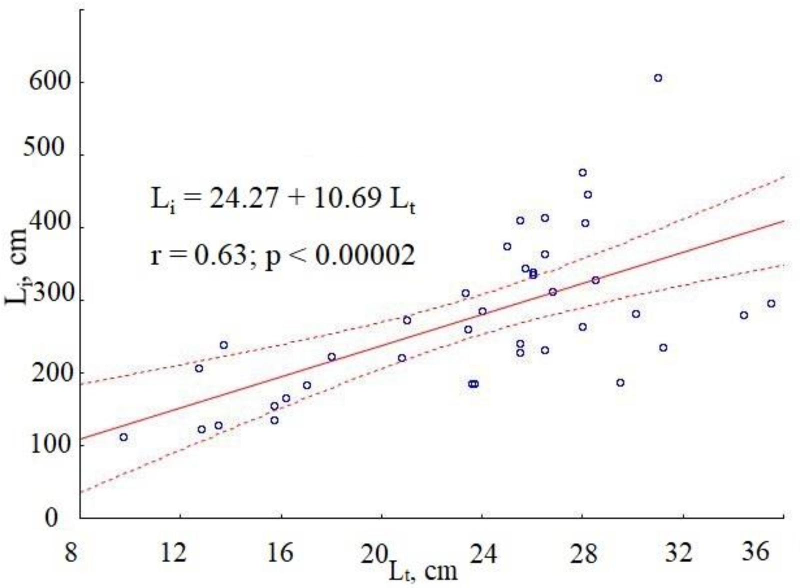

Figure 3.11: Regression dependence of the intestinal length (Li, cm) on the total body length (L_t, cm) of suckermouth armored catfishes.

The weight of the food bolus of the studied specimens of suckermouth armored catfish varied widely from 0.04 g to 34.35 g (on average ~ 5 g). The food spectrum of suckermouth armored catfish included mineral, plant, and animal ingredients. The main components of the food bolus were mostly represented by detritus. The results showed that fish have eaten typical detritus (plant debris). The main mineral and plant components of the food bolus were mostly represented by small fractions of bottom sediments (clay, pelitic, and powdery sands), microscopic and larger detritus particles of uncertain origin, and plant remains. Sediments and fine various detrital particles prevailed in the diet of most studied specimens. However, a high content of relatively large (> 5 mm) remains of higher semiaquatic and aquatic vegetation, as well as medium and large fractions of sand, which constituted 1–3% of the total mass of the fish food bolus, was found in the food bolus of some fish.

The content of animal food in the digestive tract of armored catfishes was relatively low. Benthic, planktonic, and amphibiotic groups of organisms were found. The total biomass of animal food components of suckermouth armored catfishes was on average ~ 0.1% of the total weight of the fish food (in some specimens, 0.3 – 1.0%). Nematodes (P = 50%), cladocerans of the genus Bosminopsis (*Bosminopsis* sp.; P = 50%), cyclopoids (P = 50%) and ostracods *Cypria cf. furfuracea* (P = 75%) and chironomid larvae (P = 75%) prevailed in respect to the frequency of occurrence in the animal food of suckermouth armored catfishes. Copepods and the ostracod *C. cf. furfuracea* prevailed in the bolus in terms of abundance 139 and 75 individuals/sample, respectively (Table 3.5). The average size of invertebrates in fish diet did not exceed 3–5 mm.

The detection of different ecological groups of animal origin in the food spectrum of suckermouth armored catfish showed that these groups were mainly benthic and zooplankton. In addition, groups of amphibians and terrestrial animals were also found in the food spectrum of suckermouth armored catfish.

Table 3.5. The bolus components of suckermouth armored catfishes *Pterygoplichthys* spp. from different habitats and total sample.

Component	Dinh River (*n* = 9)			Suoi Trau R (*n* = 7)			Am Chua Canal (*n* = 7)			Serepok Canal (*n* = 7)			Ea Kao (*n* = 10)			Total (*n* = 40)		
	P	*N*	w_i	*P*	*N*	w_i	*P*	*N*	w_i	*P*	*N*	w_i	*P*	*N*	w_i	*P*	*N*	w_i
Detritus, mineral, plant and other undetermined remains	**100**	**–**	**5674**	**100**	**–**	**1355**	**100**	**–**	**4987**	**100**	**–**	**12896**	**100**	**–**	**3473**	**100**	**–**	**5511**
Sand	**22**	**–**	**31**	**–**	**–**	**–**	**14**	**–**	**7**	**–**	**–**	**–**	**–**	**–**	**–**	**8**	**–**	**8**
Nematoda (ind.)	78	6	<1	14	<1	<1	86	3	<1	43	22	<1	30	1	<1	**50**	6	<1
Acari (aquatic; ind.)	11	<1	<1	–	–	–	–	–	–	–	–	–	–	–	–	3	<1	<1
Acari (terrestrial; ind.)	56	9	<1	14	<1	<1	–	–	–	14	<1	<1	–	–	–	18	2	<1
Cladocera (*Bosminopsis* sp.)	11	<1	<1	57	3	<1	29	1	<1	–	–	–	90	17	<1	**40**	5	<1
Cladocera (Chidoridae gen. spp.)	–	–	–	43	1	<1	–	–	–	43	13	<1	10	<1	<1	18	3	<1
Cladocera (Macrothricidae gen. spp.)	11	1	<1	–	–	–	43	<1	<1	14	<1	<1	–	–	–	13	<1	<1
Cladocera (*Ilyocryptus* spp.)	11	1	<1	29	1	<1	57	2	<1	–	–	–	30	1	<1	25	1	<1
Cladocera (Sididae gen. spp.)	–	–	–	–	–	–	43	1	<1	14	95	1	60	1	<1	25	17	<1
Cladocera (ind.)	33	1	<1	–	–	–	–	–	–	14	1	<1	40	2	<1	20	1	<1
Copepoda (Cyclopoidae gen. spp.)	100	4	<1	43	2	<1	100	47	1	100	2897	17	60	2	<1	**80**	517	3
Copepoda (nauplii)	–	–	–	–	–	–	14	<1	<1	14	1	<1	–	–	–	5	<1	<1
Copepoda (Diaptomidae gen. spp.)	11	<1	<1	–	–	–	–	–	–	14	45	4	–	–	–	5	8	1
Copepoda (Harpacticoida; ind.)	11	<1	<1	–	–	–	–	–	–	29	<1	<1	20	<1		13	<1	<1
Ostracoda (*Cypria* cf. *furfuracea*)	44	2	<1	86	56	1	100	215	1	57	14	<1	90	356	4	**75**	139	1
Ostracoda (ind.)	22	3	1	43	<1	<1	71	6	<1	29	1	<1	50	2	<1	43	3	<1
Trichoptera (ind.)	11	1	<1	–	–	–	–	–	–	–	–	–	–	–	–	3	<1	<1
Ephemeroptera (ind.)	22	<1	<1	–	–	–	–	–	–	14	1	<1	–	–	–	8	<1	<1
Hemiptera (Pleidae gen. sp.)	22	<1	<1	–	–	–	–	–	–	–	–	–	–	–	–	5	<1	<1
Hemiptera (*Micronecta* spp.)	11	<1	<1	14	<1	<1	57	1	<1	29	1	<1	60	3	<1	35	1	<1
Coleoptera (larvae; ind.)	22	1	<1	–	–	–	–	–	–	–	–	–	–	–	–	5	<1	<1
Culicidae gen. spp.	11	<1	<1	–	–	–	–	–	–	–	–	–	–	–	–	3	<1	<1
Ceratopogonidae gen. spp	44	73	5	–	–	–	29	<1	<1	43	2	<1	–	–	–	23	17	1
Chironomidae gen. spp.	67	13	2	71	6	<1	86	1	<1	57	18	<1	90	13	2	**75**	10	1
Chironomidae (pupae; ind.)	11	<1	<1	–	–	–	–	–	–	–	–	–	10	<1	<1	5	<1	<1
Diptera (Dolichopodidae gen. spp.?)	11	11	4	–	–	–	–	–	–	–	–	–	–	–	–	3	2	1
Diptera (larvae; ind.)	33	20	4	–	–	–	–	–	–	–	–	–	–	–	–	8	5	1
Insecta (Formicidae)	22	4	<1	–	–	–	–	–	–	–	–	–	–	–	–	5	1	<1
Insecta (terrestrial; ind.)	11	<1	<1	–	–	–	–	–	–	–	–	–	–	–	–	3	<1	<1

Component	**Dinh River (*n* = 9)**			**Suoi Trau R (*n* = 7)**			**Am Chua Canal (*n* = 7)**			**Serepok Canal (*n* = 7)**			**Ea Kao (*n* = 10)**			**Total (*n* = 40)**		
	P	*N*	w_i	*P*	*N*	w_i	*P*	*N*	w_i	*P*	*N*	w_i	*P*	*N*	w_i	*P*	*N*	w_i
Mollusca (*Hippeutus* sp.?)	–	–	–	–	–	–	14	<1	<1	–	–	–	–	–	–	3	<1	<1
Mollusca (Pisidiidae gen. spp.)	11	<1	<1	–	–	–	14	<1	<1	–	–	–	–	–	–	5	<1	<1
All animal components	**100**	**151**	**17**	**100**	**747**	**11**	**100**	**278**	**2**	**100**	**3111**	**23**	**90**	**398**	**6**	**98**	**739**	**10**
Benthic	89	33	29	100	65	1	100	229	1	71	71	1	90	376	6	90	165	3
Planktonic	100	5	<1	57	5	<1	100	49	1	100	3039	22	90	22	<1	90	548	4
Amphibiotic	33	100	13	–	–	–	–	–	–	–	–	–	–	–	–	8	22	3
Terrestrial	56	13	1	14	<1	<1	–	–	–	14	<1	<1	–	–	–	18	3	<1

Note: P – Occurrence (%); N – average abundance (ind.); Wi – the average recovered weight of the bolus (mg); n – sample size; "–" indicates absence in the bolus; <1 – is the average abundance and average recovered weight, < 1 ind. and 1 < mg, respectively.

CHAPTER 4. CONCLUSIONS AND RECOMMENDATIONS

4.1. CONCLUSIONS

1. Suckermouth armored catfishes, genus *Pterygoplychthys* (Loricariidae) have appeared in most of the river basins of Southern Vietnam such as the down Mekong River (Cuu Long River), Dong Nai River, Serepok River, Da Rang River and other small river systems. They occurred in many types of water bodies such as rivers, streams, reservoirs, ponds, canals, and rice fields and in many different geographical areas from lowland to highland areas with a rate up to 66.67%. Suckermouth armored catfishes are distributed in estuary areas where salinity ranges from 4–25 PSU.

2. Suckermouth armored catfishes collected from the waters of Southern Vietnam, have from 10 to 13 soft fin rays and belong to the genus *Pterygoplichthys*. The ventral pattern of the Suckermouth armored catfishes was not only expressed by *P. pardalis* and *P. disjunctivus*, but also had a hybrid between the two species mentioned above. Morphological and molecular genetics analysis gave reliable results on the species composition of the armored catfishes (including two species of *P. pardalis* and *P. disjunctivus*), each represented by different phylogenetic branches.

3. The ratio of suckermouth armored catfish (*P. disjunctivus*) males and females was 1:1. The matured fish presented all year round, but with a higher percentage during April-October and peaked in July, August. The total length at 50% maturity of females was ≈ 18 cm for fish from Suoi Trau Reservoir and ≈ 23,4 cm for fish from Dinh River. They had a relatively large reproductive capacity and were positively correlated with body mass. There was a difference in absolute fecundity in Dinh River and Suoi Trau Reservoir, respectively 4812 ± 383 eggs/individual and 841 ± 91 eggs/individual ($p < 0.05$). However, there was no difference in relative fecundity in the two water bodies, reaching about 13 eggs/g and fish can spawn one or more times a year. Egg diameter in fishes from Dinh River was significantly bigger than Suoi Trau Reservoir, 2.94 ± 0.03 mm and 2.58 ± 0.01 mm, respectively ($p < 0.05$). Fish tend to grow faster in length than in weight with the growth parameter b from 2.66 to 2.82.

4. Suckermouth armored catfishes have eaten detritus typically with long digestive tracks of mean 277 cm and long digestive tracks were greater by 12 to 17 times than the total length. Animalistic ingredients in the food spectrum of suckermouth armored catfishes with high-frequency groups were Nematodes, Cladocera, crustaceans (Cyclopoidae), Ostracods and Chironomidae. Although, the biomass of animalistic ingredients was low, accounting for only about 0.1% of the weight of the food spectrum, the suckermouth armored catfishes will compete for food with native fish species and affect the food web in the water bodies.

4.2. RECOMMENDATIONS

1. Next study the mechanisms of adaptation to the salinity of suckermouth armored catfishes under experimental and field conditions.

2. Proving the phenomenon of interspecific hybrid requires further studies on genetics and experimental studies of crossbreeding in artificial reproduction.

3. Next study on the rate of biomass of suckermouth armored catfishes and converted change native species fish composition in water bodies. Since then, there are important evidence about the harmful effects of the suckermouth armored catfishes.

4. The government and organizations on alien species should pay more attention to the management and take measures to prevent the invasion of the suckermouth armored catfishes.

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