Research Article

Morphometric Landmark Measurements and Length Weight Relationship of Sail Fin Cat Fishes, *Pterygoplichthys* Species (Family Loricariidae) in Victoria and Kalawewa Reservoirs, Sri Lanka

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Abstract

Species of genus Pterygoplichthys; Pterygoplichthys disjunctivus and Pterygoplichthys pardalis, have been reported from seven provinces of Sri Lanka which have significant impacts on fish fauna and fisheries activities. The length weight relationship of P. disjunctivus in the current study is useful in identifying growth and mortality parameters where prediction of yields and corresponding exploitation rates. Fish specimen of P. disjunctivus were collected from Kalawewa and Victoria reservoirs during the period of January to December 2015, 2016 and 2017 for length weight analysis. Pterygoplichthys species were also collected for morphometric landmark measurements. Samples were preserved in ice and transferred to the laboratory. Fish specimens were grouped into five categories according to their abdominal spots and vermiculations. Thirty two morphometric landmarks measurements of fish specimen were measured with the use of digital venire caliper to the nearest 0.1 mm. Length weight relationships of both females and males of P. disjunctivus in Kalawewa & Victoria reservoirs indicated an allometric growth for both sexes. Condition factor value of both sexes was significantly different indicating a healthy condition. There was no significant difference between the five fish categories based on the 32 morphometric measurements, one-way multivariate analysis of variance (MANOVA) and canonical variate analysis were performed. MANOVA resulted a significantly different value for the morphometric landmark measurements. Canonical variate analysis and 95% significance regions showed five clusters for the five fish categories in Victoria and four clusters in Kalawewa. This shows five Pterygoplichthys fish categories are present in the Victoria reservoir and at least four fish groups were present in Kalawewa reservoir. The results should be further addressed using molecular approach.

Keywords: *Pterygoplichthys species, morphometric landmark measurements*

1. Introduction

Pterygoplichthys species are endemic to neo-tropical South America (Armbruster, 2004). This widespread invasive species are common in the tropical fish trade and has been introduced to different regions of the world such as North America, Asia and Europe (Nico, 2009; Simonovic, 2010; Hui Wei, 2017) by aquarium release or escaping from aquaculture farms (Page & Robins, 2006). Accidental introduction of South American sail fin catfish through ornamental fish industry is one of the main environmental concerns in Sri Lankan reservoirs (Marambe *et al.,* 2011). Species of genus *Pterygoplichthys*; *P. disjunctivus, Pterygoplichthys pardalis,* have been reported from seven provinces of Sri Lanka (Epa, 2014). *Pterygoplichthys* spp. have significant impacts on fish fauna and fisheries activities in Sri Lankan reservoirs such as competition for food niche and entangling into gillnets (Sumanasinghe & Amarasinghe, 2014; Wijethunga & Epa, 2008). Hence identification of its growth pattern and possible hybrids are essential in controlling/ eradication these species. Length weight relationships of fishes are important to study the growth pattern of fish stocks and are of practical importance. The length weight relationship is useful in studies of fish population dynamics (Pauly, 1993), and to estimate fish yields (Beverton & Holt, 1979; Ricker, 1975). Length and weight data are further useful in assessing the fish landings and provide baseline information in linking the populations over space and time (Chanchal *et al.,* 1978). Length weight relationship also provides evidence on fish growth, gonadal maturation and fish condition (Le Cren, 1951) and consequently, suitable for assessment of body forms of diverse groups of fishes. Rendering to the cube law of length-weight relationship, the weight of the fish may differ as per the cube of length. Le Cren, (1951) suggested comparative condition factor (K) which reflects all the differences of K like those connected with food and feeding, sexual maturity, etc., when the exponent value is equal to 3. Therefore 'K' factor acts on the differences from a model fish, which holds the cube law of the length-weight relationship (Dar *et al.,* 2012). Significance in length weight relationship has also been discoursed by Pauly, (1980); Koutrakis & Tsikliras, (2003); Abdurahiman *et al.,* (2004); Olurin & Aderibigbe, (2006); Hossain *et al.,* (2012); etc.

The morphometric characteristic analysis of fish is an essential key in the study of biology of fish (Murta, 2000). Growth of different body parts of change at different development stages especially at sexual maturity (Bhuiyan & Islam, 1990). Mekkawy & Mahmoud, (1992a, b), stated that growth of the body parts is proportional to the growth of the total length. Thus, in taxonomic studies, fish morphometric measurements with study of statistical relationship among these measurements are essential for studying species (Tandon *et al.,* 1993, Turan *et al.,* 2005; Randall & Pyle, 2008). Bookstein, (1982) also stated the importance of landmark based measurements. The concept of landmarks based measurements has been found to be usable in identification of fish stocks in fisheries (Cadrin, 2000).

Individual shapes of fish can be detected using landmarks which denote to some randomly selected points of a fish body (Cadrin & Silva, 2005). Hence, these measurements also falls into morphometric characteristics of fishes. These measurements may vary with natural and sexual selection within a species. Hossain *et al.*, (2010), Trajano & Britski, (1992), also identified that landmark points are powerful tools which can be used for the stock identification of fish species.

Landmark measurements which allow visualizing of shape changes through diagrams have also been developed recently for Gonia, *Labeo gonius* (Begum *et al.*, 2013), Kalibaus, *Labeo calbasu* (Hossain *et al.*, 2010), Rohu *Labeo rohita* (Hasan & Bart, 2007) and Thai Pangas, *Pangasius hypophthalmus* (Barua *et al.*, 2004). Landmark measurements can be paired with molecular techniques which can be used for the study of cave fishes (Cobolli-Sbordoni *et al.*, 1996; Borowsky & Mertz, 2001; Espinasa & Borowsky, 2001; Strecker *et al.*, 2003).

The length weight relationship of *P. disjunctivus* in the current study is useful in identifying the growth pattern of it (isometric or allometric growth) and estimating the condition factor value for different localities/reservoirs which can be compared to identify better locality with high condition factor value which in turn be useful in identifying the reasons for establishment/expansion of its populations in different reservoirs. It also provides the basement to estimate the growth and mortality parameters where prediction of yields and corresponding exploitation rates.Literature on *Pterygoplichthys* species/stock identification are lacking in Sri Lanka. So by analysing landmark based measurements, the fish species and fish stocks can be identified. Furthermore it provides the baseline information for genetic population structure of *Pterygoplichthys* species which help in constructing its phylogeny. The possible hybrids of *Pterygoplichthys* may be more successful in invading Sri Lankan water bodies. So identification of possible hybrids is essential in controlling/eradication of *Pterygoplithys* species.

2. Materials and Methods

Length Weight relationships - Fish specimens of *P. disjunctivus* were collected monthly from Kalawewa and Victoria reservoirs during the period of January to December 2015, 2016 and 2017, (Female specimens: Victoria - 337; Kalwewa - 325: male specimens Victoria - 231; Kalawewa - 292). The two reservoirs are morphometrically different and located in two climatic zones where exists a fishery with records of by catch of *P. disjunctivus*. Fish were correctly identified as *P. disjunctivus* using the keys of Ambruster and Page, (2006). Length and weight of the fish specimens of two reservoirs were measured to the nearest 0.1 cm and 0.1 g respectively. The length-weight relationships were estimated for each reservoir using the linear form of the formula as follows,

$$W = aL^b \text{ (Le Cren, 1951)} \quad (1)$$
$$\log W = \log a + b \log L$$

where,

W - Denotes the weight of the fish,

L - Denotes the total length of the fish

a and b are constants

Condition factor value (C) was calculated using the following equation assuming the b value is equal to 3 i.e. isometric growth patterns.

$$C = (W/L^3) \text{ (Fulton, 1902)} \quad (2)$$

Above procedure was followed for separate sexes in Victoria and Kalawewa reservoirs from January to December 2015, 2016 and 2017, respectively.

Morphometric landmarks - Fish samples from commercial landings of Kalawewa (138) and Victoria (111) were collected monthly during the period of January to December 2015, 2016 and 2017. Samples were preserved in ice and transferred to the laboratory. Fish specimens were grouped into five categories according to their abdominal spots and vermiculations (Plate 1). Thirty two morphometric landmarks measurements of fish specimens were made with the use of digital venire caliper to the nearest 0.1 mm. Table 1 indicates the different morphometric landmark measurements and the Figure 1 shows the distribution of different landmarks of fish body according to Armbruster, (2003) one-way multivariate analysis of variance (MANOVA) statistical test was performed for the raw data of morphometric landmark measurements, since all fishes were matured. Here to test the null hypothesis, there was no significant difference between the five fish categories based on the 32 measurements, one-way multivariate analysis of variance (MANOVA) was performed to check the significance of the morphometric landmarks measurements for the two reservoirs separately.

A follow up analysis to determine which fish groups were significantly different from each other canonical variate analysis was performed using the morphometric landmarks measurements. A two-dimensional scatter plot of the first two canonical variables was used in the process of identifying group separation. Analyses were performed using the university edition of SAS® software, GLM and CANDISC procedures. The graphs were created using Microsoft Excel 2016.

Table 1: Definitions of morphometric landmarks (Armbruster, 2003)

	Morphometric landmark
1	Tip of snout
2	Posteromedial border of posterior left naris
3	Posteromedial border of posterior right naris
4	Anterior border of left orbit
5	Posterior border of left orbit
6	Posterior border of right orbit
7	Posteromedial margin of supra occipital
8	Posterior margin of right cleithrum
9	Posterior margin of left cleithrum
10	Anterior margin of dorsal-fin spinelet
11	Tip of dorsal-fin spine
12	Insertion of pectoral-fin spine
13	Insertion of pelvic-fin spine
14	Insertion of anal-fin spine
15	Posterior margin of last lateral plate of ventral plate series
16	Insertion of last dorsal-fin ray
17	Insertion of adipose-fin spine
18	Tip of adipose-fin spine
19	Posterior margin of last lateral plate of dorsal plate series
20	Posterior margin of last lateral plate of median plate series
21	Posterior angle of right maxillary barbel and lip
22	Posterior angle of left maxillary barbel and lip
23	Tip of left barbel
24	Posteromedial margin of lip
25	Lateral margin of dentary tooth cup
26	Medial margin of dentary tooth cup
27	Lateral margin of premaxillary tooth cup
28	Medial margin of premaxillary tooth cup
29	Tip of pectoral-fin spine
30	Tip of pelvic-fin spine
31	Tip of anal-fin spine

Table 2: Morphometric landmark measurements based of Figure 1

	Distance	Landmark measurement
1	1–20	Standard Length
2	1–10	Predorsal Length
3	1–7	Head Length
4	7–10	Head-dorsal Length
5	8–9	Cleithral Width
6	1–12	Head-pectoral Length
7	12–13	Thorax Length
8	12–29	Pectoral-spine Length
9	13–14	Abdominal Length
10	13–30	Pelvic-spine Length
11	14–15	Postanal Length
12	14–31	Anal-fin spine Length
13	10–12	Dorsal-pectoral Depth
14	10–11	Dorsal spine Length
15	10–13	Dorsal-pelvic Depth
16	10–16	Dorsal-fin base Length
17	16–17	Dorsal-adipose Depth
18	17–18	Dorsal Adipose-caudal Depth
19	15–19	Caudal peduncle Depth
20	15–17	Ventral adipose-caudal Depth
21	14–17	Adipose-anal Depth
22	14–16	Dorsal-anal Depth
23	13–16	Pelvic-dorsal Depth
24	5–7	Head-eye Length
25	4–5	Orbit Diameter
26	1–4	Snout Length
27	2–3	Internares Width

28	5–6	Interorbital Width
29	7–12	Head Depth
30	1–24	Mouth Length
31	21–22	Mouth Width
32	22–23	Barbel Length

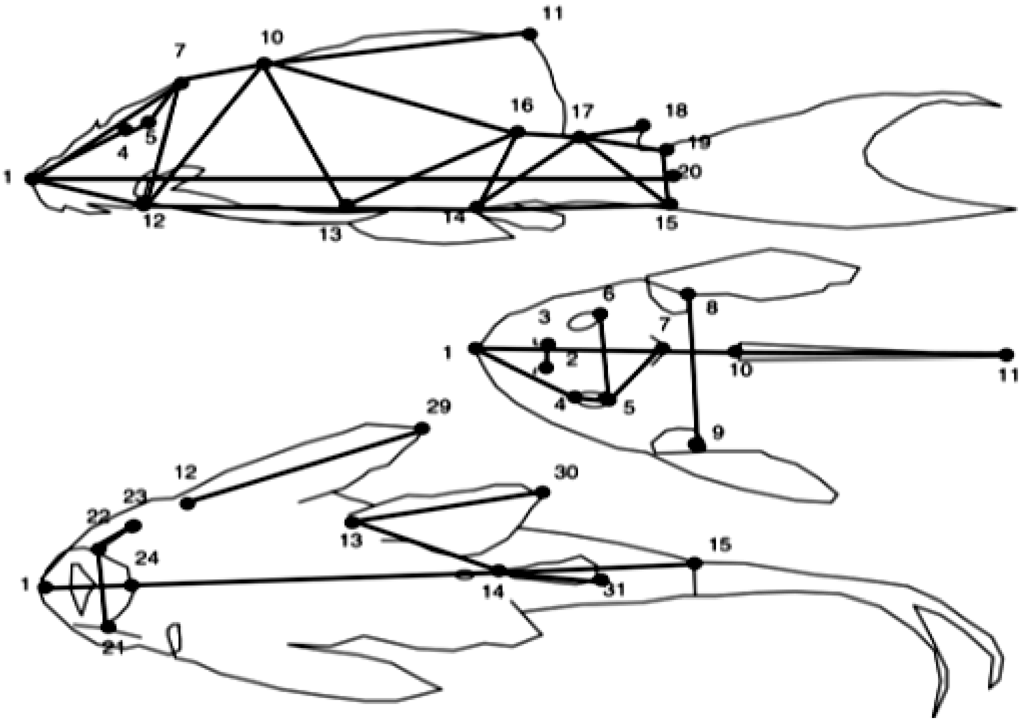
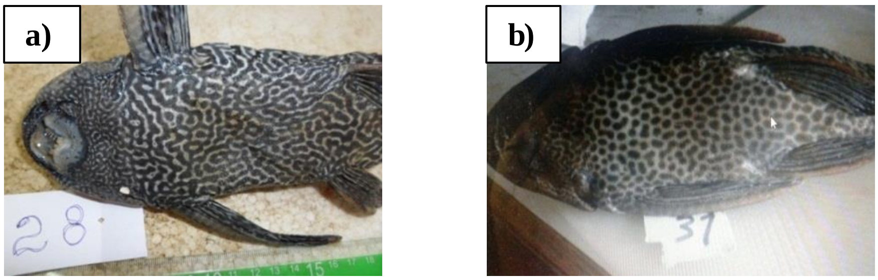


Figure 1: Morphometric landmarks of *Pterygoplichthys* sp.



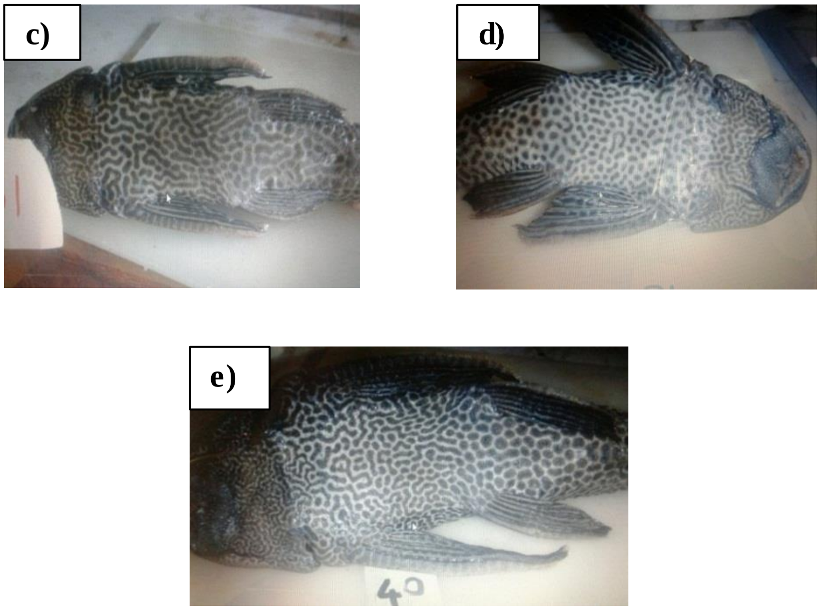


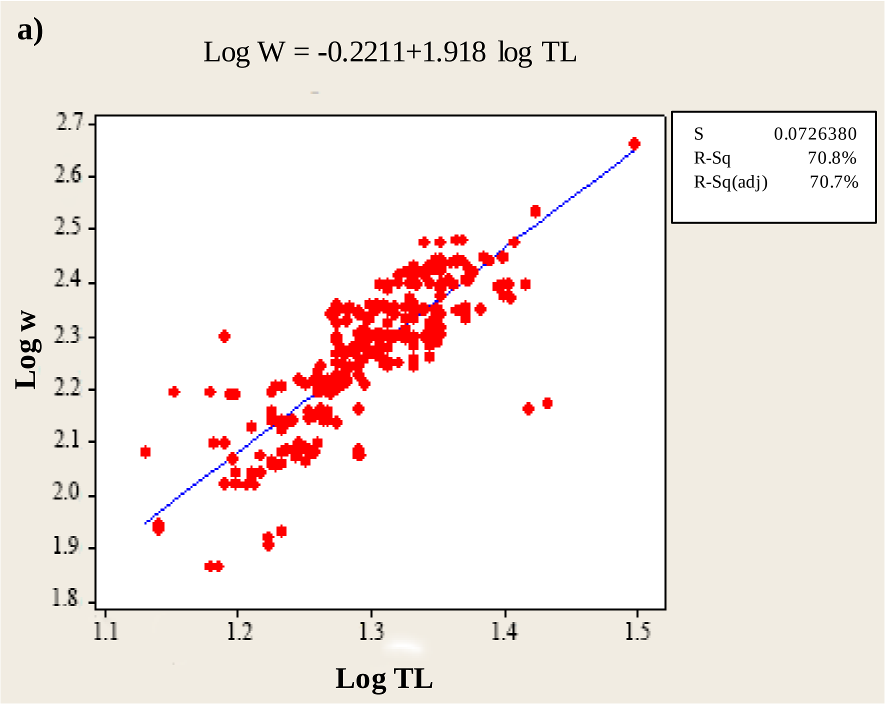
Plate 1: Different color patterns observed in the fish samples from Kalawewa and Victoria reservoirs. Fish were grouped into different abdominal spots and vermiculations as (a) *P. pardalis* (b) *P. disjunctivus* (c) intermediate specimen (d) close to *P. pardalis* (e) close to *P. disjunctivus*

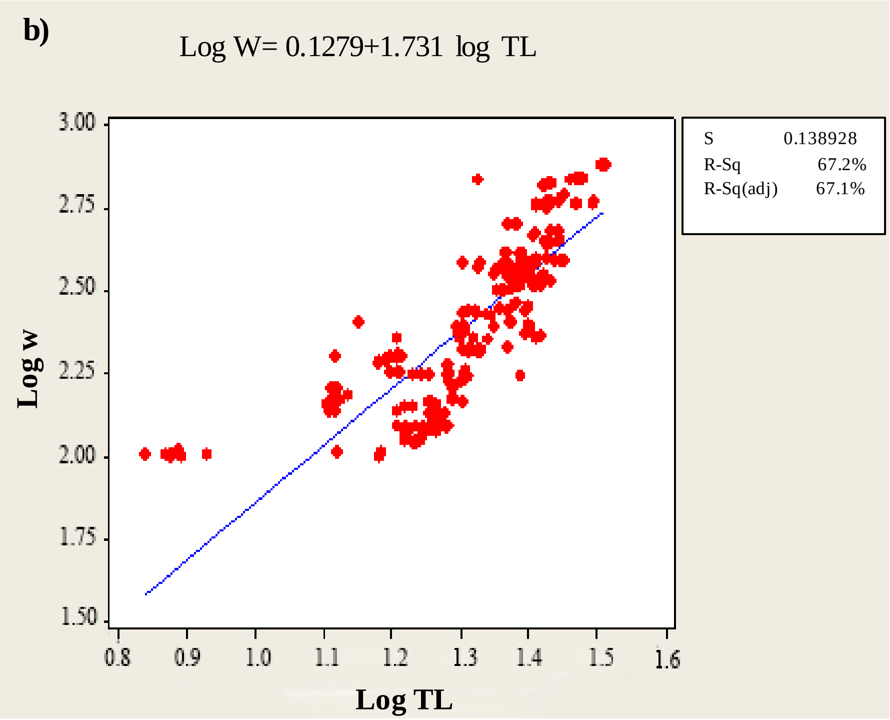
3. Results

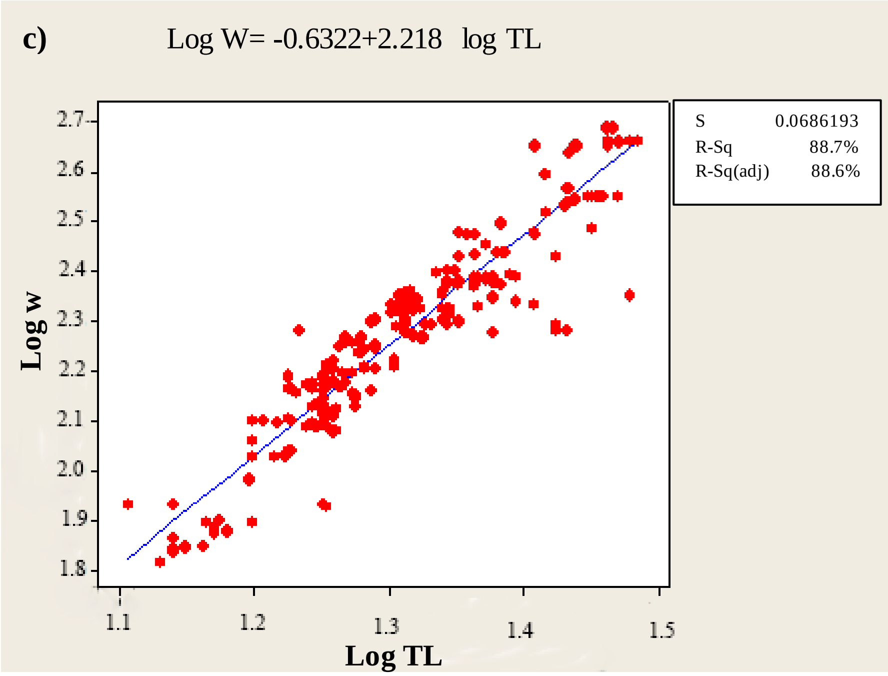
Length weight relationships of both females and males of *P. disjunctivus* in Kalawewa & Victoria reservoirs indicated an allometric growth ($b \neq 3$) for both sexes (Table 3 and Figure 2). Condition factor value of females of Kalwewa reservoir is significantly different from that of females of Victoria reservoirs.Condition factor values of males of both the reservoirs ($p < 0.05$) indicating a healthy condition (Table 3).

Table 3: Length weight relationships, condition factor values of both females and males of *P. disjunctivus* in Kalawewa & Victoria reservoirs

	Gender (F/M)	Kalaweva	Victoria	Probability
Length weight relationship	Females	log W = 0.1279 + 1.731 log TL	log W = -0.2211 + 1.918 log TL	<0.05
	Males	log W = -0.6540 + 2.298 log TL	log W = -0.6322 + 2.218 log TL	<0.05
Condition factor	Females	0.033 ± 0.032	0.024 ± 0.005	<0.05
	Males	0.0244 ± 0.006	0.022 ± 0.004	<0.05


a)
Log W = -0.2211+1.918 log TL
S 0.0726380
R-Sq 70.8%
R-Sq(adj) 70.7%
Log w
Log TL
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.1
1.2
1.3
1.4
1.5


b)
Log W= 0.1279+1.731 log TL
S 0.138928
R-Sq 67.2%
R-Sq(adj) 67.1%
Log w
Log TL
3.00
2.75
2.50
2.25
2.00
1.75
1.50
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6



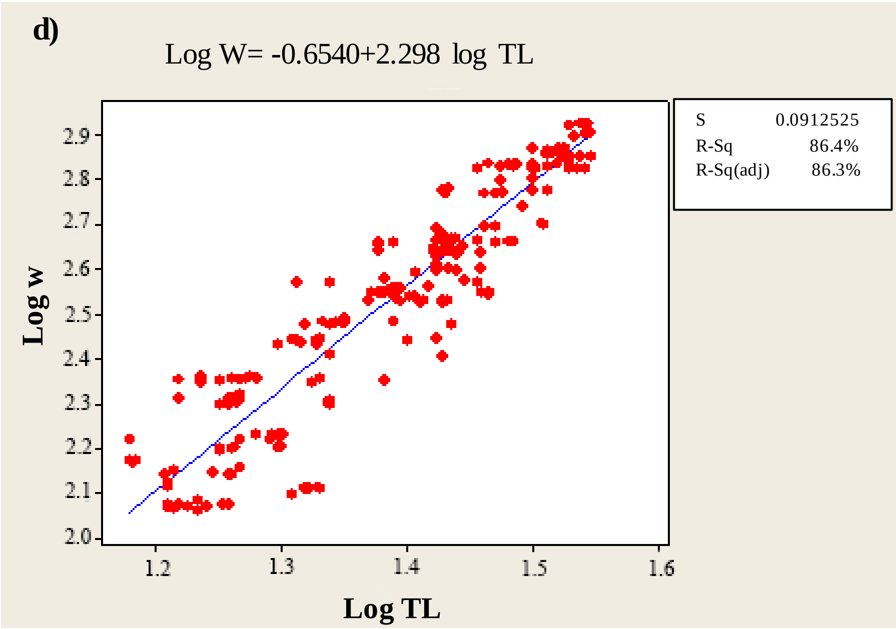


Figure 2: Length weight relationships of females and males of Victoria and Kalawewa reservoir. (a). Females – Victoria reservoir (b). Females – Kalawewa reservoir (c). Males - Victoria reservoir (d). Males - Kalawewa reservoir

Morphometric landmark measurements of fish specimens which were categorized into fish specimen groups (Plate 1) harvested from Victoria and Kalawewa reservoirs were analysed using MANOVA and canonical variable analysis. Table 4 indicates MANOVA test output for morphometric landmarks measurements of Victoria reservoir.Based on results of the MANOVA output, it could be seen that all 4 test statistics are significant (Table 4: $p<0.05$). This concludes that at least two of the groups in the morphometric landmarks measurements for the five fish categories (Plate 1) of Victoria reservoir were significantly different from each other. (The most commonly referred test statistic is Wilk's Lambda).

Canonical variate analysis method generates a new set of independent variables (called canonical variables) using the original 31 variables of morphometric landmarks measurements, that maximized the difference between groups. As more than one of the generated canonical variables were significant (having non-zero roots), the first two (most important) canonical variables were graphed using scatter plots and the 95% confidence region around the centroids for each fish group was plotted to identify the level of separation of the original groups. According to the data a maximum of 4 (the number of groups minus one) canonical variables were created. With regard to the data of Victoria reservoir all 4 of the created canonical variables became significant. Since the distinction of differences in a four-dimensional space was difficult to visualize, a two-dimensional scatter plot of the first two canonical variables were used in the process of identifying group separation. Figure 2 shows the scatter of individual observations according to group {1: *P. pardalis*, 2: *P. disjunctivus* , 3: Intermediate specimen, 4: Close to *P. pardalis*, 5: Close to *P. disjunctivus* (Plate 1)}

Table 4: MANOVA test output for morphometric landmarks measurements of Victoria reservoir

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall FISGRUP Effect

H = Type III SSCP Matrix for FISGRUP
E = Error SSCP Matrix

S=4 M=14.5 N=35.5

Statistic	Value	F Value	Number DF	Den DF	p > F
Wilks' Lambda	0.00110563	9.77	136	293.25	<.0001
Pillai's Trace	2.99078157	6.62	136	304	<.0001
Hotelling-Lawley Trace	31.38509026	16.52	136	239.84	<.0001
Roy's Greatest Root	23.84893929	53.31	34	76	<.0001

Note: F Statistic for Roy's Greatest Root is an upper bound.

Figure 4 indicates that the 95% confidence regions of each group (according to Plate 1) from their centroids (the mean coordinates of each group was based on canonical variable 1 and 2). As none of the 95% confidence regions overlap with each other, it is possible to state that all 5 groups of fish are distinctly different from each other based on the morphometric landmark measurements for the five fish categories (Plate 1).

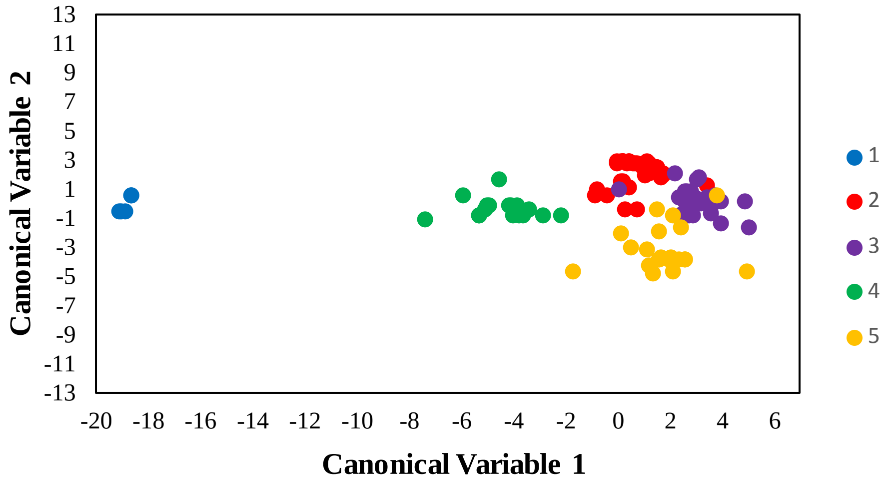


Figure 3: Scatter plot of 1st two canonical variables of morphometric landmarks measurements in Victoria for the five fish categories (Plate 1) (legend: - 1: *P. pardalis*, 2 : *P. disjunctivus* , 3: Intermediate specimen, 4: close to *P. pardalis*, 5. Close to *P. disjunctivus*)

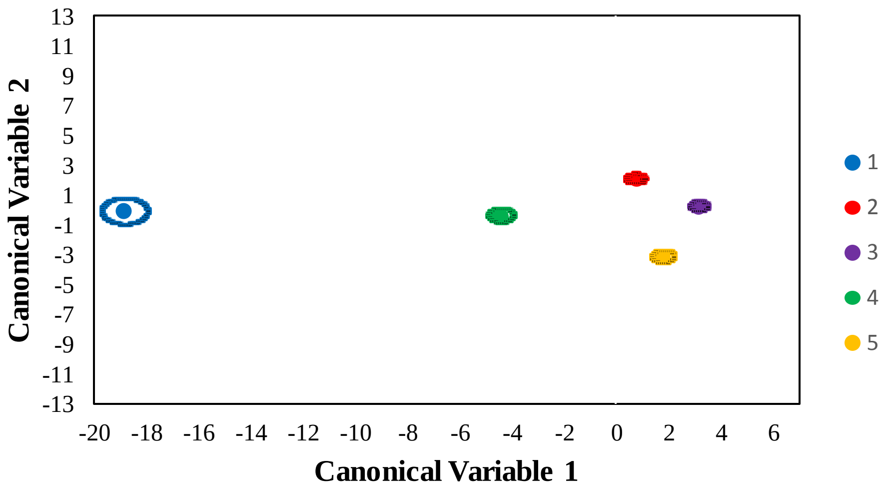


Figure 4: Group 95% confidence regions for first two Canonical Variables of morphometric landmarks measurements in Victoria for the five fish categories (Plate 1) (legend: - 1: *P. pardalis*, 2 : *P. disjunctivus* , 3: Intermediate specimen, 4: Close to *P. pardalis*, 5. Close to *P. disjunctivus*)

All of the 4 statistics used in MANOVA to test whether there is a difference between fish groups to show a probability level less than the cut-off 0.05 for Kalawewa (Table 5). This means that at least two of the fish of the groups in the morphometric landmarks measurements of groups for the five fish categories (Plate 1) from the Kalawewa are significantly different from each other. Table 5 shows MANOVA test output for morphometric landmarks measurements of Kalawewa reservoir.

In the follow-up canonical variate analysis 3 out of the possible 4 created canonical variables became significant. Figure 5 shows the scatter plot of individual observations according to group. Graph in Figure 6 indicates the 95% confidence regions of each group is from their centroids (the mean coordinates of each group based on canonical variable 1 and 2). As the 95% confidence regions of groups 1 and 4 overlap each other, it is possible to state that groups 1 and 4 (Plate 1) are not significantly different from each other based on the morphometric measurements. The other 3 groups are different from each other as well as from groups 1 and 4 (Plate 1).

Table 5: MANOVA test output for morphometric landmarks measurements of Kalawewa reservoir

MANOVA Test Criteria and F Approximations for the Hypothesis of No Overall FISGRUP Effect

H = Type III SSCP Matrix for FISGRUP
E = Error SSCP Matrix

S=4 M=14.5 N=49

Statistic	Value	F Value	Number DF	Den DF	p > F
Wilks' Lambda	0.04700277	3.41	136	400.73	<.0001
Pillai's Trace	2.05805879	3.21	136	412	<.0001
Hotelling-Lawley Trace	4.94147919	3.58	136	335.2	<.0001
Roy's Greatest Root	2.13720606	6.47	34	103	<.0001

Note: F Statistic for Roy's Greatest Root is an upper bound.

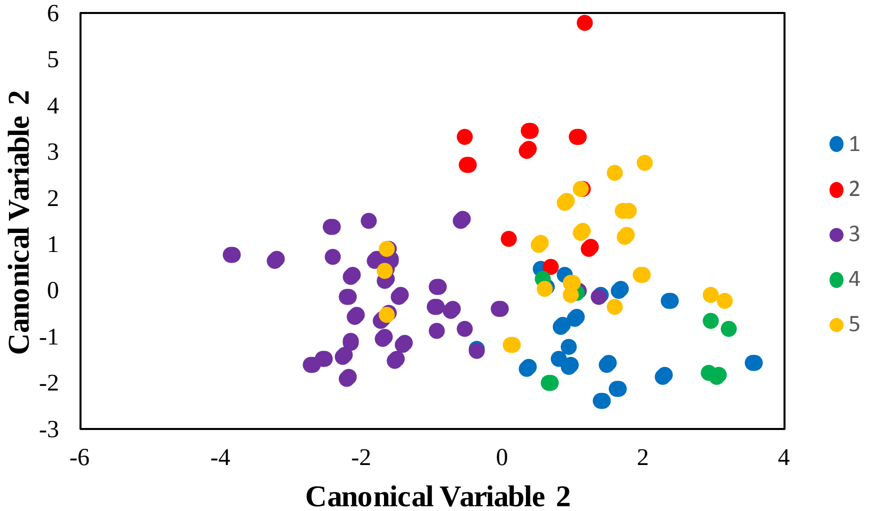


Figure 5: Scatter plot of 1st two canonical variables of morphometric landmarks measurements in Kalawewa for the five fish categories (Plate 1) (legend: - 1: *P. pardalis*, 2 : *P. disjunctivus* , 3: Intermediate specimen, 4: close to *P. pardalis*, 5. close to *P. disjunctivus*)

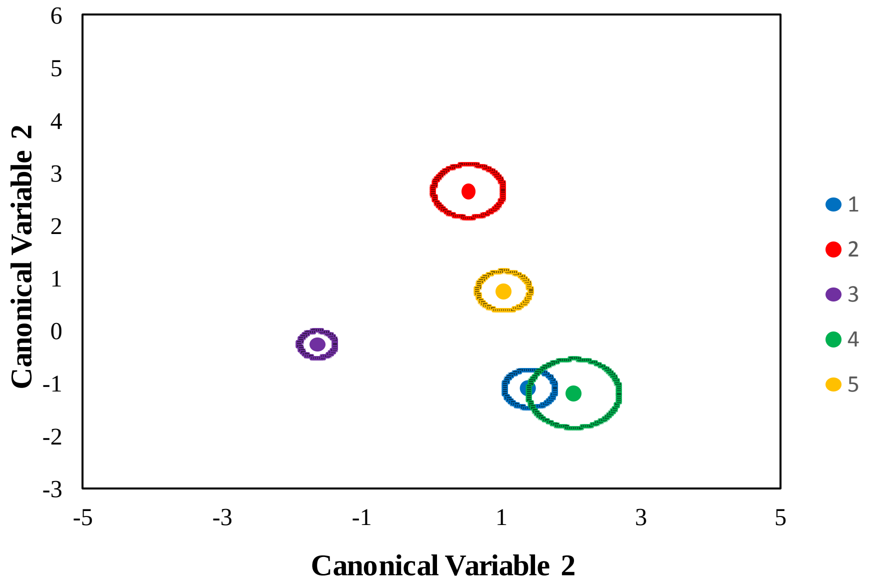


Figure 6: Group 95% confidence regions for first two Canonical Variables of morphometric landmarks measurements in Kalawewa for the five fish categories (Plate 1) (legend: - 1: *P. pardalis*, 2 : *P. disjunctivus* , 3: Intermediate specimen, 4: close to *P. pardalis*, 5. close to *P. disjunctivus*)

3. Discussion

The length weight relationships for females and males for Victoria and Kalawewa reservoirs were highly correlated (r > 70%: $p < 0.05$). Length weight relationships in the current study (Table 3) are consistent with the results of Liang, *et al.,* (2005); Samat *et al.,* (2008) and Wakida-Kusunoki and Amador-del Angel, (2011). Differences in regression coefficient (b) values can be endorsed to the combination of one or more aspects: (i) number of specimens examined; (ii) area ⁄ seasonal effect; (iii) habitat; (iv) degree of stomach fullness; (v) gonadal maturity; (vi) sex; (vii) health and general fish condition; (viii) preservation technique; and (ix) differences in the observed length ranges of the specimens caught (Tesch,1971; Wooten, 1998; Samat *et al.,* 2008), all of which were not accounted for in the present study. While discussing the seasonal effect on length-weight relationship of *Clarias batrachus,* Mitra & Naser, (1987) found that higher metabolic activity with spawning season dropped the 'b' value whereas fewer metabolic activities, gathering of fat, weight of gonad etc., throughout the pre spawning period increased the values and higher regression coefficients in female *Schizopyge esocinus* may perhaps be endorsed to the greater fat accumulation and greater gonadal weight once related to its male counterpart. And there is possibility that local climatic conditions effect 'b' values. The regression coefficient (b) of *P. pardalis* in the Langat River also affected by water levels (Samat *et al.,* 2008). Condition factor value of females of Kalwewa reservoir is significantly different from that of females of Victoria reservoirs as well as condition factor values of males of both the reservoirs showing a healthy condition.

A morphological approach was undertaken to analyze the differences in the genus *Pterygoplichthys* in the two reservoirs which pave future studies including genetic investigations.

According to Trajano & Britski, (1992), Burr *et al.,* (2001) morphometric landmarks which overcome the traditional morphometrics and truss geometric procedures because the configuration of the constructed landmarks covers the entire fish body with no loss of information, and it is more sensitive to change because the configuration of the constructed landmarks covers the entire fish body with no loss of information, and it is more sensitive to change (Trajano & Britski, 1992). Landmark measurements are complementary to molecular studies (Strecker *et al.,* 2003). Study on Baltic sea herring (*Clupea harengus*) have shown that MANOVA and canonical variable tests were sensitive enough to check morphometric variations (Jørgensen *et al.,* 2008). Livingston & Schofield, (1996) successfully discriminate the stocks of hoki (*Macruonus novaezelandiae*) in New Zealand using MANOVA and discriminant function. De Oliveira *et al.,* (2012) and Armbruster *et al.,* (2015) recorded several new Loricariid species using similar morphometric measurements.

MANOVA and canonical variable tests verified five *Pterygoplichthys* fish categories are present in the Victoria reservoir (Figure 4) and at least four fish groups were present in Kalawewa reservoir (Figure 6), but this should be further addressed using molecular approach. In Victoria reservoir highest number of specimen were categorized into group 3 (intermediate fish specimen) which possessed both *P. pardalis* and *P. disjunctivus* abdominal patterns, in Kalawewa second highest number of specimens recorded in the same group indicating possible hybrids in the two reservoirs. According to the results of current study it can be recommended to perform DNA patterns of the fish species and possible hybrids. This may be useful to check whether the hybridization of these two fish species improved their invasive ability.

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